No. 212

FOOD AND ENVIRONMENT PROTECTION ACT, 1985, PART III

Control of Pesticides Regulations 1986

Evaluation of Fully Approved
or Provisionally Approved Products

Evaluation on: **FIPRONIL (HORTICULTURAL USES)**

APRIL 2004

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SAFETY FOR PEOPLE AND THE ENVIRONMENT

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DISCLOSURE DOCUMENT

EVALUATION ON FIPRONIL

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Appendices

ABBREVIATIONS

A:G ratio	albumin:globulin ratio
ACP	Advisory Committee on Pesticides (UK)
ADI	Acceptable daily intake
ADME	Adsorption, distribution, metabolism and excretion
ADR	European agreement concerning the International carriage of dangerous goods by road
ALT	alanine aminotransferase (SGPT)
AOEL	Acceptable operator exposure level
AR	Applied radioactivity
ARfD	Acute reference dose
a.s.	active substance
AST	aspartate aminotransferase (SGOT)
ASTM	American Society for Testing and Materials
at. wt.	atomic weight
b.p.	boiling point
BBA	Federal Biological Research Centre for Agriculture and Forestry, Germany
BCF	bioconcentration factor
bfa	body fluid assay
BOD	biological oxygen demand
bw	body weight
°C	degree centigrade
CAC	Codex Alimentarius Commission
CAS	Chemical Abstracts Service
CCPR	Codex Committee on Pesticide Residues
CDA	controlled drop(let) application
CEC	cation exchange capacity
CIPAC	Collaborative International Pesticides Analytical Council Ltd
CL	confidence limits
Cmax	Maximum concentration
CNS	central nervous system
COPR	Control of Pesticides Regulations (1986)
cv	coefficient of variation
d	day
DAT	days after treatment
DMSO	dimethylsulfoxide
DNA	deoxyribonucleic Acid
DO	dissolved oxygen
DOC	dissolved organic carbon
DT50	degradation time to 50%
EbC50	Effective concentration (biomass) - 50%
ErC50	Effective concentration (growth rate) - 50%
EC	European Commission
EC	emulsifiable concentrate
EC_{50}	effective concentration
ECD	electron capture detector
EEC	European Economic Community
EINECS	European Inventory of Existing Commercial Chemical Substances
ELISA	enzyme linked immunosorbent assay
EMDI	estimated maximum daily intake
EPA	Environmental Protection Agency (USA)
EPPO	European and Mediterranean Plant Protection Organization
ETE	Estimated Theoretical Exposure
EU	European Union
f.p.	freezing point

F_0	parental generation
F_1	filial generation, first
F_2	filial generation, second
FAO	Food and Agriculture Organisation
FC	field capacity
FG	Fine granule
FID	flame ionization detector
FIFRA	Federal Insecticide, Fungicide and Rodenticide Act
FOB	Functional observation battery
FS	Flowable concentrate for seed treatment
GABA	γ-amino butyric acid
GAP	good agricultural practice
GC	gas chromatography
GC-EC	gas chromatography with electron capture detector
GC-MS	gas chromatography-mass spectrometry
GEP	good experimental practice
GFP	good field practice
GIFAP	Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques
GI(T)	gastro-intestinal (tract)
GLC(-FID)	gas liquid chromatography (-flame ionization detector)
GLP	good laboratory practise
GR	granules
h	hour(s)
ha	hectare
HDPE	High density polyethylene
HGPRT	Mammalian gene mutation assay
hl	hectolitre
HPLC	high performance liquid chromatography
HPTH	Hypothalamic-pituitary-thyroid-hepatic
HSE	Health and Safety Executive (UK)
IC50	median immobilisation concentration (ecotoxicology context) inhibitory concentration 50% (toxicology context)
i.d.	Internal diameter
ID	ionization detector
ip	intraperitoneal
IPM	integrated pest management
IR	infra red
ISO	International Organisation for Standardisation
IUPAC	International Union of Pure and Applied Chemists
iv	intravenous
JMPR	Joint FAO/WHO meeting on pesticide residues
K	Kelvin
Kd	soil/water sorption coefficient
Koc	organic carbon adsorption coefficient
Kom	organic matter adsorption coefficient
LC	liquid chromatography
LC50	the theoretical lethal concentration for 50% of a group of organisms
LC_{Lo}	lethal concentration low
LD50	the theoretical lethal dose for 50% of a group of animals
LD_{Lo}	lethal dose low
LDPE	low density polyethylene
LOQ	Limit of quantification
LOAEL	lowest observable adverse effect level
LOD	limit of determination

LOEC	lowest observable effect concentration
LOEL	lowest observable effect level
LSC	liquid scintillation counting
LT	lethal threshold
M	molar
ε	molar extinction coefficient
m.p.	melting point
MAC	maximum allowable concentration
MAFF	Ministry of Agriculture, Fisheries and Food (UK)
µg	microgram
min	minute(s)
MLD	minimum lethal dose
MMAD	mass median aerodynamic diameter
mp	melting point
MRE	maximum residue expected
MRL	Maximum Residue Limit
MS	mass spectrometry
-MSD	with mass-selective detection
MSDS	Material safety data sheet
MTD	maximum tolerated dose
MWHC	maximum water holding capacity
n	normal (defining isomeric configuration)
NAEL	no adverse effect level
NEDI	national estimate of daily intake
NEL	no effect level
NERL	no effect residue level
NMS	Northern Member States
NMR	nuclear magnetic resonance
NOAEL	no observed adverse effect level
NOEC	no observed effect concentration
NOED	no observed effect dose
NOEL	no observed effect level
OECD	Organisation for Economic Co-operation and Development
Pa	Pascal
PC	paper chromatography
PCE: NCE	Polychromatic erythrocytes: normochromatic erythrocytes
PEC	predicted environmental concentration
PHI	pre harvest interval
POEM	predictive operator exposure model
Pow	partition coefficient (n-octanol/water)
PPE	personal protective equipment
ppm	parts per million
PSD	Pesticides Safety Directorate (UK)
PTU	propylthiouracil
r^2	coefficient of determination
Rf	radio of fronts
RID	European Agreement Concerning the International Carriage of Dangerous Goods by Rail
RL_{50}	residual lifetime
RPE	Respiratory protective equipment
RSD	relative standard deviation
SC	suspension concentrate
SCPH	Standing Committee on Plant Health
SD	standard deviation
SE	standard error
SETAC	Society of Environmental Toxicology and Chemistry

SI	Systeme International d'Unites
SMS	Southern Member States
SOHD	single oral high dose
SOLD	single oral low dose
sp/spp.	species (only after a generic name)
TADI	temporary acceptable daily intake
TC_{Lo}	toxic concentration, low
TD_{Lo}	toxic dose low
TER	toxicity exposure ratio
TLC	thin layer chromatography
TMDI	theoretical maximum daily intake
TMRL	temporary maximum residue limit
TOC	total organic carbon
TRR	Total radioactive residues
TSH	Thyroid stimulating hormone (thyrotropin)
TWA	time weighted average
µ(g)	micro(gram)
UBA	German Department of Environment
UDS	unscheduled DNA synthesis
UK	United Kingdom
ULV	ultra low volume
UV/vis	ultra violet/ visible
v/v	volume ratio (volume per volume)
Vd	Volume of distribution
w/v	weight per volume
w/w	weight per weight
WG	water dispersible granule
WHC	water holding capacity
WHO	World Health Organisation
WIIS	Wildlife Incident Investigation Scheme (UK)
wk	week
WPPR	Working Party on Pesticide Residues (UK)
wt	weight
wt/vol	weight per volume
yr	year

1. INTRODUCTION

Fipronil is a phenyl pyrazole insecticide, for horticultural use to control vine weevil in non-edible ornamentals. It is formulated as a 1 g/kg a.s fine granule, 'Vi-Nil GR', for compost incorporation at a rate of 1 kg product/m^3, prior to planting container-grown ornamentals.

1.1 Identity of the active substance

1.1.1 Applicant:

Aventis CropScience UK Ltd (formerly Rhône-Poulenc Agriculture Limited).

1.1.2 Common name and synonyms (IIA 1.3)

Fipronil

1.1.3 Chemical name (IIA 1.4)

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

CA: 5-amino-1-{2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1R,S)-(trifluoromethyl)sulfinyl]-1H-pyrazol-3-carbonitrile

1.1.4 Manufacturer's development code number (IIA 1.5)

M&B 46030 or MB 46030

1.1.5 CAS, EEC and CIPAC numbers (IIA 1.6)

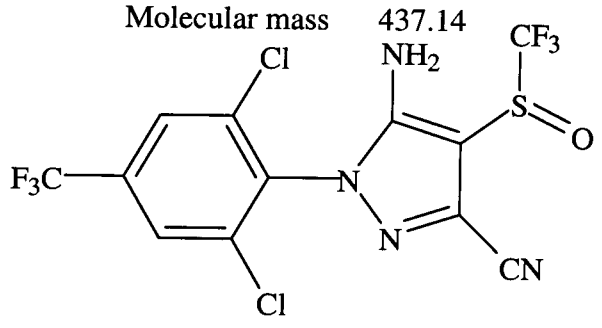
CAS number:	120068-37-3
EINECS number:	Not available
CIPAC:	581

1.1.6 Molecular formula and structural formula, molecular mass

Molecular formula $C_{12}H_4Cl_2F_6N_4OS$

Structural formula:

Molecular mass 437.14



(DP 82764)

1.1.7 Specification of purity of the active substance (IIA 1.9):

95 % w/w minimum.

(DP 82751, DP 86151)

1.1.8 Function (IIA 3.1, IIIA 1.6)

Insecticide

1.1.9 Effects on harmful organisms (IIA 3.2, IIIA 3.2)

The applicant has stated, "*Contact and ingestion activity. Some slight systemic action in plants via translocation* [in xylem] *from the roots*".

(DP 82764)

1.1.10 Field of use (IIA 3.3, IIIA 3.1)

Horticulture

1.1.11 Harmful organisms controlled and crops protected (IIA 3.4, IIIA 3.3)

Black Vine Weevil (*Otiorhynchus sulcatus*). Container-grown hardy ornamental nursery stock and non-edible ornamentals, both protected and outdoors.

(DP 82764)

1.1.12 Mode of action (IIA 3.5)

Fipronil inhibits the chloride flux regulated by the γ-amino butyric acid (GABA) receptor by binding at a site within the chloride channel, interfering with the central nervous system (CNS).

(DP 82764)

1.1.13 Information on the occurrence or possible occurrence of the development of resistance and appropriate management strategies (IIA 3.6)

See Section 3.5.

1.1.14 Procedures for destruction or decontamination (IIA 3.8)

The applicant has stated, "*In case of contamination of water with fipronil (this is also true with any other plant protection product), always try to isolate and protect the contaminated area. Where feasible, the contaminated water can be pumped out and then isolated for further treatment as appropriate (activated charcoal etc.). If accidentally contaminated water is normally used to produce drinking water, ensure with the competent authorities that the level of contamination does not reach the Parametric Value for Drinking Water*".

(DP 82764)

1.2 Identity of the plant protection product

1.2.1 Current, former and proposed trade names and development code numbers (IIIA 1.3)

Trade name: 'Vi-Nil GR' or 'Regent 1GR' [identical formulation].

Manufacturer's Development Code: EXP 60818A

(DP 82842)

1.2.2 Type of the preparation and code (IIIA 1.5)

Fine granule, FG

1.2.3 Packaging (type, materials, size etc.), compatibility of the preparation with proposed packaging materials (IIIA 4.1)

1. 20 kg sacks of fipronil granules for mixing with compost by a compost manufacturer. The sacks will be either foil-lined paper sacks or laminated plastic (low-density polyethylene) sacks.
2. 10 kg sacks of fipronil granules for mixing with compost by nurseries. The sacks will be either foil-lined paper sacks or laminated plastic (low-density polyethylene) sacks.

The applicant has stated, "*Accelerated storage stability tests have been conducted on both the foil-lined paper sack and the laminated plastic* [low density polyethylene] *sack and results have shown that both types of packaging are compatible with the product.*" See Section 2.2.14-15.

(DP 82842, 82773)

1.2.4 Application rate (IIIA 3.4)

1 kg of product per cubic metre (m^3) of compost.

1.2.5 Concentration of active substance in material used (IIIA 3.5)

1.0 g/kg

1.2.6 Method of application (IIIA 3.6)

The applicant has stated, "*product should be used only in compost incorporation. It should be evenly mixed into the compost by hand or by mixing machine. Application rate is 1 kg of product per cubic metre of compost*".

(DP 82842)

1.2.7 Number and timing of applications and duration of protection (IIIA 3.7)

The applicant has stated, *"Maximum number of treatments is 1 per batch of compost. The product should be mixed with the compost before plants are planted in the treated compost. The product must be incorporated into fresh compost each time a plant is re-potted. Compost treated with the product will give up to two years' protection against vine weevil. If untreated liners/plugs are potted into treated compost, control of vine weevil grubs cannot be guaranteed in the untreated portion"*.

(DP 82842)

1.2.8 Necessary waiting period or other precautions to avoid phytotoxic effects on succeeding crops (IIIA 3.8)

See Section 3.7.

1.2.9 Summary of intended uses

Table 1.1 Summary of UK intended uses

Crop/situation	Rate: (maximum per application) (kg a.s./cu m compost)	Rate: (maximum per season) (kg a.s./cu m compost)	Spray conc. (g as/hl)	No. of applications (maximum per season)	Spray interval (days)	Pre-harvest interval in days
Container-grown ornamentals, outdoors and under glass	0.001	0.001	N/A	One per batch of compost	N/A	Application to be made before any planting in treated compost

1.2.10 Proposed instructions for use (IIIA 3.9)

See draft label at Appendix D.

1.2.11 Procedures for cleaning application equipment (IIIA 4.2)

The applicant has stated, *"At the end of each working day, machinery used for either mixing fipronil and compost, or for potting, should be brushed down. Any spilt compost can be placed back into the hopper for further use"*.

(DP 82842)

1.2.12 Re-entry periods, necessary waiting periods or other precautions to protect man, livestock and the environment (IIIA 4.3)

The applicant has stated, *"As product is only for use on non-edible ornamental plants, no harvest intervals or withholding periods are specified. No re-entry or other restrictions are required when product is used in a glasshouse"*.

(DP 82842)

1.2.13 Recommended methods and precautions concerning handling, storage, transport or fire (IIIA 4.4)

The applicant has stated the following:

"Handling precautions: Wear suitable protective gloves when handling the product or admixing with compost. Avoid all contact by mouth. Wash hands and exposed skin before meals and after work. Keep out of reach of children.

Storage precautions: Keep in original container, tightly closed, in a safe place. Empty container completely and dispose of safely. Never transfer the product into another container for storage. Never re-use empty containers.

Transport precautions: Product is not classified as dangerous for transport.

Fire precautions: Not combustible. Danger of toxic gases in smoke in case of fire: Carbon and Nitrogen oxides. Recommended fire-fighting media: foam, carbon dioxide, dry powders, spray water. Contain the spread of fire-fighting media. Wear self-contained breathing apparatus".

(DP 82842)

1.2.14 Emergency measures in case of an accident (IIIA 4.5)

The following advice is quoted directly from that provided by the applicant. The medical basis of these proposals has not been assessed in this evaluation. (However, it is generally considered that induction of vomiting following ingestion is <u>not</u> good advice). It is recommended that the information should not be used as a basis for treatment advice in the event of a poisoning incident. Specialist advice should be sought from an appropriate source such as a National or Regional Poisons Unit or similar organisation.

"Sweep up spilled granules wearing suitable protective gloves, transfer into a marked container, and dispose via an approved waste disposal contractor.

For de-contamination of water: as for active substance; always try to isolate and protect the contaminated area. Where feasible, the contaminated water can be pumped out and then isolated for further treatment as appropriate (activated charcoal etc.). If accidentally contaminated water is normally used to produce drinking water, ensure with the competent authorities that the level of contamination does not reach the Parametric Value for Drinking Water.

First Aid Measures:

Ingestion: *Give 1 or 2 glasses of water and induce vomiting by touching back of throat with finger. Do not induce vomiting or give anything by mouth to an unconscious person. Seek medical advice.*

Skin contact: *Wash with plenty of soap and water.*

Eye contact: *Flush eyes with plenty of water. Seek medical advice if irritation persists.*

Medical Advice: There is no specific antidote. All treatment should be based on observed signs and symptoms of distress in the patient".

(DP 82842)

1.2.15 Procedures for destruction or decontamination of the plant protection product and its packaging (IIIA 4.6)

The applicant has stated the following:

"Controlled incineration of the formulation: as for active substance. Incineration at 800 °C and 1200 °C, under excess oxygen, with three minutes' retention time for the solids and approximately two seconds' retention time for the gas, results in a Destruction Removal Efficiency (DRE) of ≥ 99.99% for the active ingredient. A large number of decomposition products were identified especially at 800 °C. A lesser number of decomposition products were identified at 1200 °C. Hydrogen fluoride was detected in the exhaust gas with a concentration of approximately 330 mg/m^3. 1.63% of the product stayed in the furnace after three minutes of incineration at 800 °C, but only 0.54% stayed at approximately 200 °C. In both cases, less than 0.3% of the active ingredient was measured in the residue".

No alternative procedures for destruction are recommended.

(DP 82842)

1.2.16 Information on authorisations in EU Member States (IIIA 12.1)

Table 1.2 Authorisations and Registrations in the EU

Country	Type of Authorisation	Crops/Uses	Authorisation Details
Austria **	Not required	Gel and bait formulations used by professional and amateur users for cockroach control.	Formulations containing 0.05 % w/w a.s.
	Scotts Celaflor registration	A bait formulation used by professional and amateur users for ant control.	A formulation containing 0.02 % w/w a.s.
Belgium *	Provisional	A GR formulation used by professional users on sugar beet.	A formulation containing 1.4% w/w a.s and 8.6% w/w aldicarb.
Denmark *	Provisional	A WG formulation for use by professional users on ornamentals.	A formulation containing 80% w/w a.s.
France * **	Not required	A gel formulation used by professional and amateur users for cockroach control.	A formulation containing 0.05 % w/w a.s.
	Not required	EC & WG formulations used by professional users for termite control (soil & wall applied.	Formulations containing 2.5 and 80% w/w a.s.
	Scotts Celafor registration	An aerosol formulation used by professional users for wasp control (outdoor).	A formulation containing 0.08 g/l a.s.
	Scotts Celaflor registration	A powder formulation for use by professional and amateur users for ant control (outdoor).	A formulation containing 0.02% w/w a.s.
	Provisional	A GR formulation used by professional users on maize, sunflower & sugar beet.	A formulation containing 2% w/w a.s.
	Provisional	A GR formulation used by professional users on banana.	A formulation containing 0.5% w/w a.s
	Provisional	An FS formulation used by professional users on maize & sunflower.	A formulation containing 50% w/w a.s.
	Full	An FS formulation used by professional users on cereals.	A formulation containing 25% w/w a.s.
	Full	An FS formulation used by professional users on cereals.	A formulation containing 12.5% w/w a.s, 20% w/w guazatine & 1.25% w/w triticonazole.
	Provisional	A GR formulation used by professional users on maize & sunflower.	A formulation containing 2% w/w a.s & 8.2% w/w aldicarb.
	Provisional	A GR formulation used by professional users on sugar beet.	A formulation containing 1.4% w/w a.s. & 8.6% w/w aldicarb.

Country	Type of Authorisation	Crops/Uses	Authorisation Details
Germany **	Not required Scotts Celaflor registration	A gel formulation used by professional and amateur users for cockroach control. A bait formulation used by professional and amateur users for ant control.	A formulation containing 0.05 % w/w a.s. A formulation containing 0.02% w/w a.s.
Greece *	Provisional	A FS formulation used by professional users on maize for control of wireworms.	A formulation containing 50% w/w a.s.
Italy * **	Full Full Full	A GR formulation used by professional users on maize, sunflower, sugar beet, tomato and potato. A FS formulation used by professional users on maize. Gel and bait formulations used by professional and amateur users for cockroach control.	A formulation containing 0.2% w/w a.s. A formulation containing 50% w/w a.s. Formulations containing 0.05% w/w a.s.
Portugal **	Full	Gel and bait formulations used by professional and amateur users for cockroach control.	Formulations containing 0.05 % w/w a.s.
Spain * **	Full Full Full	Gel and bait formulations used by professional and amateur users for cockroach control. EC & WG formulations used by professional users for termite control (soil treatment). A WG formulation used by professional users on potato.	Formulations containing 0.05 % w/w a.s. Formulations containing 2.5 and 80% w/w a.s. A formulation containing 80% w/w a.s.
United Kingdom **	Provisional	Gel and bait formulations used by professional and amateur users for cockroach control. **[See SC 10435/ACP 70 (260/98)]**	Formulations containing 0.05 % w/w a.s.

* agricultural
** non-agricultural

(DP 85251, DP 86390)

2 PHYSICAL AND CHEMICAL PROPERTIES

2.1.1 Physical and chemical properties of the active substance fipronil

Table 2.1 Summary of the physical and chemical properties of the active substance (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise)

Section (Annex point)	Study	Purity	Method	Results	Comment	Reference
2.1.1 (IIA 2.1)	Melting point	96.6% 97.4% 99.4%	Differential Thermal Analysis	195°C 203°C 202.7 - 203°C	Method is considered acceptable	DP 70963 + Encl. 4 of DP 82772
2.1.2 (IIA 2.1)	Boiling point			Not determined as bp measured and solid at room temperature	Acceptable	
2.1.3 (IIA 2.1)	Temperature of decomposition or sublimation			Not determined as b.p. measured	Acceptable	
2.1.4 (IIA 2.2)	Relative density	96.6% 97.4%	EEC method A 3	1.477 1.626		DP 70963
2.1.5 (IIA 2.3)	Vapour pressure	95.2%	EEC method A 4	Stated to be 3.7×10^{-7} Pa at 25°C based on Clausius Clapeyron equation. Using this equation vapour pressure is calculated to be 1.58×10^{-7} Pa at 20°C	Non volatile. Sample used was of low purity. Case presented to address pure based on ideal vapour pressure model calculations and is acceptable.	DP 70969 + DP 85254
2.1.6 (IIA 2.3)	Volatility, Henry's law constant			3.7×10^{-5} Pa m^3 mol^{-1} (dimensionless Henry's Law coeff. of 1.49×10^{-8} at 20°C)	very low volatility	
2.1.7 (IIA 2.4)	Appearance: physical state	96.6% 97.4% 99.4%	ASTM methods	Powder at 23°C Powder at 23°C Powder (temp unknown)		DP 70963 + Encl. 4 of DP 82772
2.1.8 (IIA 2.4) 2.1.8 (IIA	Appearance: colour	96.6% 97.4%	ASTM methods	White at 23°C White at 23°C		DP 70963 + Encl. 4 of DP 82772

Section (Annex point)	Study	Purity	Method	Results	Comment	Reference
2.4) [cont'd]		99.4%		White (temp unknown)		
2.1.9 (IIA 2.4)	Appearance: odour	96.6% 97.4% 99.4%	ASTM methods	Mouldy smell at 23°C Mouldy smell at 23°C Not determined		DP 70963 + Encl. 4 of DP 82772
2.1.10 (IIA 2.5)	Spectra Fipronil	99.4%	UV/vis. IR and MS NMR	Main absorbance peaks at 220 nm and 279.5 nm. Spectra submitted. Molecular ion at m/z 436. Characteristic m/z were 367 & 213 H, F and C NMR spectra submitted	Data were from establishment of a reference standard.	Encl. 4 of DP 82772
2.1.11 (IIA 2.6)	Solubility in water	95.4%	EEC method A 6	**pH** **solubility** purified water (5.7-6.1) 1.9 mg/l 5 2.4 mg/l 9 2.2 mg/l	Slightly soluble	DP 70988
2.1.12 (IIA 2.7)	Solubility in organic solvents (technical active substance)	96.7%	CIPAC MT 157	Acetone 545.9 g/l Dichloromethane 22.3 g/l Ethyl acetate 264.9 g/l Hexane 0.028 g/l Methanol 137.5 g/l 1-octanol 12.2 g/l 2-propanol 36.2 g/l Toluene 3.0 g/l	Slightly to readily soluble	DP 70989
2.1.13 (IIA 2.8)	Partition co-efficient	99.3%	EEC method A 8	Log_{10} Pow 4.00 at 20°C in distilled water	Effect of pH not investigated but see 2.1.11 above	DP 70990
2.1.14 (IIA 2.9) 2.1.14 (IIA	Stability in water	> 98.6 (phenyl label)	US EPA Guideline No 161-1	**pH** **temp** **hydrolysis** 5 25°C none observed 7 25°C none observed	See also Section 7.4.1. Hydrolysis product was RPA 200766 (amide	DP 70991

Section (Annex point)	Study	Purity	Method	Results	Comment	Reference
2.9) [cont'd]				9 25°C DT_{50}: 28 days	analogue)	
2.1.15 (IIA 2.9)	Hydrolysis rate	> 98.6 (phenyl label)	US EPA Guideline No 161-1	See 2.1.14 above		DP 70991
2.1.16 (IIA 2.9)	Photochemical degradation	Radio labelled	US EPA Guideline No 161-2	Sterile pH5 solution, 0.545 days Florida sun, DT_{50} was calculated to be 3.6 hours (≡ 0.33 days Florida summer sunlight). After 6 hours, 43% of radioactivity was metabolite MB 46513 + RPA 104615 at 8.2% AR).	See section 7.4.2 a)	DP 71138
2.1.17 (IIA 2.9)	Quantum yield	99.4%	UBA test guideline	Estimated to be 3 hours (June) to 99 hours (December) in top mm of natural aquatic systems @ 52°N:	See section 7.4.2 b)	DP 82480
2.1.18 (IIA 2.9)	Dissociation constant (pKa)		OECD Test Guideline 112	Unable to determine because of low solubility; + spectra of parent and metabolites v. similar.		DP 82842
2.1.19 (IIA 2.10)	Stability in air, photo degradation			After 12 days' continuous sunlight, fipronil content of technical material dropped by < 30 g/kg with ~ 10 g/kg MW 46513 formed.		DP 70992
2.1.20 (IIA 2.11)	Flammability and auto-flammability (technical active substance)	96.2%	Flammability: EEC method A 10 Auto-flammability: EEC method A 16	Not highly flammable Not auto flammable. Melted at 220°C and test stopped:		DP 70993
2.1.21 (IIA 2.12)	Flash point (technical active substance)			Not determined as melting point > 40°C	Acceptable	
2.1.22 (IIA 2.13)	Explosive properties (technical active substance)	96.6%	BBA guideline	Minimum ignition energy is > 10J, lower explosion limit is 30g/m^3 and no autoignition seen for 24 hours at 140°C in 10 cm^2 basket.	No data on effects of thermal and mechanical shock presented	DP 70994
2.1.23 (IIA 2.15)	Oxidising properties (technical active substance)	97.4%	FIFRA guidelines	Unreactive with: tapwater, ammonium dihydrogen phosphate, metallic zinc and dilute neutral potassium permanganate		DP 70999
2.1.24 (IIA 2.14)	Surface tension		EEC method A 5	72.5 mN/m		DP 70998

2.1.2 Physical and chemical properties of fipronil metabolites MB 46136, MB 45950, RPA 200766 and RPA 200761

Table 2.2 Summary of requested physical and chemical properties of the fipronil metabolites (studies were completed to an acceptable standard and results were considered to be valid unless specified otherwise)

Section (Annex point)	Study	Purity	Method	Results	Comment	Reference
2.1.5 (IIA 2.3)	Vapour pressure (MB 46136)	99.7%	EEC method A 4	Vapour pressure determined at 50°C and 46°C. Vapour pressure at 25°C calculated using Clausius Clapeyron equation. Using this equation vapour pressure is calculated to be 7.6 10^{-7} Pa at 25°C	Non volatile.	DP 112731
2.1.5 (IIA 2.3)	Vapour pressure (MB 45950)	95.2%	EEC method A 4	Vapour pressure determined at 50, 40 and 30°C. Vapour pressure at 25°C calculated using Clausius Clapeyron equation. Using this equation vapour pressure is calculated to be 2.3 10^{-6} Pa at 25°C	Non volatile.	DP 112732
2.1.6 (IIA 2.3)	Volatility, Henry's law constant (MB46136)		Calculation	1.56 x 10^{-3} Pa m^3 mol^{-1} at 20°C	very low volatility	DP 112734
2.1.6 (IIA 2.3)	Volatility, Henry's law constant (MB 45950)		Calculation	3.56 x 10^{-4} Pa m^3 mol^{-1} at 20°C	very low volatility	DP 112735
2.1.10 (IIA 2.5)	Spectra MB 45950	99.2%	UV/vis.	Main absorbance peaks at 202.7 nm and 277.7 nm.	Data were from establishment of a reference standard for analysis of fipronil.	DP 70984
			IR and MS	Spectra submitted. Molecular ion at m/z 420. Characteristic m/z were 351, 255 & 213		
			NMR	H, F and C NMR spectra submitted		
	MB 46136	98.3%	UV/vis.	Main absorbance peaks at 204.1 nm and 272.8 nm.	Data were from establishment of a reference standard	DP 70987

Section (Annex point)	Study	Purity	Method	Results	Comment	Reference
2.1.10 (IIA 2.5) [cont'd]			IR and MS	Spectra submitted. Molecular ion at m/z 452. Characteristic m/z were 383, 335, 255 & 213		
			NMR	H, F and C NMR spectra submitted		
	RPA 200766	98.7%	UV/vis.	Main absorbance peaks at 209 nm and 287 nm.	Data were from establishment of a reference standard	
			IR and MS	Spectra submitted. Characteristic m/z were 438 & 385		
			NMR	H NMR spectra submitted		
	RPA 100344	97.4%	UV/vis.	Main absorbance peaks at 192.5 nm, 203 nm and 279 nm.		
			IR and MS	Spectra submitted. Molecular ion at m/z 420. Characteristic m/z were 401, 366, 332 & 297		
			NMR	H, F and C NMR spectra submitted		
2.1.11 (IIA 2.6)	Solubility in water (MB 46136)	99.9%	EEC method A 6	0.16 mg/l at pH 7, (20°C). Effect of pH not examined as molecule not ionisable and does not form ions in water. Preliminary determinations at pH 4.9 and 8.8 indicated solubility <10 mg/l	Slightly soluble	DP 112725
2.1.11 (IIA 2.6)	Solubility in water (MB 45950)	98.8%	EEC method A 6	(20°C) **pH** **solubility** purified water 0.33 mg/l 5 0.42 mg/l 9 0.57 mg/l	Slightly soluble. Report states solubility not significantly affected by pH.	DP 112727
2.1.13 (IIA 2.8)	Partition co-efficient (MB 46136)	99.9%	EEC method A 8	Log_{10} Pow 3.80 at 20°C in distilled water	Effect of pH not investigated but see 2.1.11 above	DP 112728
2.1.13 (IIA 2.8)	Partition co-efficient (MB 45950)	98.8%	EEC method A 8	Log_{10} Pow 3.70 at 20°C in distilled water (pH 6.49)	Effect of pH not investigated but see 2.1.11 above	DP 112730

2.2 Physical, chemical and technical properties of the plant protection product

Product name: Vi-Nil GR (fine granule formulation containing 1 g/kg fipronil).

Table 2.3 Summary of the physical and chemical properties of the plant protection product

Section (Annex point)	Study	Method	Results	Comment	Reference
2.2.1 (IIIA 2.1)	Appearance: physical state	Visual inspection	Granular solid		DP 71235
2.2.2 (IIIA 2.1)	Appearance: colour	Visual inspection	Grey		DP 71235
2.2.3 (IIIA 2.1)	Appearance: odour	Visual inspection	No odour detected		DP 71235
2.2.4 (IIIA 2.2)	Explosive properties	EEC method A 14	Case for non explosivity based on properties of formulation components	Case acceptable.	DP 82842
2.2.5 (IIIA 2.2)	Oxidising properties	MRI SOP No. LS-512	No reaction seen with tapwater, hexane, monoammonium phosphate or zinc.	Tier II summary states no coformulants capable of exothermic reaction . This is acceptable	DP 71235, DP 82842
2.2.6 (IIIA 2.3)	Flammability	Penskey-Martens closed cup	No flash point observed up to 110°C		DP 71235
2.2.7 (IIIA 2.3)	Auto-flammability		No data presented (see 2.2.6 above)	Case is acceptable.	DP 82842
2.2.8 (IIIA 2.3)	Flash point		Not determined as formulation is a solid		
2.2.9 (IIIA 2.4)	Acidity/alkalinity		Not determined		
2.2.10 (IIIA 2.4)	pH	MRI SOP No. LS-512	pH of 1% aqueous dilution 8.06 @ 24.2°C		DP 71235
2.2.11 (IIIA 2.5)	Surface tension		Not required for solids		
2.2.12	Viscosity		Not required for solids		

Section (Annex point)	Study	Method	Results	Comment	Reference
(IIIA 2.5)					
2.2.13 (IIIA 2.6)	Density	CIPAC MT 3	1.79 g/ml		DP 71235
2.2.14 (IIIA 2.6)	Bulk (tap) density		No specific data presented: see 2.2.13 above		
2.2.14 (IIIA 2.7)	Storage stability	CIPAC Method MT 46	**After storage (2 weeks at ambient): Plastic bag/ foil lined paper bag** Appearance = Grey granules/ Grey granules Active assay = 0.98 g/kg/ 0.93 g/kg pH (1% dilution) = 7.38/ 7.48 Sieve analysis Mesh size (µm) % retained 850 4.16/ 3.86 710 18.03/ 14.11 500 65.03/ 68.94 425 9.91/ 10.84 355 2.34/ 1.78 250 0.41/ 0.34 150 0.03/ 0.03 Pan 0.09/ 0.09 Dustiness 0.0001% w/w/ 0.0001% w/w **After storage (2 weeks at 54°C):** **Plastic bag/foil-lined paper bag** Appearance = Grey granules/ Grey granules Active assay = 0.92 g/kg/ 0.90 g/kg pH (1% dilution) = 7.33/ 7.32	No data presented for samples prior to storage. However, the samples stored at ambient temperatures for 14 days can be considered as initial data. See 2-year ambient data assessed as 2.2.15 below.. 6% loss of a.s. on storage was attributed to hydrolysis and identified as RPA 200766. The applicant states this was an artefact of the high storage temp. This is acceptable for PA, however, this implies that water may be present which may have an effect at ambient temperatures.	DP 71238
2.2.14 (IIIA 2.7) [cont'd]			Sieve analysis Mesh size (µm) % retained 850 3.45/ 3.88 710 16.52/ 17.48		

Section (Annex point)	Study	Method	Results	Comment	Reference
			500 65.72/ 65.84 425 11.73/ 10.62 355 2.05/ 1.77 250 0.44/ 0.34 150 0.03/ 0.03 Pan 0.06/ 0.03 Dustiness 0.0002% w/w/ 0.0001% w/w Report also includes a statement that no lumps or caking were seen during the study and packaging did not deteriorate		
2.2.15 (IIIA 2.7)	Shelf life		**Plastic bag** **Initial** **24 months** Appearance Grey granules Active assay 0.98 g/kg 0.90 g/kg pH (1% dilution) 7.38 7.30 Sieve analysis Mesh size (μm) % retained 851 4.16 3.70 711 18.03 22.17 500 65.03 60.17 425 9.91 11.33 355 2.34 2.13 250 0.41 0.43 150 0.03 0.03 Pan 0.09 0.03 Dustiness 0.0001% w/w/ 0.0003% w/w Attrition resistance after storage:99.73% **Foil lined paper bag** **Initial** **24 months**	Under previous submission with accelerated storage data, the fipronil content declined by 6%. Analysis showed that the breakdown product was RPA 200766 (hydrolysis product). The applicant stated this was an artefact of the high temperature storage. This was considered acceptable for PA and envisaged that this was less likely to occur during storage at ambient temperatures. The ambient data indicate that the fipronil content declined by 8.2%. The applicant has stated that the results have arisen through sampling error given that this is a 1 g/kg formulation and possibly adsorption onto the packaging walls. Additional chromatograms do not identify any additional components after storage over pre storage ones.	DP 117236

Section (Annex point)	Study	Method	Results	Comment	Reference
			Appearance: Grey granules Active assay: 0.94 g/kg 0.89 g/kg pH (1% dilution): 7.48 7.35 Sieve analysis Mesh size (µm) % retained 850: 3.86 3.90 710: 14.11 23.14 500: 68.94 59.41 425: 10.84 11.12 355: 1.78 1.93 250: 0.34 0.43 150: 0.03 0.03 Pan: 0.09 0.03 Dustiness 0.0001% w/w/ 0.0001% w/w Attrition resistance after storage: 99.91% Report also includes a statement that no lumps or caking were seen during the study and packaging did not deteriorate	The fipronil content has declined by ~5% and similar to the material stored in the plastic bag shows a greater loss than was seen in the accelerated study. Applicant has attributed this to sampling error. The available chromatograms have not shown any additional peaks over pre storage analysis. Method of analysis used to quantify the fipronil differs to that considered under SC 10698 (COP 98/01208). However, validation data for the method as used has been submitted. The previous application saw a 6% loss which was identified as RPA 200766 and stated to be an artefact of storage at 54°C.	
2.2.16 (IIIA 2.8)	Wettability		Not determined as formulation incorporated into compost		
2.2.17 (IIIA 2.8)	Persistent foaming		Not determined as formulation incorporated into compost		
2.2.18 (IIIA 2.8)	Suspensibility		Not determined as formulation incorporated into compost		
2.2.19 (IIIA 2.8)	Suspension stability		Not determined as formulation incorporated into compost		
2.2.20 (IIIA 2.8)	Dilution stability		Not determined as formulation incorporated into compost		
2.2.21 (IIIA 2.8)	Dry sieve test		See 2.2.14 above		DP 71238
2.2.22 (IIIA 2.8)	Wet sieve test		Not determined as formulation incorporated into compost		

Section (Annex point)	Study	Method	Results	Comment	Reference
2.2.23 (IIIA 2.8)	Particle size distribution	CIPAC MT 58.3	See 2.2.14 above		DP 71238
2.2.24 (IIIA 2.8)	Content of dust/fines	CIPAC MT 171	See 2.2.14 above		DP 71238
2.2.25 (IIIA 2.8)	Attrition and friability		Not normally required as COPR application and granules are compost incorporated. However, fipronil is toxic. See 2.2.15 above	Data on granule integrity acceptable	
2.2.26 (IIIA 2.8)	Emulsifiabilty, re-emulsifiabilty and emulsion stability		Not determined as formulation incorporated into compost		
2.2.27 (IIIA 2.8)	Stability of dilute emulsion		Not determined as formulation incorporated into compost		
2.2.28 (IIIA 2.8)	Flowability		Not required as COPR application and granules not applied through applicator but compost incorporated		
2.2.29 (IIIA 2.8)	Pourability (rinsibility)		Not determined as formulation incorporated into compost		
2.2.30 (IIIA 2.8)	Dustability		Not determined as formulation incorporated into compost		
2.2.31 (IIIA 2.8)	Adherence and distribution to seeds		Not determined as formulation incorporated into compost		

2.2.32 Summary of physical and chemical compatibility with other products (IIIA 2.9)

'Vi-Nil GR' is intended for use as a compost-incorporated granule; therefore no tank mixes are proposed.

2.3 Summary of physical and chemical properties

2.3.1 Active substance

Fipronil is a white powder which melts at 195 to 203°C.

Fipronil exhibits very low volatility from aqueous solutions and is only slightly soluble (1.9 mg/l in purified water) with solubility highest at pH 5 and 9 (2.4 mg/l and 2.2 mg/l). Fipronil is slightly- to readily-soluble in organic solvents with solubility highest in acetone and lowest in hexane. The log octanol-water partition coefficient of 4.00 in distilled water suggests bioaccumulation may occur. The low solubility precluded a determination of the pKa. Fipronil is hydrolytically stable at 25°C at pH 5 and 7. However at pH 9 the DT_{50} is 28 days with the amide analogue formed (RPA 200766). Photolytic degradation occurs with a DT_{50} of 3.6 hours in water exposed to Florida summer sun (see Section 7.4.2).

The technical active substance was not auto-flammable, flammable, explosive or oxidising.

The following data requirements were set for provisional approval when fipronil was considered under a previous application (COP 98/01208) and have been addressed in this evaluation.

i) Data on the solubility in water of the following metabolites: MB 46136, MB 45950, RPA 200766 and RPA 200761.
ii) Data on the octanol/water partition coefficient for the following metabolites: MB 46136, MB 45950, RPA 200766 and RPA 200761.
iii) Data on the vapour pressure (together with a calculation of Henry's Law constant) for the following metabolites: MB 46136, MB 45950, RPA 200766 and RPA 200761.
iv) Data on the integrity of the 'Vi-Nil GR' granule in the sales pack before and after storage.

No physical/ chemical property data have been submitted for metabolites RPA 200766 or RPA 200761. The applicant has stated that the need for such data on these metabolites arose through the ecotoxicological risk assessment. The applicant states that a risk assessment for these two metabolites has been possible and as such these data are not required. The submission also maintains that the risk assessment for these metabolites indicates that they are not of ecotoxicological significance (see section 8.2.5.6 and 8.2.6).

Metabolite MB 46136 is very slightly volatile from aqueous solutions and is slightly soluble (0.16 mg/l) in purified water. The effect of pH was not determined as the applicant states that MB 46136 is not ionisable and does not form ions in water. Preliminary data at pH 4.4 and 8.8 indicated the solubility was <10 mg/l. The log

octanol-water partition coefficient of 3.80 in distilled water suggests potential for bioaccumulation.

Metabolite MB 45950 is very slightly volatile from aqueous solutions and is slightly soluble in purified water (0.33 mg/l) with solubility highest at pH 5 and 9 (0.42 mg/l and 0.57 mg/l). The log octanol-water partition coefficient of 3.70 in distilled water suggests potential for bioaccumulation.

2.3.2 Plant protection product

'Vi-Nil GR' is a fine granule formulation (FG) containing 1 g/kg fipronil (95% purity). The preparation generally showed satisfactory physical and chemical properties before and after storage at ambient temperatures for 2 years in both the low density polyethylene bag and the foil lined paper sack. However, in both packs, the fipronil content dropped by 8.2% and 5.3% respectively. A 6% drop in fipronil content was also seen in the low density polyethylene bag during accelerated storage. Under the previous evaluation, the applicant stated this was due to hydrolysis with the RPA200766 metabolite formed and attributed this to the high temperatures of accelerated storage at 54°C. This was considered acceptable for provisional approval.

However, a greater decline was seen in the ambient study for both the LDPE bag and the foil lined paper sack (8.6% and 5.2% respectively). The applicant was asked to comment on this in light of the results seen at elevated temperature. The applicant has stated that the octanol water partition coefficient of 4.00 suggests that the material may be adsorbing onto the LDPE material and also that given the very low content, sampling errors are a likely contributor to the results seen. In addition, the applicant has also cited chromatographic data which shows no additional peaks after storage compared to the initial runs. The fipronil content in the ambient study was determined using a different, but validated, method of analysis to that seen in the accelerated study and the use of different methods could add to the different results seen before and after storage. Taking all these contributory factors and that FAO tolerances for active ingredients at the levels seen here are ± 25% of declared content, the explanation is considered acceptable and approval from a storage stability perspective may be proposed.

The formulation was not flammable or oxidising. An acceptable case to address the auto flammability and explosivity of the formulation was presented indicating the formulation to be neither auto flammable or explosive.

Data have been submitted on granule integrity after storage in both pack types and the data are acceptable.

2.4 Conclusions

The information submitted on physical and chemical properties of fipronil and of the formulation is sufficient to support approval of 'Vi-Nil GR' for use on non-edible ornamentals. The following further data are required:

For full approval:

(i) A further explanation of the degradation of fipronil observed in the storage stability studt conducted at ambient temperature is required.

(ii) Data on the solubility in water of pure fipronil are required. These data are to include measurements at pH 4 as well as neutral and pH 9/10.

3 EFFICACY

Fipronil in 'Vi-Nil GR' is a compost treatment for the control of vine weevil (*Otiorhynchus sulcatus)* in container-grown ornamental plant production. Vine weevil is a major pest of horticultural crops; adults damage plant foliage by ingesting leaf material reducing quality and marketability of affected plants. However, the major damage is caused by larvae feeding on roots causing plants to wilt and plant growth to slow, particularly producing stunting of root and eventually killing the plants. Adult females emerge in late spring or summer. Eggs which are laid on the plant or the soil hatch within two to three weeks with larvae feeding on the roots until pupating the following spring.

3.1 Efficacy data

Evaluation was made from the Biological Dossier presented by the applicant. Additional data were made available by reference to the applicant's trials report.

3.1.1 Testing organisations (IIA 2.1-2.3)

Three Testing Organisations were used. All trials series were begun before the UK had initiated its Official Recognition procedures and therefore those trials begun before 1998 do not need to show compliance. All three organisations used have registered and have Officially Recognised status to cover those trials begun in 1998.

3.1.2 Assessment

The organisations used and the data produced by them and reported in the efficacy overview are acceptable.

3.1.3 Test conditions and guidelines (IIIA 6.2)

Methodologies used were drawn from EPPO guidelines PP 1/111 (2) except that the guidelines suggest using 15 plants per plot with four replicates per treatment. One organisation used only five plants in four replicates per treatment and the other two organisations used 10 plants as individual replicates. Assessments were made at only one time during each trial instead of the three assessments suggested in the guidelines. Assessments of larval numbers are made by destructively pulling the plants roots apart and looking for numbers of weevil larvae. The applicant stated that deviations were made from the guidelines because of the very high demand for numbers of replicates that are required. In addition the applicant stated that because the trials were artificially inoculated and good numbers of larvae per pot were guaranteed, a reduction in the number of pots per treatment to either four replicates of five pots or 10 fully randomised pots per treatment was possible. They propose that the degree of success they encountered with the artificial inoculations in the range-finding tests (77% infestation rate) showed that there were sufficiently high numbers present at the assessments. The applicant further stated that inoculating with too high numbers of eggs would produce populations of larvae which were likely to be cannibalistic.

Plants were inoculated with vine weevil eggs between late July and September. Between 10 and 15 eggs per pot were put on the soil surface and covered with 2 cm of fresh compost. Two inoculations were made at intervals of three to four weeks. Assessments of control were made by removing each plant from its pot, tearing apart its roots and counting the number of viable larvae. Crop damage was also assessed by taking fresh root and shoot weights. Crop safety assessments were made by taking fresh weights of plants and scoring for root growth.

The standard used in all trials was 'SuSCon Green' (containing chlorpyriphos-methyl).

3.1.4 Assessment

The case the applicant makes for deviating from the EPPO guideline is acceptable. It is acknowledged that some changes to the guideline are warranted. The use of 'SuSCon Green' is fully approved for vine weevil control as a soil incorporated granule and is therefore acceptable as a standard.

3.1.5 Location

All trials were carried out in the UK under one of the following conditions:

- polythene tunnels in the summer and either a cool or a heated glasshouse in the winter
- outdoors in the summer and polythene tunnel in the winter
- polythene tunnels all year
- cool glasshouses all year

3.1.6 Assessment

The trials were located in types of conditions where ornamental plant production are found in commercial situations and therefore are acceptable.

3.2 Preliminary test (IIIA 6.1)

3.2.1 Range-finding tests

The overview describes preliminary tests to determine the appropriate dose of fipronil to give good control of vine weevil. An experimental 20% granular formulation was peat-incorporated in outdoor pot-grown *Thuja plicata* at five doses from 2.5 to 75 g as/m^3. Twenty single pot replicates were set up. Two inoculations of eggs were made to each pot in June 1996, nine-11 weeks after plants were potted in treated compost. Interim assessments of five pots per replicate were made 21 weeks later showing no larvae in fipronil-treated pots, a mean of 0.8 larvae per chlorpyriphos-treated pots and a mean of 3.1 larvae per pot in the untreated pots. Final assessments on the remaining 15 pots per replicate, 72 weeks after inoculation, showed that there were no larvae in the fipronil-treated pots, a mean of 3.1 larvae per chlorpyriphos-treated pots and 2.8 larvae per living plant in the untreated pots. All plants survived in the treated pots but 28% died in the untreated pots. Fresh weights of roots and shoots of the untreated plants were approximately half those of the treated plants.

3.2.2 Assessment

Only one test is described which showed good control of larvae of vine weevil, although the methodology given for the test was minimal. No information was given on the method of re-infestation of weevils between generations. The test showed that the lowest dose used gave total control of larvae but did not demonstrate that lower doses would have been equally effective.

3.3 Dose justification

Although the applicant gives no justification for the label-recommended dose there are data in all the trials reports from treatments using doses of 'Vi-Nil GR' at half, a quarter and one eighth of the label-recommended dose. Results showed that in only five of the 19 trials were larvae recorded following treatments at lower doses. Larvae were found after a second inoculation of eggs in only one trial. The highest number of larvae (1.1 larvae per pot) was found in a trial using an eighth of the label dose. The highest number of larvae found in compost treated at half the recommended dose was 0.01 larva per pot.

3.3.1 Assessment

Results from a few trials showed that at one eighth of the proposed dose there was still good levels of control of larvae. Data from all 19 trials showed that lower doses could give commercially acceptable levels of control. The applicant must justify more fully the need for the proposed dose.

3.4 Effectiveness (IIIA 6.2)

3.4.1 Proposed label recommendations

Rate: *1 kg of Vi-Nil GR per cubic metre of compost*

Vi-Nil GR must be incorporated into fresh compost each time the plant is re-potted. If untreated liners/plugs are potted in treated compost, control of vine weevil grubs can not be guaranteed in the untreated portion.

3.4.2 Pest control

Reports from 19 trials carried out between 1996-1998 were presented. Eleven trials were in peat media and eight in peat/bark media. Plants used were *Azalea, Fuchsia* and *Cyclamen spp, Euonymus fortunei*, strawberry and polyanthus. All treatments of 'Vi-Nil GR' were made at the potting stage using cuttings, rooted cuttings, rooted plugs, young plants or plug-raised plants. Trials investigated the effectiveness of treatments over one or two seasons (Table 3.4).

a) One-season trials

Two trials on fuchsia were carried out in either peat-based or in peat/bark-based potting media. Rooted cuttings were potted into small pots containing treated potting media and re-potted within 20 days into one litre pots also containing treated compost. Vine weevil eggs were added to the pots immediately following final potting. A single assessment of infestation was made 101 days following incorporation of the treatments into the compost. This represented an assessment of larval numbers approximately 78 days after infestation. Results showed 100% control of larvae using 'Vi-Nil GR' at the label-recommended dose. Mean larval numbers in the untreated controls were 6.6 larvae per pot and 7.0 larvae per pot. Mean control using 'SuSCon Green' in both trials was 0.1 larvae per pot. Fresh weights of top growth of plants varied considerably within and between treatments. Mean weights of treated material were always greater than the untreated controls. The applicant stated that shoots were trimmed during the period of the trials. Root weights of plants treated by both 'SuSCon Green' and 'Vi-Nil GR' and assessed after 12 weeks showed approximately twice the weight compared with the untreated control plants (Table 3.1).

Table 3.1 Fresh root weights (g) of fuchsias following infestation of vine weevil larvae 12 weeks after infestation

Trial	Trial 1	Trial 2
Untreated control	2.4	3.0
Vi-Nil GR	5.8	5.4
SuSCon Green	5.4	8.0

Three trials were carried out on azaleas using peat (one trial) or peat/bark potting media. In two trials assessments were made 90 days following two inoculations of eggs and 120 days after potting up. Assessments showed mean numbers of weevils in the untreated plants were 4.9 and 1.1 larvae per pot, no larvae in the 'Vi-Nil GR' treatments and means of 0.3 and 1.8 larvae per pot for 'SuSCon Green' treatments. Fresh weights showed no consistent differences between treatment and between treated and untreated plants. Assessments of vigour and colour showed variability between treatments and between treated and untreated plants. In the third trial, inoculations of plants were made 93 days after cuttings were potted up. Assessments made 183 days after inoculation and 276 days after plants were potted up showed that mean numbers of larvae in the untreated control were 0.14 larvae/per pot. In the 'Vi-Nil GR' treatments larval numbers were assessed as 0.08 larvae per pot and no larvae were found in the 'SuSCon Green' treated compost. At the assessment times given by the applicant for this trial, the majority of larvae should have pupated and emerged as adults. Top growth scores for both treatments were twice those of the untreated plants. Root volume scores were ten times greater in the 'Vi-Nil GR' treatments compared to the untreated controls and nearly eight times in the 'SuSCon Green-treated compost.

Two trials on Polyanthus using plant plugs were done over a six month period. Inoculations of eggs were made over a four week period following potting up in peat compost. An assessment of larval numbers was made nine weeks after the second inoculation and 12-13 weeks after plants were potted up in treated composts. Mean numbers of weevil larvae were assessed as being 10.7 and 7.6 larvae per pot in the

untreated controls, none in the 'Vi-Nil GR' treatments and 1.7 and 0.1 larvae per pot in the 'SuSCon Green' treatments. Fresh weights of plants from either treated composts were approximately twice those of the untreated plants. In one trial where fresh root weight was measured treated plants had approximately four times the weight of roots compared to the untreated controls. Vigour scores assessed in one trial were also higher for the plants in treated composts. It was stated that in one of the trials eight of the ten untreated plants were wilting at assessment whilst no plants in treated compost had wilted. Small differences were noted between the two treatments.

A single trial was reported using strawberry plants grown in one-litre pots (compost material not given). Eggs were inoculated on to soil surfaces three and five weeks after planting. Assessments of larval numbers were made 10 weeks after the second inoculation and 14 weeks after potting up. There was a mean number of 8.83 larvae per pot on the untreated controls and no larvae on the 'Vi-Nil GR' treated plants. There were insignificant differences in phytotoxicity assessments, vigour scores and plant fresh weights.

In a trial involving seedling cyclamen, weevil eggs were inoculated 28 days after the seedlings were potted up into peat–based compost. Assessments were made 16 weeks after potting the seedlings and 11 weeks after the second inoculation. Results showed no larvae in the 'Vi-Nil GR'-treated composts and mean larval numbers per pot of 1.4 in 'SuSCon Green'-treated compost and 7.9 in the untreated controls.

Three trials were set up using *Euonymus fortunei*; one trial, using peat only compost was divided into two so that assessments could be made 32 and 58 weeks after the potting up of the plants (see section b) below for the longer assessment details). In the other two trials one used peat and the other used peat/bark composts. Assessments were made between 24 and 32 weeks after potting up and between 16 and 24 weeks after being inoculated with weevil eggs. The mean number of larvae in the untreated controls for each trial were 1.2, 3 and 12 larvae per pot. No larvae were found in any of the plants in the 'SuSCon Green'-treated composts in all of the trials. A mean of one larvae per pot was found in the 'Vi-Nil GR'-treated compost in one of the trials and no larvae in the other two trials. Top growth vigour scores showed small increases in the treated plants in two trials whereas in the third, using peat/bark compost, there was at least a 1.5 times increase in vigour in both treated composts compared with the untreated. Root volume scores between trials showed variability in results (table 3.2).

Table 3.2 Vigour and root volume scores of *Euonymus fortunei* in treated and untreated composts

Treatment	Vigour score	Root volume score
Trial 1 (Peat)		
Untreated	4.5	2.75
Vi-Nil GR – 1 kg/m^3	4.6	3.65
SuSCon Green 1 kg/m^3	3.28	2.55
Trial 2 (Peat/bark)		
Untreated	2.85	1.3
Vi-Nil GR – 1 kg/m^3	4.8	3.7
SuSCon Green 0.75 kg/m^3	5.0	2.55
Trial 3 (Peat)		
Untreated	4.48	2.1
Vi-Nil GR – 1 kg/m^3	4.78	3.15
SuSCon Green 0.75 kg/m^3	4.88	3.4

b) Trials over two seasons

Four trials on *Fuchsia* were carried out in either peat-based or in peat/bark-based potting media. Two trials used peat potting medium and two used a peat/bark mixture. In two of the trials rooted cuttings were potted into small pots containing treated compost and re-potted within 20 days into one litre pots also containing treated compost. In the other two trials rooted cuttings were potted directly into 1.5 litre pots. Pests were added to the soil as eggs approximately 52 weeks (four trials) following final potting. A single assessment of infestation was made 56-60 weeks following incorporation of the treatments into the compost. This represented an assessment of larval numbers approximately 12-16 weeks after inoculation. Results showed 100% control of larvae using 'Vi-Nil GR' at both the label-recommended dose and half the recommended dose. Mean larval numbers in the untreated controls ranged from 2.5 larvae per pot to 8.1 larvae per pot. Control using 'SuSCon Green' ranged from a mean of 0.1 larvae per pot to 0.2 larvae per pot. Fresh weights for "tops" varied considerably within and between treatments. Mean weights of treated material were always greater than the untreated controls. The applicant stated that shoots were trimmed during the period of the trials. Root weights of plants assessed 56-60 weeks after potting up and 12 weeks after inoculation were variable with 'SuSCon Green' having greater weights than the untreated control in three of the four trials and 'Vi-Nil GR' showing increases in weight compared to the untreated controls in two trials (Table 3.3).

Table 3.3 Fresh root weights (g) of fuchsias following infestation of vine weevil larvae 56-60 weeks after infestation

Trial	Trial 1	Trial 2	Trial 3	Trial 4
Untreated control	16.5	23.7	17.0	19.6
Vi-Nil GR	23.0	20.4	20.8	16.3
SuSCon Green	19.7	25.2	19.9	15.7

Two trials were carried out on azaleas using peat or peat/bark potting media with the first inoculations of eggs, made about four weeks after potting up and a second inoculation after the first generation of weevils had emerged, 48 weeks after potting up. Assessments of larval numbers, vigour scores and phytotoxicity were made 66 weeks after the inoculations. Some of the control plants had to be replaced with uninoculated plants after 24 weeks as first generation weevil larvae had destroyed the plants. Mean larval numbers in untreated plants were assessed as 6.6 and 0.4 larvae per pot for each trial. No larvae were found in the 'Vi-Nil GR' treatments. No larvae were found in the 'SuSCon Green' treatment in one trial and a mean of 1.0 larvae per pot in the other. Assessments of vigour, colour and fresh plant weights showed a similar variability to the other azalea trial described above except that vigour scores of the untreated through the season changed as the plants died and were replaced. Comparisons between treated and untreated plants were therefore difficult to make. There were no differences between the two treatments in one trial. In the second trial, which had shown a mean of one larva per pot in the 'Vi-Nil GR'- treated compost, 'SuSCon Green'-treated compost vigour scores throughout the season were lower than the treatment; this trial also showed a mean of one larva per pot.

One trial was set up using *Euonymus fortunei* using a peat compost and divided into two so that assessments could be made 32 and 58 weeks after the potting up of the plants (see a) above for details of shorter period trial). Assessments made 58 weeks after potting up and 14 weeks after being inoculated with the second batch of weevil eggs, found on average 6.8 larvae per pot in the untreated control pots and no larvae in the treated plants. Top growth vigour scores showed no consistent differences between plants raised in treated and untreated composts. Root volume scores showed 2.8 and 2.7 times the untreated scores for 'Vi-Nil GR' and 'SuSCon Green' respectively.

Over all trials, numbers of larvae in the untreated control at assessment ranged from 0.4 to 28 (mean 6.8) larvae per pot. Numbers of larvae in treated composts were mean 0.21 and 0.37 per pot for 'Vi-Nil GR' and 'SuSCon Green' respectively (see Table 3.4 for results of individual trials).

3.4.4 Assessment

The data show good control of vine weevil larvae over the periods used in the trials. Control was consistently better than the standard 'SuSCon Green' and particularly so in the trials which ran over two seasons. The label claims that 'Vi-Nil GR' can give two years' protection against vine weevil. The longer intervals from treatment to assessment were less than two years (between 423 and 468 DAT). However, some ornamentals may be potted up early in the season so that, prior to a second egg-laying period in August/September in the following year, compost could have been treated over 468 days previously. Presented data cover only 468 DAT. However the data show almost a complete kill of weevil larvae even into the second year and data also exist in the trials reports, although not presented in the overview, showing good control at lower than label-recommended doses (see Section 3.3 on dose justification).

Crop effects noted in effectiveness trials over one season showed increases in root weights in all trials, (up to 10 times the weight of the untreated control), although similar assessments of foliage weights were more variable due in some cases to the need to trim foliage during the trials. Assessments made after two egg-laying seasons showed lesser effects on plant size and weights. Although in most cases treated plants showed increases in root size or plant weights. With regard to the devastating effects that vine weevil can have on the roots of plants, it is not surprising that effects were noted more in root development rather than foliage weights.

The standard used in all trials, 'SuSCon Green', has label recommendations which differ depending on the composition of the potting compost with higher doses (1.0 kg/m^3) of product being recommended for peat/bark mixtures compared with peat-only composts (0.75 kg/m^3). There was no indication that compost type affected the control using 'Vi-Nil GR' at the recommended doses. The trials reported by the applicant showed that control of weevil larvae is good in all the usual circumstances that could be expected to occur in commercial situations, from outdoor to under heated glass.

The label recommends a maximum period of 30 days before use following mixing of the compost with 'Vi-Nil GR'. No data were provided to substantiate this recommendation. Of the 19 trials presented five gave no indication of the length of time of incorporation before use, in seven trials composts were used immediately following incorporation and in seven trials composts were left for 12 – 14 days before use. No effects on control levels were noted in these trials. However, it is not known what effect leaving treated compost on potting benches, as long as the label-recommended 30 days, would have and therefore it is recommended that a label amendment be made to reduce the time treated compost is left before use.

Approval for control of larvae is recommended with the following label amendments:

i) "Fipronil will give control of larvae into the second year after treatment."

ii) "Treated compost should be used within two weeks of mixing."

Table 3.4 Trials details and control of vine weevil (mean numbers of larvae per trial) using Vi-Nil GR and SuSCon Green

Treatment	Plant sp	Treatment date	Mean number of larvae per pot at assessment (percent control)			Assessment (date)	Assessment (DAT)
Trial (compost type)			Untreated	Vi-Nil GR	SuSCon Green		
LA6L2 (peat)	Polyanthus	30.8.96	10.7	0	1.7	2.12.96	94
Saynor VW2 (peat/bark)	Fuchsia	1.9.96	7.0	0	0.1	9.12.96	100
Saynor VW1 (peat)	Fuchsia	26.8.96	6.6	0	0	6.12.96	102
LA97719 (peat)	Strawberry	5.9.97	8.83	0	*	18.12.97	104
LA6L30 (peat/bark)	Azalea	29.8.96	4.9	0	0.3	17.12.96	110
LA6L32 (peat)	Azalea	29.8.96	1.1	0	1.8	18.12.96	111
Saynor VW3 (peat)	Polyanthus	1.8.96	7.6	0	0.1	21.11.96	113
Saynor VW7 (peat)	Cyclamen	30.7.97	7.9	0	1.4	23.01.97	116
HRI trial C (peat)	*Euonymus*	14.7.97	0.15	0	0	30.01.98	201
HRI trial C (peat/bark)	*Euonymus*	14.7.97	0.6	0.15	0	30.01.98	201
HRI trial B (peat)	*Euonymus*	19.8.96	1.2	0	0	7.04.97	231
HRI trial A (peat/bark)	Azalea	19.8.96	1.4	0.15	0	22.05.97	276
Mean no. of larvae (assessed up to 276 DAT)			4.832	0.025 (99.48)	0.491 (89.84)		
Saynor VW6 (peat/bark)	Fuchsia	20.9.96	2.7	0		17.11.97	423
Saynor VW5 (peat)	Fuchsia	20.9.96	8.1	0	0.2	29.11.97	435
HRI trial B (peat)	*Euonymus*	19.8.96	6.83	0	0	19.11.97	457
Saynor VW4 (peat/bark)	Fuchsia	1.9.96	2.7	0	0.1	3.12.97	458
Saynor VW3 (peat)	Fuchsia	26.8.96	2.5	0	0	29.11.97	461
LA6L31 (peat/bark)	Azalea	29.8.96	6.6	0	0	10.12.97	467
LA6L33 (peat)	Azalea	29.8.96	0.4	0	1.0	11.12.97	468
Mean number of larvae (assessed more than 423 DAT)			4.261	0 (100)	0.186 (95.64)		
Overall mean number of larvae			4.62	0.016 (99.66)	0.35 (92.37)		

* figure not given

3.5 Resistance (IIIA 6.3)

3.5.1 Laboratory data/field information e.g. baseline monitoring

The applicant states that fipronil has been used commercially for control of various insect pests of cotton and rice since 1993. Some of the pests controlled are known to be resistant to organophosphorus, carbamate and pyrethroid insecticides. No known cases of resistance to fipronil have been recorded. As fipronil acts on the same site as cyclodiene insecticides cross resistance studies were instituted. Studies have shown that there is a degree of cross-resistance between dieldrin and fipronil albeit at low levels, (details not given of degree of cross-resistance). The applicant further states that it is reasonable to assume that as maximum resistance to dieldrin will have already been achieved further increases in resistance to fipronil are unlikely. No indication of resistance has so far been observed in control programmes using fipronil against resistant diamond back moth.

No resistance to any insecticide has been found in weevil population in the UK. The applicant does not state if resistance in vine weevil has been reported elsewhere in the world.

Although the applicant considers the risk of resistance development is low in vine weevil, a fipronil resistance monitoring programme is being established to generate baseline data for fipronil susceptibility in UK populations of wine weevil.

3.5.2 Resistance management strategy

The applicant's dossier contains no resistance management strategy.

3.5.3 Assessment

The applicant makes a case for suggesting that the use of fipronil against vine weevil constitutes a low risk of resistance development. The facts used are the lack of evidence of resistance in vine weevils against any pesticide and the lack of resistance to fipronil which has developed elsewhere in the world in any pest species. The case that use of 'Vi-Nil GR' on vine weevil constitutes a low risk situation can be accepted, although the idea that resistance is unlikely to develop to fipronil because cross-resistance tests to dieldrin show only low levels of decreased sensitivity at the GABA site is questionable. There is no reason to suppose that evolution will not continue to develop further changes in amino acid sequences which may confer higher levels of resistance. The acceptance of the applicant to commence a baseline monitoring scheme is to be commended.

3.6 Yield, quantity and quality(IIIA 6.4)

3.6.1 Yield (IIIA 6.4.3)

Ornamental plants do not usually have yield assessments made. However assessments were made of root and plant or foliage weights during effectiveness trials (Section 3.4)

and these showed increases in most of the trials, up to ten time root increases in some cases.

3.6.2 Transformation processes (IIIA 6.4.2)

Not required.

3.6.3 Quality (IIIA 6.4.1)

Not required for ornamental plant production although assessments of phytotoxicity (Section 3.7) showed no reductions of plant quality.

3.6.4 Assessment of quality, quantity and yield data

See Section 3.7.1 on assessment of phytotoxicity data.

3.7 Phytotoxicity (IIIA 6.5)

Data were supplied from five trials on the safety of fipronil to pest-free plants. Plants used included four azalea cultivars (cvs), five rhododendron cvs, seedlings of three cyclamen cvs, cuttings of six poinsettia cvs and 38 cvs of 25 species of ornamental plants. In four trials the potting medium used was split and one portion was "thoroughly hand mixed" with 2 kg of 'Vi-Nil GR' per m^3 of growing medium (twice the label-recommended quantity). In the fifth trial Poinsettia cuttings were dipped in a mixture of rooting compound and fipronil granules before being potted into Jiffy plugs. Growing media for all trials consisted of either peat/bark mix or peat.

In a trial, using a peat/bark mixture young plants of Azalea and Rhododendron cvs were potted into 'Vi-Nil GR'-treated compost, at twice the label-recommended dose, as nine single plant replicates of each cultivar and compared to plants grown in the same but untreated compost. Assessments were made between 106 and 164 DAT of plant vigour, growth differences and phytotoxicity. No differences were noted in any of the assessments between treated and untreated.

In a second trial using three varieties of cyclamen, 63 seedlings of each cv were potted into peat-based seedling growing medium. Half of the compost had previously been treated with 'Vi-Nil GR' at twice the label recommended dose. Assessments were made of phytotoxicity and plant vigour. Growth differences were assessed as differences between treated and untreated in plant height, growth habit and root development. No effects were seen in any of the parameters assessed between 7 – 28 DAT.

In a third trial, between 5 and 10 plants each of 27 varieties of 25 plant species were potted into either 'Vi-Nil GR'-treated (at twice the label-recommended dose) or untreated peat compost. Assessments of plant height and root vigour were made. Nine of the 27 varieties showed reduction (2%-14%) in mean plant height compared to the untreated and 15 varieties showed increases. Two varieties showed reductions in

assessments of root development whereas 20 varieties showed increases. Plants used included conifers, heathers, fuschias, deciduous shrubs and herbaceous species.

In a fourth trial between 10 and 20 plants each of 11 species of plug-raised ornamental shrubs were potted into compost treated with twice the recommended dose of 'Vi-Nil GR' or the standard dose of 'SuSCon Green'. No untreated controls were used. Assessments of plant height were made at regular intervals with final assessment being made 34 weeks later which include an assessment of root development on a 0-5 scale (0= poorest and 5= most vigorous root development. Four out of the 11 species showed reduced mean plant height when grown in 'Vi-Nil GR'-treated compost compared with 'SuSCon Green' and 7 species showed greater mean heights than 'SuSCon Green'. Root development assessments showed either an increase in root development (seven spp) when potted in 'Vi-Nil GR' compared to 'SuSCon Green' or no differences were noted (four spp).

Six cultivars of Poinsettia cuttings were dipped in a mixture of rooting powder and Vi-Nil GR granules before being potted up to allow rooting. Approximately 10-12 granules of Vi-Nil GR adhered to each cutting which is equivalent to the dose which could be expected in 5 ml of treated potting medium. Assessments were made of roots/bases of the cuttings (16 DAT) and plant vigour, leaf colour and establishment of plants (34 DAT). Comparisons between treated and untreated showed no differences in either assessments.

3.7.1 Assessment of phytotoxicity data

Although not required for an insecticide, all crop effect studies used fipronil at twice the recommended label rate. No information was given on the relative concentration of fipronil in the trial using Poinsettia. Two of the trials produced data showing reduction in plant height in some of the species.

There was no consistency in the species showing the reductions and only one plant species affected also showed a reduction in root development. All the other cultivars used in these two trials showed either no differences in or else improvements in plant height when grown in treated compost compared to those grown in untreated compost.

Although many of the tests did not last as long as the duration of the effects of fipronil are claimed, it is expected that signs of phytotoxicity would be visible during the earlier days following treatment when plants are still young and tender. Very few plants used in tests were of the more tender, herbaceous type; cyclamen, poinsettias and phlox being the only species tested.

The occasions that the use of 'Vi-Nil GR' showed deleterious effects on the crop safety of a number of plant species were far outweighed by the improvements in the same trial to other species. In addition 'Vi-Nil GR' had been used in the trials at twice the label-recommended dose and the results of the phytotoxic assessments during effectiveness trials showing no ill effects on the plants. The label has a statement that growers should test species not listed on the label in small numbers to check for crop safety. Approval of the crop safety of 'Vi-Nil GR' is recommended.

3.8 Undesirable side-effects (IIIA 6.6)

3.8.1 Succeeding crop (IIIA 6.6.1)

The applicant makes no reference in the dossier to the issue of following crops. While the use of the a.s. is as a treatment for potting media the applicant suggests that spent medium will be used on soil that could be used for agricultural crops. Although information from crops safety trials described above in Section 3.6 suggests that safety to following crops should not present a problem, the issue of following crops has to be considered

(A confined semi-protected rotational crop study at 3N application rate, using carrot or radish, lettuce or mustard and wheat or sorghum has been submitted and assessed in the Residues Section 6.1).

(DP 82842)

3.8.2 Adjacent crops (IIIA 6.6.2)

Fipronil used as a granule incorporated into containers of compost will not affect adjacent crops.

3.8.3 Plant or plant parts for propagation (IIIA 6.6.3)

No evaluation of plants propagated from parent material grown in fipronil-treated growing media were presented. Although some of the plant material used in the crop safety trials (Section 3.6 above) were un-rooted cuttings and could be interpreted as being propagation material.

3.8.3.3 Assessment

No problems were noted in crop safety trials on plant material used for propagation, including poinsettia a plant group known to be particularly sensitive and therefore the safety to plant material for propagation can be accepted. Although there are no data, a case can be made for accepting that 'Vi-Nil GR' is unlikely to have any effects on following crops. Spent soil containing 'Vi-Nil GR' will be diluted to a great extent when treated as wastage from propagation houses and in addition no phytotoxic effects were noted when 'Vi-Nil GR' was used on a range of plant species at twice the label-recommended rate.

3.9 Effects on beneficial and non-target organisms (IIIA 6.6.4)

The label makes no statement claiming safety to beneficial organisms. The applicant makes the case that although fipronil is a broad-spectrum insecticide field experience has demonstrated it is less harmful to beneficials than many other commercially-used insecticides. No data are presented to support this contention although a graph is presented as a summary of data generated between 1988 and 1995 which shows the impact of fipronil on beneficial insects and mites by foliar application. Using data which were not presented the applicant lists the success which biological agents are

likely to have when used in fipronil-treated situations. Beneficial soil-inhabiting or soil-applied predatory mites are predicted as being at risk, whilst those agents considered safe are soil-applied nematodes.

3.9.1 Assessment

No data on the effects of 'Vi-Nil GR' on beneficial insects were presented by the applicant. As beneficial insects are in common use in glasshouses, a phrase must be added to the label stating that the effect of 'Vi-Nil GR' on the safety to beneficial insects has not been demonstrated.

3.10 Summary (IIIA 6.7)

Conclusions

The information submitted on efficacy is sufficient to support approval of 'Vi-Nil GR' for use on container-grown ornamentals.

Further data are required for full approval:

(i) With respect to efficacy, the dose of the product must be fully justified.

Label Amendments:

(i) In the section headed "General Information", the sentence, "Compost treated with Vi-Nil GR ...against vine weevil", must be changed to, "Compost treated with Vi-Nil GR will give control of larvae into the second year after treatment".

(ii) The phrase, "The effect of Vi-Nil GR on the safety to beneficial insects has not been demonstrated" must be added to the label.

(iii) The sentence beginning ,"Treated compost should be used within 30 days of mixing", must be amended to read, "Treated compost should be used within 12 days of mixing".

4 METHODS OF ANALYSIS

4.1 Technical active substance and plant protection product (IIA 4)

4.1.1 Technical active substance (IIA 4.1)

F-735-09-93 (E) & F-735-06-96 (E)
Samples were extracted with acidified methanol/acetonitrile/water (pH 3). Analysis was by reverse phase HPLC (C18 column) with UV detection at 220 nm, with external calibration.

Validation data are presented in Table 4.1.

(DP 70950 & DP 71011)

4.1.2 Impurities (IIA 4.1)

4.1.2.1 Organic impurities

[MB45950, MB46136, RPA 200766, MB45897, RPA100344, RPA097965 and chlorobenzene] also [RPA200060, RPA098028 and, RPA109263]

Samples were extracted with acetonitrile. Analysis was by reverse phase HPLC (C18 column) with UV detection at 220 nm, with external calibration.

Validation data are presented in Table 4.1. No validation data were presented for RPA200060, RPA098028 and, RPA109263 as the report states that these impurities were found below 0.1% w/w. The LOQ was claimed to be 0.02% w/w of technical fipronil and the accuracy was stated to be determined by the standard addition technique.

(DP 70950, DP 71013 & DP 70943)

This method is also described as being suitable for the determination of other minor impurities: MB46513 & MB46058. However, no other details/validation are presented.

[Ethanol]
Samples were extracted with acetonitrile. Analysis was by GLC-FID (Porapak Q packed column) with external calibration.

Limited validation data were presented as the report states that ethanol was not detected in any batch analysis. However, a chromatogram submitted for technical fipronil spiked with 0.01 g/l ethanol confirms the LOD is sufficient to confirm ethanol content is below 0.1% w/w.

(DP 70943)

4.1.2.2 Other impurities (sulphur)

Sulphur was extracted with a mixture of acetonitrile/toluene (90/10). Analysis was by reverse phase HPLC (C18 column) with UV detection at 270 nm, with external calibration.

Validation data are presented in Table 4.1.

(DP 71016)

4.1.2.3 Water

Water content was determined by the Karl Fischer method. No validation data were presented as the report states that water is not present above 0.1% w/w.

(DP 70943)

4.1.3 Plant protection product (IIIA 5.1)

a) (Accelerated storage stability study method)
Samples were extracted with acetonitrile. Analysis was by reverse phase HPLC (C18 column) with UV detection at 220 nm with internal calibration using butyrophenone.

Validation data are presented in Table 4.1.

(DP 71235)

b) (Ambient storage stability study method)
Samples were extracted with methanol and water. Analysis was by reverse phase HPLC (Spherisorb 5 ODS 2 column) with UV detection at 280 nm with internal calibration using diphenyl phthalate.

Validation data are presented in Table 4.1.

(DP 71238)

Table 4.1 Summary of method description and validation (active substance and plant protection product)

Substrate	Analyte	Method	Accuracy (%) (mean recovery)	Interference	Precision - reproducibility (n)	Precision - repeatability RSD (%) (n)	Linearity demonstrated (range g/l)	Reference
	Fipronil	F-735-06-96 (E)	100%-102% 101%	None, chromatogram peaks well resolved		0.2% (6)	0.4 – 0.6	DP 70950
Technical material (Elbeuf source)	Impurities: MB45950	F-863-12-97 (E)	100% - 101% (101%)	None, chromatogram peaks well resolved		0.4% (6)	0.003 – 0.153	DP 70950
	MB46136	F-863-12-97 (E)	101% - 101% (101%)			0.7% (6)	0.003 – 0.162	
	RPA 200766	F-863-12-97 (E)	100% - 100% (100%)			1.8% (6)	0.003 – 0.164	
	MB045897	F-863-12-97 (E)	100% - 102% (101%)			10.1% (6)	0.003 – 0.164	
	Chlorobenzene	F-863-12-97 (E)	103% - 104% (104%)			-	0.003 – 0.166	
	RPA100344	F-863-12-97 (E)	93% - 100% (96%)			0.7% (6)	0.003 – 0.1	
	RPA097965	F-863-12-97 (E)	96% - 98% (97%)			2.2% (6)	0.003 – 0.163	
Technical material	impurities: sulphur		101% - 105% (103%)	None, chromatogram peaks well resolved	0.7% (12)	0.7% (6)	0.001 - 0.1	DP 71016
Formulation: 'Vi-Nil GR'	Fipronil	(accelerated study method)	(94.2-104.8) (100%)	None, chromatogram peaks well resolved		3.29% (8)	0.11 – 1.1	DP 71235
Formulation: 'Vi-Nil GR'	Fipronil	(ambient study method)	(98.0-99.3) (98.6)	None, chromatogram peaks well resolved		0.69% (6)	30-150% of nominal	DP 71238

4.2 Residues in treated plants, plant products, foodstuffs and feedingstuffs (IIA 4.2.1)

An analytical method for the determination of fipronil and metabolites, MB45950, MB46136, MB46513 & RPA 200766 in non-fatty vegetables, cereal and fruit has been submitted. However, as no data on residues in plant materials (either from direct application or for following crops) have been presented no further consideration of this method is made at this time.

Consideration of this method and appropriate validation data will be required if use on edible crops is sought at a later date.

4.3 Residues in the environment (IIA 4.2)

4.3.1 Residues in soil (IIA 4.2.2)

a) DAG 1504 (fipronil, MB46513, MB45950, MB46136 & RPA 200766)

Samples were extracted with acetonitrile/acetone (70/30 v/v) and concentrated. The samples were cleaned up using an activated charcoal column and eluted with acetonitrile. The solvent was evaporated and the sample was dissolved in toluene. Analysis was by GLC-ECD with external calibration and DB 210 column. The LOQ for each compound was 0.002 mg/kg.

Validation data are presented in Table 4.3.

(DP 71024)

b) RPA/FIP/92062 (RPA 104615)

Samples were extracted with methanol and the solvent evaporated. The residuum was cleaned up by partition into dichloromethane followed by C18 column cleanup. The methanol was evaporated and the samples were dried and made into an azeotrope with acetonitrile. The azeotropic mixture was heated with concentrated sulphuric acid and the RPA 104615 is hydrolysed to MB45897. Analysis was by GLC-ECD with external calibration against MB45897. The LOQ for MB45897 was 0.002 mg/kg. Additional validation data have been submitted specific to the RPA 104615 with an LOQ 0.001 mg/kg.

Validation data are presented in Table 4.3.

(DP 71024 & DP 86154)

c) RPA/FIP/94051 (RPA 200761)

Samples were extracted with methanol and Sohxlet extraction and the methanol was evaporated and the sample hydrolysed with concentrated hydrochloric acid. Acetonitrile was added and the solvents evaporated to ensure all traces of moisture were removed. The dried residue was dissolved in methanol and toluene and methylated with diazomethane. Excess diazomethane was removed with acetic acid, the solvents evaporated and the residues taken up in de-ionised water/acetonitrile

(90/10). The samples was cleaned up on a C18 column, eluted with de-ionised water/acetonitrile, dried and taken up in toluene.

Analysis was by gas liquid chromatography - mass selective detection (GLC-MSD). The LOQ for RPA 200761 was 0.005 mg/kg.

Validation data are presented in Table 4.3.

(DP 71024)

4.3.2 Residues in water (IIA 4.2.3)

a) D.Ag. 1749 (fipronil) – surface water

Samples were extracted using C18 SPE after filtration and eluted with acetonitrile. The solvent was evaporated and the residue dissolved in ethyl acetate. Analysis was by GLC-ECD with external calibration. The LOQ was 0.1 µg/l in distilled water and 0.2 µg/l in surface water.

Validation data are presented in Table 4.3.

(DP 71026)

b) AR 163-98 (fipronil, MB45950, MB46136 and MB46513 – mineral and tap water

Samples were extracted using a fipronil affinity column and eluted with methanol. The solvent was evaporated and the residue dissolved in toluene. Analysis was by GLC-ECD with external calibration. The LOQ was 0.1 µg/l for all analytes in mineral water (tradename Evian) and tap water. (Further details will be required)

Validation data are presented in Table 4.3.

(DP 86157)

4.3.3 Residues in air (IIA 4.2.4)

Study Number 96-21

Samples were collected and desorbed from ORBO™-44 tubes with toluene (ORBO™-44 tubes are flame sealed glass adsorption tubes from Supelco, sufficient details have been submitted). Analysis was by GLC-ECD with external calibration. The LOQ was 0.05 µg/m³. The method was validated to determine extractability and breakthrough of the tubes using fortified adsorbent material at two temperature/humidity combinations.

Validation data are presented in Table 4.3.

(DP 71028)

Table 4.3 Summary of method description and validation (environmental samples)

Substrate	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (range)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
Soil [DAG 1504]	Fipronil	0.002	0.002 0.01 0.1	90 (65-115) 85 (69-100) 88 (81-99)	18.9 (12) 10.8 (12) 7.0 (12)	Yes	None	DP 70124
	MB46513	0.002	0.002 0.01 0.1	100 (80-125) 92 (59-126) 89 (76-104)	15.4 (12) 21.5 (12) 10.0 (12)	Yes	None	DP 70124
	MB45950	0.002	0.002 0.01 0.1	95 (75-115) 98 (68-120) 95 (98-105)	33.0 (12) 16.5 (12) 5.85 (12)	Yes	None	DP 70124
	MB46136	0.002	0.002 0.01 0.1	99 (65-125) 94 (58-123) 91 (78-112)	18.0 (12) 22.8 (12) 13.4 (12)	Yes	None	DP 70124
	RPA 200766	0.002	0.002 0.01 0.1	109 (75-135) 83 (64-112) 102 (79-130)	22.1 (12) 17.0 (12) 18.4 (12)	Yes	None	DP 70124
Soil [RPA/FIP/92062]	RPA 104615 (as MB45897)	0.002	0.002 0.01 0.1	96 (80-125) 90 (74-110) 80 (74-92)	16.9 (10) 14.7 (10) 8.4 (10)	Yes	None	DP 70124
	RPA 104615	0.001	0.001 0.002 0.005 0.01	82 (70-92) 79 (71-88) 90 (77-105) 92 (71-105)	10.8 (6) 7.1 (6) 11.0 (6) 14.5 (6)	-	-	DP 86154
Soil [RPA/FIP94051]	RPA 200761	0.005	0.005 0.01	87 (74-110) 95 (83-105)	10.5 (20) 10.2 (8)	Yes	None	DP 70124

Table 4.3 Summary of method description and validation (environmental samples) continued

Substrate	Analyte	Limit of quantification (µg/l)	Recovery fortification level (µg/l)	Mean recoveries % (range)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
Surface water [D.Ag. 1749]	fipronil	0.2	0.1 1.0	102 (79-124) 83 (66-93)	11.6 (4) 15.4 (4)	Yes	None	DP 71026
Distilled water [D.Ag. 1749]	fipronil	0.1	0.2 0.4 0.5	83 (67-97) 77 (61-92) 68 (63-73)	16.3 (4) 20.2 (3) - (2)	Yes	None	DP 71026
Mineral water [AR 163-98]	fipronil	0.1	0.1 1.0	101 (100-105) 95 (92-97)	2.0 (5) 2.0 (5)	Yes	None	DP 86157
Tap water [AR 163-98]	fipronil	0.1	0.1 1.0	103 (97-113) 87 (83-90)	5.9 (5) 4.7 (5)	Yes	None	DP 86157
Mineral water [AR 163-98]	MB45950	0.1	0.1 1.0	105 (103-108) 95 (92-97)	2.0 (5) 2.2 (5)	Yes	None	DP 86157
Tap water [AR 163-98]	MB45950	0.1	0.1 1.0	104 (102-109) 90 (85-95)	2.9 (5) 5.1 (5)	Yes	None	DP 86157
Mineral water [AR 163-98]	MB46136	0.1	0.1 1.0	99 (97-100) 92 (89-95)	1.5 (5) 2.8 (5)	Yes	None	DP 86157
Tap water [AR 163-98]	MB46136	0.1	0.1 1.0	97 (91-101) 84 (75-92)	4.2 (5) 10.2 (5)	Yes	None	DP 86157
Mineral water [AR 163-98]	MB46513	0.1	0.1 1.0	101 (97-103) 96 (92-99)	2.3 (5) 2.8 (5)	Yes	None	DP 86157
Tap water [AR 163-98]	MB46513	0.1	0.1 1.0	102 (95-107) 94 (84-101)	4.6 (5) 7.2 (5)	Yes	None	DP 86157
Air [22.2°C + 47.3% RH]	fipronil	0.05 µg/m3	0.05 µg/m3	87	-	Yes	None	DP 71028
Air [21.9°C + 39.3% RH]	fipronil	0.05 µg/m3	0.05 µg/m3	89 (84-93)	-	Yes	None	DP 71028

4.4 Residues in human and animal tissues and fluids (IIA 4.2.5)

4.4.1 Animal tissues and milk

Method GC/ECD 12/95 (fipronil, MB46136 and MB45950)
Samples were extracted with 30% acetone in acetonitrile and filtered. The samples were then cleaned up by column chromatography (florisil/silica gel/charcoal). Analysis was by GLC-ECD with external calibration.

The evaluation under application COP 98/01208, (SC10698), identified that the recovery figures appeared to show a pattern for some of the milk recoveries which was repeated for all analytes (DP 71032). The applicant was requested to submit all raw data for the validation of this method together with all the chromatograms as part of any future submission.

The applicant has submitted a revised report which has been 'amended' with changes/additions requested by the United States EPA. In the report it states that some of the recovery values reported were incorrectly transcribed from the reports in which they were generated. The applicant maintains that with the revised report it is now not necessary to submit the raw data and chromatograms as requested.

However, in the revised report (July 2001, DP 112739), some of the recovery data for the milk analyses have been omitted (9 data points omitted at 0.01mg/kg, none at the 0.05mg/kg, 4 at the 0.1 mg/kg and 2 at the 0.5 mg/kg fortifications). The example chromatograms for milk indicate some dilution of the spiked samples has been necessary (so levels fall within linearity range). Additional recovery data have also been included for cow kidney and fat at the 0.5 mg/kg fortification level.

Amended validation data are summarised in Table 4.4

(DP 71032 & DP 112739)

4.4.2 In support of therapeutic and diagnostic regimes

Fipronil (plus metabolites MB46513, MB45950 and MB 46136)
Fipronil and metabolites were extracted from plasma by purification over a C18 column. The columns were conditioned with 2 washes of methanol followed by 2 washes of ultra pure water. The plasma was applied to the column and the column was then washed twice with methanol/ultra pure water (25/75) and the cartridge allowed to dry. The analytes of interest were eluted with 2 washes of methanol/ultra pure water (75/25). The samples were collected and toluene added to the samples. The samples were then shaken and centrifuged to allow partition of the analytes into the toluene. The toluene phase was collected and analysis was by GLC-ECD with external calibration.

The report submitted was a stability report for the analytes in plasma when stored at 4°C for 2-14 days. The report concluded that whilst recoveries were always within ± 20% of the spiked concentration a gradual decline was observed for all analytes such

that the recommended storage temperature is –20°C. The fipronil and metabolite MB45950 eluted very close together, however, they were distinguishable in all sample chromatograms provided.

Validation data are summarised in Table 4.4

(DP 112738)

Table 4.4 Summary of method description and validation (animal tissues)

Substrate	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (range)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
Cow liver	Fipronil	0.01	0.01 0.05 0.10 0.50	95 92 102 (80-103) 91 (86-95)	- - 12.8 (3) -	Yes	None	DP 71032
	MB46136	0.01	0.01 0.05 0.10 0.50	104 103 96 (85-114) 92 (85-99)	- - 16.1 (3) -	Yes	None	DP 71032
	MB45950	0.01	0.01 0.05 0.10 0.50	91 86 90 (82-99) 88 (86-90)	- - 9.4 (3) -	Yes	None	DP 71032
Cow kidney	Fipronil	0.005	0.50	90 (86-92)	2.7 (6)	Yes	None	DP 71032 & DP 112739
	MB46136	0.005	0.50	96 (90-101)	4.2 (6)	Yes	None	DP 71032 & DP 112739
	MB45950	0.005	0.50	84 (72-90)	7.4 (6)	Yes	None	DP 71032 & DP 112739
Cow fat	Fipronil	0.005	0.50	86 (77-96)	7.0 (7)	Yes	None	DP 71032 & DP 112739
	MB46136	0.005	0.50	91 (84-100)	5.4 (7)	Yes	None	DP 71032 & DP 112739
	MB45950	0.005	0.50	84 (77-90)	5.2 (7)	Yes	None	DP 71032 & DP 112739
Cow muscle	Fipronil	0.01	0.01 0.05 0.10	91 (88-94) 98 93 (81-104)	3.3 (3) - -	Yes	None	DP 71032
	MB46136	0.01	0.01 0.05 0.10	98 (94-105) 112 103 (92-114)	6.2 (3) - -	Yes	None	DP 71032
	MB45950	0.01	0.01 0.05 0.10	95 (94-96) 92 89 (81-97)	1.2 (3) - -	Yes	None	DP 71032

Items in *Italics* – unchanged from SC 10698 (COP 98/01208)

Table 4.4 Summary of method description and validation (animal tissues) (continued)

Substrate	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (range)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
Cow milk	Fipronil	0.01	0.01 *0.05* 0.10 0.50	88 (84-91) *90 (85-96)* 90 (81-95) 88 (81-92)	2.9 (8) *5.4 (4)* 7.6 (4) 6.7 (3)	Yes	None	DP 71032 & DP 112739
	MB46136	0.01	0.01 *0.05* 0.10 0.50	99 (94-106) *96 (90-104)* 100 (94-108) 93 (87-98)	4.1 (8) *6.3 (4)* 6.2 (4) 6.1 (3)	Yes	None	DP 71032 & DP 112739
	MB45950	0.01	0.01 *0.05* 0.10 0.50	95 (86-102) *90 (86-96)* 91 (84-97) 92 (87-95)	5.8 (8) *4.8 (4)* 5.9 (4) 4.5 (3)	Yes	None	DP 71032 & DP 112739
Poultry liver	Fipronil	0.01	0.01 0.05 0.10 0.50	85 (68-102) 92 105 (92-117) 88 (85-90)	- - - -	Yes	None	DP 71032
	MB46136	0.01	0.01 0.05 0.10 0.50	100 (84-116) 99 96 (92-100) 91 (86-96)	- - - -	Yes	None	DP 71032
	MB45950	0.01	0.01 0.05 0.10 0.50	97 (87-106) 92 90 (88-91) 88 (86-90)	- - - -	Yes	None	DP 71032

Items in *Italics* – unchanged from SC 10698 (COP 98/01208)

Table 4.4 Summary of method description and validation (animal tissues) (continued)

Substrate	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (range)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
Poultry skin & fat	fipronil	0.01	0.01 0.05 0.10 0.50	94 (90-98) 91 (86-95) 90 85 (84-85)	- - - -	Yes	None	DP 71032
	MB46136	0.01	0.01 0.05 0.10 0.50	99 (95-103) 94 (89-98) 93 87 (86-88)	- - - -	Yes	None	DP 71032
	MB45950	0.01	0.01 0.05 0.10 0.50	96 (94-98) 90 (90) 88 89 (87-91)	- - - -	Yes	None	DP 71032
Poultry muscle	fipronil	0.01	0.01 0.05 0.10 0.50	88 (84-92) 93 (87-98) 90 86 (84-88)	- - - -	Yes	None	DP 71032
	MB46136	0.01	0.01 0.05 0.10 0.50	93 (84-102) 90 (84-95) 94 86 (85-86)	- - - -			DP 71032
	MB45950	0.01	0.01 0.05 0.10 0.50	91 (89-92) 89 (87-90) 93 91 (90-92)	- - - -			DP 71032

Table 4.4 Summary of method description and validation (animal tissues) (continued)

Substrate	Analyte	Limit of quantification (mg/kg)	Recovery fortification level (mg/kg)	Mean recoveries % (range)	Repeatability %RSD (n)	Linearity demonstrated	Interference	Reference
Poultry eggs	fipronil	0.005	*0.005*	*81 (76-86)*	*5.1 (7)*	Yes	None	DP 71032 & DP 112739
			0.01	96 (85-105)	7.3 (17)			
			0.025	*93 (88-100)*	*4.9 (5)*			
			0.05	93 (85-100)	5.5 (6)			
			0.10	88 (84-89)	2.2 (6)			
			0.50	90 (86-95)	3.5 (5)			
	MB46136	0.005	*0.005*	*92 (83-102)*	*9.4 (7)*	Yes	None	DP 71032 & DP 112739
			0.01	106 (96-127)	7.2 (17)			
			0.025	*99 (92-108)*	*6.7 (5)*			
			0.05	102 (93-116)	7.7 (6)			
			0.10	95 (87-96)	4.2 (6)			
			0.50	97 (94-102)	3.8 (5)			
	MB45950	0.005	*0.005*	*93 (85-103)*	*7.3 (7)*	Yes	None	DP 71032 & DP 112739
			0.01	98 (87-113)	8.5 (17)			
			0.025	*86 (84-89)*	*2.4 (5)*			
			0.05	94 (89-99)	4.7 (6)			
			0.10	90 (86-91)	3.1 (6)			
			0.50	89 (85-93)	3.0 (5)			
Human Plasma	Fipronil	50µg/l	50µg/l	102, 106	-	Yes	None	DP 112738
			200µg/l	91, 99	-			
			400µg/l	92, 106	-			
	MB46513	2µg/l	2µg/l	105, 110	-	Yes	None	DP 112738
			4µg/l	110, 110	-			
			8µg/l	105, 116	-			
	MB46136	50µg/l	50µg/l	104, 108	-	Yes	None	DP 112738
			200µg/l	105, 110	-			
			400µg/l	101, 115	-			
	MB45950	50µg/l	50µg/l	96, 100	-	Yes	None	DP 112738
			200µg/l	93, 97	-			
			400µg/l	91, 104	-			

Items in *Italics* – unchanged from SC 10698 (COP 98/01208)

4.5 Evaluation and assessment

Acceptable and validated HPLC methods with UV detection have been submitted for fipronil and impurities in the technical material. A combination of these methods were used to perform five batch analyses which support the revised technical specification submitted during the evaluation.

A number of methods were used with various reference numbers, F-735-09-93 (E) and F-735-06-96 (E) for fipronil and B-658-10-91 (E) and F-863-12-97 (E) for the impurities. The corresponding a.s. and impurity methods appear the same from the descriptions. The applicant has clarified that the differences between these methods were minor (column diameter and extension of analytes) and this is acceptable.

Acceptable reverse phase HPLC methods with UV detection were submitted for analysis of fipronil in the proposed formulation.

Methods of analysis have been submitted for residues in plants. However, use on edible crops is not sought at present, there are no studies presented which rely on these methods and no indications of residues in either crops or following crops. These methods, together with appropriate validation data, will require consideration should use on edible crops be sought and residues in crops be considered likely.

Three methods of analysis for soil were presented using GLC-ECD (2) and GLC-MSD. LOQs were validated at 0.002 and 0.005 mg/kg. The recoveries seen with the method validation vary. However, these data have been used as part of the fate and behaviour evaluation and are considered acceptable (see Section 7.1.3.1). The second GLC-ECD method for metabolite RPA 104615 involved hydrolysis of this metabolite to MB45897 with external calibration against MB45897. Acceptable validation data have been presented for both the RPA104615 and the MB45897.

A GLC-ECD method was submitted for analysis of fipronil in de-ionised and surface water. The methods are validated with an LOQ at 0.1 µg/l for the de-ionised water but only 0.2µg/l for the ground water.

A second GLC-ECD method was submitted for the analysis of fipronil, MB45950, MB46136 and MB46513 in mineral and tap water with validated LOQ's of 0.1 µg/l for all analytes. This method employed extraction/cleanup with a fipronil affinity column. Further details of the underlying principles of the technique will be requested from the applicant.

An acceptable GLC-ECD method for the analysis of air samples has been submitted with an LOQ of 0.05 µg/m^3.

A method of analysis was submitted for animal tissues under the original application. At that time no data were presented on likely intakes by or residues in livestock. However, the toxicological profile of fipronil and its metabolites warranted consideration of this method. Although the method appeared acceptable, further

clarification of the recovery data was requested. The applicant was requested to submit all the raw data and chromatograms for the submitted analysis.

The applicant has submitted another report which has been amended in accordance with request made by the EPA. A number of recovery data points have been removed (e.g. milk and eggs) with some additions (cow kidney and fat). However, these changes in the report do not address the original request by PSD. The submission of the rotational crop study is reassuring in that intakes by domestic animals will be <0.1 mg/kg diet as received. As such, the need for a method of analysis for animal tissues becomes less acute. Therefore, it is acceptable for the applicant to submit the necessary raw data and chromatograms for full approval.

A validated method of analysis for the determination of fipronil and metabolites in plasma and urine was required. The applicant has submitted a study report looking at the stability of fipronil and three metabolites (MB 46513, MB46136 & MB45950) in human plasma. These data include validation (linearity, specificity and accuracy) of the method used and are considered acceptable. This method in conjunction with the animal tissues method is considered sufficient to indicate that analysis of urine would also be achievable.

4.6 Conclusions and data requirements

Methods of analysis have been submitted and considered acceptable, such that provisional approval may be proposed. However, some further information regarding the methods of analysis for animal tissues are required for full approval. Whilst these data were originally requested for provisional approval, the additional rotational crop metabolism data indicate that intakes by animal will be <0.1 mg/kg diet AR. Therefore, the method of analysis for animal tissue is no longer a requirement for provisional approval, but may be submitted for full approval. Some sample chromatograms have been submitted and it appears that these may have been diluted to bring the analysis within the linearity range. Referring to the milk samples, a peak around 20-21 minutes appears to reflect dilutions for determining untreated, 10 μg/kg and 100μg/kg respectively. However, a peak is seen at ~13 minutes in the untreated and 100μg/kg samples at about the same response but is absent from the 10μg/kg sample. The cow liver samples also show a prominent responder at ~22-23 minutes. The sample chromatograms for fat and muscle also show a strong responder at ~19 minutes for which the peak size does not seem to match the dilutions which are suggested by the chromatogram. The data submitted and evaluated are still unclear as some data points have been removed and other included apparently at the request of the US EPA. The further information will be required for full approval:

The information submitted on methods of analysis is sufficient to support approval of 'Vi-Nil GR' for use on non-edible ornamentals. The following further data are required for full approval:

(i) Full details of the fipronil affinity column used for the quantification of fipronil and metabolites in water.

(ii) For the method of analysis for animal tissues all raw data and chromatograms for all analyses are required together with a fuller explanation of why certain recovery data points have simply been deleted and additional ones included. The raw chromatograms are required for all determinations with clear annotations as to the matrix/analyte and any dilution factors applied. Please suggest the identity of the peak at ~20-21 minutes for the milk samples, the peak ~22-23 minutes on the cow liver samples and 19 minutes on the cow fat and muscle sample chromatograms.

5 TOXICOLOGICAL AND METABOLISM STUDIES

Fipronil (see structure in Section 1.1.6) is a member of a class of insecticides known as the phenylpyrazoles. Its putative mode of insecticidal action is through blockage of the passage of chloride ions through the γ-aminobutyric acid (GABA)-regulated chloride ion channel, which results in uncontrolled central nervous system activity and subsequent death of the insect. Although fipronil is selectively toxic to insects, some of the toxicity of fipronil observed in mammals also appears to involve interference with the normal functioning of the GABA receptor.

All studies below were conducted to GLP, and to appropriate OECD and EPA guidelines unless otherwise stated.

5.1 STUDIES ON ABSORPTION, DISTRIBUTION, EXCRETION AND METABOLISM IN MAMMALS (IIA 5.1)

5.1.1 Absorption, distribution and excretion

a) Sprague-Dawley rats (5 animals/sex/group) were administered either single doses of 4 or 150 mg/kg (^{14}C) phenol ring-labelled fipronil (batch number IHR 1465, re-purified to 98.6-99.5% radiochemical purity, specific activity 45 μCi/mg), or 14 daily doses of 4 mg/kg non-labelled fipronil (batch number AJK 232, 99.3% purity) followed 24 h after the last dose by a single dose of (^{14}C) phenol ring-labelled fipronil. Doses were administered by gavage as a suspension in aqueous methylcellulose (0.5%w/v) and 'Tween 80' (0.01% w/v). Following administration of (^{14}C) fipronil the animals were placed in a metabowl where urine was collected 0-6, 6-24 and then every 24 h until 168 h post radioactive dose. Faeces were collected every 24 h until 168 h post-dose. Given the low recovery of radioactivity via exhaled air in the pilot study, no collection via this route was conducted. In addition 5 animals/sex/group were administered a single gavage dose of either 4 or 150 mg/kg (^{14}C) phenol ring-labelled fipronil (98.6-99.5% purity) and approximately 0.1 ml of blood was sampled from the lateral tail vein at 0.5, 1, 2, 4, 6, 24 h and then every 24 h until 168 h post-dose.

The radioactive dose recovered was 97.6-106.5% of that administered with no sex differences noted in elimination routes. Radioactive distribution at 168 h is noted in Table 5.1.

Table 5.1 % Distribution of radioactivity collected 0-168 h.

Regimen	Urine	Faeces	Tissues/Carcass	Totals
4 mg/kg – single dose	6	46	46	98
4mg/kg – repeat dose	14-16	56-61	20-23	90-100
150mg/kg – single dose	22-29	67-75	3-5	92-99

Following a single 4 mg/kg dose, renal elimination was broadly constant, with 0.4-1.5% of the dose recovered at each sampling point. A mean of 20% of the dose was noted in faeces at 24 h, with 3-6% recovered at the subsequent time points. At necropsy abdominal fat contained 15-19 μg equiv./g of the dose with 1-6 μg equiv./g noted in; adrenals, pancreas, skin, liver, kidney, ovaries and uterus. In blood Cmax

was 0.6-0.7 μg equiv./g and was reached in 4-6 h. Concentrations at 168 h were ≈40% of Cmax and elimination half-lives were calculated to be 150 h in males and 200 h in females.

Animals repeatedly dosed with 4 mg/kg/d fipronil displayed renal elimination which was rapid up to 72 h (11-13% of the dose) but which persisted until 168 h (≈0.3% of the dose 144-168 h). A similar pattern was noted for the faecal route with 45-47% of the dose being eliminated up to 72 h and 1.7% collected between 148-168 h. At necropsy the distribution of radioactivity was similar to that in the single dose group with the highest concentration noted in fat (6 μg equiv./g) and 1-2μg equiv./g noted in pancreas, adrenals, skin, liver and uterus.

Following a single 150 mg/kg dose, renal elimination comprised 22-29 % of the dose which was mostly eliminated between 24-96 h. There was a minimal (0.1-0.4%) portion of the dose collected at 168 h. Faecal elimination followed a similar pattern with 59-63% of the dose collected up to 96 h and 0.6-1.3% eliminated at 168 h. At necropsy, concentrations of radioactivity were higher in females but with similar organs affected in both sexes. Abdominal fat contained 29 μg equiv./g in males and 54 μg equiv./g in females, with adrenals, pancreas, skin and liver containing 6-9 μg equiv./g in males and 11-17 μg equiv./g in females. The ovaries also contained a relatively high level of activity (16 μg equiv./g). There were no sex differences in blood pharmacokinetic parameters, with Cmax being ≈20 μg equiv./g at 48-72 h. At 168 h 10-12% of the dose was still present in blood and elimination half-lives were calculated to be 51-54 h.

(DP 71035)

b) In this rat (Sprague Dawley) biliary metabolism study conducted in 1995 (Japanese MAFF guidelines, GLP compliant), the excretion balance and metabolism of radiolabelled fipronil (^{14}C -phenyl ring) was examined at single oral high dose (SOHD, 40 mg/kg bw) and single oral low dose (SOLD, 4 mg/kg bw). Excreta was collected from 4 cannulated animals/ sex/ dose for up to 72 hours after dosing. Radioactivity for the excretion balance was determined by liquid scintillation counting.

The results are shown in Tables 5.2 to 5.4

Recoveries were satisfactory ranging from 82% in females to 108 % in males. As in non-cannulated rats in the study at (a) above a greater proportion of the administered dose was excreted via the urine and feces at SOHD than at SOLD (See Tables 5.2 and 5.3). The same is true of biliary excretion. Fecal excretion in cannulated rats represents unabsorbed material, and can be used to estimate total absorption. At SOLD total absorption was approximately 90%. As in the study at (a) elimination of administered radioactivity was slow. Significant amounts of radioactivity were still being excreted after 48 hours and the majority of administered radioactivity remained in tissues at 72 hours after treatment. Table 5.4 shows greatest concentrations in body tissues (especially the GIT) rather than GIT content.

To conclude, this biliary excretion study demonstrates that absorption is approximately 90% and a lag in the elimination of radioactivity is due to slow release of material partitioned to body tissues (in particular the GIT).

Table 5.2 Excretion of radiolabelled fipronil (phenyl ring) in SOHD male and female rats as a percentage of administered dose up to 72 hours.

	Males			Females		
Hours	0-24	0-48	0-72	0-24	0-48	0-72
Urine	0.2	0.8	4.7	0.4	1.5	2.6
Feces	14.6	18.9	21.4	30.4†	31.8†	35.5†
Bile	2.5	12.7	24.9	3.1	6.7	11.6
Cage wash			1.2			1.3
Total excreta			52.2			50.9
Total tissues and GIT contents*			55.8			66.3
Recovery			108.0			81.8

Data are means of four animals. *Intestinal tract contents, intestinal tract, stomach contents, stomach, residual carcass, blood, plasma, skin & fur. † excludes fourth animal which appeared constipated.

Table 5.3 Excretion of radiolabelled fipronil (phenyl ring) in SOLD male and female rats as a percentage of administered dose at up to 72 hours.

	Males			Females		
Hours	0-24	0-48	0-72	0-24	0-48	0-72
Urine	0.3	0.5	0.9	0.8	1.3	1.6
Feces	9.8	11.8	13.7	8.6	8.6	9.7
Bile	3.6	5.8	7.6	4.0	5.1	6.8
Cage wash			0.1			0.4
Total excreta			22.3			18.5
Total tissues and GIT contents*			80.2			83.4
Recovery			102.4			101.9

Data are means of four animals. *Intestinal tract contents, intestinal tract, stomach contents, stomach, reseal carcass, blood, plasma, skin & fur. † excludes fourth animal which appeared constipated.

Table 5.4 Distribution of radioactivity in tissues or organs (µg equivalents benfuracarb/ g tissue) following SOLD or SOHD to male or female rats

	SOLD		SOHD	
Tissue	Males	Females	Males	Females
Intestinal tract contents	0.6	0.8	32.0	30.6
Intestines	5.0	5.7	19.5	23.5
Stomach contents	0.6	0.9	95.6	135.5
Stomach	4.0	3.5	81.9	101.6
Residual carcass	3.5	3.4	17.6	18.6
Blood	0.4	0.4	5.8	4.3
Plasma	0.5	0.5	9.9	7.3
Skin & Fur	4.9	6.5	15.9	27.7

5.1.2 Metabolism

a) An investigation in to the metabolism of fipronil was incorporated into the study on absorption, distribution and excretion at 5.1.1 (a).

After solvent extraction for faeces and tissues and deconjugation with 13-glucuronidase and aryl sulfatase for urine, metabolites were characterised by HPLC

elution profiles against authentic standards and MS in selective ion and/or scan modes. An initial HPLC method using filtered urine noted that all labelled material was associated with very polar components eluting with the void volume of the column. Only after deconjugation were the components resolved, which suggests the presence of glucuronide or sulfate conjugates.

There were no apparent sex-based differences in metabolic profiles. For urinary and faecal samples 50-90% of the metabolites were identified. A large part of the unidentified faecal metabolites were polar in nature. The proposed metabolic pathway for fipronil is noted in Figure 5.1. The principal urinary metabolites were conjugates of RO/l, RO/2 with increasing amounts of parent molecule, RPA 10548 and RPA 200766 noted with repeat dosing and at the higher dose level. Precise comparison between dosing regimens was not possible as the pooled samples analysed were made up from variable time points. All faecal samples were pooled and showed no dose-related change in metabolic profile. The principal faecal components were parent compound (6-18% of the dose), MB 46136 (4-12% of the dose) and MB 45950 (1-2.5% of the dose). In all tissues the principal metabolite was MB 46136 (94-100% of the extracted dose). The test laboratory made no comment as to the likely positions of conjugation (Tables 5.5 and 5.6).

The single radiolabel position used in this study was considered to be adequate as known metabolites accounted for the majority (80-90%) of the radioactivity recovered in repeat low-dose animals. Unidentified radioactivity consisted of highly polar metabolites eluting in the void volume of the HPLC column after deconjugation. Also there was no evidence of cleavage of the molecule between the two rings from the phenol ring-labelled metabolites recovered.

Table 5.5 Urinary excretion of metabolites of phenyl ring radiolabelled fipronil as a percentage of administered dose.

Metabolite	Single dose 4 mg/kg		14 d repeat dose 4 mg/kg		Single dose 150 mg/kg	
	Male (0-72 hour sample)	Female (0-24 hour sample)	Male (6-72 hour sample)	Female (0-96 hour sample)	Male (6-96 hour sample)	Female (6-120 hour sample)
Parent	0.1	0.03	0.68	1.2	1.0	2.0
RPA 200766	0.4	ND	ND	0.5	ND	1.9
RPA 105048	ND	0.1	0.3	0.7	ND	7.4
RO/1 RO/2	0.8	1.1	5.6	5.3	13.3	17.0
Unidentified	0.2	0.2	6.8 (up to 7 components)	6.8 (up to 7 components)	14.7	2.8
Total	1.5	1.4	13.5	14.5	28.0	19.8

ND = not detected

Table 5.6 Faecal excretion of metabolites of phenyl ring radiolabelled fipronil as a percentage of administered dose.

Metabolite	Single dose 4 mg/kg		14 d repeat dose 4 mg/kg		Single dose 150 mg/kg	
	Male (0-120 hour sample)	Female (0-120 hour sample)	Male (0-120 hour sample)	Female (0-120 hour sample)	Male (0-120 hour sample)	Female (0-120 hour sample)
Parent	13.1	10.5	8.3	6.4	10.6	18.6
RPA 200766	ND	ND	0.1	ND	0.8	ND
MB 45950	1.6	1.2	3.0	1.0	1.3	2.5
MB 46136	11.7	9.1	7.2	7.8	3.8	4.4
Unidentified	3.8	4.4	14.5 (up to 7 components)	15.8 (up to 7 components)	20.6 (up to 7 components)	14.5% (up to 7 components)
Unextractable	8.3	13.2	19.5	25.9	25.34	29.6
Total	30.1	25.1	33.2	31.0	37.1	40.0

ND = not detected

b) An investigation in to the metabolism of fipronil was incorporated into the biliary excretion study at 5.1.1 (b).

Metabolites were separated and quantified only in bile. This was done using HPLC, TLC and enzymic hydrolysis to determine conjugates. Individual metabolites separated are displayed in Table 5.7. The most prominent metabolites were BMET 3, 5 and 7. As in the study at (a) above, a proportion of the radioactivity could not be identified as specific metabolites. Although no sex difference in metabolism was apparent from the study above, a sex difference was apparent in the biliary metabolic profiles at both SOLD and SOHD: This was particularly evident with metabolites BMET 3, 5 RPA 200766, and 10. Changes in the ratios of metabolites (e.g. BMET 3: RPA 200766) from SOLD to SOHD also indicate a possible saturation or induction of pathways.

Enterohepatic recirculation and further metabolism of biliary metabolites is likely to account for the reduction in the number of unknown components as seen in Tables 5.2 and 5.3 above.

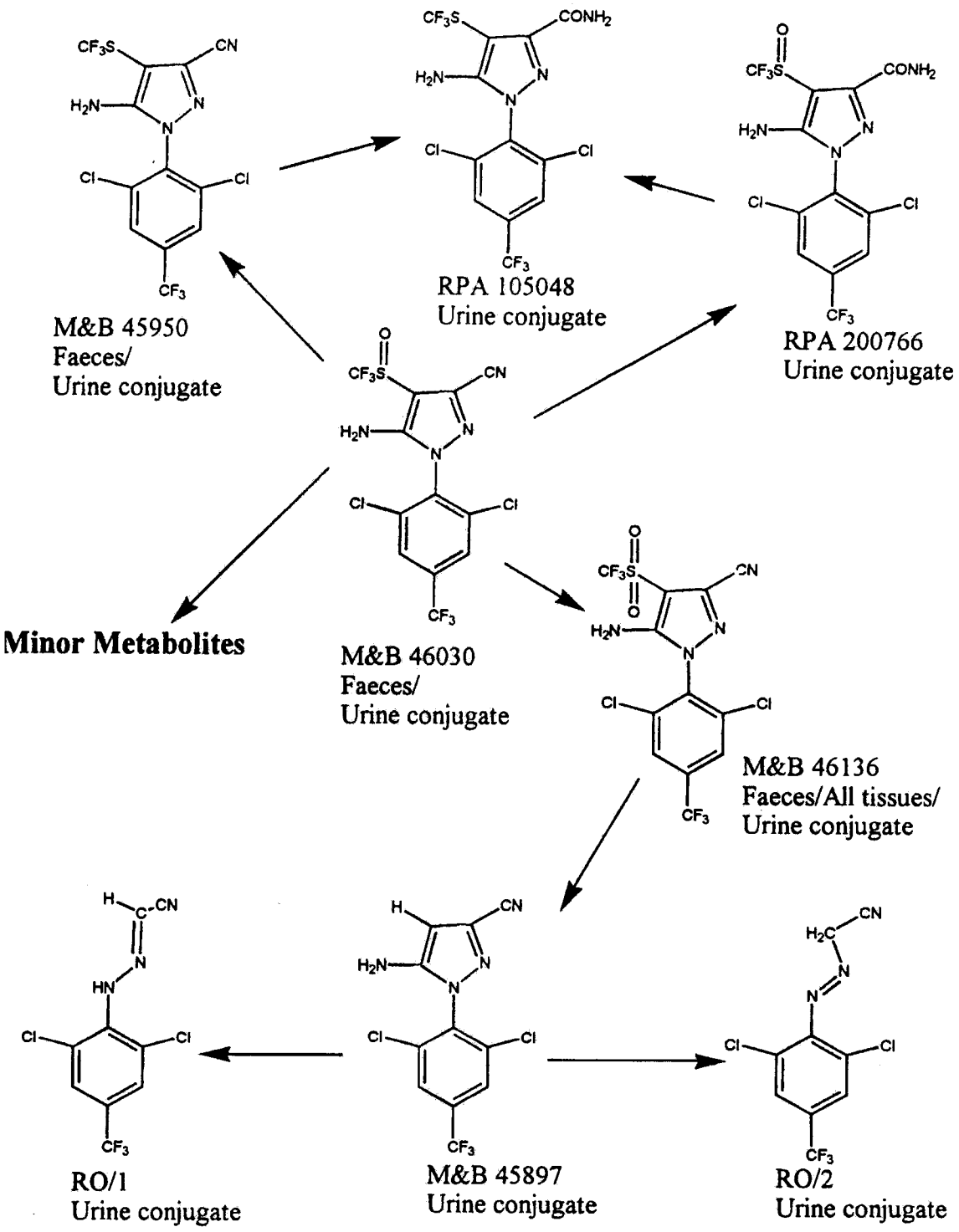
Table 5.7 Metabolites in bile of rats (% administered radiolabelled fipronil) at SOLD or SOHD – 0-72 hour.

		SOLD		SOHD	
BMET /-	metabolite name	Male	Female	Male	Female
1	ni	0.10	nd	0.30	nd
2 (c?)	ni	0.16	0.01	0.54	nd
3 (c)	**ni**	**3.14**	**1.35**	**21.97**	**8.08**
4	ni	nd	nd	0.03	0.24
5 (c)	**ni**	**0.90**	**2.98**	**0.73**	**1.70**
6 (c)	ni	0.01	nd	0.31	0.33
7	**RPA 200766**	**1.37**	**0.69**	**0.77**	**0.57**
8	ni	0.10	0.20	0.05	0.07
9	ni	0.12	0.26	0.06	0.10
10 (c?)	ni	0.22	0.11	0.09	0.05
11	MB 45897	0.24	0.33	0.09	0.10
12	ni	0.27	0.23	0.09	0.07
13	ni	0.25	0.14	0.12	nd
14	ni	0.02	nd	0.01	nd
15	Fipronil	0.25	0.26	0.09	0.15
16	MB 46136	0.47	0.21	0.05	0.15

ni = not identified. nd = not detected. (c) = conjugates. (c?) = possible conjugates.

(DP 114688)

Figure 5.1 Proposed metabolic pathway of fipronil in the rat



(DP 71035, DP 71037)

5.1.3 Dermal penetration

a) The *in vitro* absorption of ^{14}C fipronil (98.7% purity) was compared to that of ^{14}C hydrocortisone (batch number GHS 634 A, 97.1% purity, specific activity 154

μCi/mg) using human (autopsy derived), rat (Sprague-Dawley) and rabbit (New Zealand White) epidermal membrane.

Radiolabelled fipronil and hydrocortisone were formulated with 'EXP 601 45A' (a formulation mixture containing propylene glycol, ethoxylated polyaryl phenol, ethoxylated alkyl phenol, ethoxylated fatty alcohol, silicone oil emulsion, de-waxed maize oil, blend emulsifier and water) to give a base concentration of 200 g/l (20% w/v). After sonication in the water and oil phases of 'EXP 601 45A', the particle size for fipronil and hydrocortisone was 1-2 μm. The base concentration was further diluted in distilled water as required.

Rat and rabbit membranes were prepared by blunt dissection and freezing of the dermal side to a steel plate followed by dermatoming to 300 μm. Human membranes were prepared by immersing whole skin in water at 60°C for 45 seconds after which the epidermis was peeled away and taken up onto filter paper. Membranes were placed as a barrier in horizontal glass diffusion cells which were kept at a constant temperature (37.0+0.5°C). Membrane areas used and receptor chamber volumes were noted for each cell. Membrane integrity was measured using tritiated water on the first experimental day. In the experiments, 100 μl/cm^2 of the test solution was placed on the skin surface and 200 μl samples taken from the receptor chamber which had previously been filled with a known volume of ethanol.

The results showed that in 'EXP 601 45A' the flux rates (including the lag phase) at 24 h and 4 g/l for fipronil were 0.99+0.84, 0.8±0.41 and **0.03±0.04 μg cm^2/h** in rat, rabbit and human epidermis respectively. At 24 h 0.18 ± 0.26% of the applied dose penetrated human epidermis. The study notes that hydrocortisone is a relatively poor permeant and that the flux rates (4 g/l at 24 h) were 6.44+1.12, 2.79±1.47 and 0.41+0.43 μg cm^2 /h in rat, rabbit and human epidermis respectively. Compound flux rates at application concentrations of 0.2 and 200 g/l at 8 and 24 h, and 4 g/l at 8 h showed the same relative order as at 4 g/l at 24 h. This study was not conducted to GLP or a recognised guideline.

(DP 71251)

b) In further *in vitro* studies evaluated by HSE in 1999 (not submitted to PSD), three formulations containing ^{14}C fipronil (90-95% purity) were applied to human and rat (Wistar) epidermis at 10 μl/cm in a static chamber and the dermal absorption compared to that of ^{14}C testosterone (Ref CFA 129 batch 60, 96.2% purity, specific activity 196 μCi/mg). Human membrane was prepared as above and rat membrane was prepared by soaking in sodium bromide. The formulations comprised : 50 g/l fipronil in a suspension concentrate (corn oil, water, propylene glycol, emulsifier, dispersant and wetting agent); 25 g/l fipronil in an oil based mixture (rapeseed oil, diacetone alcohol and esterified rapeseed oil); and 300 g/l fipronil in an emulsifiable concentrate (n-alkl-2-pyrrolidone, cyclohexanone and emulsifiers). Membrane integrity was measured using tritiated water.

In humans the absorption of all three formulations was comparable with **mean flux rates in the range 0.04-0.2 μg cm^2 h (steady state kinetics)**. At 24 h, 0.15-0.3% of the dose had penetrated human epidermis for all the formulations. Dilution of the formulations (suspension concentrate 1:100 in water, oil mixture 1:10 in oil and

emulsifiable concentrate 1:50 in water) decreased the flux rate and increased the percentage of the mean dose penetrating human skin to 1-3%. This protocol demonstrated that fipronil in these formulations penetrated rat epidermis (1-15% of the dose undiluted, 10-35% of the dose diluted at 24 h) to a greater extent than human epidermis.

(DP 71251)

c) The extent of absorption of ^{14}C-fipronil was studied following application of 'Regent 80 WDG' (containing 79% fipronil) spiked with ^{14}C-fipronil (lot GHS-826, 98% radiopurity) to the skin (approximately 12.5 cm^2) of male rats.

This study conducted in 1995 was compliant to GLP but no guideline was specified in the study report.

A preliminary phase consisting of two groups of four animals each was conducted to evaluate and establish test material application and skin washing techniques. In the definitive phase, male rats were dermally dosed at three levels: 0.876 mg/animal, (0.070 mg/cm^2), 8.35 mg/animal (0.668 mg/cm^2) and 48.5 mg/animal (3.88 mg/cm^2) 'suspended' in a vehicle of 1.0% carboxymethylcellulose solution. Control animals received vehicle alone. No rationale was presented for selection of the doses used. Application site skin of four animals/group per time point was washed just before sacrifice at 0.5, 1, 2, 4, 10, and 24 hours after application. Urine and feces were collected throughout the test periods.

Table 5.8 Absorption of radioactivity from an 80% w/w granular formulation of fipronil in male rats up to 24 hours after application.

Dose	0.070 mg/cm^2						0.668 mg/cm^2						3.88 mg/cm^2					
Time hours	0.5	1	2	4	10	24	0.5	1	2	4	10	24	0.5	1	2	4	10	24
Application site skin	1.14	1.51	2.45	1.86	1.87	1.82	0.6	5.75*	0.85	1.58	1.57	3.29	0.35	0.80	0.35	0.76	0.69	0.49
Carcass	ND	0.07	0.46	ND	0.65	0.36	ND	0.06	0.05	ND	ND	0.38	ND	0.64	0.05	0.07	0.18	0.07
Urine	<.005	ND	ND	<.005	<.005	0.01	ND	ND	<.005	ND	<.005	0.01	ND	ND	ND	ND	<.005	<.005
Feces	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.10	0.01	0.01	ND	ND	ND	ND	<0.005	ND
Enclosure rinse	0.3	0.22	0.17	0.11	0.29	0.09	0.19	0.27	0.21	0.09	0.19	0.18	0.06	0.15	0.07	0.11	0.16	0.11
Total absorped (sum of above rows)	**1.4**	**1.8**	**3.1**	**2.0**	**2.8**	**2.3**	**0.8**	**6.1***	**1.1**	**1.8**	**1.8**	**3.9**	**0.4**	**1.6**	**0.5**	**0.9**	**1.0**	**0.7**
Skin Wash	98.8	98.7	97.9	97.8	96.2	96.8	101	95.4	101	100	101	97.1	105	101	103	101	103	103
Non occlusive cover	ND	ND	ND	ND	ND	ND	0.01	0.09	<0.005	0.01	0.01	0.01	0.01	ND	0.01	0.01	0.01	0.01
Total Recovery	100.2	100.5	101.0	99.8	99.0	99.1	101.8	101.6	102.1	101.8	102.8	101.0	105.4	102.6	103.5	102.0	104.0	103.7

*high value due to an outlier. ND = not detected. No radioactivity detected in blood.

The mean total recovery of radioactivity among all treated groups ranged from 99.8% to 116%. The majority of radioactivity was not systematically absorbed and was removed by washing. Amount of radioactivity found in excreta (cage wipe and cage wash included) was less then 0.02% of the total applied radioactivity for all treated groups. Amounts of radioactivity found in blood, eliminated in exereta, retained in carcass (including application site skin), and found in cage washes and wipes and enclosure rinse were considered as absorped material. The total % of fipronil absorped did not vary from 0.07 to 0.668 mg/cm^2, nor was it proportionally lower at 3.88 mg/cm^2. The majority of absorped radioactivity was recovered from application site skin and was typically >70%, and the flux of material into the animal was not discernible from 4 to 24 hours.

To conclude
Among the test groups, total absorption was typically less than 3%. The proportion of material absorbed did not vary linearly with dose.

(DP 116914)

5.1.4 Summary of ADME studies

At low and high doses fipronil is rapidly absorbed, extensively distributed and relatively slowly excreted ($t_{½}$ in blood = 150-200 hrs), mostly in the faeces. From the biliary excretion study oral absorption was estimated to be approximately 90%. Only a small proportion of the parent compound was excreted unchanged (6-18%) and the identified metabolites were conjugates of glucuronide or sulfate. A large proportion of the metabolites were identified, >99% of administered dose being identified in the single low dose group, 80-90% in the repeat dose group and >65% in the high dose group.

In an *in vitro* study fipronil was less penetrative than hydrocortisone and testosterone through human, rat and rabbit epidermis. All compounds were applied in EXP 601 45A (a formulation mixture containing propylene glycol, ethoxylated polyaryl phenol, ethoxylated alkyl phenol, ethoxylated fatty alcohol, silicon oil emulsion, dewaxed maize oil, blend emulsifier and water). In addition it was noted that fipronil was an order of magnitude less penetrative than hydrocortisone (applied at 4 g/l, measured at 24 h) which is considered a relatively poor penetrant.

In further *in vitro* studies, three fipronil formulations (oil, organic solvent and oil/water based) showed flux rates in the range 0.04-0.2 µg/cm^2/hr. At 24 h, 0.15-0.3% of the dose had penetrated human skin for all the formulations. Dilution of the formulations increased the mean percentage of the dose penetrating human skin to 1-3%. The comparable results for very different liquid formulations indicate it is unlikely that the dermal penetration in humans of the fipronil formulations tested would be greater than 1-3%.

For the dry granule formulation containing 80% fipronil, absorption in rats was <3%. The *in vitro* dermal absorption study at 5.1.3a showed that the flux rate of fipronil across human skin was at least ten times less than that for rat skin hence dermal penetration in operators from granules is expected to be less than 1% (see also Section 5.12).

5.2 ACUTE TOXICITY, IRRITANCY AND SENSITISATION

5.2.1 Acute oral toxicity (IIA 5.2.1)

In a preliminary study, CD rats (2 animals/sex/dose) were dosed with fipronil (batch number IGB444, 93% purity) at either 25 or 100 mg/kg and observed for 5 d. The LD_{50} was estimated to be 100 mg/kg. No further examination was conducted.

In the main study conducted in 1988, CD rats (5 animals/sex/dose, Crl:CD (SD) BR strain) were administered fipronil (batch number IGB444, 93% purity) at either 50, 80, 126 or 200 mg/kg in corn oil by gavage at 10 ml corn oil/kg bw. Animals were observed for 14 d post-dose. The LD_{50} (determined by probit analysis) was found to be 92 mg/kg (95% CI 64-128) in males and 103 mg/kg (95% CI 73-141) in females. Mortalities were reported from 4 h to 3 d post-dose at ≥80 mg/kg, in both males (2/5, 4/5 and 5/5 at 80, 126 and 200 mg/kg respectively) and females (2/5, 4/5 and 4/5 at 80, 126 and 200 mg/kg respectively). The common signs of toxicity reported in all animals were piloerection, hunched posture, abnormal gait and diarrhoea. Lethargy was reported in animals receiving ≥80 mg/kg. Decreased respiratory rate and pallor of the extremities and/or ptosis were reported in one male receiving 80 mg/kg and all animals receiving ≥126 mg/kg. At 200 mg/kg clonic convulsions and prostration were reported prior to death in two males and one female. At 50 mg/kg signs of toxicity were absent at 3 d, and at higher doses were absent at 6 d. No abnormal macropathology findings were reported at necropsy.

To conclude the acute oral LD_{50} in rats was 92 mg/kg. The test material is classifiable as toxic via the oral route according to current EC classification criteria.

(DP 71039)

5.2.2 Acute dermal toxicity (IIA 5.2.2)

In a limit study conducted in 1988, shaved CD rats (5 animals/sex) were exposed to a paste of 2000 mg/kg fipronil (batch number IGB444, 93.0% purity) in distilled water (fipronil concentration 90.9% w/v) on clipped dorsal skin under an occlusive dressing. The area exposed was approximately 10% of the total body surface. After 24 h the dressing was removed and the treated area washed with water and blotted dry. The dermal LD_{50} was >2000 mg/kg in both sexes, no deaths being reported in the 14-d observation period. No local or systemic signs of toxicity were noted. Anticipated body weight gains were made at the end of the observation period and no treatment-related findings were noted at necropsy. The NOEL for this study was 2000 mg/kg.

To conclude, the acute dermal LD50 in rats was >2000 mg/kg bw. The test material is not classifiable via the dermal route according to current EC classification criteria.

(DP 71040)

5.2.3 Acute inhalation toxicity (IIA 5.2.3)

In a 4-h exposure study conducted in 1990 (no guideline specified), CD rats (5 animals/sex/dose) were exposed nose only to atmospheres containing particles of fipronil (batch number PGS 963, 95.4% purity) at nominal concentrations of 0.5, 0.8 or

2.1 mg/l and observed for 14 d. The test system achieved measured concentrations of 0.24, 0.52 and 0.93 mg/l (gravimetric analysis), with the percentage of particles with a mean equivalent aerodynamic diameter of <6 μm being 30, 45 and 40% respectively. The LC_{50} was calculated to be 0.68 mg/l (95% confidence limits 0.43-0.94 mg/l) based on 7 deaths (4 male, 3 female) at 0.93 mg 1, 3 deaths (1 male, 2 females) at 0.52 mg/l and 1 female death at 0.24 mg/l noted up to 2 d after treatment. Immediate signs of toxicity after treatment were a hunched posture, tremors and vocalisation when handled. Subsequently piloerection (up to 10 d at 0.52 mg/l, up to 14 d at 0.93 mg/l) and hunched posture (females up to 9 d at 0.52 mg/l, up to 10 d at 0.93 mg/l) were noted. In addition, two females exhibited convulsions (2 and 3 d) at 0.52 mg/l. Body weight loss or no body weight gain was noted in all animals up to 2-3 d post treatment. Thereafter, anticipated body weight gain was achieved. At necropsy the study noted that decedent lung weights (except 1 male and 1 female at 0.93 mg/l) were increased, but no other effects related to treatment were noted.

To conclude, the acute LC_{50} in rats was 0.68 mg/ml. The test material is classifiable as 'toxic' by inhalation according to current EC classification criteria.

(DP 71042)

5.2.4 Skin irritancy (IIA 5.2.4)

A skin irritation study conducted in 1988 was available, in which 500 mg of fipronil (batch number IGB 444, 93% purity) was moistened with 0.5 ml of distilled water and applied to the intact, clipped, skin of 3 rabbits (male New Zealand White) for 4 h under an occluded patch. Observations for both erythema and oedema were made at 30 minutes and 1, 2, 3 and 4 d post treatment. No dermal irritation was reported at any time point therefore, fipronil is not classified as a skin irritant on the basis of this study.

(DP 71044)

5.2.5 Eye irritancy (IIA 5.2.5)

In an eye irritation study, 0.1 ml (82 mg solid) fipronil (batch number IGB 444, 93% purity) was instilled into the conjunctival sac of one eye of each of 3 male New Zealand White rabbits, with the other eye serving as a control. Animals were observed 1 h and 1, 2, 3, 4 and 7 d post instillation for eye damage, after which the study was terminated. Mild conjunctival redness (grade 1) was noted in all animals at 1 h. Based on readings at 1, 2 and 3 d, mean conjunctival redness scores were 0.3, 0.3 and 0.7. Redness had resolved in all animals by 72 h. On the basis of this study fipronil is not classified as an eye irritant.

(DP 71046)

5.2.6 Skin sensitisation (IIA 5.2.6)

a) In a preliminary irritation screen for a modified Buehler study, 0.25 ml of up to 30%w/v fipronil (batch number PGS 963, 95.4% purity) as a suspension in paraffin oil did not elicit a treatment-related dermal response on Dunkin-Hartley guinea pigs at 24 or 48 h after 6 h occluded treatment. The study considered that 30%w/v fipronil was the highest practical concentration for topical application, but no further detail was

provided. Given the solubility of fipronil in organic solvents, it seems reasonable to assume the technical limitation was the solubility of fipronil in paraffin oil.

In the main study, Dunkin-Hartley guinea pigs (10 animals/sex/group) were allocated to test and negative control groups. Nine animals (5 male and 4 female) were allocated to a positive control group and treated with dinitrochlorobenzene. At induction (days 1, 8 and 15), 0.25 ml of 30% w/v fipronil in paraffin oil was placed under an occlusive dressing on the clipped flank of the test animals for 6 h. The positive control animals were induced similarly but 3% dinitrochlorobenzene in absolute ethanol was substituted for the test chemical. The negative control animals were not treated at this stage. At challenge (day 29), 0.25 ml of 30 and 5% w/v fipronil in paraffin oil were applied to both test and negative control animals under an occlusive dressing for 6 h. Positive controls were similarly treated but 0.1% dinitrochlorobenzene in acetone was substituted for the test compound. The guidelines under which this study was conducted (EPA 81-6 or OECD 406) specify a patch size and note that this should be 'fully loaded'. The study states that the saturating volume of fipronil was 0.25 ml.

No treatment-related changes in body weight gain or signs of toxicity were noted during the study. No dermal reaction was noted at induction. At challenge 'very faint usually non-confluent' erythema was noted in 1 treated animal at 30%w/v fipronil, 4 treated animals at 5%w/v fipronil and 5 negative control animals. Positive control animals showed a 'faint usually confluent' erythema in 4/9 animals and a 'very faint usually non-confluent' response in a further 4 animals.

Under the conditions of this study, fipronil is not a skin sensitiser. However, the weight that can be given to this result is decreased since the strain response to dinitrochlorobenzene was less than would be expected from this strong sensitiser.

(DP 71069)

b) In a preliminary screen for a Magnusson-Kligmann study, a range of doses of fipronil (batch number PGS 963, 95.4% purity) in propylene glycol were investigated for intradermal and dermal irritation potential. Indications were that intradermal injection of 5%w/v fipronil in propylene glycol (a systemic dose of 50-57 mg/kg) was irritant and was therefore selected for induction. For topical induction, the preliminary screen indicated potential for systemic toxicity at 50% w/v. Multiple topical dosing used in the preliminary screen may confounded dose selection. The study selected the non-irritant dose of 5%w/v fipronil in propylene glycol for topical dermal induction, but it was not explained why (30% w/v produced just perceptible erythema in some animals). The maximally non-irritant dose of 10%w/v fipronil in propylene glycol for dermal challenge. Dermal challenge was also conducted at 3%w/v.

In the main study, Dunkin-Hartley guinea-pigs (10/sex/group) were induced by intradermal injection (0 d) with 5%w/v fipronil in propylene glycol or vehicle only. Animals were administered three pairs of injections comprising : (i) 0.1 ml of FCA; (ii) 0.1 ml of 5%w/v fipronil in propylene glycol (controls 0.1 ml vehicle); and (iii) 0.1 ml of 5%w/v fipronil in propylene glycol and FCA 1:1 (controls 0. 1 ml vehicle and FCA 1:1). At dermal induction (8 d), 0.6 ml of 5%w/v fipronil in propylene glycol (control vehicle only) was topically applied under an occlusive dressing for 48 h. Sodium lauryl sulphate was not applied. At challenge (22 d), 0.03 ml of each of

vehicle and 10 and 3%w/v fipronil were applied under an occlusive dressing for 24 h to both test and control animals. Sites were examined after 24 and 48 h.

The laboratory strain sensitivity was confirmed by routine 6-month testing using the mild sensitiser benzocaine in propylene glycol.

No treatment-related changes in body weight gain were noted. At intradermal induction, injection of FCA produced a 'slight' to 'moderate' erythema in all animals. 5%w/v fipronil in propylene glycol administered intradermal did not produce an irritant response. However, the test animals were agitated and overactive for up to 4 d after the injection when compared to control animals. At dermal induction no irritant response was noted but, treated animals were more agitated than controls when handled. Erythema responses at challenge for 24 h and 48 h are detailed in Table 5.9.

Table 5.9 Numbers of animals showing erythema responses at 24 h and (in brackets) 48 h

		Severe	**Moderate**	**Slight**	**Barely Perceptible**
Test group	10% w/v fipronil	2 (2)		2 (0)	1 (0)
	3% w/v fipronil				3 (0)
	Propylene glycol				3 (1)
Control group	10% w/v fipronil				2 (0)
	3% w/v fipronil				1 (0)
	Propylene glycol			1 (0)	2(1)

Less than 30% (2/20) of animals showed a more marked response than controls. Fipronil is not classifiable as a skin sensitiser according to current EC classification criteria.

(DP 71070)

5.2.7 Summary of acute toxicity, irritancy and sensitisation

Study	Batch and purity	Result	Classification
Acute rat oral LD_{50}	IGB444, 93%	LD_{50} = 92 mg/kg	Toxic if swallowed
Acute rat dermal LD_{50}	IGB444, 93%	LD_{50} >2000 mg/kg	Not classified
Acute rat inhalation LC_{50}	PGS 963, 95.4%	LC_{50} = 0.68 mg/l	Toxic by inhalation
Eye irritation (rabbit)	IGB 444, 93%	-	Not classified
Skin irritation (rabbit)	IGB 444, 93%	-	Not classified
Skin sensitisation (Guinea pig, Buehler)	PGS 963, 95.4%	-	Not classified
Skin sensitisation (Guinea pig, M&K)	PGS 963, 95.4%	-	Not classified.

Fipronil is acutely toxic in the rat via the oral and inhalation route. The respirable component in the inhalation study was only 30-45% of the measured chamber concentration. In both these studies, deaths were noted from 80 mg/kg and from 0.239 mg/l with common symptoms of hunched posture and piloerection. Convulsions were

reported at 200 mg/kg and 0.523 mg/l. Fipronil was of low dermal toxicity to the rat and was not irritant to the skin or eye. Fipronil was not classified as a skin sensitiser.

5.3 SHORT-TERM TOXICITY (AII 5.3.2)

5.3.1 28 day dietary administration to rats.

Technical-grade fipronil (batch number IGB 464, purity, 93%) was administered in the diet for four weeks to groups of five Crl:CD (SD) BR rats of each sex at concentrations of 25, 50, 100, 200, or 400 ppm, equal to 3.4, 6.9, 13, 24, or 45 mg/kg bw per day for males and 3.5, 6.7, 13, 25, or 55 mg/kg bw per day for females. Although there were no clinical signs of toxicity, one female at 400 ppm died, but with no accompanying clinical or pathological findings. Decreased body-weight gain seen in animals of each sex at doses ≥ 100 ppm in the first week (26, 60, 87% and 33, 54, 79 % at 100, 200 and 400 ppm for males and females respectively) was temporary and possibly due to unpalatability, since food consumption was also temporarily decreased by over 50% in these groups during the same period. The platelet counts of animals at 200 and 400 ppm were marginally increased. The results of urinalysis were negative. Increased total protein and globulin were seen in all treated animals, and these increases were statistically significant; however, they were small in comparison with the values in controls and were poorly correlated with dose. Cholesterol levels were increased in females at all doses and in males at the high dose.

The target organs were the liver and thyroid. Liver weights were significantly increased in females at all doses and in males at 200 and 400 ppm. At necropsy, liver enlargement was observed in one or both sexes starting at 50 ppm, and five males and three females at 400 ppm had enlarged livers. Generalised hepatocyte enlargement was observed microscopically in one male at 100 ppm, with increasing incidence in animals of each sex at 200 and 400 ppm. Thyroid follicular-cell hypertrophy, generally of minimal severity but of moderate severity in several males at 200 and 400 ppm, was found in almost all treated animals but not in the controls.

Table 5.10 Treatment-related effects in the 28 day rat dietary study

	Males						Females					
mg/kg bw	**0**	**3.4**	**6.9**	**13**	**24**	**45**	**0**	**3.5**	**6.7**	**13**	**25**	**55**
Platelets $\times 10^3/mm^3$	945	1027	1145	1125	1211*	1531**	1213	1277	1315	1247	1422	1439
Total proteins g/dl	6.5	7.0**	7.0**	7.0**	6.9**	7.0**	6.3	6.6**	7.0**	6.9**	7.1**	6.9**
Globulins g/dl	3.2	3.6**	3.6**	3.6**	3.6**	3.7**	2.9	3.2*	3.6**	3.6**	3.9**	3.8**
GOT mU/ml	53	49	46*	43*	47*	42**	59	55	50*	46**	46**	47**
Cholesterol mg/dl	85	83	93	86	81	110*	61	84**	106**	105**	115**	139**
Body weights g	372	378	379	363	345	337	252	240	245	230	232	230
Liver weight g	18.6	20.4	21.6	21.2	21.7*	25.6**	10.5	12.1*	13.8**	14.3**	16.1**	17.3**
Minimal hepatocyte enlargement	0/5	0/5	0/5	1/5	3/5	5/5	0/5	0/5	0/5	0/5	2/5	4/5
Thyroid follicular-cell hypertrophy	Min 0/5	5/5	4/5	5/5	3/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5
	Mod 0/5	0/5	0/5	0/5	2/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5

* 0.05≥P, ** 0.01≥P0

No NOAEL was identified because of changes in blood chemistry in one or both sexes, increased liver weights in females, and thyroid follicular-cell hypertrophy in animals of each sex at the lowest dose (Peters et al., 1990).

(DP 71076)

5.3.1 90-day dietary administration to rats

In a 13-week study, rats (CD strain 10/sex/group) received dietary administration of either 1, 5, 30 or 300 ppm fipronil (batch number PGS 963, 95.4% purity). This was equivalent to 0.07, 0.3, 2.1 or 22 mg/kg/d. Doses were selected after a preliminary 14-d study showed deaths (3/10 animals by 5 d) and muscular spasms at 30 mg/kg/d. A neurological examination was performed at week 12 on animals from control and top dose groups. The test laboratory stated that the results showed no evidence of abnormalities, and that these results were not presented in the study report. They are held in archives.

No deaths were reported in the main study. Tail trauma (5/10) and tail encrustations (3/10) were reported in females receiving 300 ppm. Body weight gains were reduced in both sexes 40-50% during the first week at ≥30ppm. Thereafter, body weight gains were comparable to controls.

Haematological findings revealed a treatment-related increase in platelet numbers with a concomitant decrease in prothrombin time in females. A treatment-related increase in platelet numbers was reported in males at ≥5 ppm (12.5% at 300 ppm).

In females decreases in ALT and AST levels were reported (20 and 35% at the top dose respectively) which reached statistical significance. Levels of serum proteins were significantly increased in females at all dose levels and in males at ≥5 ppm (8% at 300 ppm). Urinalysis was found to be normal.

In males, the absolute liver weight was significantly increased at the top dose only (42%). In females liver weights were elevated in all treatment groups (4.6-35% at 1-300 ppm), achieving statistical significance at ≥5 ppm. Absolute thyroid weights were elevated (4.2-100% at 5-300 ppm) achieving statistical significance at ≥30 ppm in females and at 300 ppm in males.

Histopathological examination found treatment-related effects at the top dose in the thyroids and livers of both sexes. Oil red O staining revealed a high incidence of fat deposits in all liver samples, including controls. A statistically significant increase in panacinar hepatic fatty vacuolation (controls 0/10 and 7/10 at 300 ppm) was reported in males only.

Table 5.11 Treatment-related effects in the 90 day rat dietary study

	Males					Females				
ppm	**0**	**1**	**5**	**30**	**300**	**0**	**1**	**5**	**30**	**300**
Mg/kg bw	**0**	**0.07**	**0.3**	**2.1**	**22**	**0**	**0.07**	**0.3**	**2.1**	**22**
Body weights	540.5	563.9	540.3	546.8	539.1	304.9	324.6	330.6	325.8	298.0
Liver weights	19.1	21.0	19.4	21.8	27.2*	10.8	11.3	12.7*	13.4**	16.6**
Thyroid weights	0.024	0.024	0.025	0.030	0.048**	0.019	0.019	0.021	0.023*	0.032**
Prothrombin time (s)	15.0	15.8*	14.7	15.2	14.7	14.2	14.4	14.0	13.7*	13.5**
Platelets $\times 10^3/mm^3$	852	858	911	948*	926	913	937	933	993	1028
Panacinar hepatic fatty vacuolation	0/10	2/10	0/10	1/10	7/10*	0/10	0/10	0/10	0/10	1/10
peri-acinar hepatic fatty vacuolation	0/10	0/10	0/10	0/10	2/10	0/10	0/10	0/10	0/10	2/10
Hepatic congestion	4/10	2/10	3/10	3/10	6/10	2/10	0/10	1/10	0/10	5/10
thyroid follicular-cell hypertrophy	3/10	1/10	0/10	5/10	8/10	1/10	0/10	0/10	0/10	10/10*
thyroid follicular-cell hyperplasia	2/10	0/10	0/10	1/10	6/10	0/10	0/10	1/10	1/10	2/10

* P<0.01, **P<0.001

Although there was an increase in liver weight in females at 5 and 30 ppm and thyroid weight effects at 30 ppm these were dismissed in the absence of histopathology. A NOAEL of 30 ppm (equivalent to 2.1 mg/kg/d) was established based on histopathological changes in the liver. The applicant considered 1 ppm to be the NOEL and 30 ppm to be a non-toxic effect level.

(DP 71079)

5.3.1 13-week dietary administration to dog.

In this 1991 study, beagle dogs (4/sex/dose) received either 0.5, 2 or 10 mg/kg/d fipronil (batch number PGS 963, 94.4-96.5% purity) in gelatine capsules for 13 weeks. Haematology and clinical chemistry observations were made at week 6 and study termination. Urinalysis was at weeks 5 and 11. Deaths were reported at the top dose only; 1 male and 3/4 females died or were killed intercurrently during the first two weeks of the study. No signs of toxicity were reported at the low dose. At the intermediate dose, 2/4 females exhibited reduced food consumption from weeks 1-4 of the study. Signs of toxicity reported from week 1-3 at the top dose were loss of appetite, hunched posture and inactivity. Further signs, suggestive of neurotoxicity were also reported at this dose (weeks 1-7). These were convulsions, body tremors and head nodding. In surviving animals these signs resolved during the study, with only slight loss of appetite being reported at study termination. Urinalysis and haematology were unaffected at both time points.

Changes in some clinical chemistry parameters were reported in animals receiving 10 mg/kg/d. In males, serum cholesterol levels were decreased at 6 (33%) and 12 weeks (14%). Increases in ALP (24% and 42% at 6 and 12 weeks respectively) and AST (24% and 17% at 6 and 12 weeks respectively) were reported.

Information on the cause of death of decedents is lacking. However, examination of individual animal gross and histopathology data revealed no unusual findings. At necropsy an increase in absolute liver weight was apparent in all treated males (approximately 16% in all groups). No abnormal gross or histopathological observations were reported.

A NOEL was established in this study at 2 mg/kg/d based on deaths and signs of neurotoxicity reported at the top dose.

(DP 71081)

5.3.2 1 year dietary administration to dog.

a) Groups of six beagle dogs/sex received fipronil (batch number PGS 963, 96.8% pure) by capsule in 1991, at dosages of 0.2, 2.0 or 5.0 mg/kg/day for 52 weeks. From day 16 of treatment the test material was supplied as a triturate in lactose. A similarly constituted control group received empty gelatin capsules during the first 15 days and thereafter received capsules containing lactose at a dosage of 100 mg/kg/d.

At 5 mg/kg/d two males were killed following marked signs of ill-health during weeks 31 and 34 of treatment respectively. Signs observed prior to sacrifice included convulsions, abnormal gait, nervous behaviour, muscle tremors, inappetence and weight loss. Similarly in the 2.0 mg/kg/d dose group, one male was killed during Week 11 of treatment. Signs observed in the three days before sacrifice included body tremors, stiffened limbs, unsteady gait and lack of co-ordination, head nodding, muscle twitching, bodyweight loss and inappetence. Another male and one female in this dose group showed convulsions.

Signs seen in all animals from Week 2 of treatment at 5 mg/kg/d included convulsions in two males, and muscular twitches or tremors, nervous behaviour, exaggeration of

limb rigidity and abnormalities of gait and stance in these and other animals. In addition, changes in activity patterns, vocalisation, head nodding, aggression and resistance to dosing occurred. Transitory periods of inappetence occurred in some dogs which showed these signs, and low bodyweight gain was noted for one female in the first 26 weeks of the study. Similar signs of reaction to treatment were also seen at 2 mg/kg bw/d.

Veterinary examination from Week 12 showed tenseness, nervous behaviour, hyperaesthesia, stiffness and abnormal positioning of the limbs and twitching of the facial muscles at 5 mg/kg/d. Occasional tenseness and nervousness was seen in both sexes at 2 mg/kg bw/d. This was also considered to be related to treatment.

Specific neurological examinations during the course of the treatment period revealed effects in three males and five females at 5 mg/kg/d. Changes included tenseness, gait or stance abnormalities, exaggeration of the hopping and gag reflexes and depressed foot sliding reaction. Neurological examination at 2 mg/kg/d showed tenseness in females from week 25.

There was no clear effect of treatment at 0.2 mg/kg bw/d.

There was no associated histopathological change detectable in any of the tissues examined in any dose group.

The NOAEL, in this study, was 0.2 mg/kg/d based on mortality and signs of neurotoxicity.

(DP 112755)

b) Groups of five beagle dogs/sex received fipronil (batch number PGS 963, 96.8% pure), in 1992 via the diet, at concentrations designed to achieve dosages of 0.075, 0.3 or 1.0 mg/kg bodyweight/day for a scheduled period of 52 weeks. A similarly constituted group received a dosage of 3.0 mg/kg/d for the first 38 days of treatment; the dosage for this group was subsequently reduced to 2.0 mg/kg/d in view of significant toxicity. Doses were initially selected on the basis of the results from the capsule study above.

One female given 3.0 mg/kg/d was killed on Day 32, having displayed marked signs of ill-health and inappetence, suggestive of possible 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. Examination of blood samples indicated high packed cell volume, haemoglobin concentration, erythrocyte counts, plasma alkaline phosphatase activity and total plasma protein and cholesterol concentration.

Other animals in the 3/2 mg/kg/d group also displayed signs suggestive of neurological disturbance from Week 4 of treatment. These included convulsions, head nodding, extensor rigidity of limbs and twitching or tremors. Overall, signs indicative of a response to treatment were noted in three males and two females.

Signs of reaction to treatment at 1 mg/kg/d 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.

There were no findings that could be related to treatment at 0.3 or 0.075 mg/kg/d.

There was no treatment related histopathology in any dose group.

Analysis of plasma obtained after 1, 13, 24, 38 and 50 weeks of treatment showed fipronil and a metabolite, MB 46136, in the plasma of animals from all treated groups. In each case, the concentration of MB 46136 noted in the plasma was higher than that of fipronil. For each material, plasma levels showed a clear dose-relationship but there was no evidence of accumulation as the study progressed.

The NOAEL in this study was considered to be 0.3 mg/kg/d based on clinical signs of toxicity suggestive of neurological involvement in two females at the next dose up.

(DP 112756)

5.3.3 Overall NOAEL for the 90 day and 1 oral year dog studies.

An overall NOAEL is expressed for all the dog studies (90 d and 2 x 1 year) as some effects seen in the 1 year dog studies occurred at or below the NOAEL for the 90 day dog study, and within 90 days of commencing the 1 year dog studies. The overall NOAEL for these studies is hence 0.5 mg/kg bw/day from the 90 day dog study based on clinical signs of neurotoxicity in a female animal during week 13 of the second 1 year dog study (5.3.2 b.). See Table 5.12 below for a summary of effects in the oral dog studies.

Table 5.12 Summary of effects in the oral studies in dogs with fipronil.

mg/kg bw/day	0.75	0.2	0.3	0.5	1	2	3 for 38 days, then reduced to 2 due to toxicity.	5	10
13 wk dog study. 4 dogs/ sex/ dose. Capsule administration.				NOAEL		NOAEL			Deaths and signs of neurotoxicity
52 week dog study. 6 dogs/ sex/ dose. Capsule administration.		NOAEL				One male killed in extremis **week 11** (tremors stiffened limbs, unsteady gate and lack of co-ordination, head nodding, muscle twitching, body weight loss and inappetence). Another male and one female also showed convulsions. Signs of neurotox also seen in remainder of animals similar to those at 5 mg/kg.		Two males killed in extremis **weeks 31 and 32**, (convulsions, inappetance, weight loss etc). Signs of neurotox in other animals were, twitches, tremors, nervous behaviour, exaggeration of limb rigidity, altered gait or stance, vocalisation, head nodding, aggression, resistance to dosing.	
52 week dog study. 5 dogs/ sex/ dose. Dietary administration	NOAEL		NOAEL		Twitching of the body in **week 13** for one female and extensor rigidity of the limbs at week 20 in another female.		One female killed in extremis **day 32** with marked signs of neurotox and weight loss. Other animals showed convulsions and other neurotox signs throughout.		

5.3.4 Short-term dermal toxicity

In a 1993 21-d dermal study, fipronil (lot number 78/GC/90, 96.7% purity) suspended in 0.5% carboxymethyl cellulose was applied to the clipped backs of New Zealand White rabbits (6 animals/sex/dose) at 0.5, 1.0, 5.0 or 10.0 mg/kg/d. A total of 15 occluded applications (6 hours per day, 5 days per week) were made. Clinical observations were made daily, body weights were measured at 8, 15, 21 d and immediately preceding sacrifice; food consumption was noted approximately every 2 days. Haematological and clinical chemical investigations were conducted at necropsy.

No deaths were reported and clinical signs were limited to 1 male and 1 female in the top dose group exhibiting hyperactivity (20-21 d) from which both animals recovered. No local erythema or oedema was noted at any treatment site in any group. At 10 mg/kg/d mean body weights of males were significantly decreased 7% at 21 d. Mean body weight gain of females at this dose was non-significantly decreased by 25, 38 and 51% at 8, 15 and 21 d. In addition at 10 mg/kg/d mean food consumption was significantly decreased in males (44%) and non-significantly in females (22%).

At necropsy no treatment-related changes in organ weights or gross and microscopic findings were noted. In addition, there were no treatment-related changes in haematological or clinical chemistry parameters.

Apart from being conducted for 21 d, this study is considered to be compliant with Annex V of Council Directive 67/548 (EEC). The NOEL for this study was 5.0 mg/kg/d based on the body weight decreases, decrease in food consumption and hyperactivity seen at 10 mg/kg/d.

(DP 71083)

5.3.5 Summary of short-term toxicity

In the rat 90-d study, the key target organs were the thyroid gland and the liver with follicular cell hyperplasia and hypertrophy; and fatty vacuolation and congestion observed in these respective organs. Although muscular spasms were observed at 30 mg/kg/d in the preliminary study, no evidence of neurotoxicity was seen in the main study (top dose 10 mg/kg bw). In the dog studies, mortality and signs indicative of neurotoxicity were observed. An overall NOAEL is expressed for all the dog studies (90 d and 2 x 1 year) as some effects seen in the 1 year dog studies occurred at or below the NOAEL for the 90 day dog study, and within 90 days of commencing the 1 year dog studies. The dog appeared to be more sensitive to the neurotoxic effects of fipronil than the rat. Although it is noted that convulsions were observed at doses as low as 0.06 mg/kg/d following long-term administration of fipronil to rats. Decreased body weights and reduced food consumption, and hyperactivity were the only effects noted in the 21 day dermal study and were used to set the NOEL.

5.4 GENOTOXICITY

5.4.1 *In vitro* testing (AII 5.4.1)

a) A bacterial point mutation study was conducted with fipronil (batch number IGB 438, 95-97% purity) in 1988 using *Salmonella typhimurium* strains TA 100, 98, 1535 and 1537. This study was broadly compliant with Annex V [Council Directive 67/548 (EEC)], however bacterial cell counts were not presented. An S9 fraction was prepared from the livers of Aroclor 1254 induced male rats. Unless stated otherwise the solvent used was DMSO. The positive controls used were 2-nitrofluorene for TA98 without S9; sodium azide (aqueous) for TA100 and 1535 without S9; 9-aminoacridine for TA1537 without S9; and 2-aminoacridine for TA98 and 100 with S9. The laboratory criteria for a positive response were a two-fold increase in the number of revertants with TA100 and 98 and a three-fold increase with TA1535 and 1537, with such increases confirmed.

A range-finding test was carried out with TA 100 only, at concentrations of 8-5000 µg/plate fipronil (95-97% purity) with and without S9. Bacteriotoxicity was reported at ≥1000 µg/plate, although cell counts were not provided. A mutagenicity test was then carried out using three plates per dose with fipronil and four plates per dose with the relevant controls. The doses selected for the first test were 0-500 µg/plate for all strains, with and without S9. Bacteriotoxicity was reported at 500 µg/plate. No increase in the mutation frequency was reported in those plates treated with fipronil; the positive controls responded appropriately. Because of the toxicity observed in the first test, the dose range was altered in the second test to 0-400 µg/plate. No increase in the mutation frequency was reported in those plates treated with fipronil; the positive controls responded appropriately. Bacteriotoxicity was not reported in the second test.

As the positive controls gave appropriate responses, fipronil did not cause bacterial point mutations under the conditions of the study.

(DP 71084)

b) Fipronil (batch number JJW092/1, 97.2% purity) was tested with and without metabolic activation for the potential to cause gene mutations at the HGPRT locus in cultured Chinese hamster ovary cells. The positive controls used were ethylmethanesulphonate in non-activation assays and 7,12-dimethylbenzanthracene in activation assays. Cytotoxicity was determined by comparing the cloning efficiencies of the solvent controls to those of the treated cells (%). The negative control was DMSO containing medium. No information was provided concerning the criteria for assay acceptance or a positive result.

A range-finding experiment using 0-500 µg/ml with and without S9 was carried out. None of these doses were found to be cytotoxic. Although precipitation was reported at ≥100 µg ml, the same dose range was used in the following gene mutation experiments.

In the first experiment no increase in cytotoxicity or mutation frequency was reported either with or without S9. However, in the second experiment cytotoxicity was reported at 100 and 500 µg ml (47 and 62.7% of the solvent control values

respectively). No increase in the mutation rate was reported in the second experiment. As the positive controls gave appropriate responses, fipronil was not considered to be mutagenic at the HGPRT locus under the conditions of the study.

(DP 71088)

c) The ability of fipronil (batch number IGB 438, 95-97% purity) to cause chromosome aberrations was investigated using human lymphocytes. The study was not quoted as being adherent to any guideline. A single harvest time only and no repeat test was performed. At least two harvest times and a repeat test would have been required by current guidelines (OECD 473 1997; 92/69/EEC). The positive controls used were cyclophosphamide (with S9) and methylmethanesulphonate (without S9). A vehicle (DMSO) containing negative control was used. Lymphocytes were obtained from a single male and female donor and assayed separately. The criteria for a positive response were defined as a statistically significant increase in aberrations (preferably dose-related) which exceeded the normal range. The S9 fraction was obtained from the livers of Aroclor 1254 induced male rats. The exact dose levels to be examined for aberrations were selected from a range-finding study to determine the effect of dose on mitotic rate. The doses were in the range 0-300 µg/ml plus appropriate controls. The mitotic indices were not determined below 75 µg/ml, the remaining data can be seen in Table 5.13. No statistical significance was determined.

Table 5.13 Mitotic indices

	+S9		-S9	
Treatment	**Male**	**Female**	**Male**	**Female**
Vehicle Control	3.9	2.9	2.6	4.1
75µg/ml	2.7	4	2	2.4
150 µg/ml	3.7	2.4	1.7	2.6
300 µg/ml	1.1	0.9	1.5	0.8

As these doses gave discernible decreases in mitotic indices, these concentrations were selected for determination of chromosomal aberrations.

Duplicate lymphocyte preparations were cultured for 44 h prior to the addition of test solutions and S9 fraction if required. Cells were then incubated for a further 3 h, washed, and allowed to grow on for 25 h. One hour prior to harvest cells were arrested with colchicine. After harvest, metaphase spreads were prepared and one hundred examined per preparation.

No increases in chromosomal aberrations were reported with cells treated with fipronil or vehicle control. Both positive control substances gave appropriate responses. Therefore fipronil did not cause chromosome aberrations under the conditions of the study, although this was not confirmed in a repeat assay.

(DP 71087)

d) Chinese hamster lung cells were treated at different concentrations of fipronil (batch number 1747, 98.3% pure) in 1995 in duplicate cultures to examine its potential to induce chromosomal aberrations in the presence or absence of S9 rat liver metabolic activating fraction (derived from Arochlor induced rats). The concentrations tested (see Table 5.14) were chosen on the basis of preliminary cytotoxicity tests. The

solvent was DMSO and the positive controls were cyclophosphamide and mitomycin C. The study was compliant to GLP and OECD 473 (1997) except that the wrong positive control was used at the 6 hour harvest time in the absence of S9. The results are shown in Table 5.14.

Cyclophosphamide, which requires metabolic activation, was erroneously used to control the 6 hour harvest time without metabolic activation (-S9). This experiment hence lacks a suitable positive control. However, given that suitable controls were used for subsequent experiments and gave satisfactory results, and that an increase in aberrations was seen with the test material, it is considered likely that the conduct of the 6 hour experiment –S9 was satisfactory.

Table 5.14 % Cells with aberrations excluding gaps (cytotoxicity - cell counts as % of control) exposed to fipronil.

Harvest time	6 hours	6 hours	24 hours	48 hours
Metabolic activation	-S9	+S9	-S9	-S9
Vehicle control	0.5	2.0	0.5	1.0
7.5 µg fipronil/ml			1.0 (65)	2.0 (80)
15 µg fipronil/ml		0.5 (96)	0.5 (81)	0.5 (70)
22.5 µg fipronil/ml				0.5 (46)‡
30 µg fipronil/ml	0.5 (81)	1.0 (87)	1.5 (47) †	
45 µg fipronil/ml	3.5* (76)			
60 µg fipronil/ml	14.5*** (44)	5.5 (72) †		
Cyclophosphamide (10 µg/ml)	1.0 (117)	62***		
Mitomycin C (0.05 µg/ml)			29.3***	59***

† There were no scorable metaphases at the next concentration up (double this concentration). ‡ no scorable metaphases at 30 µg/ml. Blank cell = no metaphases counted or no culture conducted. *P<0.05, **P<0.01, ***P<0.001

There was a dose related increase in aberrations at six hours in the absence of S9 (statistically significant at the top two doses), and a possible increase in the presence of S9 (although the increase at 60 µg fipronil/ml was not statistically significant). The increases in aberrations were due to increases in chromatid breaks and chromatid exchanges. The mechanism is not considered to be due to cytotoxicity (i.e. as outlined by **Kirkland**[1]) as large proportion of the aberrations seen were chromatid exchanges, whereas only breaks usually characterise the mechanism relating to cytotoxicity.

Where appropriate positive controls were used satisfactory results were obtained.

To conclude fipronil was clastogenic under the conditions of this assay.

(DP 112757)

5.4.2 *In vivo* genotoxicity in somatic cells (AII 5.4.2)

A 1995 mouse micronucleus test is available. In a preliminary test, animals (CD-I, 2 animals/dose/sex) received either 25, 50, 100 or 200 mg/kg of fipronil (batch number

[1] Kirkland D. (1998) Chromosome aberration testing in genetic toxicology - past, present and future. *Mutation Research* , **404**, 173-185

DA 832 (6 ADM), 97.2% purity) by gavage in a 0.5% aqueous methyl cellulose suspension. The positive control used was chlorambucil. One male from each of the 100 and 200 mg/kg dose groups died and all remaining animals in these dose groups were sacrificed 18 h post-dose due to excessive toxicity. The signs of toxicity reported at ≥100 mg/kg were convulsions and increased motor activity. No signs of toxicity were reported at <100 mg/kg, with the exception of piloerection immediately post-dose at 25 mg/kg only. The remaining animals were sacrificed after 72 h and bone marrow samples examined for signs of toxicity (decreased PCE:NCE ratios). At both 25 and 50 mg/kg the PCE:NCE ratio was 0.5, compared to the supplied historical control value of 0.9, indicating toxicity to the bone marrow.

The preliminary test was used to determine the dose levels to be used in the main test, the dose levels and numbers used can be found in Table 5.15.

Table 5.15 Dosing schedule in main test

Treatment	Dose (mg/kg)	Numbers
Vehicle control	-	15/sex
Chlorambucil	30	5/sex
Fipronil	1	5/sex
Fipronil	5	5/sex
Fipronil	25	15/sex

No deaths or signs of toxicity were reported during the study. Five animals/sex were sacrificed after 24 h, with a further 5/sex sacrificed from the vehicle control and 25 mg/kg fipronil groups after 48 h and 72 h. Bone marrow samples were taken immediately post sacrifice and 1000 PCE examined for micronuclei; the PCE:NCE ratio was then determined.

No increases in the numbers of micronuclei or change in PCE:NCE ratio were reported in animals receiving vehicle control or fipronil at any time point. The positive control gave appropriate responses.

These data suggest that the dose range could have been inadequate as no depression of bone marrow activity was detected. Therefore, the slides prepared from animals receiving 50 mg/kg in the preliminary test were re-examined to determine the numbers of micronuclei and PCE:NCE ratio. No increases in the numbers of micronuclei were reported. The PCE:NCE ratio was again found to be 0.5; however, only 1 time point (72 h) was used.

These data indicate that fipronil does not cause an increase in the numbers of micronuclei under the conditions of the study.

(DP 71091)

5.4.3 Summary of genotoxicity studies

Fipronil gave negative *in vitro* results in a bacterial point mutation study (Ames), a mammalian gene mutation study (HGPRT) and a chromosome aberration study (human lymphocytes). A single harvest time only and no repeat test were performed. At least two harvest times and a repeat test would have been required by current guidelines (OECD 473 1997; 92/69/EEC). A second chromosome aberration assay

conducted in Chinese hamster lung cells gave positive results at the 6 hour harvest interval in the absence of S9 and possibly in the presence of S9. Fipronil was negative in an *in vivo* mouse micronucleus assay. Although the *in vitro* cytogenetics assay in Chinese hamster lung cells, was positive (there was an increase in breaks and sister chromatid exchanges) the mouse micronucleus test indicated that the test material was not actually clastogenic *in vivo*. It is considered unlikely that a second *in vivo* assay in a different tissue is likely to produce anything other than a negative result. The lack of any tumours that could be attributed to direct genotoxic action in the rat and mouse chronic studies also support the lack of *in vivo* genotoxicity by fipronil.

5.5 ORAL LONG-TERM TOXICITY AND CARCINOGENICITY (AII 5.5)

5.5.1 2-year dietary study in rats

The carcinogenic potential of fipronil was determined in a 2-year study carried out in the rat (CD strain). Animals (50/sex/dose) received dietary administration of fipronil (batch number PGS 963, 95.4% purity) at either 0.5, 1.5, 30 or 300 ppm; equivalent to 0.02, 0.06, 1.3 and 13 mg/kg/d (males) and 0.03, 0.08, 1.6 and 17 mg/kg/d (females). A similarly constituted group received untreated diet. Further animals (15 animals/sex/dose) were included to cover the interim kills at week 52. Separate groups (15 animals/sex/dose) were treated as above for 52 weeks, at which time they were transferred to control diet for a further 13 weeks to assess recovery. The toxicity phase of the study was terminated early, after 88 weeks in males and after 91 weeks in females, due to poor survival. No mortality rates were provided. Termination for the oncogenicity phase of the study was carried out at 91 weeks for both sexes and was not below that specified in guidelines (mortality rates at Table 5.16). Blood samples were taken for clinical chemistry and haematological analysis at weeks 24, 50, 76 and at study termination. A blood sample was also taken from animals in the recovery group after 12 weeks. Analysis of serum for levels of TSH, T4 and T3 was conducted at weeks 1, 4, 12, 24 and 50 only. Samples were also analysed for thyroid hormones at weeks 2, 4, 7 and 11 of the recovery period. Urinalysis was carried out at weeks 23, 49, 75 and 87 (males only) and at weeks 23, 49, 75 and 90 (females), plus at weeks 6 and 11 of the recovery period.

Deaths were reported throughout the study period and the overall survival rate was not treatment-related.

Table 5.16 Mortality (%) during the oncogenicity phase in the rat chronic tox/carc study with Fipronil

Dose (ppm)		0	0.5	1.5	30	300
	Wk					
Male	81	44	50	32	48	48
	82	44	54	32	48	50
	88	54	66	54	60	70
	91	60	72	56*	60	76*
Female	80	42	28	32	50	19
	81	42	32	34	54	38
	88	46	44	54	62	44
	91	54	58	58	74	56

* includes animals dying during the terminal sacrifice.

Signs of toxicity reported throughout the treatment period were irritability, over-activity, vocalisation, grinding of teeth and aggressive behaviour from ≥1.5 ppm. The severity of these signs increased with dose. Convulsions were also noted at ≥1.5 ppm (3 males), 30 ppm (1 male and 3 females) and 300 ppm (8 males and 12 females). In some animals these convulsions were the direct cause of death. The earliest incidence of convulsions was week 23 at 1.5 ppm, and week 1 in females at 30 ppm, and week 1 in both sexes at the top dose. Body weight was significantly decreased in both males and females receiving 300 ppm, and in females only at 30 ppm. Food consumption was initially diminished at the top dose, but was indistinguishable from control levels by study termination. Food utilisation was unaffected by the treatment regimen.

Clinical chemistry investigations weeks 24, 50, 76 and 88/90 (study termination) showed a significant decrease in serum albumin:globulin ratios (A:G ratios) and bilirubin level, and elevations in cholesterol levels at the two highest dose levels. The reported decreases in serum albumin and total protein levels were within the range of supplied historical control data. There was a significant increase in platelet numbers in males at 30 and 300 ppm at study termination (29 and 45 %). Although other significant haematological changes were observed, these were not toxicologically significant.

After 52 weeks followed by 12 weeks on control diet, no effects were reported in males. In females cholesterol levels was still increased (98%) and remained outside the range of supplied historical control data.

In treated animals placed on control diet for 12 weeks to assess recovery, T4 and TSH levels in females were indistinguishable from controls throughout the recovery period, but T3 levels were significantly increased at 300 ppm at week 4 (22%) and at 30 and 300 ppm at weeks 7 and 11 (3 1% and 48% respectively at 300 ppm). In males, T3 levels were similar to controls through the recovery period. T4 levels had also reverted to the control range by week 7, but TSH levels remained significantly increased at 300 ppm throughout the recovery period (121% after 11 weeks).

Throughout the study, from week one, TSH levels were significantly elevated in males at ≥30 ppm, and females at 300 ppm only. T4 levels were also significantly decreased

in all treatment groups in males 300 ppm, and females at ≥30 ppm. T3 was decreased in week one in males only (13% at 300 ppm). (Table 5.17.)

There were no toxicologically-significant observations in the urinalysis data from this study.

At 52 weeks of treatment, liver weights were significantly increased in males (16%) and females (42%) receiving fipronil at 300 ppm. Thyroid weights were increased at ≥1.5 ppm in males and all treated females, achieving significance at 300 ppm (42% and 66%, males and females respectively). In animals sacrificed after the recovery period, thyroid weights were significantly increased to the same extent at 30 ppm and 300 ppm (8% and 13%, males and females respectively). No further effects were observed.

At study termination, absolute thyroid weights were significantly increased in all male treatment groups and in females receiving ≥30 ppm. Adrenal gland and kidney weights were increased 300 ppm in males only, achieving statistical significance at ≥30 ppm. Liver weights were significantly increased in both sexes receiving 300 ppm. Thymus weights were decreased in all female groups, significantly so at 300 ppm, and uterus weights were decreased at 300 ppm only. Observed increases in relative organ weights (relative to body weight) reflected the noted decreases in body weight. In animals dying or sacrificed intercurrently, enlarged kidneys were noted in both sexes and enlarged livers, thyroids and adrenals in males only (Table 5.17.). In males the incidence of pale and enlarged kidneys was significant at ≥30 ppm. The incidence of granular kidneys in males was not affected by treatment. At the top dose both the liver and thyroids were found to be enlarged. In females the macropathology findings were confined to a statistically significant increase in the number of pale kidneys at ≥30 ppm.

Histopathological examination revealed no abnormal non-neoplastic findings in animals killed or dying intercurrently, or in animals from interim kills at week 52 and animals in the 13-week recovery group. At study termination histopathological findings were confined to a statistically significant increase in progressive senile nephropathy in males and thyroid "follicular cysts" (growth anomaly) at ≥30 ppm in females.

Neoplastic findings were confined to the thyroid and are presented in Table 5.18. No tumours were observed at the sacrifice of animals at 52 weeks, indicating the observed thyroid tumorigenesis was a relatively late event. The increase in follicular cell adenomas is not deemed to be biologically significant until 300ppm as there is no dose response between 1.5 and 30 ppm.

Table 5.17 Treatment-related effects in the 24 month rat dietary study

ppm		0	0.5	1.5	30	300	0	0.5	1.5	30	300
Mg/kg bw		0	0.02	0.06	1.3	13	0	0.03	0.08	1.6	17
Animals displaying convulsions during the study		0/50	0/50	3/50	1/50	5/50	0/50	0/50	0/50	2/50	11/50
	Week										
Body weight (% of control)	88/91	-	100	112	93	82	-	93	97	77	75
		Blood chemistry (24wk)									
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	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***
	88/90	0.7	0.7	0.6*	0.5***	0.5***	0.9	0.8	0.7*	0.6**	0.6***
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*
Total bilirubim mg%	24	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1*	0.1***	0.1***
	50	0.2	0.1	0.1	0.1	0.1*	0.2	0.2	0.2	0.1**	0.2
	76	0.2	0.2	0.2	0.2	0.2**	0.2	0.2	0.2	0.2	0.2
	88/90	0.2	0.2	0.2	0.2*	0.1**	0.1	0.1	0.1	0.1	0.1
Platelets $\times 10^3/mm^3$	88/90	918	917	1050	1185*	1338***	921	1133*	956	1017	1061
TSH	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***
T3	24	0.69	0.73	0.74	0.70	0.64	0.93	0.92	0.84	0.82	0.84
	50	0.7	0.67	0.84*	0.82*	0.69	0.87	0.88	0.83	0.84	0.87
T4	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**	3.9***	23.07***	3.31	3.46	3.00	2.06***	1.38***
Organ weights (g) and macropathology.		**89 weeks males, 91 weeks females**									
Thyroid weight	89/91	0.042	0.051*	0.053*	0.063**	0.094**	0.036	0.038	0.036	0.044	0.072*
Enlarged	89/91	0/20	0/14	1/22	3/20	5/12**	1/23	0/21	0/21	1/13	3/22
Adrenal gland weight	89/91	0.074	0.088	0.086	0.092	0.109**	0.108	0.138	0.123	0.125	0.128
Kidney weight	89/91	6.32	7.29	7.24	8.56**	9.86*	4.23	4.51	4.15	5.75	4.89
Pale	89/91	1/20	1/14	3/22	8/20*	7/12**	0/23	2/21	0/21	4/13*	4/22*
Enlarged	89/91	3/20	2/14	6/22	11/20*	10/12***	0/23	2/21	0/21	5/13**	2/22
Granular	89/91	2/20	1/14	2/22	3/20	1/12	0/23	1/21	0/21	4/13	4/22
Thymus weight	89/91	1.871	0.76	0.923	0.610	0.617	0.678	0.608	0.602	0.564	0.448**
Histopathology		**89 weeks males, 91 weeks females**									
Incidence of progressive senile nephropathy	89/91	8/20	11/14*	17/22*	17/20**	11/12**	6/23	12/21	5/21	9/13*	12/22
Thyroid "follicular cysts"	89/91	0/20	1/14	0/22	1/20	0/12	0/23	0/21	0/21	3/13*	3/22

*P<0.05, **P<0.01, ***P<0.001

Table 5.18 Thyroid tumours observed in rats scheduled for all animals in the 24 month rat carcinogenicity study.

Pathology	Males						Females					
ppm	0	0.5	1.5	30	300	HCD	0	0.5	1.5	30	300	HCD
Follicular cell carcinoma	0/49	0/48	0/50	0/50	5/50*	1.1%	0/50	1/50	0/50	1/50	2/50	1.6%
Follicular cell adenoma	0/49	1/48	5/50	3/50	12/50	6.1%	0/50	0/50	0/50	0/50	8/50**	1.4%

* $p<0.05$, ** $p<0.01$

A NOEL for non-neoplastic end-points was not established due to the increased thyroid weight in all treated males at study termination. However, histopathological correlates of the thyroid weight increases were reported at ≥30 ppm. Therefore, it is possible to establish a NOAEL of 0.5 ppm based on convulsions at 1.5 ppm. This is equivalent to 0.02-0.03 mg/kg/d in males and females respectively. The NOAEL for neoplastic findings was established at 30 ppm based on increased thyroid tumours at 300 ppm, equivalent to 13-17 mg/kg/d, males and females respectively.

(DP 71094)

5.5.2 18-month dietary study in mice

A 78-week dietary carcinogenicity study was carried out in the mouse (52 animals/sex/group, CD-1 strain). Animals received fipronil (batch number PGS 963, 95.4% purity) at either 0.1, 0.5, 10 or 30 ppm; equivalent to 0.02, 0.06, 1.02 and 3.5 mg/kg/d in both sexes. Further animals (20/sex/group) were included to be sacrificed after 53 weeks as the interim kill. An additional dose level of 60 ppm was included but terminated after 10 weeks due to extreme toxicity (convulsions) and excessive mortalities. The animals which died before the study was terminated prematurely were necropsied, the only effect seen was increased relative liver weight. The rest of the animals in this group, which were killed at week 10, were not necropsied. No signs of toxicity were reported in the remaining groups. A differential blood count was carried out at week 52 and 76; no further diagnostic tests were carried out. Survival was not affected by treatment and was adequate for the duration of the study. Only survival in control males dipped below 50% to 54% at week 76 but no further loss from this group was reported up to study termination.

At termination at 30 ppm, food consumption was decreased by 7% (males) and 14% (females), whilst bodyweight gain was decreased by 14% (males), and 19% (females, $P<0.05$). Body weights of animals sacrificed at 53 weeks were significantly decreased in males only at 30 ppm. At study termination a decreases in mean body weight was noted in both males and females (≤10%) at 30 ppm but these decreases were not statistically significant. Food efficiency was calculated for the first 14 weeks of the study only. There may have been a decrease at 10 and 30 ppm (3.3% control vs. 2.9 and 2.3%) in males only but this was not indicated as statistically significant.

Significant decreases in neutrophil (30%) and leukocyte numbers (28%) were reported in females receiving 30 ppm at week 76. These effects were apparent at week 52, but were not significant.

Necropsy of males after 53 weeks showed statistically significant changes in kidney and liver weights at 30 ppm. In females the absolute liver weight was increased in all treatment groups, achieving statistical significance at ≥10 ppm. A variable decrease in spleen weight was reported in both sexes in all treatment groups, and was significant at 30 ppm.

At study termination the absolute liver, heart and kidney weights were significantly different to controls in all treated males at 30 ppm. No effects upon absolute organ weight were reported in females, with the exception of a slight, but non-significant increase in absolute liver weight at ≥10 ppm. Relative organ weights were unaffected, with the exception of liver weight. Increases were reported in all treated males which were significant at ≥10 ppm, and in females at ≥10 ppm. These increases may reflect the previously noted slight decreases in body weight.

Macropathological findings were confined to a significant increase in "areas of change" in the liver of males receiving 30 ppm fipronil (8/52 and 0/52 30 ppm and control respectively) at study termination.

At 53 weeks and study termination, significant increases in "microvesicular peri-acinar vacuolation" were reported in males at ≥10 ppm and females at 30 ppm. No further significant histopathological findings were reported.

Table 5.19 Treatment-related effects in the 18 month mouse fipronil carcinogenicity study.

Ppm		**0**	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
Mg/kg bw		**0**	**0.02**	**0.06**	**1.02**	**3.5**	**0**	**0.02**	**0.06**	**1.02**	**3.5**
	week	Males					Females				
Body weight (g)	78	49	48.5	49.2	46.9	46.6	45.3	43.3	47.3	43.0	40.8
Absolute Heart weight (g)	78	0.318	0.288	0.286	0.284	0.279*	0.201	0.203	0.219	0.195	0.202
Absolute spleen weight (g)	53	0.178	0.157	0.14	0.127*	0.143	0.113	0.113	0.129	0.102	0.092*
	78	0.163	0.15	0.171	0.153	0.158	0.163	0.143	0.132	0.147	0.149
Absolute kidney weight (g)	78	0.941	0.926	0.904	0.904	0.820**	0.535	0.548	0.554	0.522	0.512
Absolute liver weight (g)	78	2.77	2.78	2.92	3.3	3.81**	1.99	1.93	2.06	2.02	2.13
Relative liver weight (% of body weight)	78	5.634	5.744	5.977	7.008*	8.261**	4.535	4.575	4.451	4.799	5.294**
Microvesicular peri-acinar vacuolation	53	0/14	2/15	2/19	7/16**	12/18***	1/18	1/19	4/15	1/17	4/13
	78	5/24	7/31	7/26	13/26*	13/26*	0/32	0/32	4/26*	3/37	7/38*

*P<0.05, **P<0.01

Table 5.20 Tumour incidence for all animals in the 18 month mouse fipronil carcinogenicity study

Ppm	**0**	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
Mg/kg bw	**0**	**0.02**	**0.06**	**1.02**	**3.5**	**0**	**0.02**	**0.06**	**1.02**	**3.5**
	Males					**Females**				
Hepatocellular carcinoma	1/52	1/52	2/52	1/52	5/52	0/52	0/52	0/52	0/52	0/52
Hepatocellular adenoma	10/52	3/52	2/52	6/52	6/52	0/52	0/52	0/52	0/52	1/52

No treatment-related increases in tumour incidence or alteration in tumour type were reported in this study.

In conclusion, the NOAEL for non-neoplastic events was established at 0.5 ppm in males and 10 ppm in females (equivalent to 0.055 and 1.23 mg/kg/d) based on the previously noted hepatic histopathological findings. A NOEL for neoplastic events was established at 30 ppm in males and females, equivalent to 3.43 and 3.62 mg/kg/d respectively.

(DP 71096)

5.5.3 Summary of chronic toxicity/carcinogenicity

Low survival rates were observed in the rat carcinogenicity study with <50% survival in all dose groups including controls; fipronil itself had no effect on survival. Historical data from the test laboratory indicate that they typically see a low survival rate in control-animals (16-53%). A significant increase was observed in the incidences of thyroid follicular cell adenomas and carcinomas in both sexes at the top dose (300 ppm); these exceeded the historical control incidence. No tumours were observed at 52 weeks. The NOAEL for non-neoplastic effects was 0.5 ppm (0.02 mg/kg/d) based on signs of neurotoxicity (convulsions) at 1.5 ppm. In mice, survival was satisfactory, and no treatment-related increases in tumour incidence were observed after 78 weeks administration of fipronil at up to 3.5 mg/kg d. The NOEL was 0.06 mg/kg d in males and 1.2 mg/kg d in females based on hepatic microvesicular vacuolation.

5.6 REPRODUCTIVE TOXICITY

5.6.1 Dietary two-generation study in rats (AII 5.6.1)

In a two-generation study, CD rats (30 animals/sex/dose) were administered fipronil (batch number PGS 963, 95.4% purity) in the diet at 3, 30 or 300 ppm, equivalent to 0.25, 2.5 or 26 mg/kg bw per day for males and 0.27, 2.7 and 28 mg/kg bw per day for females. F0 adults were treated for 71 d before mating (1 male to 1 female pairing), throughout the mating period (up to 21 d) and then throughout gestation and lactation of the litter (F1a). Approximately 10 d after weaning (25 d *post partum*) of the F1a pups, animals administered 3 or 30 ppm, as well as the control group, were paired a second time to produce an F1b generation. The F1a litters were randomly adjusted to 8 pups on 4 d *post partum* and, after weaning, 30 animals/sex/dose were selected to form the Fl parents. Fl parents were treated from weaning for a minimum of 10 weeks and then paired (1 male to 1 female) to produce an F2 generation. F1 adult treatment continued until F2 pups were weaned.

Gross pathological examination was conducted on F0 and Fl adults and all weanlings except those selected to be the Fl adults. Histopathology was conducted on all adults dosed at 0 and 300 ppm and in addition, the liver and thyroid were examined from animals dosed at 3 and 30 ppm. All decedents and abnormal tissues noted at the gross examination were also examined.

There were 7 deaths or sacrifices in the F0 adults at the top dose, 2 males (1 and 24 weeks) and 5 females (1 animal 1 week, 4 animals 17 d post mating to 17 d *post partum*); at 30 ppm, 1 male (23 weeks); and in controls, 1 female (4 d *post partum*). Necropsy and histopathology did not note any treatment-related findings attributable to

the cause of death. However, two of the top dose females exhibited convulsions and the male at 30 ppm exhibited decreased muscular control. Two further top dose females exhibited convulsions but survived to scheduled termination.

In the Fl adults, 5 female deaths and sacrifices were noted : 3 at the top dose (from 26 d post mating to 16 d *post partum*); and 1 each at 30 ppm (20 d post-mating) and in the control group (18 d post-mating). Necropsy and histopathology did not note any treatment-related findings attributable to the cause of death. However, one top dose female exhibited convulsions.

In F0 males at 300 ppm, body weight gains were significantly lower during weeks 1-10 of treatment (9% by 10 weeks). Female F0 body weight gains were also significantly lower than controls at 300 ppm before pairing (9% by 10 weeks) and during gestation (18% at 300 ppm) and lactation (40% at 300 ppm).

In Fl males at 300 ppm, body weight was significantly lower than control at selection (14%). This percentage weight difference was maintained until scheduled sacrifice. In F1 females at 300 ppm, body weights were also significantly lower than in the controls at selection (17%). Subsequent body weight gain was comparable to controls during maturation and gestation. A significant change in body weight loss was noted throughout the lactation period (0.3% loss in controls, 2% loss at 300 ppm).

For F0 and F1 adults, food consumption was only measured during the 10-week pre-treatment period. A significant decrease in food consumption was noted in both F0 sexes at 300 ppm (4% at 10 weeks). In Fl males a 17% decrease was noted at 10 weeks. Calculated compound intakes at 10 weeks are noted in Table 5.21:

Table 5.21 Achieved fipronil intakes for F0 and Fl adults at 10 w (mg/kg/d).

		3 ppm	30 ppm	300 ppm
F0	Male	0.16	1.68	16.97
	Female	0.20	2.00	20.76
Fl	Male	0.16	1.69	17.72
	Female	0.20	2.09	23.04

In F0 and Fl females the distribution of regular, irregular cycles and acyclic females showed no treatment-related variation and was within the historical range for this strain. No treatment- related effect was noted in the mating performance and fertility in the F0 'a' and 'b' pairings. In Fl adults the numbers of animals mating was decreased to 83% at 300 ppm (outside historical control data for females) and hence, the fertility index was decreased to 80%. No effect was noted at 3 or 30 ppm. Pre-mating interval was comparable to controls in F0 and Fl adults.

In F0 females, one female at 3 and 30 ppm gave birth at 24 d gestation after the second pairing. However, there was no other evidence of dystocia or a treatment-related effect on gestation index. In F1 females no dose-related effect on parturition or gestation index was noted.

In Fl litters at 300 ppm the following parameters were significantly decreased: mean litter size (13%); the live birth index (98% in controls, 83% at 300 ppm); and the

viability index (97% in controls, 89% at 300 ppm). There were no treatment-related effects on these parameters at 3 or 30 ppm and, subsequent to culling, survival was comparable in all groups. No treatment-related effect was noted in sex ratio or lactation index.

In F2 litters, post implantation survival index was significantly lower at 3 and 300 ppm (90, 84, 85, 81% at 0, 3, 30, 300 ppm). Given the lack of dose response, the effect at 3 ppm was not considered toxicologically significant. In addition the following parameters were significantly decreased at the top dose; live birth index (100% in controls, 78% at 300 ppm), and viability index (98% in controls, 73% at 300 ppm). There were no treatment-related effects on these parameters at 3 or 30 ppm and the lactation index was not affected at any dose.

At 300 ppm convulsions were seen in 13 Fl pups in 9 litters (14-20 d *post partum*) and 4 F2 pups in 3 litters (15-18 d *post partum*). On external examination there was no increase in abnormalities noted when compared to controls for any dose/generation. Body weights of top dose Fl and F2 pups were significantly lower than controls (6-10% 1 d *post partum*, 10-14% 4 d *post partum* and 19-22% 25 d *post partum*).

At 300 ppm there was a slight significant delay in the onset of tooth eruption in Fl litters (9.7 d *post partum* in controls, 10.4 d *post partum* at 300 ppm). There was a slight delay in the timing of pinna unfolding in a number of litters at 300 ppm, however this was not statistically significant. There was no other treatment-related effect on pup development in the F2 generation.

At necropsy no treatment-related macroscopic changes were noted in adults or pups. In adults there were increases, often significant, in absolute and relative liver (10-15% and 15-40% at 30 and 300 ppm) and thyroid weights (15-25% and 25-50% at 30 and 300 ppm).

In addition, F0 female ovary weights were significantly decreased (30% absolute,) at 300 ppm. Also in Fl adults, decreases were noted in the following absolute organ weights female pituitary (19 and 25% at 30 and 300 ppm); ovary (14% at 300 ppm); testis (7% at 300 ppm); and epididymis (8% at 300 ppm). Given the body weight changes and lack of relevant histopathology, these weight changes are not considered toxicologically significant. Histopathological examination of F0 adults showed a significant increase in the incidence of liver centrilobular fatty vacuolation in females (0/26 in controls, 9/26 at 300 ppm) and thyroid follicular hypertrophy in both sexes (males 0/30 in controls, 10/29 at 300 ppm and females 0/29 in controls, 6/26 at 300 ppm).

In Fl adults histopathological examination showed a significant increase in the incidence of liver centrilobular fatty vacuolation in females (1/29 in controls, 6/27 at 300 ppm) and thyroid follicular hypertrophy in both sexes (males 0/28 in controls, 9/30 at 300 ppm and females 0/29 in controls, 15/27 at 300 ppm).

In conclusion:

(i) The NOEL for fertility and developmental effects was 1.7-2.0 mg/kg/d (30 ppm) based on the following effects at 17.0-23 mg/kg/d (300 ppm) : convulsions (Fl/F2

pups); low birth weight (FI/F2 pups); decreases in body weight gain (Fl/F2 pups); a delay in onset of tooth eruption (Fl pups); a decrease in the number of animals mating (Fl adults); decreases in live birth index and viability index (FI/F2 litters); and a decrease in post implantation survival index (F2 litters).

(ii) The NOEL for parental toxicity was 0.16-0.20 mg/kg/d (3 ppm) based on the following effects at 1.7-2.0 mg/kg/d (30 ppm): 1 male death (F0); and increases in thyroid and liver weights with associated histopathology (F0/F1 adults).

(DP 71100)

5.6.2 Developmental toxicity studies (AII 5.6.2)

a) Oral teratology study in rats

CD rats (25 animals/dose) were administered fipronil (batch number JJW 2070, 93% purity) in methylcellulose by gavage at 1, 4 or 20 mg/kg/d during 6-15 d of gestation. Control animals received vehicle alone. Doses were derived from a range-finding study and were adjusted for body weight at 8, 10, 12 and 14 d of gestation. Necropsy of the dams and examination of the foetuses was performed at 20 d of gestation. Half of the foetuses in each litter were examined for visceral abnormalities and half for skeletal abnormalities. Statistical analysis was carried out on litter parameters only.

No deaths or treatment-related signs of toxicity were noted. At 20 mg/kg/d food consumption was decreased (10-25%) from 6-11 d gestation but was comparable to controls for the rest of the study. In addition, at 20 mg/kg/d, body weight gain was decreased by 10% at 20 d and terminal body weights were 4% lower than controls. Water consumption was increased (20%) from 8 d gestation to the end of treatment at 20 mg/kg/d.

At necropsy no treatment-related changes were noted in macroscopic abnormalities, pregnancy rate, number of implantations, post implantation loss, numbers of early and late embryonic deaths, litter size, mean fetal body weight and sex ratio.

There were no treatment-related effects on the incidence of malformations and skeletal or visceral anomalies.

Fipronil did not cause developmental toxicity under the conditions of this study. The NOAEL for maternal toxicity was 20 mg/kg/d based on the limited changes in water/food consumption and body weight gain at 20 mg/kg/d. Although the signs of maternal toxicity were not large, the effects seen at 24 mg/kg/d in the rat sub-chronic study and deaths noted at 30 mg/kg/d in the preliminary 14-d study suggest that the highest dose tested was appropriate.

(DP 71104)

b) Oral teratogenicity study in rabbits

In an adequately conducted study, inseminated New Zealand White rabbits (22 animals/dose) were administered fipronil (batch number PGS 963, 95.4% purity) in

0.5%w/v aqueous methylcellulose mucilage and 0.5%w/v 'Tween 80' by gavage. Doses of 0.1, 0.2, 0.5 or 1.0 mg/kg/d were administered during 6-19 d gestation. Dams were necropsied at 29 d gestation and uterine contents examined.

The numbers of animals with viable young were reported as 19, 19, 21, 18 and 18 at 0 to 1mg/kg/d. No deaths were reported, but one animal was killed *in extremis* at 26 d gestation. Necropsy of this animal revealed evidence consistent with an intra-peritoneal infection. From 6-18d gestation significant decreases in body weight gain were noted in the top 3 doses (30, 43, 60% at 0.2, 0.5, 1.0 mg/kg/d respectively). In addition, body weight loss (1%) was noted in the top two doses 16-20 d gestation. Body weight gains after treatment were broadly comparable to controls, but the deficit compared to controls incurred during dosing (5%) at 1.0 mg/kg/d was maintained until the end of the study. Food intake was significantly decreased at the top two doses during dosing (20 and 32% at 0.5 and 1.0 mg/kg/d 13-19 d gestation) but comparable to controls after treatment. At necropsy no treatment-related macroscopic changes were noted. However, total litter resorption was noted in 1 female at each of 0, 0.1 and 1.0 mg/kg/d and in 1 female at 0.1 mg/kg/d aborted at 23 d gestation. There were no treatment-related effects on the numbers of corpora lutea, implantations, resorptions and viable young, or on sex ratio, mean fetal weight and mean placental weight. Fetal examination showed no treatment-related increase in the incidence of external malformations, visceral or skeletal malformations. Skeletal ossification was comparable to study and historical controls.

Fipronil did not cause developmental toxicity under the conditions of this study. The NOEL for maternal toxicity was 0.1 mg/kg/d based on the decreases in body weight gain at ≥0.2 mg/kg/d and the decreases in food intake at ≥0.5 mg/kg/d.

(DP 71106)

5.6.3 Summary of reproductive toxicity

In a rat 2-generation reproductive study, effects on fetal toxicity and reproductive parameters were noted only at parental toxic doses. The NOEL for fertility and developmental effects was 1.7-2.0 mg/kg/d (30 ppm) based on convulsions (Fl/F2 pups); low birth weight FI/F2 pups); decreases in body weight gain (Fl/F2 pups); a delay in onset of tooth eruption (Fl pups); a decrease in the number of animals mating (Fl adults); decreases in live birth index and viability index (FI/F2 litters); and a decrease in post implantation survival index (F2 litters) at 17.0-23 mg/kg/d (300 ppm). The NOEL for parental toxicity was 0.16 - 0.20 mg/kg bw, based on 1 male death (F0); and increases in thyroid and liver weights with associated histopathology (F0/FI adults) at 1.7 - 2.0 mg/kg bw.

In two developmental toxicity studies fipronil did not cause any increase in skeletal or visceral malformations in the rat or rabbit. Maternal toxicity based on food intake and body weight gain decreases was demonstrated at 20 and ≥0.2 mg/kg/d in the rat and rabbit respectively.

5.7 DELAYED NEUROTOXICITY STUDIES (AII 5.7)

No delayed neurotoxicity studies were submitted.

5.8 OTHER TOXICOLOGICAL STUDIES (AII 5.8)

5.8.1 Neurotoxicity

a) In a single exposure (gavage) study, Sprague-Dawley rats (15 animals/sex/group) were administered 0.5, 5.0 or 50 mg/kg fipronil (batch number 78/GC/90, 96.7% purity) in corn oil. A Functional Observation Battery (FOB) and motor activity evaluation were performed prior to and approximately 7 h, 7 d and 14 d after dosing. Body weights were noted weekly. At necropsy, 15 d post treatment, 10 animals/sex/group were perfusion fixed and examined for gross pathological changes. Histopathological examination was conducted on 6 animals at 0 and 50 mg/kg.

There were 6 deaths (5 male, 1 female) noted in the top dose group (at 2-5 d). Of these animals, 4 (3 male, 1 female) exhibited convulsions and 5 (4 male, 1 female) exhibited diffuse brain haemorrhage. No histopathology was conducted. In addition at the top dose the following signs of toxicity were noted: a further 4 males with convulsions (2-6 d); dehydration in 6 males and 6 females including 3/5 male decedents (2-5 d); and urine stains on 4 males and 6 females (2 - 5 d). Mean body weight was decreased in top dose males by 10% at 7 d and 6% at 14 d.

In top dose males, observation in the FOB at 7 h noted: drooping or half shut eye lids; previously noted convulsions; tremors (fine, coarse and 2 animals exhibiting both); head bobbing (1/15); myoclonic movement (1/15); splayed gait; and significantly decreased static hind limb splay (30%). Static hind limb splay was also significantly decreased at 5.0 mg/kg by l0%.

Mean overall motor activity was decreased to 90% of control values in top dose males at 7 h. In addition, open field activity was affected with decreased arousal and significantly decreased rearing. Significant decreases were also noted in the approach response and air righting reflex in males at 7 h. In addition the tail pinch response was non-significantly decreased at the top dose.

In males at 7 h significant effects were also noted in muscle tone, pupil size and body temperature (3°C decrease at the top dose.

At 7 h in top-dose females the following significant effects were noted: an increase in fine tremors; a decrease in rear events; decrease in muscle tone; a decrease in body temperature (3°C at the top dose); and a decrease in static hind limb splay (30%). In addition static hind limb splay was significantly decreased at 5.0mg/kg.

At 7 d, FOB observations were limited to an apparent stimulation of activity in top-dose males. The observations included a significant increase in mean rear events and non-significant increases in exaggerated responses to startle and tail pinch tests. A significant correlation was noted between dose level and level of arousal with top dose males producing greater numbers at the two highest response levels.

Table 5.22 Observations in the single gavage dose neurotoxicity study in rats with fipronil.

ppm	0	0.5	5.0	50	0	0.5	5.0	50
	Males				Females			
	7 hours after treatment							
Total number of animals	15	15	15	15	15	15	15	15
Drooping or half shut eyelids	1	0	1	4	0	0	0	3
Convulsions clonic	0	0	0	4 (days1-2)	0/15	0/15	0/15	1/15 (day 2)
Convulsions tonic	0	0	0	2/15 (day 2)	0/15	0/15	0/15	0/15
Tremors								
Coarse	0	0	0	5*	0	0	0	1
Fine	0	0	0	6*	0	0	0	6*
Gait Normal	15	15	15	5**	11	14	14	7
Splayed	0	0	0	8	0	0	0	4
Hypotonic	0	0	0	2	-	-	-	-
Walking on toes	-	-	-	-	4	1	1	4
Decreased static hind limb splay (cm)	7.92	7.18	7.00*	5.72**	7.54	7.64	6.55*	5.34**
Decreased arousal	7	2	5	11	0	1	0	12
Mean overall motor activity (sum of all counters)	557.4	613.9	531.2	56.0**	981.5	945.3	1008.5	68.9**
Decreased rearing (events)	3.67	6.53	3.93	0.2*	18	9.07**	10.53**	0.6**
Approach response								
Noticeable	15	15	13	9*	-	-	-	-
Absent	0	0	2	6	-	-	-	-
Tail pinch response								
Noticeable	13	14	14	9	14	15	15	12
None	1	1	1	6	0	0	0	3
Exaggerated	1	0	0	0	1	0	0	0
Decreased muscle tone	0	0	0	5*	1	0	1	10**
Pupil size Dilated	0	0	0	9*	-	-	-	-
Decreased	-	-	-	-	5	4	2	8
Body temperature	37.95	38.08	38.23**	35.21**	38.75	38.48	38.45	35.79**
Air righting								
Feet/coordinated	14	15	15	7*	-	-	-	-
Feet/uncoordinated	1	0	0	1	-	-	-	-
Back	0	0	0	4	-	-	-	-
Side	0	0	0	3	-	-	-	-
Found dead	0/15	0/15	0/15	5/15 (days2-6)	0/15	0/15	0/15	1/15 (day 2)
Total number of animals	15	15	15	10	15	15	15	14
	7 days after treatment							
Increase in mean rear events	3.53	3.13	6.6	10.2**	15.8	11.33	13.8	16.36
Exaggerated response to startle	0	0	0	3	0	0	0	1
Exaggerated response to tail pinch tests	0	1	0	2	0	1	0	1
Level of arousal	6	5	9	10	15	12	15	13

*P<0.05, **P<0.01, - not measured

No effects were noted in motor activity at 7 d. At 14 d no treatment-related effects were noted in FOB or motor activity.

At necropsy there were no treatment-related gross pathological changes and no treatment-related histopathological changes of CNS and PNS.

The NOAEL for this study was 5.0 mg/kg, based on the toxicologically significant effects noted at 50 mg/kg.

(DP 71111)

b) In a 90-d dietary study Sprague-Dawley rats (15 animals/sex/dose) were provided with food containing fipronil (batch number 78/GC/90, 96.7% purity) at doses of 0.5, 5.0 or 150 ppm fipronil (equivalent to 0.03, 0.3 or 8.9 mg/kg bw per day for males, and 0.03, 0.3 or 11 mg/kg bw per day for females). During the study body weight and food consumption were noted weekly, prior to neurobehavioral observation and prior to sacrifice for neuropathological examination. Behaviour was evaluated in 10 animals/sex/dose using a FOB, and motor activity in all 15 animals/sex/dose using an automated recording apparatus. Observations were carried out one week prior to dosing and during weeks 4, 9 and 13 of the study. At 14 weeks, 10 animals/sex/dose (11 top dose females) were perfusion fixed and examined for gross pathological abnormalities. Selected neurological tissues of 6 animals/sex in control and top dose groups were histopathologically examined.

No treatment-related deaths or signs of toxicity were noted. Body weight gain was significantly decreased (62% in males, 70% in females) during the first week of treatment at the top dose. Body weights were comparable to controls at 13 weeks. Food consumption was decreased (23% in both sexes) at the top dose during the first week of treatment, but was comparable to controls thereafter.

In the FOB several minor statistically significant changes were noted at 4 weeks in top-dose males. These were an increase in the number of animals with urine present in the cage, an exaggerated startle reflex and an exaggerated tail pinch reflex. These findings were not noted in later evaluations and, with regard to the reflex changes, were transient at 4 weeks.

There were no treatment-related effects on motor activity or habituation rates.

At necropsy, absolute brain weights were significantly increased (5%) in top dose males but, gross and histopathological examination did not show any treatment-related findings.

Based on this study, the NOAEL for neurotoxicity is the top dose tested, 8.9-10.8 mg/kg/d (150 ppm). The NOEL for general toxicity is 0.3-0.35 mg/kg/d (5 ppm) based on the body weight and food consumption changes.

(DP 71125)

c) In a study not conducted to a recognised protocol, 4 female beagles were administered 20 mg/kg/d fipronil (batch number PGS 963, 95.4% purity) in gelatine capsules for up to 14 d or until signs of neurotoxicity' were noted. When neurotoxicity signs were noted, a 28-d reversibility phase started. One control animal was treated with gelatine capsules alone. Neurological examinations were performed 3 times per week and consisted of:

(i) Cranial nerve reflexes : pupillary light; consensual light; palpebral; blink and corneal; gag and a general examination of the head to assess other cranial nerves.

(ii) Segmental reflexes: flexor (withdrawal) including crossed flexor and patellar extensor tone.

(iii) Postural reactions : placing reactions - visual and tactile; extensor postural thrust; and righting reactions - tonic neck reactions, hopping reflex.

(iv) General observations: behavioural changes (aggression, sedation); abnormalities of gait stance; presence of tremor or other dyskinesias.

(v) Obstacle avoidance and hearing tests.

At necropsy cerebellum, forebrain, medulla, sciatic nerves (distal and proximal), spinal cord (cervical and thoracic swellings) and tibial nerves (distal and proximal) were examined after perfusion fixation.

Following 5-13 d of treatment (T days) and the subsequent 28-d recovery period (R days), there were no deaths reported. The recovery periods for each animal started after 13 d (control) and 13, 5, 5 and 7 d of treatment. Food consumption was reduced in treated animals (20-50%) despite dietary supplements from T1-2. All animals were feeding normally by R10. Body weight loss was noted in treated animals from T3 (12-20% at RO compared to weights at T0). By R17 initial body weights were regained and overall body weight gains (1-13%) were noted by the end of the respective recovery periods.

Routine observation of signs and neurological examination noted a large degree of individual variation in the effects noted and in their size and timing. The recovery periods for each animal started after 13, 13, 5, 5 and 7 d of treatment. Observations included : ataxia (4/4 started T6-13 and resolved after R10); convulsions (2/4 on RI and R6); reflex abnormalities (4/4 RI-26); and gait/limb abnormalities (3/4 RI-4).

One animal displayed visual impairment (R2-1 1) which was associated with depressed pupillary, consensual light and blink reflexes. In addition the corneal response was exaggerated. The same animal was non-responsive to the whistle test and startle response (R18 and R20), but did respond R25 and R27. All animals apparently habituated to the whistle test with a decrease in response times noted as the study progressed.

At necropsy no treatment-related macroscopic or histological effects were noted.

Based on this study, 20 mg/kg/d fipronil caused CNS effects in beagles which resolve in 28 d with no pathological changes observed after recovery.

(DP 71126)

5.8.1.1 Summary of neurotoxicity

In an acute rat study the NOEL was 0.5 mg/kg based on the significant decreases in hind limb splay at 5.0 mg/kg. The NOAEL for this study was 5.0 mg/kg, based on the

toxicologically significant effects noted at 50 mg/kg. Effects noted in both sexes at 50 mg/kg included death and at 7 h convulsions, tremors, decreased motor activity and decreased muscle tone. In a rat 90-d repeat dose study the FOB noted only several minor statistically significant changes and there were no treatment-related effects on motor activity or habituation rates. The NOAEL for neurotoxicity was 8.9-10.8 mg/kg/d. The NOEL for general toxicity was 0.3-0.35 mg/kg/d based on body weight and food consumption changes. Identical neurobehavioural and neuropathological endpoints were examined in both the rat 90 day dietary neurotoxicity study and the rat single-dose gavage study.

In a study not conducted to a recognised protocol, female beagles were dosed with 20 mg/kg/d fipronil until neurotoxicity developed. A large degree of individual variation in the effects, and in their size and timing were noted. Observations included : ataxia; convulsions; reflex abnormalities; and gait/limb abnormalities. However, CNS effects resolved in 28 d with no pathological changes observed after recovery.

5.8.2 Studies with metabolites of fipronil

A series of acute studies and genotoxicology studies were submitted for certain technical impurities and metabolites of fipronil, which were MB 45950, MB 46136, RPA 200766. All these materials are technical impurities, plant, environmental, and rat metabolites. The toxicity of another environmental metabolite (RPA 200761), not found in rats is also considered.

5.8.2.1 Studies with MB 45950

a)

Study Type:	**Acute Oral LD_{50} with MB 45950**		
Reference:	82798	GLP Certified:	Yes
Substance Purity:	Batch number OP5502, purity 98.9%.	Guideline Compliance:	OECD 401 (1987)
Test System:	Rat: Sprague - Dawley (5/sex/dose)	Year(s) of Conduct:	1994
Doses:	50, 65, 90 and 120 mg/kg by gavage	Vehicle:	As a suspension in corn oil at 10 ml corn oil per kg animal.

All mortalities occurred within the seven days of treatment and are displayed in Table 5.23. Clinical signs of toxicity observed were excessive jumping, increased or reduced motor activity, clonic convulsions, tremors, curling up, and subdued behaviour, were noted in all animals at doses ≥ 50 mg/kg bw.

Table 5.23 Mortality in the first week after a single gavage administration of MB 45950

Dose level (mg/kg)	Males	Females	Combined
50	0/5	0/5	0/10
65	3/5	0/5	3/10
90	4/5	3/5	7/10
120	5/5	3/5	8/10

The applicant calculated the LD_{50} as follows (Table 5.24) according to Litchfield and Wilcoxon

Table 5.24 Acute oral LD_{50} for MB 45950

Animal	Calculated LD_{50} (mg/kg bw)	95% confidence interval (mg/kg)
Male	69	52-90
Female	100	77-129
Combined	83	67-101

To conclude the oral LD_{50} in rats for MB 45950 is 69 mg/kg bw. MB 45950 would be classifiable as toxic via the oral route according to current EC classification criteria.

(DP 82798)

b)

Study Type:	**Acute Dermal LD_{50} with MB 45950**		
Reference:	82801	GLP Certified:	Yes
Substance Purity:	Batch number : DXH 1379/3, Purity not stated	Guideline Compliance:	OECD 402 (1987)
Test System:	Rat: Sprague - Dawley	Year(s) of Conduct:	1988
Doses:	4000, 500, 250 mg/kg	Vehicle:	Applied as supplied (powder)
Area Covered:	10% of body surface area on the back, clipped with an electric clipper.	Exposure Duration:	24 hours under an occlusive dressing.

In the first instance 4000 mg/kg of the test material was applied to the animals under an occlusive dressing. It was not moistened. As significant toxicity was observed at this dose level, two other dose levels, 500 and 250 mg/kg bw were investigated.

Mortality for all three dose levels is displayed in Table 5.25.

Table 5.25 Mortality in dermal toxicity study with MB 45950

Dose level (mg/kg bw)	Males	Females
4000	5/5	4/5
500	0/5	0/5
250	0/5	0/5

Clinical signs noted at 4000 mg/kg bw were, perinasal staining, chromodacryorrhoea, piloerection and convulsions in up to 2 animals of each sex. Symptoms appeared from 4 hours after dosing, and on day 3, four males and three females displaying these symptoms died. The surviving animals displayed piloerection, perinasal staining, and hypoactivity and the remaining male and 1 of the females died on day 5. The remaining female displayed increased chromodacryorrhoea on days 4, 5 and 6, but activity was normal from day 7 of the study with piloerection and perinasal staining remaining until day 10 of the study. Decedents showed fur staining around the mouth of 3 rats of each sex, and general fur staining was seen in two male and one female rat. Staining of the fur with urine was seen in the inguinal region of 2 female rats. Necropsy results for the surviving animals are not stated.

Clinical signs at 500 and 250 mg/kg were chromodacryorrhoea in one male on day 4 at 500 mg/kg bw and perinasal staining in one female on day 2 at 250 mg/kg bw. These may or may not be related to treatment. No other clinical signs were seen on other days of the study or other animals.

Necropsy: no abnormalities were seen at 500 and 250 mg/kg except for reddening of the kidneys in 1 male and female at 250 mg/kg bw.

Body weight gain was normal in all survivors, except the female administered 4000 mg/kg bw which lost weight in the first week after administration.

c)

Study Type:	**Acute Dermal Irritation / Corrosion with MB 45950**		
Reference:	82802	GLP Certified:	Yes
Substance Purity:	Batch number : DXH 1379/3, Purity not stated	Guideline Compliance:	OECD 404 (1981)
Test System:	Rabbit: New Zealand White	Year(s) of Conduct:	1987
Solvent / Vehicle:	Material used as supplied.	No. of Animals:	6 females
Dose & Nature:	0.5 g (powder) over 6 sq. cm of dorsal clipped skin, under a semi-occlusive dressing for four hours.	pH:	-

No irritation or oedema was seen at any time point between 1 hour and 7 days after treatment.

To conclude, the test material would not be classifiable as irritating to skin according to current EC classification criteria.

d)

Study Type:	**Acute Eye Irritation with MB 45950**		
Reference:	82804	GLP Certified:	Yes
Substance Purity:	Batch number : DXH 1379/3, Purity not stated	Guideline Compliance:	OECD 405 (1987)
Test System:	Rabbit: New Zealand White	Year(s) of Conduct:	1987
Solvent / Vehicle:	None	No. of Animals:	9 females
Dose & Nature:	0.1 ml (powder) - equivalent to 97 mg of test material.	pH:	-

The test material was instilled into the right conjunctival sac of each animal and the eyelids held together for a few seconds and moved about gently to distribute the test material around the surface of the eye and lids. The treated eyes of the first group of six rabbits were not rinsed after dosing. Two minutes after dosing the treated eyes of the second group of three rabbits were rinsed for one minute using distilled water at approximately 37 °C.

Unrinsed animals:
Although there was some conjunctival redness and chemosis (scores of 1 and 2) in all of these animals at one hour after dosing, there were no significant eye effects remaining in the group as a whole over the 24 to 72 hour observation period.

Rinsed animals:
At the one hour observation period there were no eyes effects except for conjunctival redness scores of 1 in 2/3 animals at the one hour observation period. No eye effects were still apparent at the 24-72 hour observation points.

To conclude the test material would not be classifiable as irritating to eyes under current EC classification criteria.

e) **Ames test**

In this 1994 study the mutagenic potential of MB 45950 (batch number OP5502, purity 98.9%) was assessed towards *Salmonella typhimurium* strains TA 98, 100, 1535, 1537 in two independent assays. Concentrations of 10, 25, 50, 100, 250 µg MB 45950/plate were tested in triplicate in the absence and presence of metabolic activating fraction, S9, from 'Aroclor 1254' induced rat liver. 250 µg/plate was the maximum concentration tested due to cytotoxicity and precipitation at this concentration and on certain plates at 50 and 100 µg/plate depending on the presence or absence of S9.

Sodium azide, 2-nitrofluorene, 9 aminoacridine and 2-aminoanthracene were the positive control materials used in accordance with strain and presence or absence of S9.

The test material did not induce any increase in revertant numbers at any concentration in any strain tested.

The positive controls produced satisfactory results.

The test material is not considered to be mutagenic under the conditions of this study.

(DP 82809)

f) ***In vitro*** **cytogenetics study**

In this 1987 mammalian cytogenetic study, MB 45950 (lot number 37JJW1898, purity >99%) was investigated as to its clastogenic potential in human lymphocytes from 1 male and 1 female donor), in the presence and absence of 'Aroclor 1254' induced rat liver S9 fraction. The study was not quoted as being adherent to any guideline. A single harvest time only and no repeat test was performed. At least two harvest times and a repeat test would have been required by current guidelines (OECD 473 1997; 92/69/EEC). Concentrations of test material used and exposure periods were as in Table 5.26. The positive control materials were methylmethanesulphonate (MMS) in the absence of S9, and cyclophosphamide (CPA) in the presence of S9.

Table 5.26 Concentrations of test material used, exposure period and toxicity results.

		Frequency of mitosis as a % of control at doses tested respectively.			Exposure period
Sex	S9	Dose (µg MB 45950/ ml)			
		25	50	100	3 hour exposure period +25 hours' recovery
M	No	84.6	84.6	69.2	
	Yes	56.3	75.0	37.5	
F	No	76.5	47.1	64.7	
	Yes	100.0	137.5	100.0	

At 200 µg MB 45950/ml there was severe depletion in cell numbers accompanied in most cases by >80% mitotic inhibition.

After treatment, cells from each donor were arrested in metaphase (colchicine) and fixed on slides for counting after staining with Gurr's Giemsa.

Cells were first counted to assess mitotic index (based on 1000 cells counted). The top dose for analysis was one in which 50-80% reduction in M.I. has occurred. Twenty five cells from positive controls were then assayed for structural aberrations to ensure the system was operating adequately. Where possible 100 metaphases from each treatment were analysed for chromosome aberrations. Cells with 44 or more chromosomes were considered acceptable for scoring.

No treatment related increase in chromosome aberrations were observed. The positive controls produced satisfactory results.

To conclude, the material produced no chromosome aberrations in the absence or presence of S9 under the conditions of this study.

(DP 82812)

5.8.2.1.1 Summary of studies with MB 45950

MB 45950 showed an acute oral LD_{50} of <200 mg/kg bw in rats, however apart from these studies no further concerns were identified.

Study	Batch and purity	Result	Classification	DP number
Acute rat oral LD_{50}	OP5502, 98.9%	LD_{50} = 69 mg/kg	Toxic if swallowed	82798
Acute rat dermal LD_{50}	DXH 1379/3, Purity not stated	>1000mg/kg	Not classified	82801
Acute rabbit dermal Irritation / Corrosion	DXH 1379/3, Purity not stated	Not irritant	Not classified	82802
Acute rabbit eye irritation with MB 45950	DXH 1379/3, Purity not stated	Not irritant	Not classified	82804
Ames test	OP5502, 98.9%	Negative with and without S9	-	82809
In vitro cytogenetics assay	lot number 37JJW1898, purity >99%	Negative with and without S9	-	82812

5.8.2.2 Studies with MB 46136

a)

Study Type:	**Acute Oral LD_{50} with MB 46136**		
DP ref.:	82814	GLP Certified:	Yes
Substance Purity:	Batch no. WAB 212 98% purity	Guideline Compliance:	79/831/EEC B1 OECD guideline 401
Test System:	Rat: Crl/CD (SD) BR (5/sex/dose, except for dose 160 and 250 where there were 10/sex/dose)	Year(s) of Conduct:	1988
Doses:	64, 100, 160, 250, 400, 640 mg/kg bw by gavage	Vehicle:	Corn oil

All deaths occurred on days 2 or 3. The incidences were 0/10, 4/10, 11/20, 7/20, 7/10, 9/10 at doses 64, 100, 160, 250, 400 and 640 mg/kg bw. Apart from pilo-erection that became apparent 20 minutes after dosing, most clinical signs were delayed until day 2. These clinical signs were: hunched posture and abnormal gait amongst rats treated at all dose levels, lethargy amongst rats dosed at ≥100 mg/kg, pallor of the extremities and/or diarrhoea amongst rats dosed at ≥ 160 mg/kg, decreased respiratory rate and ataxia for a single male treated at 100 mg/kg, and increased salivation and clonic convulsions, preceding death for a single male and female rates dosed at 250 mg/kg. Surviving rats had completely recovered by day 5. Low bodyweight gains were recorded on day 8 for most male rats dosed at ≥ 100 mg/kg and for up to three female rats per dose level. All rats reached normal bodyweight gains by the second week.

Terminal autopsy findings were normal.

The oral LD_{50} in male rats was calculated to be 184 mg/kg bodyweight, and 257 mg/kg in females. A combined LD_{50} was calculated to be 218 mg/kg. As there is a large difference in the LD_{50} between the sexes the lower LD_{50} for females is taken as the LD_{50} for this material.

To conclude, the oral LD_{50} in rats was 184 mg/kg. The test material would be classifiable as toxic via the oral route according to current EC classification criteria.

b)

Study Type:	**Acute Dermal Limit Test with MB 46136**		
DP ref.:	82815	GLP Certified:	Yes
Substance Purity:	Batch number: WAB 212 Purity 98%	Guideline Compliance:	79/831/EEC B3 OECD guideline 402
Test System:	Rat: Crl/CD (SD) BR 5 males and 5 females	Year(s) of Conduct:	1988
Doses:	2000 mg/kg	Vehicle:	Distilled water
Area Covered:	10% of body surface area on the back, clipped with an electric clipper.	Exposure Duration:	24-hours under a semi-occlusive dressing.

The test material was applied under a semi-occlusive dressing to the intact clipped dorsal skin of each of 5 rats/sex. After 24-hours, the dressing was removed and the test site was cleaned with lukewarm water and dried with absorbent paper.

There were no deaths and 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 for four males and two female rats on day 8, and for one male and one female on day 15. The terminal autopsy findings were normal.

Conclusion:
The acute dermal LD_{50} in rats under the conditions of this study is >2000 mg/kg bw. The test material would not be classifiable via the dermal route, according to current EC classification criteria

c)

Study Type:	**Acute Dermal Irritation/Corrosion with MB 46136**		
DP ref.:	82816	GLP Certified:	Yes
Substance Purity:	Batch number: WAB 212 Purity 98%	Guideline Compliance:	79/831/EEC B4 OECD guideline 404
Test System:	Rabbit: New Zealand White	Year of conduct:	1988
Solvent / Vehicle:	Distilled water	No. of Animals:	3 males
Dose & Nature:	0.5 g (powder)	pH:	-

An area of approximately 10 sq. cm was clipped on the dorso-lumbar region of the animals approximately 24-hours before treatment. A gauze patch (2.5 cm) bearing 0.5g of the test material moistened with 0.5 ml distilled water was applied to an intact skin site on the animal. Each treatment site was occluded with an elastic adhesive dressing for 4 hours. At the end of the exposure period the elastic dressing and gauze were removed and the treatment site was washed with water to remove any residual test substance.

None of the animals showed any response to treatment.

Conclusion:
The test material would not be classifiable as an irritant according to current EC classification criteria.

(DP 82816)

d)

Study Type:	**Acute Eye Irritation with MB 46136**		
DP ref.:	84610	GLP Certified:	Yes
Substance Purity:	Batch number: WAB 212 Purity 98%	Guideline Compliance:	79/831/EEC B5 OECD guideline 405
Test System:	Rabbit: New Zealand White	Year of conduct:	1988
Solvent / Vehicle:	None	No. of Animals:	3 males
Dose & Nature:	0.1 ml (powder)	pH:	-

0.1 ml of dry test material was placed in the lower everted lid of one eye of each animal, the other eye serving as a control. The eyelid was held together for about one second immediately afterwards to prevent loss of test material.

There was a temporary dulling of the normal corneal lustre in one animal. Obvious swelling of the conjunctivae with partial eversion of the eyelids was seen in one animal 24 hours after instillation. Transient mild conjunctival irritation was seen in the other two animals. The eyes were normal three to four days after instillation.

Conclusion:
The test substance elicited a irritant response in one of the three treated animals according to OECD test criteria. The test material however is not classifiable as irritating to eyes according to current EC classification criteria.

e) **Ames test**

MB 46136 (batch number WAP 202/1A, purity 98.7%) was investigated as to its potential to induce reverse mutation in histidine-requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535 and TA1537) both in the absence and presence of metabolic activation by an Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9), in two separate experiments. The concentrations of the test material tested ranged from 0.32 to 500 µg/plate (the range-finding study had shown toxicity at the following doses: 200, 1000 and 5000 µg/plate and thus the doses were lowered in the main test). The vehicle was DMSO. Suitable positive controls were conducted with each bacterial strain, and produced satisfactory results.

Despite the reduction in test concentration, toxicity was again observed in experiment 1, with and without S-9, at the top doses (500 and 200 µg/plate respectively). The doses were then reduced again (to 400 and 150 µg/plate).

No treatment of any of the four tester strains resulted in increases in revertant numbers sufficient to indicate an induction of mutation.

To conclude, the test material is not a bacterial mutagen under the conditions of this study.

(DP 82818)

f) ***In vitro* cytogenetics study**

In this 1988 study, MB 46136 (lot no. MAB 202/1A, purity 98.7%) was investigated as to its clastogenic potential in human lymphocyte cultures from a male and a female donor, cultured *in vitro* and treated in the presence and absence of Aroclor 1254 induced rat liver S9 fraction. The study was not quoted as being adherent to any guideline. A single harvest time only and no repeat test was performed. At least two harvest times and a repeat test would have been required by current guidelines (OECD 473 1997; 92/69/EEC). The cells were exposed for three hours after which they were washed twice with sterile saline and resuspended for an additional 25 hours in fresh medium. Concentrations of the test substance and frequency of mitosis as compared to the controls (%) are shown in Table 5.27.

Table 5.27 Concentrations of test material used and toxicity results.

Concentrations of MB 46136 (µg/ml)	**Frequency of mitosis as a % of control**			
	-S-9		**+S-9**	
	Male	**Female**	**Male**	**Female**
4.69	84.6	137.9	111.5	85.4
75	61.5	103.5	76.9	92.7
150	15.4	41.4	38.5	78
300	20.5	44.8	20.7	17.1
300*	-	-	28.6	31

* the experiment with S-9 homogenate was repeated because there were not enough analysable cells in the first trial.

The highest concentration used was 300 µg/ml due to solubility limitations. Negative controls were treated with solvent, DMSO. Positive controls were methyl methanesulphonate (MMS) without S-9, and cyclophosphamide (CPA) with S-9 in the following concentrations: MMS – 50, 75 and 100 µg/ml; and CPA – 12.5, 25 and 50 µg/ml.

As seen in the table mitotic inhibition ranged from approximately 65-83% at 300 µg/ml. Cells receiving this dose and the next two lowest doses were analysed for chromosome aberrations. However, there were not enough analysable cells amongst the ones exposed to 300 µg/ml fipronil in the presence of S-9 to meet the acceptance criteria (only 115 out of a minimum target of 160), and thus the experiment was repeated with appropriate negative and positive controls.

Treatment with fipronil in the absence of S-9 did not cause aberration frequencies that were significantly different from concurrent control frequencies, and fell within historical solvent control ranges.

In the presence of S-9 the number of structural and numerical aberrations, and structural aberrations alone, exceeded the historical control ranges in the culture from the male donor treated with 75 µg/ml. However, more than half of these were clustered in two cells, and in addition the effect was not seen in the female replicate nor at higher doses. The observation was therefore considered by the applicant to be of no biological significance. In the repeat experiment the numbers of aberrations was higher in treated cells than in concurrent negative controls. The increase was only statistically significant

when gaps were included however, and all aberrations frequencies (with or without gaps) fell within historical solvent control ranges.

In conclusion MB 46136 is not considered to be able to induce chromosome aberrations under conditions of this study.

(DP 82820)

5.8.2.2.1 Summary of studies with MB 46136

MB 46136 showed an acute oral LD_{50} of 184 mg/kg bw in rats, however apart from these studies no further concerns were identified.

Study	Batch and purity	Result	Classification	Reference
Acute oral, rat	WAB 212, 98% purity	LD_{50} = 184 mg/kg	Toxic if swallowed	82814
Acute dermal, rat	WAB 212, 98% purity	LD_{50} > 2000 mg/kg	Not classified	82815
Skin irritation, rabbit	WAB 212, 98% purity	–	Not classified	82816
Eye irritation, rabbit	WAB 212, 98% purity	–	Not classified	84610
Ames test	WAP 202/1A, 98.7%	+S9 – negative -S9 – negative	-	82818
Cytogenetic study in human lymphocyte cultures	MAB 202/1A, 98.7%	+S9 – negative -S9 – negative	-	82820

5.8.2.3 Studies with RPA 200766

a)

Study Type:	**Acute Oral LD_{50} with RPA 200766**		
DP ref.:	82826	GLP Certified:	Yes
Substance Purity:	Batch no. 57TDS62 98% purity	Guideline Compliance:	OECD guideline 401 EPA/FIFRA 81-1
Test System:	Rat: Sprague Dawley, five males and five females.	Year(s) of Conduct:	1993
Doses:	2000 mg/kg bw by gavage	Vehicle:	Corn oil

A range-finding study was performed with two males and two females per dose (1000 and 2000 mg/kg bw) before the main study. As there were no mortalities after seven days a limit test was performed.

There were no mortalities during the study. The only clinical sign recorded was chromodacryorrhoea which was observed in all animals the day after treatment. Body weight evolution was not affected in any of the animals. The only macroscopic observation was a moderately enlarged liver in one male animal.

The acute oral LD_{50} of RPA 200766 was found to be greater than 2000 mg/kg bw in rats. The test material would not be classifiable as harmful via the oral route according to current EC classification criteria.

(DP 82826)

b) RPA 200766 (purity, 96.2%) was administered in the diet for 28 days to 10 male and 10 female Sprague-Dawley rats at doses of 0, 50, 500, 5000, or 15000 ppm (equal to 0, 3.8, 38, 390, or 1100 mg/kg bw per day for males and 0, 4.4, 44, 390, or 1100 mg/kg bw per day for females).

The animals were examined for general health, clinical signs of toxicity, body weight, food consumption, urinary and ophthalmological changes, haematological and clinical chemical parameters, and macroscopic changes at necropsy.

Histopathological examinations were not conducted on animals at 15 000 ppm, because the dose was found to be too high, inducing excessive body-weight loss.

Microscopic examinations were carried out on all animals that died, all those at 5000 ppm, and all controls, on the liver, lungs, and kidneys of animals in all groups except those at 15 000 ppm, and on the adrenals and thyroid (the target organs), as considered necessary to establish a no-effect level.

No treatment-related deaths were seen in other groups, and no clinical or ophthalmological alterations were reported.

The body weights of males and females at 5000 and 15 000 ppm were significantly decreased on days 8-28.

The mean body-weight gain over the course of the study was decreased by 27% in males at 5000 ppm, 61% in males at 15 000 ppm, 46% in females at 5000 ppm, and 77% in females at 15 000 ppm. Mean food consumption over the course of the study was decreased by 11% in males at 5000 ppm, 25% in males at 15 000 ppm, 22% in females at 5000 ppm, and 33% in females at 15 000 ppm.

Mean haemoglobin concentrations were decreased in males and females at doses ≥500 ppm and mean haematocrit values were decreased in animals at doses ≥ 5000 ppm, significantly except in females at 15 000 ppm. Mean corpuscular haemoglobin values were decreased in males and females at 15 000 ppm and in males at 5000 ppm.

The mean cholesterol levels were significantly increased in animals at doses ≥500 ppm, mean triglyceride values were increased in animals at 5000 and 15 000 ppm, urea nitrogen was increased in females at 5000 and 15 000 ppm, and creatinine values were increased in males at doses ≥ 500 ppm. The results of urinalysis showed no changes.

Dose-related increases in absolute and relative liver weights were seen in males and females at doses ≥500 ppm, and the liver:brain weights were also increased in these groups.

Dark livers were observed in males at ≥ 500 ppm and in females at 5000 and 15 000 ppm. Significantly increased relative adrenal weights and adrenal:brain weights were seen in all treated males. The group mean thyroid weights were increased in males at doses ≥50 ppm. However, the increases were not found consistently, and the individual values were reported to be generally within the expected range for animals of this age and strain. As there were also no microscopic changes in the thyroid, these findings are of questionable toxicological significance, even though the target organ of the parent compound, fipronil, is the thyroid.

Microscopic examination showed slight-to-moderate, centrilobular or diffuse hepatocellular hypertrophy in the livers and slight-to-moderate extramedullary haematopoiesis in the adrenals of males and females at 5000 ppm.

A dose-related increase in the incidence of fine or coarse vacuolation of the zona fasciculata of the adrenal gland was observed in males at doses ≥50 ppm, with incidences of 0/10 in controls, 2/10 at the low dose, 5/10 at the intermediate dose, and 10/10 at the high dose.

The severity was slight at 50 and 500 ppm and mild to marked at 5000 ppm.

A similar change was seen in seven females at 5000 ppm, with slight-to-mild severity; the incidence in the female controls was 0.

Increases in the weights of the adrenals and thyroids and vacuolation of the adrenal zona fasciculata in males at 50 ppm were considered to be marginal and of questionable toxicological significance.

The NOAEL was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of decreased haemoglobin concentration, increased cholesterol levels, and increased liver weights in animals of each sex at the next dose.

(DP 82830)

c) **Ames test.**

RPA 200766 (batch 57 TDS 62, 98% purity), a metabolite of fipronil, was investigated as to its potential to induce reverse mutation in histidine requiring strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA1538) in two independent mutagenicity tests.

In the first mutagenicity test RPA 200766 was tested in triplicate at concentrations of 50, 100, 250, 500, 1000 and 2500 µg/plate in the presence of a rat liver homogenate metabolic activation system (S9 mix) obtained from rats pre-treated with Aroclor 1254, and 50, 100, 250, 500 and 1000 µg/plate in the absence of S9 mix. The solvent was DMSO. Positive controls used were sodium azide for TA100 and TA1535, 2-nitrofluorene for TA98 and TA1538, and 9-aminoacridine for TA1537 – all these in the absence of S9. 2-aminoanthracene was used towards all *Salmonella* strains in the presence of S9 mix. The number of revertant colonies observed on negative- and positive control plates were in concordance with historical control ranges.

A precipitate was noted on all plates, containing test substance concentrations of 1000 µg/plate and above. A cytotoxic effect, seen as a thinning of the background lawn, was not noted on any plate at any of the test substance concentrations studied.

RPA 200766 did not induce increases in revertant numbers sufficient to indicate an induction of mutation in any of the tester strains at any of the test substance concentrations studied, with or without S9-mix.

To conclude, the test material is not a bacterial mutagen under the conditions of this study.

5.8.2.3.1 Summary of studies with RPA 200766

The acute oral LD_{50} of RPA 200766 in rats was >2000mg/kg bw. The NOAEL in the 28 day rat study with RPA 200766 was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of decreased haemoglobin concentration, increased cholesterol levels, and increased liver weights in animals of each sex at the next dose. The clinical chemistry effects seen were similar to those seen for fipronil. RPA 200766 was not neurotoxic. No other concerns were identified from the Ames study submitted and RPA 200766 contains no structural alerts for genotoxicity. RPA 200766 was also identified as an intermediate metabolite in the rat metabolism study (>1.4% of SOLD, Table 5.7) and has hence been present in the animals during toxicity studies with the parent. Overall due to its presence in toxicity studies, lack of genotoxic potential and neurotoxicity, RPA 200766 is of no toxicological relevance.

5.8.2.3.2 RPA 200761

Due the to similarity of this molecule to RPA 200766 (substitution of –COOH group for the $-CONH_2$ group), the overall assessment for RPA 200766 also stands for RPA 200761. RPA 200761 is of no toxicological relevance.

5.8.2.4 MB 46513 (MB 46513)

No studies were submitted for MB 46513 which is an environmental metabolite of fipronil. Examination of JMPR toxicity evaluations of MB 46513 is summarised below.

MB 46513 is a photolytic product of fipronil which can be formed in the presence of sunlight and is produced in the environment or on treated surfaces. It is not a mammalian metabolite of fipronil. The LD_{50} of MB 46513 in rats was much lower than that of fipronil, and it has much greater binding affinity for GABA receptor chloride-ion channel sites in rat brain. It is neurotoxic. The toxicity of MB 46513 is qualitatively similar to that of fipronil, but the dose-effect curve for neurotoxic effects is steeper (see below) for MB 46513.

In 1997, JMPR set a separate ADI of 0.00003 mg/kg bw for MB 46513 (fipronil-desulfinyl). This was based on data of 90 days duration or less, indicating that this metabolite was of similar or greater toxicity than parent, on repeat dosing and more potent on single exposure, (NOAEL of 0.029 mg/kg bw per day in rats). An additional

assessment factor of 10 was used (giving a total assessment factor of 1000) as there was uncertainty about long-term findings. Fipronil produced thyroid lesions on chronic exposure and thyroid effects were noted with MB 46513 after 90 days.

In 2000, the JMPR reviewed a 2 year rat study with MB 46513 and concluded that the NOAEL was 0.025 mg/kg bw/d, giving an ADI of the same order as for the parent compound. Thus in 2000, a combined ADI for the parent compound and the metabolite MB 46513, (alone or in combination), was determined at 0.0002 mg/kg bw.

Given the toxicity profile indicated by the JMPR evaluation[2], and the occurrence of MB 46513 in the wheat straw at >0.01 mg/kg in a rotation crop study (see Metabolism in Succeeding Crops - Section 6.1), it is clear that MB 46513 is a toxicologically relevant metabolite. It is proposed that MB 46513 be included in the residue definition for risk assessment.

Derivation of the same ADI for MB 46513 as for fipronil.

The 2000 JMPR Meeting concluded that the NOAELs in the long-term studies of toxicity and carcinogenicity with fipronil and MB 46513, both based on clinical signs of neurotoxicity, were comparable (see Table 5.29). The Meeting therefore established a group ADI of 0—0.0002 mg/kg bw for fipronil and MB 46513, in accordance with that established for fipronil in 1997. This value was supported by the NOAEL of 0.025 mg/kg bw per day for MB 46513 in the 24 month rat chronic toxicity and carcinogenicity study in rats, with a safety factor of 100.

Although MB 46513 is more acutely toxic via the oral route than fipronil (based on acute toxicity studies, see Table 5.28), the dose-response curves for neurotoxicity in the 24 month rat chronic toxicity studies do not start for either chemical until after the first dose level, which was 0.5 ppm for both studies. The LOAELs were also similar for both studies (see the comparison of the 24 month rat chronic toxicity study NOAEL/LOAELs in Table 5.29). This is the reason why the same ADI could be set for both fipronil and MB 46513. Although the neurotoxicity dose response curves for both fipronil and MB 46513 in the 24 month rat chronic toxicity studies start in approximately the same narrow dose interval (0.5 - 1.5 ppm), the curve for MB 46513 would appear to have a much steeper gradient. It is this that is responsible for; the lower acute oral LD_{50}s (Table 5.28) of MB 46513; the occurrence of convulsions at lower doses in females in the 2 year chronic toxicity study (Table 5.30), and limiting the maximum dose that could be administered to females in the MB 46513 24 month rat chronic toxicity study (due to a substantial numbers of deaths at the highest dose level (10ppm), this was reduced to 6 ppm at week 26).

In addition, the JMPR stated that a comparison of the NOAEL for the developmental toxicity of MB 46513 in rats (1 mg/kg bw per day) with the NOAEL in the long-term study of toxicity and carcinogenicity (0.025 mg/kg bw per day) shows a 40-fold difference (Table 5.29). The proposed ADI for MB 46513 would hence protect developing organisms.

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[2] JMPR (1998) Pesticide residues in food - 1997 evaluation.
JMPR (2001) Pesticide residues in food - 2000 evaluation.
JMPR (2002) Pesticide residues in food - 2001 evaluation.

Apart from the studies mentioned above, there were no other comparable studies that provided useful information on the relative neurotoxicities of fipronil and MB 46513. The 90 day rat dietary studies listed in Table 5.29 give no useful indication of relative toxicities (although a NOAEL for neurotoxicity in the study with fipronil was identified as being 300ppm, there were rats in the 2 year rat chronic toxicity study with fipronil which showed convulsions within 90 days of the start of the study at 30 ppm). The single dose rat neurotoxicity studies and the 28 day rat dietary toxicity studies provided no usable information as the relative position of the exact neurotoxicity NOAEL/LOAEL boundaries for fipronil or MB 46513 in these studies.

At present it is considered acceptable to rely on the JMPR evaluation of MB 46513 to make an estimation of its ADI. This is because the level of this photolysis product formed at UK latitudes is extremely small (see Section 7.8) and consumer exposure has been calculated to be <<0.000025mg/kg bw (see Section 6.2.2). The current application is for use on ornamentals only. If a future application were to be received for use of fipronil on a fodder or edible crop, exposure of consumers may become more significant. In this case a full UK evaluation of the available data on MB 46513 would be required for verification of the currently proposed ADI. An additional element of concern was the potential for bioaccumulation of MB 46513 and/or its metabolites in ADME studies in rats, goats and hens (half life in blood quoted by JMPR 1997 as 183-195 h, and an increased fat:plasma ratio, and this would also need to be resolved). Given the very limited potential however for MB 46513 to enter the food in significant quantities at UK latitudes under the currently proposed ornamental use, this is not currently a concern.

Table 5.28 Results from acute oral and dermal toxicity studies with fipronil and MB 46513

	Study	Batch and purity	Result	DP No./ref
Fipronil	Acute rat oral LD_{50}	IGB444, 93%	LD_{50} = 92 mg/kg	71039
MB 46513	Acute rat oral LD_{50}	**not stated**	LD_{50} = ♂ 18 mg/kg ♀15 mg/kg	WHO, 2000[3]
Fipronil	Acute rat dermal LD_{50}	**IGB444, 93%**	LD_{50} >2000 mg/kg	71040
MB 46513	Acute rat dermal LD_{50}	**not stated**	LD_{50} >2000 mg/kg	WHO, 2000

[3] WHO (2000) Pesticide residues in food –2000. Toxicological and environmental evaluations. Geneva.

Table 5.29 Summary of NOAELs in comparable toxicity studies with fipronil and MB 46513.

	Study, batch number, purity	NOAEL mg/kg/d	LOAEL mg/kg/d	Effects	DP No./ref
Fipronil	28 day rat dietary, IGB 464, 93%, 25,50,100,200,400 ppm = ♂3.4, 6.9, 13, 24, 45 ♀3.5, 6.7, 13, 25, 55 mg/kg/day	None	3.4	Blood chemistry, increased liver weights, thyroid follicular-cell hypertrophy.	71076
MB 46513	28 day rat dietary, no batch or purity details, 0.5, 3, 30, 100 ppm	0.23 (3 ppm)	2.3	Decreased body weight and food consumption. Smaller thymus weights in females. Decreased serum bilirubin concentration.	WHO, 1997
Fipronil	Single dose rat acute neurotox, gavage, 78/GC/90, 96.7% 0.5, 5, 50 mg/kg	5	50	Convulsions, diffuse brain haemorrhage	711111
MB 46513	Single dose rat acute neurotox, gavage no batch/purity details 0.5, 2, 12 mg/kg	2	12	Decreased body weight gain and food consumption, decreased hind-foot splay, rectal temperature, and locomotor activity, slowed righting reflex in males and decreased grip strength in males and females at the high dose.	WHO, 1997
Fipronil	90 day rat dietary neurotox, 78/GC/90, 96.7% 0.5, 5, 150 ppm, = ♂ 0.03, 0.3, 8.9 ♀ 0.03, 0.3, 11 mg/kg/day	0.3 NOEL >8.9 neurotox	8.9	Body weight gain decrease and food consumption changes Minor effects noted at 4 weeks in top dose males.	71125
MB 46513	90 day rat dietary, FDS 97.5% pure 0.5, 3, 10, 30 ppm, = ♂0.029, 0.18, 0.59, 1.8 ♀0.035, 0.21, 0.71, 2.1 mg/kg/day	0.029	0.18	Clinical signs of toxicity in one male (irritability, aggressiveness, behavioural changes).	WHO, 1997
Fipronil	24 month rat dietary carc + chronic toxicity, PGS 963, 95.4% 0.5, 1.5, 30,300ppm, = ♂ 0.02, 0.06, 1.3, 13 ♀0.03, 0.06, 1.6, 17 mg/kg/day	0.02 non-neoplastic findings 1.3 tumours	0.06 13	Convulsions. Increased thyroid tumours.	71094
MB 46513	24 month rat dietary, , 96 – 99.2% ♂0.5, 2, 10, 0.5, 2, 10↘6 ppm = ♂0.025, 0.098, 0.50 ♀0.032, 0.13, 0.55 mg/kg/day	0.025 non-neoplastic findings 0.50 tumours	0.098 -	Clinical signs of toxicity (convulsions, irritability, aggressiveness, behavioural changes). NB Due to substantial numbers of deaths in females at the highest dose level (10ppm), this was reduced to 6 ppm at week 26. No carcinogenicity seen up to the highest dose tested.	Bigot, 1998[4]

[4] Bigot D (1998) Chronic toxicity and carcinogenicity study of MB 46513 in the Sprague Dawley rat by dietary administration. Unpublished report No. SA 95156 from Rhône-Poulenc Agrochemie. Submitted to WHO by Rhône-Poulenc, Inc., RTP, North Carolina, USA.

Table 5.29 (cont.) Summary of NOAELs in comparable toxicity studies with fipronil and MB 46513.

	Study, batch number, purity	NOAEL mg/kg/d	LOAEL mg/kg/d	Effects	DP No./ref
Fipronil	Rat gavage developmental toxicity JJW2070, 93% 1, 4, 20 mg/kg, days 6-15 gestation	20 maternal effects >20 developmental effects	-	NOAEL set at highest dose tested based on changes in water/food consumption and body weight gain at 20 mg/kg/d. No developmental toxicity observed.	71104
MB 46513	Rat gavage developmental toxicity, no batch or purity details 0.5, 1, 2.5 mg/kg, days 6-15 gestation	1 1	2.5 2.5	Reduced bodyweight gain, hair loss. Incomplete/reduced ossification of bones and slightly reduced fetal weight	WHO 1997[5]

Table 5.30 Convulsions in 24 month rat dietary studies with fipronil and MB 46513

Fipronil														
ppm	**0**	**0.5**	**1.5**			**30**	**300**	**0**	**0.5**	**1.5**			**30**	**300**
Mg/kg bw	**0**	**0.02**	**0.06**			**1.3**	**13**	**0**	**0.03**	**0.08**			**1.6**	**17**
Animals displaying convulsions during the study	0/50	0/50	3/50			1/50	5/50	0/50	0/50	0/50			2/50	11/50
MB 46513														
ppm	**0**	**0.5**		**2**	**10**			**0**	**0.5**		**2**	**10≥6†**		
Mg/kg bw	**0**	**0.025**		**0.098**	**0.5**			**0**	**0.032**		**0.13**	**0.55**		
Animals displaying convulsions during the study	7/70	2/70		9/70	10/70			5/70	8/50		13/70*	20/70**		

† Due to a substantial numbers of deaths at the highest dose level (10ppm), this was reduced to 6 ppm at week 26.
*P<0.05, **P<0.01

5.8.3 Mechanistic studies with fipronil

The following studies to elucidate the effects of fipronil on thyroid function were all carried out in the rat. Unless otherwise stated all were GLP-compliant.

a) **Iodide organification**

A 1991 study is available in which thyroid function was investigated in a perchlorate discharge test. The aim of the study was to determine the effects of fipronil on the ability of the thyroid to organify iodide, compared to a positive control of PTU. PTU is an established inhibitor of iodide organification through inhibition of several of the involved enzymes. Administration of perchlorate will cause inorganic iodide to leak out of the thyroid. The consequences of this leakage are decreasing iodide in the thyroid and increasing iodide in the blood. Therefore, it is possible to distinguish those compounds inhibiting iodide organification from those acting through alternative mechanisms to modulate thyroid function.

Animals, (males, 27 animals/group, Crl:CD (SD)BR) were administered by gavage either 10 mg/kg bw/day fipronil (batch number PGS 963, 96% purity), 200 mg/kg

[5] WHO (1997) Pesticide residues in food –1997. Toxicological and environmental evaluations. Geneva.

bw/day PTU, Noxyflex (a putative inhibitor of thyroid iodide organification) at 5 mg/kg bw per day intraperitoneally as a saline solution, or 0.5% methylcellulose (vehicle control) for 1 or 14d. On day 15, all animals received a single i.p. injection of sodium iodide (^{125}I, 0.037 MBq/kg). Six h post injection, each of the above groups was further subdivided into three subgroups of nine. Each group of nine animals were then administered either 0.9% saline, 10 mg/kg or 25 mg/kg potassium perchlorate, see Table 5.31.

Table 5.31 Numbers of animals in each treatment group administered either saline or perchlorate (at 10 or 25 mg/kg bw on day 15 of the iodide organification study.

	Vehicle	Fipronil	Noxyflex	Propylthiouracil
0.9 % saline	9	9	9	9
10 mg/kg perchlorate	9	9	9	9
25 mg/kg perchlorate	9	9	9	9

There were no deaths were reported during the study. On day 15 following saline or perchlorate administration animals were anaesthetised, a blood sample was removed via cardiopuncture and the animals were sacrificed. Signs of toxicity were only reported in those animals receiving PTU. The following indices of thyroid function were determined: radioactivity levels in whole blood and in thyroids; thyroid weights; the ratio of whole blood radioactivity: thyroid radioactivity; and the ratio of whole blood radioactivity and thyroid weight.

The levels of radioactivity in whole blood (Table 5.32) were increased in the PTU (43%) - and Noxyflex (13%) -treated animals compared to relevant controls. Perchlorate treatment resulted in a increase in activity at 10 mg/kg (75% for the PTU treated animals) and a smaller increase at 25 mg/kg (33% - PTU treated animals). Only the PTU treated animals showed significantly more radioactivity in their blood than did control animals.

Fipronil and Noxyflex both induced increases in ^{125}I accumulation in the thyroid glands, while PTU appeared to inhibit the iodide uptake. These effects were also seen in animals that received perchlorate at both concentrations, in addition the administration of perchlorate appeared to exaggerate these dose-responses. This was most marked in the case of PTU-treated animals (Table 5.33).

All mean treated group thyroid weights were significantly increased in comparison to controls, with 23, 25 and 217% increases being reported in fipronil-, Noxyflex- and PTU-treated animals respectively.

Significant decreases in blood radioactivity:thyroid weight ratio were reported in all groups of PTU-treated animals, and in animals from the fipronil- and Noxyflex- group administered 25 mg/kg perchlorate. These alterations reflect the previously-reported alterations in blood radioactivity and thyroid weight.

Significant alterations in the thyroid radioactivity:blood radioactivity ratio were reported. In comparison to saline controls, the ratio was increased (59%) in fipronil-

and Noxyflex -treated animals and decreased (88%) with PTU treatment. Following perchlorate treatment slight further increases were reported with fipronil and Noxyflex; however, these were not of biological significance. PTU treatment followed by perchlorate administration caused further significant decreases in comparison to perchlorate administration to control animals (>98%, both dose levels).

Table 5.32 Concentration of ^{125}I in whole blood - group mean values (% dose/g)

Dose group	Saline	Perchlorate (10 mg/kg)	Perchlorate (25 mg/kg)	Overall treatment group means
Control				
Mean SD	0.142 0.017	0.131 0.019	0.145 0.027	0.139
MB 46,030 (10 mg/kg/day				
Mean SD	0.147 0.017	0.143 0.015	0.142 0.027	0.144
PTU (200 mg/kg/day)				
Mean SD	0.203 0.019	0.229 0.035	0.214 0.052	0.215**
Noxyflex (50 mg/kg/day)				
Mean SD	0.160 0.018	0.142 0.014	0.155 0.026	0.152**

**P≤0.01

Table 5.33 Concentration of ^{125}I in thyroid glands - group mean values (% dose/g)

Dose group	Saline	Perchlorate (10 mg/kg)	Perchlorate (25 mg/kg)
Control			
Mean SD	3.078 0.853	3.191 0.658	2.795 0.731
MB 46,030 (10 mg/kg/day			
Mean SD	5.541** 1.565	5.245** 1.155	7.086** 1.209
PTU (200 mg/kg/day)			
Mean SD	1.388** 0.405	0.316** 0.159	0.237** 0.134
Noxyflex (50 mg/kg/day)			
Mean SD	6.355** 1.878	7.267** 1.019	7.777** 0.719

**P≤0.01

Although PTU caused an increase in thyroid weight, the above data indicate animals in this group were unable to incorporate iodide into thyroglobulin. Fipronil and Noxyflex seemed to have the same effect on thyroid weight, suggesting a slight follicular stimulation. The data on both fipronil and Noxyflex suggests that the ability of the thyroid to incorporate iodide is maintained (although high concentrations of Noxyflex *in vitro* have been shown to result in a blockade of iodide organification). In conclusion, it is likely that fipronil does not have a direct action on iodide organification.

(DP 71119)

b) **Effects on thyroxine clearance:**

In a further 1991 study, the effects of fipronil on thyroxine (T_4) pharmacokinetics were investigated. Rats (Crl:CD (SD) BR strain, 6 males/group) were administered a single a dose of fipronil, 10 mg/kg (95.4% purity) by gavage or phenobarbitone 80 mg/kg by i.p. injection. Two further groups were assigned to a 14-d repeat-dose regimen with the above concentrations of each substance. Two control groups of sham gavaged animals were also included, one for the single and one for the repeated treatment protocols. Following the final dose, all animals received an i.v. infusion of radiolabelled T_4 (^{125}I T_4, 0.037 MBq/kg). Blood samples were taken at 0.5, 1, 2, 4, 8, 12, 24 and 30 h post infusion. Following the ultimate sample, animals were sacrificed without necropsy. No signs of toxicity, body weight changes, deaths or effects upon food consumption were reported. Signs of sedation, consistent with phenobarbitone administration, were reported in those animals given this substance.

Statistically significant effects upon three pharmacokinetic parameters; half-life ($t_{1/2}$), volume of distribution (Vd) and clearance (Cl) were reported in both phenobarbitone groups and the repeat dose fipronil groups. Both fipronil and phenobarbitone caused increases in Cl and Vd and a decrease in $t_{1/2}$. These data can be found in Table 5.34. No comparisons were made between single and repeat dose treated animals, as the 1 d and 14 d control data were found to differ.

Table 5.34 Pharmacokinetic parameters of thyroxine affected by treatment with fipronil.

PK parameter		Single Dose	14-d Repeat Dose
Control	$t_{1/2}$ (h)	17.2 ±2.5	22.5 ±2.4
	Cl (ml/min)	0.055 ±0.0052	0.057 ±0.005
	Vd(ml)	80.54 ±6.55	110.05 ±2.41
Fipronil	$t_{1/2}$ (h)	15.6 ±3	11.8 ±1.5*
	Cl (ml/min)	0.061 ±0.007	0.15 ±0.15
	Vd(ml)	80.43 ±4.1	150.31 ±14.41*
Phenobarbitone	$t_{1/2}$ (h)	14.1 ±0.5*	15.5 ±2.6*
	Cl (ml/min)	0.072 ±0.0053*	0.1 ±0.019*
	Vd(ml)	87.83 ±5.91*	137.83 ±2.79*

* statistically significant, in comparison to controls

(DP 71115)

c) **Effects on thyroid hormone levels:**

A further study was carried out to determine the effects of fipronil on circulating TSH, T_3 and T_4 levels. The concentrations of the three hormones were determined by radioimmunoassay. Animals (10/sex/group, Crl:CD (SD)BR) received dietary administration of fipronil (batch number PGS 963, 98.4% purity) for 28 d at either 0.1, 1, 5 or 30 ppm, equivalent to 0.01, 0.1, 0.49 or 2.85 and 0.01, 0.1, 0.48 or 2.86 mg/kg/d, males and females respectively. One female was killed *in extremis;* no further deaths, adverse effects or signs of toxicity were reported. There were no treatment- related effects on food consumption and body weight gain. Blood samples were taken at 1 week and at sacrifice for the determination of TSH, T_3 and T_4 levels.

All animals were subject to gross necropsy, with the liver and thyroid being further investigated.

The concentrations of thyroid hormones and TSH for males and females respectively can be found in Tables 5.35 and 5.36 The data presented are corrected means, to allow an analysis of covariance to be carried out.

Table 5.35 Thyroid hormone and TSH levels in males

	Day 7			Day 28		
Dose of fipronil (ppm)	**T_3 ng/dl**	**T_4 µg/dl**	**TSH ng/ml**	**T_3 ng/dl**	**T_4 µg/dl**	**TSH ng/ml**
0	76.6	8.8	2.2	46.7	4.95	2.89
0.1	70.8	5.62	2.51	43.8	4.91	3.13
1	66.1*	5.62	2.87	43.2	4.61	3.54
5	66.5*	5.14*	3.05	47.9	4.63	4.84
30	65.3*	4.41*	334*	51.1	3.54*	6.27*

*statistically significant

Table 5.36 Thyroid hormone and TSH levels in females

	Day 7			Day 28		
Dose ppm	**T_3 ng/dl**	**T_4 µg/dl**	**TSH ng/ml**	**T_3 ng/dl**	**T_4 µg/dl**	**TSH ng/ml**
0	76.3	4.08	0.83	76.5	3.72	0.93
0.1	79.5	4.44	0.98	82.7	4	0.79
1	82.3	4.27	0.77	86.3	3.88	0.67
5	79.5	4.28	1.02	86.3	3.69	0.82
30	66.7	3.32*	1.13	91.3	3.69	1.72*

* statistically significant

It can be seen that in males, T_3 and T_4 levels were decreased in all treatment groups at day 7 with a concomitant rise in TSH. At day 28 however, T_4 levels remained decreased and TSH elevated, with T_3 levels elevated above control levels at ≥5 ppm. In females, T_3 and T_4 levels were decreased at the top dose with TSH levels elevated at ≥5 ppm. At day 28, T_3 levels were increased at all doses, T_4 levels were unaffected and TSH levels were elevated at ≥5 ppm.

At necropsy no gross pathological abnormalities were reported. Absolute liver and thyroid weights were increased in both males (15% and 6.7% liver and thyroid respectively) and females (6.7% and 16% liver and thyroid respectively). The increase in thyroid weight in females was of statistical significance. Histopathological examination revealed some minor effects. An apparent increase in the thyroid follicular epithelial height was reported in males at ≥5 ppm (1/10 and 3/10 at 5 and 30 ppm respectively) and females at the top dose (1/10). In each case no increases were reported in control animals. These increases were thought to be treatment-related. Centrilobular hepatocyte enlargement was reported in males at the top dose (controls 0/10 and 30 ppm 3/10). Staining of liver sections with Oil Red O

demonstrated an increased incidence of periportal fat deposition in females at 5 and 30 ppm (2/10 and 3/10 at 5 and 30 ppm respectively).

These data are consistent with an increase in hepatic clearance of T_4 causing a feedback increase in TSH stimulation of the thyroid gland. Increased thyroid activity should be manifested by increased serum T_4, although these increases may be hidden by increased hepatic clearance (glucuronidation). The elevated T_3 levels observed at day 28 could however be an indirect indicator of elevated serum T_4 levels.

The NOAEL for this study was 1 ppm (equivalent to 0.1 mg/kg/d), based on increased thyroid follicular epithelial height at ≥5 ppm in males.

(DP 71122)

d) **Effects of fipronil on the biliary excretion of radio-labelled thyroxine:**

In a 1993 study the ability of fipronil to modify the biliary clearance of ^{125}I-labelled thyroxine (T4) was investigated. Animals (Sprague-Dawley CD males, 3/group) were administered single or daily (14) doses of either fipronil, 1 or 10 mg/kg/d by gavage or 80 mg/kg/d phenobarbital by i.p. injection. Following the final dose animals were anaesthetised and the common bile duct cannulated and 1 mg sodium iodide (to block thyroid uptake of ^{125}I) was administered by injection into the stomach. Four hours later all animals received a single intravenous dose of about 10 µCi of ^{125}I –T4 (98.8% radiochemical purity). Blood samples were taken at 30 minute intervals and bile samples at 15 minute intervals from 0-5 h post radiolabel administration. After 5 h samples were taken, animals were sacrificed and the livers removed. Blood, bile and liver samples were assayed for radioactivity. Bile samples only were also assayed for free and conjugated T4.

The relative liver weight of animals in the fipronil 10 mg/kg/d repeat-dose group were significantly increased (20%) in comparison to control animals. A significantly higher total bile weight (29%) was reported in animals receiving for 14 d fipronil at 10 mg/kg/d or phenobarbital. The proportions of radioactivity found in liver, blood and bile can be found in Table 5.37.

Table 5.37 Percentage of administered radioactivity found in the liver blood and bile

	Single dose			Repeat dose (14 d)		
Treatment	**Blood**	**Bile**	**Liver**	**Blood**	**Bile**	**Liver**
Fipronil (a)	19.28 ± 1.62	7.55 ± 1.09	9.33 ± 0.68	12.14 ± 1.19*	10.98 ± 0.86*	8.23 ± 0.54*
Fipronil (b)	17.67 ± 2.78	8.61 ± 0.9	8.61 ± 0.38	8.4 ± 0.57	13.58 ± 0.95*	7.5 ± 1.05*
Phenobarbital	12.95 ± 2.86	9.34 ± 1.88*	8.07 ± 1.52*	12.47 ± 0.52*	14.88 ± 0.6*	12.75 ± 1.79*
Control	17.45 ± 2.48	6.48 ± 1.85	8.42 ± 0.8	8.48 ± 1.8	3.78 ± 0.46	4.58 ± 0.19

* statistically significant from controls
(a) 1 mg kg/d
(b) 10 mg kg/d

The data presented in Table 5.38 show that a single administration of fipronil has no effects on the blood, bile or liver radioactivity levels. Repeat administration of 1 and 10 mg/kg/d and phenobarbital caused significant increases in hepatic accumulation and biliary excretion of ^{125}I-T4. Increased blood levels of T4 were also apparent in animals receiving repeat administration of 1 mg/kg/d fipronil, or phenobarbital.

The biliary clearance data (T4) for both single and repeat administrations can be found in Table 5.38. These data indicate that all treatment regimens caused an increase in the biliary clearance of T4, with the high dose fipronil treatment being statistically significant.

Table 5.38 Biliary clearance data for T4

	Single dose		Repeat dose	
Treatment	**Biliary Clearance (ml/h)**	**Percentage Increase**	**Biliary clearance (ml/h)**	**Percentage Increase**
Fipronil[a]	0.991 ± 0.227	24	3.699 ± 0.77	51.5
Fipronil[b]	1.027 ± 0.3	28.5	9.83 ± 2.131	306*
Phenobarbital	1.619 ± 0.745	102.6	4.356 ± 0.633	80
Control	0.799 ± 0.016	-	2.421 ± 2.129	-

* statistically significant
(a) 1 mg kg/d (b) 10 mg kg/d

HPLC analysis of bile samples indicated that for animals in the single-dose groups, 87-93% of the radioactivity was present as conjugates and 79-90% in repeat-dose groups when compared to control animals. Of the total radioactivity in glucuronidase/ sulphatase treated bile preparations, 57-61% (single-dose) and 51-54% (repeat-dose) was present as T4. Compared to controls all treatments caused increases in T4 levels, with repeat dosing causing greater increases than a single dose (see Table 5.39). Blood clearance data for T4 was not calculated.

Table 5.39 Percentage increase in biliary radio-labelled T4 levels

	Single Dose	Repeat Dose
Fipronil(1 mg/kg/d)	48	210
Fipronil (10 mg/kg/d)	56	322
Phenobarbital	73	345

Phenobarbital causes increased biliary clearance of T4 via induction of hepatic glucuronyl transferases. These data show that repeat dosing with fipronil also causes increased hepatic clearance of T4.

(DP71124)

5.8.3.1 Summary proposed mode of action of thyroid effects

A series of studies have investigated the effects of fipronil on various components of the hypothalamic-pituitary-thyroid-hepatic axis in the rat. At 10 mg/kg/d, administration of fipronil for 14 d caused an increase in the thyroid:blood radioactivity ratio in a perchlorate discharge test. Propylthiouracil, a known inhibitor of iodide organification, caused a decrease in this ratio. In a similar study, fipronil was shown to

increase the biliary clearance of T4, increase the volume of distribution and clearance from blood and to decrease the half-life in blood. Similar effects were observed with phenabarbitone, which is regarded as a non-genotoxic thyroid carcinogen in the rat. Administration of fipronil at 3 mg/kg/d for 28 d caused an increase in serum TSH levels and less marked decreases in serum T3 and T4 levels.

Overall, these data are consistent with fipronil having an indirect action on the rat thyroid gland acting via increased hepatic clearance of T4. In the case of phenobarbitone, increased hepatic clearance of T4 is a consequence of the induction of hepatic UDPG glucuronyl transferase. However, it is noted that glucuronide has not been investigated for fipronil. In conclusion, fipronil is considered to be a non-genotoxic carcinogen in the rat. A feasible non-genotoxic mechanism for thyroid tumours is demonstrated in the rat. The mechanism is not thought to be relevant to human risk assessment as the NOAEL and LOAEL for tumours in the rat carcinogenicity study (1.3 mg/kg bw and 13 mg/kg bw) are far greater than predicted operator exposure (max 0.000032 mg/kg - see section 5.14.1.3) and long term systemic AOEL of 0.0002 mg/kg bw. The margin of safety between the NOAEL for tumours and the AOEL is 20,000 × the AOEL.

5.9 MEDICAL DATA

5.9.1 Plant personnel (AII 5.9.1)

No information on workers or others during manufacture or formulation of the products has been provided by the applicant. The applicant states that because manufacture of fipronil is conducted at a contractor site the medical surveillance records are not available to Aventis (formerly Rhône-Poulenc), but states the information can be provided directly from the contractors if required.

5.9.2 Direct observation

The applicant states that since its first launch in 1993, no human intoxications have been recorded with fipronil.

5.9.3 Observations on exposure of the general population (AII 5.9.3)

The applicant states that since its first launch in 1993, no human intoxications have been recorded with fipronil.

5.9.4 Diagnosis of poisoning (AII 5.9.4)

The applicant submits the following proposal (shown by italic text). (The ACP advised that some amendments should be made, these are as indicated by struckthrough/ bold font for deleted/new text respectively).

Fipronil is a reversible gamma-aminobutyric acid (GABA) receptor inhibitor. During intoxication, it will induce neurological stimulation with possible convulsions. Signs and symptoms which appear the most relevant for humans may be observed after acute or repeated over-exposure. These signs mainly consist of central nervous system

(CNS) hyperexcitability: over-activity, irritability, tremors, and, at a more severe stage, lethargy or convulsions. These symptoms are reversible after termination of exposure.

In the rat, clear signs of toxicity were observed following a single oral administration of fipronil at a dose of 50 mg/kg/body weight while minimal symptoms were observed at 5 mg/kg bodyweight. Due to slow absorption through the gut, symptoms of intoxication may be delayed for several hours to one day. Fipronil does not readily penetrate skin. Therefore absorption should be minimal following dermal exposure. Symptoms are expected only after repeated excessive exposure.

Measurement of fipronil and its metabolites in the blood (or in the gastric lavage) is the only way to ***definitively*** *confirm exposure. In cases of suspected intoxication evidenced by symptoms, a blood sample should be taken as soon after the alleged exposure as possible and* ***may be*** *sent to:*

Aventis ~~*Rhône-Poulenc Agro*~~
Toxicology Department
Centre de Recherche
355, Rue Dostoievski
B.P. 153
F-06903 Sophia Antipolis Cedex
FRANCE
Attention: Dr Pierre-Gerard Pontal

For this purpose *collect at least 20 ml of blood, preferably using heparin coated tubes, and centrifuge. Separate plasma, avoiding contamination with RBC. Serum is acceptable if anticoagulants tubes are not available. Plasma/serum samples can be stored temporarily at 0 to 4 °C until frozen for shipping. The following information should be provided with the sample: Name of patient; Date of sampling; Plasma or Serum; Primary route of exposure (oral, dermal, inhalation); Specific product to which patient was exposed; Estimated quantity of product to which patient was exposed; Description of treatment given following alleged exposure; Estimated time between alleged exposure and taking of blood sample; and Name and location of physician to whom results of blood analysis should be communicated. Ship frozen plasma or serum sample as soon as possible (but not on Fridays or Saturdays) by overnight air freight. The frozen serum or plasma sample and its accompanying information should be shipped in a sturdy container packed in dry ice. Samples must be kept frozen (≤ -20 °C) during shipment and until analysis.*

~~*Initial determination will allow confirmation of diagnosis and will provide a base value to follow clearance from blood.*~~ **[ACP noted: - Repeat concentration would be needed to assess clearance from the body and gain maximum information from what should be rare events. The applicant may wish to suggest appropriate intervals between samples, bearing in mind the long half-life of the compound.]**

(DP 82764)

5.9.5 Proposed treatment : first aid measures, antidotes, medical treatment (AII 5.9.5)

The following advice is as given by the applicant for fipronil. The medical basis of these proposals has not been assessed in this evaluation. (However, it is generally

considered that induction of vomiting following ingestion is not good advice). It is recommended that the information should not be used as the basis for treatment advice in the event of a poisoning incident. Specialist information should be sought from an appropriate source such as a National or Regional Poisons Unit or similar organisation.

The following advice is quoted directly from that provided by the applicant. However, the ACP did propose some changes to this advice, which are shown here as deleted text struckthrough and new text in bold font.

As is usual for acute intoxication when no specific antidote is known, treatment is based on:

Decontamination

Skin: In case of contact with skin, wash immediately with soap and water for at least 15 minutes.

Eyes: In case of contact with eyes, wash immediately with plenty of water for at least 15 minutes. Seek medical advice.

~~*Ingestion: If victim is conscious try to induce vomiting by one of the following gastric decontamination procedures:*~~

~~*1) Syrup of IPECAC (adult: 15 ml; child between 30 months and 12: 0.75 ml/kg without exceeding 15 ml; child between 12 and 30 months: 7.5 ml; not recommended under 12 months, although some authors consider it effective between 6 and 12 months) followed by a glass of water. This dose can be repeated after 20 minutes if the first dose is not efficient.*~~

[ACP note - the applicant is advised to reword the above paragraph to conform with the guidelines issued by the American Academy of Clinical Toxicology and European Association of Poisons Centres and Clinical Toxicologists (Clin Toxicol 1997; 35:711-719) e.g. Gastric aspiration should only be considered if a potentially life-threatening amount has been ingested and the procedure can be undertaken within 60 minutes of ingestion and the airway can be adequately protected.]

~~*3) If neither of the above are available, manually induce vomiting by touching the back of the throat with a finger.*~~

~~*If patient is unconscious; never try to induce vomiting, proceed immediately to tracheal intubation and decontaminate through gastric lavage.*~~

Although their use has not been evaluated during acute fipronil intoxication, activated charcoal~~, cholestyramine and catharties~~ may be useful for gut decontamination and to inhibit entero-hepatic recirculation. If used for this purpose, treatment should be continued ***at regular(eg 4 hourly) intervals with an appropriate laxative*** *for several days as follows:* ~~*Activated charcoal: 50 to 100 g (15 to 50 g for children) with a glass of water. Cholestyramine: 12 to 16 g daily in divided doses. Catharties: e.g. magnesium sulphate; 15 to 20 g (250 mg/kg for a child*~~*Specific therapy to treat symptoms*

The proposed use of anticonvulsants is based upon the observed effectiveness of treatments in laboratory animals; there is no information on the effectiveness for treating fipronil intoxication in humans. Recommendations are based on anticonvulsant therapy as routinely administered to humans.

In cases of strong clinical indications of fipronil poisoning, do not wait for analytical confirmation to start treatment. Patients may present marked resistance to the usual therapeutic doses of anticonvulsants. Exact dosage depends on the severity of the intoxication, the bodyweight and the reaction of the patient to the treatment. Therefore dosages recommended here are only indicative. The dosage can also be modified according to the ability to monitor respiration and blood pressure, since extremely high anticonvulsant therapy, especially with phenobarbitol, may lead to severe respiratory depression and drop in blood pressure. The selectivity of anticonvulsant treatments suggested below is based on their general availability and their protective effects as observed in animal studies.

~~*Phenobarbitol: Start with 10 to 20 mg/kg of phenobarbitol in rapid intravenous perfusion (about 60 mg/minute in adults) and continue according to the patient's response.*~~

Treat fits conventionally with *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*~~. ***Repeat doses of diazepam maybe required. Alternatively other intravenous anticonvulsants may be required in severe poisoning***

If the patient is not responsive to the suggested treatments, or if the drugs are not available, other benzodiazepines 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 anticonvulsant 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 anticonvulsant 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*~~*. No sequelae are expected.*

General supportive therapy

When respiratory symptoms are present (or when they are likely to be induced by treatment), give oxygen, and, when necessary, artificial ventilation~~*, if possible by mechanical means*~~*. When a patient is unresponsive and if endo-tracheal intubation is not possible* ***protect the airway****. When necessary, insert an intravenous line to maintain* ***fluid*** *balance and for the possible use in administering anticonvulsant therapy.*

(DP 82764)

5.9.6 Expected effects of poisoning (AII 5.9.6)

The applicant states that:

Fipronil is a reversible gamma-aminobutyric acid (GABA) receptor inhibitor. During intoxication, it will induce neurological stimulation with possible convulsions. Signs and symptoms which appear the most relevant for humans may be observed after acute or repeated overexposure. These signs mainly consist of central nervous system (CNS) hyperexcitability: over activity, irritability, tremors and at a more severe stage lethargy or convulsions. These symptoms are reversible after termination of exposure.

In the rat, clear signs of toxicity were observed following a single oral administration of fipronil at a dose of 50 mg/kg/body weight while minimal symptoms were observed at 5 mg/kg bodyweight.

Due to slow absorption through the gut, symptoms of intoxication may be delayed for several hours to one day.

(DP 82764)

5.10 SUMMARY OF MAMMALIAN TOXICITY, PROPOSED ADI, AOEL AND DRINKING WATER LIMIT

5.10.1 Summary of mammalian toxicity

Fipronil was acutely toxic via the oral and inhalation routes. Convulsions at 200 mg/kg and 0.523 mg/l were reported in both these studies respectively. Fipronil was of low acute dermal toxicity to rats and was not irritant to the skin or eye. Fipronil was not classified as a skin sensitiser.

In the short-term studies the key target organs in the 90 day rat study (top dose 22 mg/kg bw) were the thyroid gland and the liver, with follicular cell hyperplasia and hypertrophy; and peri-acinar fatty vacuolation and congestion observed in these respective organs. No evidence of neurotoxicity was seen the rat study. In dogs (90 day capsule, top dose 10 mg/kg bw; 52 week capsule, top dose 5 mg/kg; and 52 week dietary, top dose 3 mg/kg), mortality and signs indicative of neurotoxicity were observed. The dog may be more sensitive to the neurotoxic effects of fipronil than the rat, however convulsions were observed at doses as low as 0.06 mg/kg/d following long-term administration of fipronil to rats. Decreased body weights and reduced food consumption, and hyperactivity were the effects noted in the 21 day dermal study in rabbits. Significantly reduced body weight gain in conjunction with reduced food consumption as seen in the dietary studies should be regarded as an adverse effect.

Fipronil gave negative *in vitro* results in a bacterial point mutation study (Ames), a mammalian gene mutation study (HGPRT) and a chromosome aberration study (human lymphocytes). A single harvest time only and no repeat test was performed. At least two harvest times and a repeat test would have been required by current guidelines (OECD 473 1997; 92/69/EEC). A second chromosome aberration assay conducted in Chinese hamster lung cells gave positive results at the 6 hour harvest interval in the absence of S9 and possibly in the presence of S9. Fipronil was negative in an *in vivo* mouse micronucleus assay. **Although the *in vitro* cytogenetics assay in Chinese hamster lung cells, was positive (there was an increase in breaks and sister chromatid exchanges) the mouse micronucleus test indicated that the test material was not actually clastogenic *in vivo*. It is considered unlikely that a second *in vivo* assay in a different tissue is likely to produce anything other than a negative result. The lack of any tumours that could be attributed to direct genotoxic action in the rat and mouse chronic studies also support the lack of *in vivo* genotoxicity by fipronil.**

Low survival rates were observed in the rat carcinogenicity study with <50% survival in all dose groups including controls and casts doubts on the rigor of this study.

Fipronil itself had no effect on survival. A significant increase was observed in the incidences of thyroid follicular cell adenomas and carcinomas in both sexes at the top dose; these exceeded the historical control incidence. The increased incidences in the thyroid follicular cell adenomas at 30 ppm and below was not thought to be significant as there was no dose response, and the incidence at 30 ppm is within historical control data. The mechanism for thyroid tumours is not considered relevant to human risk assessment. No tumours were observed at 52 weeks. The NOAEL for non-neoplastic effects was less than that for thyroid adenomas and based on convulsions. In mice, no treatment-related increases in tumour incidence were observed after 78 weeks administration of fipronil. The NOEL was based on hepatic microvesicular vacuolation.

In a rat two-generation reproductive study, effects on fetal toxicity and reproductive parameters were noted only at parental toxic doses. Parental toxicity effects were 1 male death (F0); and increases in thyroid and liver weights with associated histopathology (F0/FI adults). Fertility and developmental effects were convulsions (Fl/F2 pups); low birth weight FI/F2 pups); decreases in body weight gain (Fl/F2 pups); a delay in onset of tooth eruption (Fl pups); a decrease in the number of animals mating (Fl adults); decreases in live birth index and viability index (FI/F2 litters); and a decrease in post implantation survival index (F2 litters) at 17.0-23 mg/kg/d (300 ppm).

In two developmental toxicity studies fipronil did not cause an increase in skeletal or visceral malformations in the rat or rabbit. Food intake and body weight gain decreases were observed maternally in both the rat and rabbit.

In an acute rat neurotoxicity study the main effects observed were significant decreases in hind limb splay at the second highest dose, and convulsions, tremors, decreased motor activity and decreased muscle tone at the highest dose. In a rat 90-d dietary neurotoxicity study several minor statistically significant changes were noted in the FOB but there were no treatment-related effects on motor activity or habituation rates. The NOAEL for neurotoxicity was greater than that for general toxicity which was based on body weight and food consumption changes. Female beagle dogs were dosed with fipronil until neurotoxicity developed. The effects were variable in degree and time of onset. The observations were ataxia; convulsions; reflex abnormalities; and gait/limb abnormalities. CNS effects resolved in 28 d with no pathological changes observed after recovery. Identical neurobehavioural and neuropathological endpoints were examined in both the rat 90 day dietary neurotoxicity and the rat single-dose gavage study.

A number of acute toxicity and genotoxicity studies were conducted with fipronil rat metabolites MB 45950, MB46136, and RPA 200766. MB 45950 and MB 46136 showed acute oral LD_{50}s of 69 mg/kg bw and 184 mg/kg bw in rats respectively. No further concerns were identified from these studies. Due to its similarity to RPA 200766, environmental metabolite RPA 200761 is also considered to be of no toxicicological significance. Fipronil-desulfinyl (MB 46513) is considered to be of toxicological relevance. An ADI of 0.0002 mg/kg bw was established by JMPR for fipronil and fipronil-desulfinyl, alone or in combination. It is proposed that fipronil desulfinyl be included in the residue definition for risk assessment for this application.

The effects of fipronil on various components of the hypothalamic-pituitary-thyroid-hepatic axis were investigated in the rat. The data from these studies are consistent with fipronil having an indirect action on the rat thyroid gland acting via increased hepatic clearance of T4. In the case of phenobarbitone, increased hepatic clearance of T4 is a consequence of the induction of hepatic UDPG glucuronyl transferase. However, it is noted that this has not been demonstrated for fipronil.

Table 5.40 Summary of NOAELs in standard toxicity studies

Study, batch number, purity	NOAEL mg/kg/d	LOAEL mg/kg/d	Effects	DP No.
28 day rat dietary, IGB 464, 93%	None	3.4	Blood chemistry, increased liver weights, thyroid follicular-cell hypertrophy.	71076
Single dose rat acute neurotox, gavage, 78/GC/90, 96.7%	5	50	Convulsions, diffuse brain haemorrhage	711111
90 day rat dietary, PGS 963, 95.4%	2.1	22	Peri-acinar hepatic fatty vacuolation, and hepatic congestion in females.	71079
90 day rat dietary neurotox, 78/GC/90, 96.7%	0.3 NOEL >8.9 neurotox	8.9	Body weight gain decrease and food consumption changes Minor effects noted at 4 weeks in top dose males.	71125
90 day dog capsule, PGS 963, 94.4-96.5%	combined NOEL of 0.5	1	clinical signs of neurotoxicity in a female animal during week 13 of the second 1 year dog study	71081
52 week dog capsule PGS 963, 96.8%				112755
52 week dog dietary PGS 963, 96.8%				112756
21 day rabbit dermal, 8/GC/90, 96.7%	5 (NOEL)	10	Decrease in body weight gain, loss of appetite (decrease in food consumption)	71083
24 month rat dietary carc + chronic toxicity, PGS 963, 95.4%	0.02 non-neoplastic findings 1.3 tumours	0.06 13	Convulsions. Increased thyroid tumours.	71094
18 month mice dietary carcinogenicity, PGS 963, 95.4%	0.055 (non-neoplastic findings) 3.43 NOEL (tumours)	1.23	Hepatic microvesicular peri-acinar vacuolation, "necrosis of occasional cells" and "apoptosis", increased ploidy, hypertrophy and degeneration of peri-acinar hepatocytes, chronic inflammation and bile stasis No increase in tumour incidence at the top dose tested.	71096
2-generation rat dietary reproductive toxicity, PGS 963, 95.4%	1.7 NOEL for fertility and developmental effects.	17	Convulsions (Fl/F2 pups); low birth weight (FI/F2 pups); decreases in body weight gain (Fl/F2 pups); a decrease in the number of animals mating (Fl adults); decreases in live birth index and viability index (FI/F2 litters); a decrease in post implantation survival index (F2 litters)	71100
	0.16 NOEL for parental toxicity	1.7	1 male death (F0); and increases in thyroid and liver weights with associated histopathology (F0/FI adults)	

Study, batch number, purity	NOAEL mg/kg/d	LOAEL mg/kg/d	Effects	DP No.
Rat gavage developmental toxicity JJW2070, 93%	20 maternal effects >20 developmental effects	-	NOAEL set at highest dose tested based on changes in water/food consumption and body weight gain at 20 mg/kg/d. No developmental toxicity observed.	71104
Rabbit gavage developmental toxicity, PGS 963, 95.4%	0.1 NOEL for parental effects. >1 NOEL for developmental effects.	0.2	Decrease in body weight gain and food consumption. No developmental toxicity observed.	71106

5.10.2 Acceptable daily intake (ADI)

The ADI is based on the NOAEL (0.02 mg/kg bw) for the rat chronic toxicity/carcinogenicity study based on convulsions seen in some animals at 0.06 mg/kg day. A 100 fold assessment factor is considered appropriate.

An ADI of 0.0002 mg/kg bw is proposed.

5.10.3 Acceptable operator exposure level (AOEL)

Consideration is given to setting short-term AOELs (< 90 days' operator exposure per year, i.e. seasonal use) and long-term AOELs (>90 days' operator exposure per year). In addition, the emerging guidance suggests that account be taken of the extent of oral absorption when setting an AOEL from oral toxicity data. No correction is applied for oral absorption which was about 90% in rats.

a) Long-term systemic AOEL

It is appropriate to derive the long-term systemic AOEL in the same way as for the ADI, i.e. to apply a 100-fold assessment factor to the NOAEL from the 24 month rat chronic toxicity/carcinogenicity study. No correction for oral absorption (about 90% in rats) is necessary:

A long-term systemic AOEL of 0.0002mg/kg bw/day is proposed.

b) Short term systemic AOEL

The short term systemic AOEL is set using a 100 fold assessment factor on a NOAEL of 0.06 mg/kg bw derived from the 2 year rat chronic toxicity study. This was based on convulsions seen within one week of commencing treatment at 30 ppm. At 1 week, the convulsions at 30 ppm occurred within 13 weeks of the start of treatment and are hence at a critical timing with respect to setting a short-term systemic AOEL. However, there is a large interval between the 30 ppm dose level and the next dose level down (1.5ppm) in the 24 month rat chronic toxicity/carcinogenicity study. Reviewing the shortterm toxicity studies with fipronil in the rat, the next lowest NOAEL for convulsions, and other non-neurotoxic effects, which may be relied upon, is the 5ppm dose level (0.3 mg/kg bw/day) in both the 90 day toxicity and

neurotoxicity studies in rats (DP 71079, 71125). A 100 fold assessment factor is satisfactory. No correction for oral absorption (about 90% in rats) is necessary:

A short term systemic AOEL of 0.003 mg/kg bw is proposed.

5.10.4 Acute/short-term reference dose

The acute reference dose is set using a 100 fold assessment factor on the single-dose rat acute neurotox NOAEL of 5 mg/kg bw (based on convulsions and diffuse brain haemorrhage in decedents at 50 mg/kg bw). This study was the most appropriate study to use as the NOAEL as convulsions are the most critical single-dose endpoint. Other effects seen in other studies (liver and thyroid histopathology) require repeated exposure before manifestation and are not relevant when setting an acute reference dose.

An acute reference dose of 0.05 mg/kg bw is proposed.

5.10.5 Maximum admissible concentration in drinking water (MAC value)

To calculate the MAC for fipronil in drinking water (No suitable human data are available and there are no chronic exposure animal studies in which fipronil has been administered in drinking water) it is appropriate to divide the ADI for fipronil (0.0002 mg/kg bw) by 10. It is normal practice to propose a MAC as 10% of the ADI, and thus derive a daily intake of 0.00002 mg fipronil/kg bw. Fipronil is not considered to be mobile in soil and should not contaminate water (see Section 7.2.4).

Assuming a 60 kg person drinks 2.0 litres of water per day, a daily intake of 0.00002 mg fipronil/kg bw would be achieved by drinking water containing 0.0006mg/l. **Thus a MAC for fipronil in drinking water of 0.6 µg/l can be derived**.

5.11 ACUTE TOXICITY, IRRITANCY AND SKIN SENSITISATION OF THE PREPARATION

The formulation toxicity studies were conducted with a fine granule formulation, EXP 60819A. The application by Aventis (formerly Rhône Poulenc) however, has been made for the formulation EXP 60818A ['Vi-Nil GR']. Both formulations contain approximately 0.1% w/w fipronil technical (EXP 60818A contains 1.04 g/kg fipronil, and EXP 60819A 1 g/kg). The pertinent difference was the substitution of the granule base material in EXP 60819 A is clay-based ('Agsorb clay'). However granule base in EXP 60818 A consists of 'Biodac 20/40', a cellulose complex. The health and safety data sheet for this material has been submitted and it is judged for the purposes of this evaluation of formulation toxicity that the toxicological properties of this 'Biodac 20/40' do not differ from those of 'Agsorb Clay'.

5.11.1 Acute oral toxicity in rats (AIII 7.1.1)

Study Type:	**Acute Oral LD_{50} with EXP 60819A**		
DP ref.:	71241	GLP Certified:	Yes
Substance Purity:	Batch no. 9-MTD-7 0.1% a.i.	Guideline Compliance:	EPA/FIFRA 81-1
Test System:	Rat: Sprague Dawley, five males and five females.	Year(s) of Conduct:	1994
Doses:	5000 mg/kg bw by gavage	Vehicle:	Distilled water

This study was performed as a limit test. There were no deaths following peroral doses of 5000 mg/kg bw. There were no signs of toxicity observed and most animals had no gross lesions at necropsy. Two males had multiple black foci on the lungs and one female had dark brown mottling of the lungs.

The LD_{50} of EXP 64819A was found to be greater than 5000 mg/kg bw in rats. The test material is not classifiable according to current EC classification criteria.

5.11.2 Acute dermal toxicity (AIII 7.1.2)

Study Type:	**Acute percutanous toxicity study with EXP 60819A**		
DP ref.:	71242	GLP Certified:	Yes
Substance Purity:	Batch number: 9-MTD-7 0.1% a.i.	Guideline Compliance:	EPA/FIFRA guideline 81-2
Test System:	Rabbit: New Zealand White 5 males and 5 females	Year(s) of Conduct:	1994
Doses:	2000 mg/kg	Vehicle:	Distilled water
Area Covered:	Entire trunk clipped with an electric clipper and as large an area as possible covered with the test material.	Exposure Duration:	24-hours under a semi-occlusive dressing.

The amount of test substance /area covered ranged from approximately 50mg/cm2 (for females) to 51 mg/cm^2 (for males). None of the animals died after receiving a single cutaneous dose of 2000 mg/kg of EXP 60819A.

The dermal reactions at day 1 were erythema and oedema, and brown chemical residues. There were no signs of systemic toxicity and all rabbits exhibited a consistent weight gain over the 14-day observation period. There were no gross lesions apparent in any animal at necropsy.

The percutaneous LD_{50} for rabbits is greater than 2000 mg/kg, and is not classifiable according to current EC classification criteria.

5.11.3 Acute inhalation toxicity (AIII 7.1.3)

Study Type:	**Acute Inhalation Limit Test with EXP 60819A**		
DP ref.:	71243	GLP Certified:	Yes
Substance Purity:	Batch no.: 9-MTD-8 0.1% a.i.	Guideline Compliance:	EPA/FIFRA 81-3 OECD guideline 403
Test System:	Rat: Sprague – Dawley	Year(s) of Conduct:	1994
Doses:	5.16 (- 0.16) mg/l	Vehicle:	None
Particle Size:	MMAD 2.10 µm	Area Concerned:	Nose

No rats exposed to the dust atmosphere of EXP 60819A died during exposure or during the 14 day post-exposure period. Clinical signs observed during exposure was blepharospasm, perinasal wetness, and brown discoloration of the fur. No clinical signs of toxicity were observed during the following 14 days.

A loss of body weight was observed in 4/5 females and 1/5 males at 7 days following exposure. These animals seemed to have a normal weight gain observed during the second week of observation.

A possibly treatment related colour change, (brown in focal or multifocal area), of the lungs was observed in 2/5 males at necropsy.

In conclusion this study indicates that the 4-hr LC50 value for EXP 60819A is greater than 5.16 mg/l and is not classifiable as harmful according to the current EC classification criteria.

5.11.4 Skin irritancy (AIII 7.1.4)

Study Type:	**Acute Dermal Irritation / Corrosion with EXP 60819A**		
DP ref.:	71245	GLP Certified:	Yes
Substance Purity:	Batch number: 9-MTD-7 0.1% a.i.	Guideline Compliance:	EPA/FIFRA guidelines 81-5
Test System:	Rabbit: New Zealand White	Year of conduct:	1994
Solvent / Vehicle:	Distilled water	No. of Animals:	3 males and 3 females
Dose & Nature:	0.5 g (powder)	pH:	-

The fur was clipped from the dorsal area of the trunk of each rabbit and a dose of 0.5 g of the ground test material, moistened with distilled water, was applied directly to a 1 inch square gauze patch and secured by adhesive tape on the dose site. The animal was placed in a restraining device for the 4-hour contact period after which the coverings and as much excess of the test substance as possible was removed. Readings were made at 1, 24, 48, 72 hours and 7 days after the end of the contact period.

EXP 60819A produced minor erythema on all 6 rabbits within 1 hour after the end of the contact period. Minor oedema was apparent on 2 animals at 1 hour but subsided within day 1. There was a brown chemical residue on 4 rabbits, persisting on 1 through day 1. Erythema subsided on 4 rabbits within 1 to 2 days. There was no irritation present on any animal at 3 days.

In conclusion EXP 60819A is not classifiable as a skin irritant according to current EC classification criteria.

5.11.5 Eye irritancy (AIII 7.1.5)

Study Type:	**Acute Eye Irritation with EXP 60819A**		
DP ref.:	71246	GLP Certified:	Yes
Substance Purity:	Batch number: 9-MTD-7 0.1 % a.i.	Guideline Compliance:	EPA/FIFRA guidelines 81-4
Test System:	Rabbit: New Zealand White	Year of conduct:	1994
Solvent / Vehicle:	None	No. of Animals:	3 males and 3 females
Dose & Nature:	0.1 ml (powder)	pH:	-

The test substance was ground and 0.1 ml of test material was instilled into the conjunctival sac of one eye per rabbit. The other eye of each animal served as a control. Eye examinations were performed at 1, 24, 48, 72 hours and 7 days following instillation.

A dose of 100 mg EXP 60819A produced no corneal effects in any of the exposed rabbit eyes. Iritis and moderate conjunctival irritation were observed in all 6 rabbits within 1 hour. Iritis had however subsided in 3 of 6 eyes within 24 hours, and there was no iritis remaining in any eye after 48 hours. All eyes still had minor to moderate conjunctival irritation. Minor conjunctival redness remained in the remaining 4 rabbits. The eyes were normal, in all rabbits, after 7 days.

In conclusion EXP 60819A is not classifiable as an eye irritant according to current EC classification criteria.

5.11.6 Skin sensitisation (AIII 7.1.6)

Study Type:	**Skin sensitisation study with EXP 60819A – Buehler method**		
DP ref.:	71249	GLP Certified:	Yes
Substance Purity:	Batch number: 9-MTD-7 0.1 % a.i.	Guideline Compliance:	EPA/FIFRA guidelines 81-6 (Buehler method)
Test System:	Guinea pig: albino Hartley	Year of conduct:	1994
Solvent / Vehicle:	0.25% (w/v) aqueous methyl cellulose	No. of Animals:	40 animals, 10/group*
Dose – induction (challenge):	40% EXP 60819A (40%); 0.3% DNCB (0.1%)	Positive controls:	2,4-dinitro-1-chlorobenzene (DNCB)

* 4 groups – test group, negative control for the test group; positive control group, and negative control for the positive control group. 5 males and 5 females in each group.

On the day before application the fur was clipped from the area to be dosed. On the day of application the test substance, 0.3 ml, was placed in a plastic dome with a non-woven cotton pad to hold the test substance, which provided a dose area 25 mm in diameter. The dome was then placed securely on the animals dorsal surface. The exposure time was 6 hours.

Each of the test animals was subjected to three induction doses, spaced 1 week apart, followed by a 2 week rest period. A single challenge application for the appropriate test or positive control substance was then made. At the time of the challenge application previously untreated guinea pigs also received single applications of the EXP 60819A suspension, or the DNCB suspension.

During the induction portion of the study there was no irritation observed on any animal following any of the 3 induction doses with the test substance. Following the challenge application with EXP 60819A, slight patchy erythema was present on 1 animal at 24 hours. An area of eschar was also present at 48 hours on 1 of the animals. There was no erythema observed on the dose site of any animal at 24 or 48 hours following the application of a 40% suspension of the test substance to naïve control animals.

The positive control produced noticeable skin irritation during the induction phase of the study, and produced a well-defined skin reaction on all guinea pigs following the challenge.

In conclusion EXP 60819A did not produce irritation during induction phase of the study, and did not produce any positive scores following the challenge. Therefore, EXP 60819A was judged to have no apparent sensitising potential in the guinea pig. The positive control produced satisfactory results.

5.11.2 Summary of the toxicity of EXP 60819A

EXP 60819A is not classifiable as acutely toxic via the oral, dermal or inhalation routes based on studies in rats. It is also not classifiable as a skin or eye irritant, nor as a skin sensitiser.

Table 5.41 Summary of acute toxicity, irritancy and sensitisation of EXP 60819A

Study Type	LD_{50}	Comment	Reference
Oral, rat	> 5000 mg/kg	Not classifiable	71241
Percutaneous, rat	> 2000 mg/kg	Not classifiable	71242
Inhalation, rat	> 5.16 mg/l	Not classifiable	71243
Skin irritation, rabbit	-	Not irritating	71245
Eye irritation, rabbit	-	Not irritating	71246
Skin sensitisation, guinea pig, Buehler study.	-	Not skin sensitising	71249

5.12 Dermal absorption

For a water dispersible granule formulation containing 80% fipronil (Section 5.1.3c), absorption in rats was <3%. The *in vitro* dermal absorption study at 5.1.3a showed that the flux rate of fipronil across human skin was at least ten times less than that for rat skin hence dermal penetration in operators from granules is expected to be less than 1%. Given that %penetration did not vary greatly with dose this figure of 1% may also be extrapolated to the formulation **EXP 60819A** containing 0.1% fipronil.

5.13 Toxicology of non-active substances

The co-formulants are a carrier (Biodac 20/40, cellulose complex, 94%), a solvent (N-methyl-pyrollidone, 0.9%) and a deactivator (propylene glycol, 5%). Health and safety data sheets do not identify any further specific toxicological concerns other than that investigated below for the formulation.

5.13.1 Conclusions

The information submitted on mammalian metabolism and toxicology of the a.s. is sufficient to support provisional approval of 'Vi-Nil GR' for use on non-edible ornamentals.

For full approval:

(i) Any medical data available on manufacturing personnel or spray operators, including information on sensitisation, or from any adverse reaction schemes must be submitted (cf Section 5.9.1 of ACP 168 (274/00)).

5.14 EXPOSURE DATA

5.14.1 Operator exposure

'Vi-Nil GR' is a FG formulation (with a particle size range of 250 to 850 μm and a low (0.0002% w/w) dust content) containing a nominal 0.1% w/w fipronil. The proposed use is as a compost-incorporated, pre-planting horticultural insecticide on outdoor and protected container-grown ornamentals. The product is intended to be evenly mixed into compost by hand or using a mixing machine at an application rate of 1 kg/m^3 of compost (equivalent to 1 g fipronil/m^3 of compost). The draft product label notes that the white micro-granules are readily visible in compost as an aid to even mixing. Treated compost is to be stored in a clean, dry area and used within 12 days. 'Vi-Nil GR' is to be packaged in foil-lined paper or laminated LDPE sacks containing 10 to 20 kg of product (the 10 kg sacks are intended for supply to nurseries treating their own compost and the 20 kg sacks are intended for supply to compost manufacturers).

Fipronil is currently approved in the UK as an insecticide for cockroach control in the products 'Goliath Bait Station', 'Goliath Gel' and 'Nexa Cockroach Bait Station', all products containing 0.05% w/w fipronil (HSE evaluation SC 10435/ACP 70 (260/98)). Although the level of operator exposure to fipronil resulting from the use of these products was considered to be acceptable, it was agreed that if a future approval was likely to result in higher levels of exposure *via* the inhalation or dermal routes, it should be supported by a skin sensitisation study on the proposed formulation and an acute inhalation study with the a.s. or proposed formulation.

The applicant has proposed that 'Vi-Nil GR' should be unclassified on the basis of acute formulation toxicity data, and the evaluation of these data confirms that no classification is required (see Section 5.11.2). The draft product label recommends that operators must 'Wear suitable protective gloves when handling the product, admixing with compost or handling treated compost' (it is noted that different versions of this operator protection phrase appear in the 'statutory box' and 'PRECAUTIONS' section of the draft label).

5.14.1.1 The treatment process

The applicant has submitted details of the way in which 'Vi-Nil GR' will be used based on observations of the commercial use of similar compost-incorporated products. The applicant considers that the majority of 'Vi-Nil GR' will be used by compost manufacturers, although some nurseries will choose to incorporate the product themselves. (The sale of 'Vi-Nil GR' pre-mixed with compost in bulk bags and sacks is considered to be outside the scope of the current application. This will require a separate application, specific product label and supporting data).

Information provided to the applicant by a compost manufacturer indicates that an operator working an 8 hour shift (with a 1 hour break) is likely to handle a maximum of 3200 kg of 'Vi-Nil GR' when the treated compost is packaged in bulk bags (2 m^3), and a maximum of 2770 kg of 'Vi-Nil GR' when the treated compost is packaged in 80 litre sacks. No information was provided on the specific tasks involved in large-

scale mixing and bagging operations, the extent of automation of these processes, or the type of PPE normally worn by workers.

Three UK nurseries were visited by the applicant to investigate typical methods for compost treatment using similar incorporated insecticides, and to observe the conditions of use of treated compost.

At the first site in Leicester only pre-treated compost was used. Potting operations and the direct handling of treated compost took place over approximately 8 to 10 weeks each year, with approximately 200 000 pots (2 or 3 litres) being filled each week. The nursery used approximately 6000 m^3 of treated compost in a year. It was reported that workers always wore gloves when handling compost and worked from 8 am to 4 pm each day.

The second nursery in Stratford was reported to use mainly 2 m^3 bulk bags of compost pre-treated with insecticide. Potting operations were carried out for a period of 6 months with workers operating the potting machine from 7.30 am to 4.15 pm for 6 days a week. It was noted that the potting machine was swept clean every day. Workers using treated compost were reported always to wear gloves and dust masks.

The third nursery in Kidderminster mixed its own compost as required. An estimated total of 3000 m^3 of compost was mixed annually. Potting operations using a 'Javo' potting machine were performed for only a few days in the summer months. Gloves were always worn during potting operations and gloves and a dust mask were worn when mixing the compost.

From these observations the applicant concluded that, using a potting machine, a team consisting of 1 person mixing the compost, 2 on the potting machine, 1 unloading the conveyor belt and 2 standing out the plants would usually handle 10000 pots of 2 litres capacity or 14000 pots of 1 litre capacity in an 8 hour day. The maximum amount of compost used in a day would be 20000 litres (20 m^3) for each potting machine. If such a nursery also treated its own compost, the applicant estimated that a maximum of 28 kg of 'Vi-Nil GR' would be handled and applied in a day.

Potting by hand on very small nurseries was estimated by the applicant to use about 4 m^3 of compost in an 8 hour day. If such a nursery also treated its own compost, the applicant estimated that 4 kg of 'Vi-Nil GR' would be used in a day.

From these observations, the applicant concludes that the largest quantity of 'Vi-Nil GR' likely to be handled in a day is 3220 kg, equivalent to 3.2 kg of fipronil. Although this quantity relates to the production of pre-mixed compost (as stated earlier, pre-mixed use is considered to be outside the scope of this application), it has been used as the basis of a worst case operator risk assessment.

5.14.1.2 Field measurement of operator exposure

The applicant has submitted an operator exposure monitoring study conducted in 1994 in Cameroon investigating the potential dermal and inhalation exposure of banana plantation workers to fipronil from the use of 'Regent 20 GR' (a FG formulation

containing 2% w/w fipronil) for the control of banana weevil borer. No information was provided on the particle size or dust content of this product. Dermal exposure monitoring was by whole body dosimetry and inhalation exposure was estimated in the report from personal air samples assuming a respiratory volume of 60 l/min (a high respiratory rate associated with the performance of heavy work). Blood samples taken before the start of the monitoring programme and one week after the field study were analysed for fipronil, its main animal metabolite MB 46136, and its photo-metabolite MB 46513.

The metabolite MB 46513 seen in this study conducted in equatorial Africa is not formed in significant quantities under UK climatic conditions (see Section 7.8). It is not considered pertinent to the overall UK risk assessment for fipronil.

(DP 71250)

The product was applied using either a plastic spoon (10 operators monitored) or a Horstine Farmery Micro-Spread applicator (8 operators monitored) with a target application rate of 7.5 grams of 'Regent 20 GR' around each plant (approximately 40 kg of product/ha). Not all subjects were required to load buckets or the Micro-spread hopper with the product, and the individuals carrying out these tasks were not specifically identified. The average area treated was 0.41 ha/operator/day, the average amount of a.s. applied was 0.131 kg/operator/day (equivalent to 6.55 kg of 'Regent 20 GR'), and the average duration of daily exposure was 2 hours 36 minutes.

Geometric mean exposure values determined in this study are summarised in Table 5.42.

Table 5.42 Geometric mean exposure values from 'Regent 20 GR' study

	mg a.s./day	mg a.s./kg product	mg a.s./kg a.s.
Potential dermal *	0.65	0.10	5.06
Actual dermal **	0.077	0.012	0.598
Inhalation	0.0258	0.004	0.201

* Exposure on protective gloves and boots was not measured.

** Calculated assuming 10% transfer/penetration from coveralls as no inner clothing was worn by operators.

On the basis of the geometric mean actual dermal exposure value and geometric mean inhalation exposure value determined in this study, the applicant has calculated equivalent exposure values for the proposed use of 'Vi-Nil GR', assuming a use rate of 3.2 kg of fipronil/operator/day.

The extrapolated actual dermal exposure value is calculated as:

$$\frac{0.598 \text{ mg/kg a.s. x } 3.2 \text{ kg a.s./day}}{60 \text{ kg b.w.}} = 0.032 \text{ mg/kg b.w./day}$$

The extrapolated inhalation exposure value is calculated as:

$$\frac{0.201\ \text{mg/kg a.s.} \times 3.2\ \text{kg a.s./day}}{60\ \text{kg b.w.}} = 0.011\ \text{mg/kg b.w./day}$$

All blood samples taken before exposure produced results below the LOD (0.5 ppb). At the end of the study period all plasma levels of fipronil and its metabolites remained below the LOQ (1 ppb), although two samples contained traces of MB 46513 and three contained traces of MB 46136.

The applicant considers that exposure values for 'Vi-Nil GR' extrapolated from the 'Regent 20 GR' study may be an overestimate, as the use monitored involved more contact with the product than would arise from use as a compost treatment.

5.14.1.3 Estimation of operator exposure

The applicant has estimated operator exposure to fipronil resulting from the proposed use of 'Vi-Nil GR' using mixing/loading data from the German model [1] (geometric mean values) and soil hand contamination values from published studies [2, 3].

German model data for the mixing and loading of WG formulations using tractor-mounted/drawn equipment (the most appropriate data for a FG formulation) predict a geometric mean level of hand contamination of 2 mg/kg a.s. handled and a geometric mean inhalation exposure of 0.008 mg/kg a.s. handled. The corresponding median values are 1.8 mg hand exposure/kg a.s. handled and 0.0017 mg inhalation exposure/kg a.s. handled. The higher percentile exposure values from this data set normally used for regulatory purposes in the UK are 5.72 mg/kg a.s. handled for hand exposure (maximum value from a set of 9 values) and 0.242 mg/kg a.s. handled for inhalation exposure (75th percentile of 13 values, all at the limit of quantification). In view of the extended season of use proposed for 'Vi-Nil GR' it is considered appropriate to estimate exposure on the basis of values which represent a central tendency.

Estimates of operator exposure when handling 'Vi-Nil GR' based on geometric mean values from the German model and assuming that a 60 kg operator handles 3.2 kg of fipronil/day (the worst case assumption based on the applicant's survey information discussed above), are presented in Table 5.43. Based on *in vitro* and *in vivo* studies, a dermal penetration value for the formulation of 1% is used in the exposure estimates (see Section 5.12).

Table 5.43 Operator exposure to fipronil based on German model mixing/loading data

Hand exposure mg/kg bw/day	Inhalation exposure mg/kg bw/day	Total systemic exposure mg/kg bw/day
No PPE		
0.107	0.000427	0.00150
Gloves when handling the product*		
0.00107	0.000427	0.000438
Gloves and RPE when handling the product*		
0.00107	0.0000213	0.0000320

* Assuming a penetration/transfer factor of 1% for gloves and 5% for RPE.

Although the assumption of 95% protection from a filtering facepiece respirator (as assumed in the German model) is considered to be high for an extended period of use, the storage stability data for 'Vi-Nil GR' indicate a low level of dust (0.0002% w/w <50 μm) after storage (CIPAC Method MT 46), and inhalation exposure when handling the product is likely to be lower than the German model surrogate value based on WG formulations. Additionally, all potential inhalation data points in the German model for WG formulations are based on LOQ values and the actual values would have been lower than those assigned.

Operator exposure to fipronil when mixing 'Vi-Nil GR' into compost may also result from dermal contamination with treated compost. Field studies investigating dermal exposure to soil by direct gravimetric measurements (J.C. Kissel, K.Y. Richter, R.A Fenske. Risk Anal. 16(1), 115-125 (1996)) suggest that an appropriate hand soil loading for a worker handling growing media would be 0.44 mg/cm^2 (geometric mean peak value for farmers involved in hand weeding). A laboratory study to determine the extent of soil adherence to hands when totally immersed in a range of dry soil samples (J. H. Driver, J. J. Konz, G. K. Whitmyre. Bull. Environ. Contam. Toxicol. 43:814-820 (1989)) concluded that the mean hand loading for unsieved soil was 0.58 mg/cm^2 of skin surface. Data for sieved samples suggested that hand loading was increased when soil particle size was reduced.

Assuming a surface area of the hands of 820 cm^2 (EPA standard value) and a soil retention value of 0.44 mg/cm^2, the appropriate daily peak soil hand loading for a user of 'Vi-Nil GR' would be 361 mg. Assuming a bulk density for compost of 1.5 g/cm^3, this hand loading value equates to 0.24cm^3. This volume of compost newly treated with 'Vi-Nil GR' at the maximum application rate of 1 g a.s./m^3 of compost would contain 0.00024 mg of fipronil.

Assuming a dermal absorption value of 1% and a 60 kg body weight, the systemic exposure to fipronil for a worker handling treated compost would be 4.0 x 10^{-8} mg/kg bw/day assuming that gloves are not worn. This calculation is based on the assumption that 'Vi-Nil GR' is dispersed completely throughout the compost. In practice it is estimated that even mixing of the product will result in approximately one granule of product in every 0.3 cm^3 of compost (assuming a mean particle diameter of 675 μm corresponding to a volume of 0.16 mm^3 for a sphere, and a product density of

1.79 g/cm^3). On the basis of this calculation, it would be expected that the volume of soil adhering to the hands will contain no more than one granule.

References relied upon:

[1] Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products. Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Bundesgesundheitsamt, und Industrieverband Agrar e.V. (1992) ISBN 3489-27700-7.

[2] Kissel J.C., Richter K.Y. and Fenske R.A. (1996) Field measurement of dermal soil loading attributable to various activities : Implications for exposure assessment. Risk Analysis 16 : 115-125.

[3] Driver J. H., Konz J. J., Whitmyre G. K. (1989). Soil adherence to human skin. Bull. Environ. Contam. Toxicol. 43: 814-820.

5.14.1.4 Operator exposure risk assessment

Considering the proposed use pattern of 'Vi-Nil GR' with the possibility of daily exposure for a period of up to six months (from observations submitted by the applicant), it is appropriate to compare predicted exposures to an AOEL derived from chronic studies. The applicant has proposed a systemic AOEL for fipronil of 0.005 mg/kg bw/day based on a NOEL of 0.5 mg/kg bw/day in the 90-day dietary dog study with no correction for oral absorption (systemic availability of a dietary dose is estimated by the applicant to be 80%) and using a safety factor of 100. A transitory effect on body weight in two females during week 2 of the study was observed at the LOEL of 2.0 mg/kg bw/day. Signs of neurotoxicity were observed at the highest dose of 10 mg/kg bw/day.

The evaluator has proposed a long-term systemic AOEL for fipronil of 0.0002 mg/kg bw/day based on a NOAEL of 0.02 mg/kg bw/day in the 2 year dietary rat study with no correction for oral absorption and using a 100x assessment factor. Convulsions were observed in some animals at the LOEL of 0.06 mg/kg bw/day. An alternative short-term systemic AOEL for fipronil is 0.003 mg/kg bw/day derived from a NOAEL of 0.3 mg/kg bw/day in both the 90 day rat study and rat neurotoxicity study with no correction for oral absorption and using a 100x assessment factor (see Section 5.10.3).

Assuming a dermal absorption of fipronil of 1%, the exposure values from the field monitoring study extrapolated to reflect the rate of use of 'Vi-Nil GR' correspond to a total systemic operator exposure of 0.0113 mg/kg bw/day, assuming that gloves and coveralls are worn (56.5x the long-term systemic AOEL). If these values are adjusted to reflect the wearing of suitable respiratory protective equipment offering a high level of protection (95%), total systemic operator exposure is calculated to be 0.00087 mg/kg bw/day (4.4x the long-term systemic AOEL). As the field study was based on the unrelated use of a different product, the relevance of these extrapolated exposure values is questionable.

Exposure estimates using German model loading data in conjunction with soil hand contamination values indicate that with no PPE the level of systemic exposure to fipronil resulting from the proposed use of 'Vi-Nil GR' is likely to be unacceptable (7.5x the long-term systemic AOEL). When gloves are worn when handling the

product, the level of total systemic operator exposure is also estimated to be unacceptable (2.2x the AOEL). However, when gloves and RPE are worn when handling the product, operator exposure is estimated to be 16% of the long-term systemic AOEL. On this basis provisional approval is recommended for the approved use of 'Vi-Nil GR'.

The exposure study submitted in support of this application indicates an unacceptable level of systemic operator exposure and limited data from the German model indicate that the exposure is primarily *via* the inhalation route. Therefore, further data are required to quantify the level of potential inhalation exposure to fipronil when handling the product and mixing it into compost, using a representative range of methods and machinery.

5.14.2 Bystander exposure

The presence of bystanders during the use of 'Vi-Nil GR' by compost manufacturers and nurseries is possible. Individuals not involved in the use of the product and not involved in handling treated compost are likely to be exposed to fipronil only through the movement of dust or vapour. Storage stability data indicate that the product contains little dust (0.0002% w/w < 50 μm after storage), and the vapour pressure of fipronil is very low (1.58×10^{-7} Pa at 20 °C). Bystander exposure is, therefore, likely to be negligible. The inhalation exposure data required for full approval for the use of 'Vi-Nil GR' will allow the risk assessment for bystanders to be refined on the basis of the amount of dust generated in the mixing process.

5.14.3 Estimation of exposure when handling treated compost

Workers employed by compost manufacturers and nurseries may be exposed to fipronil when handling and using compost treated with 'Vi-Nil GR'. It can be assumed that workers involved in these activities will receive a peak daily hand loading of 0.24 cm^3 of compost, containing 0.00024 mg of fipronil (see Section 5.14.1.3). Assuming a dermal absorption value of 1% and a 60 kg body weight, the level of systemic exposure to fipronil for a worker handling treated compost would be 4.0×10^{-8} mg/kg bw/day assuming that gloves are not worn. Although the levels of airborne dust containing fipronil are likely to be low when using compost (considering the low dust content of the granules), a worst case estimate of exposure to fipronil resulting from inhalation of soil dust can be calculated using an airborne dust value of 98.6 mg/m^3 from published data relating to cultivating operations using an uncabbed tractor in California (Nieuwenhuijsen MJ, Kruisize H, Schenker MB (1998). Exposure to dust and its particle size distribution in California Agriculture. Am. Ind. Hyg. Assoc. J. 58: 34-38). Assuming an even distribution of fipronil in the dust and a breathing rate of 25 litres/minute over 8 hours for a 60 kg worker, potential inhalation exposure is estimated to be 1.31×10^{-5} mg/kg bw/day.

On this basis the risk to workers employed by compost manufacturers and nurseries is considered to be acceptable.

The level of exposure to fipronil for persons purchasing container-grown plants who may be in contact with treated compost when re-potting the plant etc. is likely to be considerably lower than that estimated for nursery workers using large quantities of treated compost on a daily basis. However, the above estimate does not consider hand to mouth transfer of treated compost. The US EPA Exposure Factors Handbook contains assumptions for daily soil ingestion of 100 mg for children, 50 mg for adults and 10 g for pica children. Assuming a body weight of 15 kg for children and 60 kg for adults, these quantities of ingested soil treated with 'Vi-Nil GR' would result in a systemic exposure to fipronil of no more than 4.5×10^{-6} mg/kg bw/day for normal behaviour or 4.5×10^{-4} mg/kg bw/day for pica behaviour. These estimated exposure levels are below the short term AOEL of 0.003 mg/kg bw/day and the ARfD of 0.05 mg/kg bw/day, indicating an acceptable risk.

5.14.4 Conclusions

The proposed use of 'Vi-Nil GR' is estimated to result in an acceptable risk to operators if suitable protective gloves and suitable respiratory protective equipment are worn when handling the product and mixing with compost. Suitable protective gloves are recommended when handling treated compost, in line with normal occupational practice.

The risks to bystanders and workers are considered to be acceptable.

The information submitted on operator, bystander and worker exposure is sufficient to support provisional approval for the use of 'Vi-Nil GR' on container-grown ornamentals.

The exposure study submitted in support of this application indicates an unacceptable level of systemic operator exposure and limited data from the German model indicate that the exposure is primarily *via* the inhalation route. Therefore, further data are required for full approval to quantify the level of potential inhalation exposure to fipronil when handling 'Vi-Nil GR' and mixing the product into compost, using a representative range of methods and machinery.

The Committee previously considered that the operator exposure modelling was not directly relevant to the proposed use: no appropriate model exists for this situation. Although only low levels of fipronil and its metabolites were detected in the operator monitoring study, there was concern that the study conditions did not reflect the UK use and further reassurance was required to confirm that the proposed use would result in an acceptable level of systemic exposure. Therefore, the Committee requested that operator exposure data, including biological monitoring, should be generated for full approval of this UK use.

Data requirements for full approval:

(i) Further data are required to quantify the level of potential inhalation exposure to fipronil when handling 'Vi-Nil GR' and mixing the product into compost, using a representative range of methods and machinery. The protocol to be agreed with PSD, who will consult HSE.

(ii) Operator exposure data, including biological monitoring, generated under UK conditions.

Label amendments:

The following operator protection phrases must appear:

'WEAR SUITABLE PROTECTIVE GLOVES AND SUITABLE RESPIRATORY PROTECTIVE EQUIPMENT * when handling the product or mixing with compost (* disposable filtering facepiece respirator to at least EN 149 FFP3 or equivalent).'

'WEAR SUITABLE PROTECTIVE GLOVES when handling treated compost'

6 RESIDUES DATA

6.1 Metabolism in succeeding crops

A confined semi-protected rotational crop study was conducted in 1994/1995 in North Carolina, USA, growing carrot or radish, lettuce or mustard and wheat or sorghum in sandy loam soil treated. (Bare ground application followed by covering with 5cm of topsoil to simulate sub surface treatment) with [^{14}C]-phenyl fipronil (radiochemical purity >99%). The radiolabelled fipronil was applied in acetonitrile at a rate of 0.162 kg/ha. The crop samples were planted 30, 153 and 365 days after application (DAT). Crop samples were taken at maturity for all crops and immature crop samples were also taken for wheat and sorghum. The concentration in the top 20cm of soil (density 1.5g/cm^3) is calculated as 0.054 mg/kg. The predicted PEC_{soil} from spreading of treated compost into agricultural land in the top 20 cm is 0.0171 mg/kg (see section 7.3). Thus this study was carried out at ca. 3N. Total radioactivity was determined by combustion LSC.

Samples were extracted using sequential extraction with acetonitrile and acetonitrile : water (1:1). Sorghum stover (30 DAT and 365 DAT) and wheat straw (153 DAT) were also extracted with acetonitrile : 0.1N HCl (1:1). Finally exhaustive extraction of 153 DAT wheat straw was performed by refluxing with 3N HCl in methanol.

The total [^{14}C] residues at harvest (expressed as parent equivalent) from application at 0.162 kg/ha (3N) are summarised in Table 6.1 below.

Table 6.1. Total radioactive residue (TRR) of radioactivity in succeeding crops following treatment with [^{14}C]-phenyl fipronil at 0.162 kg/ha

Crop (days post app'n)	Rotation interval	Days after treatment	TRR mg/kg
Lettuce leaf	30	71	<0.01
Lettuce leaf	153	349	<0.01
Lettuce leaf	365	401	<0.01
Carrot leaf	30	113	0.021
Radish leaf	153	204	<0.01
Radish leaf	365	401	<0.01
Carrot root	30	113	0.016
Radish root	153	204	<0.01
Radish root	365	401	<0.01
Sorghum forage	30	50	0.028
Wheat forage	153	204	0.017
Sorghum forage	365	401	0.014
Sorghum stover	30	142	0.036
Wheat straw	153	384	0.172
Sorghum stover	365	472	0.024
Sorghum grain	30	142	<0.01
Wheat grain	153	384	0.012
Sorghum grain	365	472	0.016

Only those plant samples with TRR ≥ 0.01 mg/kg fipronil equivalents were extracted and the extracted components were characterised and identified against reference standards and further confirmed by LC/MS/MS. For matrices other than cereals, the majority of the residues were extracted with the acetonitrile. However, the major portions of radioactivity in cereals were extracted with the acetonitrile : water solvent. Parent, metabolite RPA 200766 and MB46136 were found in all matrices except grain and parent was the dominant component in sorghum forage. In wheat straw samples from 153 days the dominant residues were RPA200766 and MB46136 (0.067 and 0.044 mg/kg respectively) with parent found at 0.02 mg/kg. RPA 200766 and RPA200761 were found in cereal grain at up to 0.001 mg/kg and 0.009 mg/kg respectively. Wheat grain from 153 days also contained an unknown metabolite at 0.002 mg/kg.

Identification and characterisation of metabolites is summarised in Table 6.2 overleaf. All unknown metabolites were more polar than parent fipronil.

(DP 112745)

Table 6.2. Characterisation and identification of radioactivity in mature succeeding crops following treatment with [14C]-phenyl fipronil

Component	30 day planting				153 day planting			365 day planting		
	Carrot leaf mg/kg (% TRR)	Carrot root mg/kg (% TRR)	Sorghum forage mg/kg (% TRR)	Sorghum stover mg/kg (% TRR)	Wheat forage mg/kg (% TRR)	Wheat straw mg/kg (% TRR)	Wheat grain Mg/kg (% TRR)	Sorghum forage mg/kg (% TRR)	Sorghum stover Mg/kg (% TRR)	Sorghum stover Mg/kg (% TRR)
RPA104615	0.001[a] (4.76)	≤0.001	0.004[b] (14.29)	0.003 (8.33)	**	**	**	0.003 (24.63)	**	**
Unknown 1	**	**	**	**	**	**	**	**	0.003 (12.50)	**
RPA200761	0.001[a] (4.76)	≤0.001	0.004[b] (14.29)	0.003 (8.33)	**	0.015 (8.72)	0.006 (50.00)	0.002 (17.50)	0.003 (12.50)	0.009 (56.25)
Unknown 2	**	**	**	0.002 (5.56)	**	**	**	**	**	**
RPA105048	**	**	**	**	**	0.003 (1.74)	**	**	**	**
Unknown 3	**	**	**	**	**	0.007 (4.07)	**	**	**	**
RPA105320	0.001 (4.76)	**	**	**	**	0.012 (6.98)	**	**	**	**
Unknown 4	**	**	**	**	0.001 (7.52)	**	**	**	**	**
Unknown 5	**	**	**	**	0.001 (6.69)	**	**	**	**	**
RPA200766	0.01 (47.62)	0.002 (47.62)	0.003 (10.71)	0.004 (11.11)	0.003 (17.65)	0.067 (38.95)	0.001 (8.33)	0.001 (7.15)	0.005 (20.83)	**
Unknown 6	**	**	**	**	0.001 (5.85)	**	**	**	**	**
Unknown 7	**	**	**	**	0.001 (5.88)	**	**	**	**	**
Unknown 8	**	**	**	**	0.002 (10.03)	**	**	**	**	**
Unknown 9	**	**	**	**	**	**	0.002 (16.67)	**	**	**
MB46513	**	**	**	0.001 (2.78)	**	0.019 (2.78)	**	**	**	**
Fipronil	0.005 (23.81)	0.005 (31.25)	0.013 (46.43)	0.003 (8.33)	0.003 (15.88)	0.020 (8.33)	**	0.001 (7.15)	0.001 (4.17)	**
MB45950	**	0.004 (25.00)	**	**	**	**	**	**	**	**
MB46136	0.001 (4.76)	0.005 (31.25)	0.003 (10.71)	0.008 (22.22)	0.003 (15.04)	0.044 (22.22)	**	0.001 (7.13)	0.004 (16.67)	**

[a] RPA104615 plus RPA200761 combined to give 0.001 mg/kg

[b] RPA104615 plus RPA200761 combined to give 0.004 mg/kg

** Not detected

6.2 Estimates of potential and actual dietary exposure through diet and other means (IIA 6.6)

Use on edible crops has not been requested. However, data on the likely residues in following crops were required to address the potential risk to consumers from residues in treated compost used to raise edible crops. The rotational crop study was carried out at 3N compared to the predicted concentration in soil following the spreading of treated compost from use on ornamental plant production.

Primary metabolism data have not been evaluated and a residue definition for primary crops has not been proposed. However, a residue definition of parent plus metabolite MB46513 has been proposed for residues in following crops (see section 5.8.2.4).

The following estimates of exposure have used this residue definition from the rotational crop study. In the study parent accounted for up to 50% of the TRR in forage for example, but less than 25% in some leafy matrices. MB46513 was found only in sorghum stover and wheat straw at up 2.78% of the TRR and in lettuce total radioactive residues were less than 0.01 mg/kg. The choice of commodities has been broad to reflect the crops used in the rotational crop study. Therefore, the following assessments are considered to be very conservative.

6.2.1 Intakes by domestic animals

Potential intakes by domestic animals are shown in Table 6.3.

An assessment of the theoretical maximum daily intakes by domestic animals from the consumption of cereal grain and straw, from beet type root and tuber crops which may contain residues of fipronil and its metabolites has been made. The following assumptions have been made:

(i) the highest likely inclusion rate of all crops which may have been treated has been used with the proviso that the aggregate does not exceed 100% diet.

(ii) all crops which may have been treated, have been treated and contain residues at the following levels:

Commodity	Fipronil + MB46513 (mg/kg)
Cereal straw	0.039
Root crops	0.005
Root crop leaves	0.005
Pasture	0.013

(iii) there is no loss of residue during transport, storage, processing or preparation of feed prior to consumption.

Table 6.3 Theoretical daily intakes of fipronil and the metabolite MB46513 by domestic animals based on residues from the rotational crop study

Animal	mg/kg diet (DM)	mg/kg diet (AR)	mg/ animal/ day	mg/kg bw/day
Dairy *	0.0611	0.0144	1.2226	0.00223
Beef	0.0552	0.0179	0.8281	0.00237
UK Sheep	0.0552	0.0179	0.1655	0.00221
Model goat *	0.0611	0.0144	0.1832	0.00259
Pig *	0.0378	0.0049	0.1134	0.00151
Chicken *	0.0100	0.0034	0.0012	0.00063
UK Turkey *	0.0000	0.0000	0.0000	0.00000

* Less than 100 % diet employed
DM=dry matter AR=as received (i.e. wet weight)

Based on the intakes calculated in Table 6.3 and given that the rotational crop study was carried out with a conservatively estimated 3N, application rate, residues in animal products are not expected to be significant (below 0.01 mg/kg in animal products [ruminants]) as intakes by domestic animals are <0.1 mg/kg diet AR.

However, if use of fipronil on edible crops is requested, then the assessment of intakes from domestic animals will require further consideration.

6.2.2 Intakes by humans.

The table below details the 97.5th percentile consumption values in kg/day for total cereals, total leafy crops and total root crops which were used to assess the risk to consumers.

Table 6.4 Consumption data for adults, children, toddlers and infants & total fipronil + metabolite MB46513

Commodity	Adult (kg/ day)	School-child (kg/ day)	Toddler (kg/ day)	Infant (kg/ day)	Fipronil + MB46513 mg/kg
Root/tuber veg total	0.0810	0.0463	0.0421	0.0427	0.005
Leaf veg/herbs total	0.0444	0.0134	0.0127	0.0049	0.005
Cereals total	0.2731	0.2357	0.0688	0.0688	0.001

The TMDIs (Theoretical Maximum Daily Intakes) for fipronil and its metabolites from the consumption of cereals, leafy vegetables and root/tuber vegetables have been calculated for adults, children, toddlers and infants (see Table 6.5). The following assumptions have been made:-

i) upper range of normal (97.5th percentile) consumption of each individual crop which may have been treated.

ii) all produce eaten which may have been treated, has been treated and contains residues at the highest levels identified from the rotational crop study which is considered to have been carried out at 3N.

iii) there is no loss of residue during transport, storage, processing or preparation of foods prior to consumption.

Table 6.5 TMDIs of fipronil + MB46513 for adults, children, toddlers and infants

Commodity	NEDI adults (mg/kg bw/day)	NEDI children (mg/kg bw/day)	NEDI toddlers (mg/kg bw/day)	NEDI infants (mg/kg bw/day)
Root/tuber veg total	0.000006	0.000005	0.000015	0.000025
Leaf veg/herbs total	0.000003	0.000002	0.000004	0.000003
Cereals total	0.000004	0.000005	0.000008	0.000008

97.5^{th} Percentile food consumption as detailed below:
Body weight of adult, schoolchild, toddler and infant taken to be 70.1, 43.3, 14.5 and 8.7 kg, respectively.
Consumption data taken from the Dietary and Nutritional Survey of British Adults 1986/1987.
Consumption data taken from the Dietary Survey of British Infants Aged 6-12 months 1986.

The total NEDIs from the combined consumption of all commodities has been calculated using the Rees/Day model and are presented in Table 6.6 below.

Table 6.6 Total TMDIs of fipronil + MB46513 for adults, schoolchildren, toddlers and infants

Consumer group	Total NEDI (mg/kg bw/day)	% ADI
Adult	0.000010	5
Child	0.000011	6
Toddler	0.000022	11
Infant	0.000033	17

Calculations of the total TMDIs of fipronil + MB46513 have been carried out using the Rees/Day model. Intakes for adults, children, toddlers and infants are 0.000055, 0.000095 and 0.000114 mg/kg bw/day, respectively.

The TMDIs (Theoretical Maximum Daily Intakes) for fipronil + MB46513 have been calculated for crop groups using potential residues in rotational crops. As the intakes from these groups comprise ≤17% of the ADI of 0.0002 mg/kg bw/day, the rotational crop study was carried out at a conservatively estimated rate of 3N and the Acute Reference Dose is 0.05 mg/kg bw (section 5.10.4 of SC10698), the acute (short term) exposure to these residues is acceptable.

6.2.3 Summary of dietary exposure

The total TMDIs for the broad crop groups in the rotational study (cereals, total root/tuber veg and total leafy veg/herbs) are 5%, 6%, 11% and 17% of the ADI of 0.0002 mg/kg bw/day. (See also section 5.8.2.4. Although the JMPR have set a much lower ADI for metabolite MB 46513, the metabolism data indicate the contribution to the diet from residues in edible crops following the proposed use is negligible. However, if approval for direct use on edible crops is sought in the future the contribution of metabolite MB 46513 will need to be reconsidered).

The ARfD is 250 x higher than the ADI. The chronic portion size for total cereals in adults is 0.2731 kg/day. The amount of cereals that would need to be eaten in a single day for intakes to approach the ARfD and trigger concerns over consumer exposure would be 68.3 kg. The potential short term consumer exposure is also acceptable.

6.3 Conclusion

Data on the likely residues in following crops were requested to address the potential risk to consumers from residues in treated compost used to raise edible crops. The rotational crop data indicate that potential residues of fipronil + the metabolite MB46513 in crops which have been grown on land upon which spent compost has been applied will be less than 0.01 mg/kg. These data are acceptable.

The information submitted on residues is sufficient to support approval of 'Vi-Nil GR' as a horticultural insecticide for ornamental plant production.

7 ENVIRONMENTAL FATE AND BEHAVIOUR

The applicant has requested a horticultural practice where one application of fipronil is made at a rate of 1g a.s./m^3 to compost that is then used to grow hardy ornamental nursery stock and non-edible ornamentals in containers. Characteristics of the soils and sediment water systems used in the studies are in Appendix C. It is assumed that composted media has a density of 1.5 g/cm^3. The following exposure assessment is made on the basis of the following information about horticultural practice:

There will be a small proportion of treated media from spillage during potting (assumed a maximum of 0.1% of a compost dump). A small proportion of treated containers (assumed a maximum of 5% of a compost dump) could be disposed to compost after 3 months as a result of quality checks grading out and rejecting some plants. In the nursery spent growing media will be disposed to compost after 18 months (assumed this represents 57.9 % of a compost dump) with the balance of the dump (37%) originating from untreated material. The compost dump is left for 12 months with mixing occurring before the composted material is used as a mulch on the horticultural holding. Some plants in pots with the media associated with the root ball will be planted out in gardens after 3 months.

(DP 82842, DP 112741 and DP112742)

All radiolabel studies used either [uniform ^{14}C-phenyl] fipronil with a radiochemical purity of greater than 95.4 % and specific activity of 1658 - 1667 kBq/mg or [^{14}C-5-pyrazole] fipronil with a radiochemical purity of greater than 97 % and specific activity of 796 kBq/mg

7.1 Route and rate of degradation in soil (IIA 7.1.1, IIIA 9.1.1)

7.1.1 Aerobic and anaerobic studies

7.1.1.1 Soil microbial studies

a) An aerobic soil degradation study was conducted with [^{14}C-phenyl] fipronil at 25°C at 75% soil moisture holding capacity (33kPa) according to US EPA (subdivision N 162-1, 1982) and BBA (Part IV, Section 4-1, 1986) guidelines on Manningtree sandy loam and Speyer 2.2 loamy sand soils. This study was evaluated previously in ACP 70 (260/98), p. 53.

Mineralisation to carbon dioxide accounted for a maximum of 3 % AR. Radioactivity not extracted by acetonitrile represented up to 15 % AR by study termination (336 days). RPA 200766 (still increasing, at 38 % AR by study termination) and MB 46136 (still increasing, at 22 % AR by study termination) were considered major metabolites. The minor metabolites MB 45950, MB 46513 and MB 45897 were also identified but none represented > 5%AR. Six further metabolites were resolved by chromatography but not identified. None represented > 4% AR at any sampling time. The DT 50 of fipronil estimated according to first order kinetics was 130 days (r^2=0.97) in the Manningtree sandy loam and 310 days (r^2=0.88) in the Speyer 2.2 loamy sand.

(DP 71129)

b) An anaerobic flooded soil degradation study was evaluated previously in ACP 70 (260/98), p. 56. This study was not submitted to support this application, as exposure to anaerobic conditions is not expected from the intended use. It should be noted that in this study the metabolite MB 45950 represented a significant proportion of the radioactivity extractable from soil.

7.1.1.1 Soil rate of degradation studies - laboratory

An aerobic soil degradation study was conducted with [^{14}C-phenyl] fipronil at 10 and 22°C at the soil moisture holding capacity (33kPa) according to Dutch (G.1.1) guidelines on Manningtree sandy loam, Speyer 2.2 loamy sand and two different French sandy clay loams. This study was evaluated previously in ACP 70 (260/98), p. 55.

Mineralisation to carbon dioxide accounted for a maximum of 3 % AR. Radioactivity not extracted by acetonitrile represented up to 14 % AR by study termination (365-7 days).

At 10°C, RPA 200766 (still increasing, at up to 38 % AR by study termination) and MB 46136 (still increasing, at 23 % AR by study termination) were considered major metabolites. The minor metabolites MB 45950, MB 46513, MB 45897, RPA 105048 and RPA 105320 were also identified but none represented > 9 % AR. Six further metabolites were resolved by chromatography but not identified. None represented > 10% AR at any sampling time. The DT 50 of fipronil was 250 days (first order, r^2=0.99) in the Speyer 2.2 loamy sand; 190 days (first order, r^2=0.97) in the Manningtree sandy loam; 62 days (√first order, r^2=0.94) in the French Sandy clay loam 1 and 120 days (first order, r^2=0.96) in the French Sandy clay loam 2.

At 22°C, RPA 200766 (still increasing, at 48 % AR by study termination but peaked at 57% AR in French sandy clay loam 1 at 157 days), MB 46136 (still increasing, at 22 % AR by study termination) and RPA 200761 (still increasing, at 21 % AR by study termination, but only in the French soils) were considered major metabolites. The minor metabolites MB 45950, MB 46233, RPA 106681, MB 46400, MB 45897 and RPA105320 were also identified but none represented > 8 %AR. Three further components were resolved by chromatography but not identified. None represented > 3% AR at any sampling time. The DT 50 of fipronil was 120 days (first order, r^2=0.97) in the Speyer 2.2 loamy sand; 120 days (first order, r^2=0.99) in the Manningtree sandy loam; 8 days (√first order, r^2=0.96) in the French Sandy clay loam 1 and 30 days (first order, r^2=0.91) in the French Sandy clay loam 2.

(DP 71133)

7.1.2 Photolysis in soil

A soil photolysis study was conducted according to EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 161-3, 1982).

Samples of fresh Manningtree clay loam soil (2 mm sieved, 50 g dry weight) were added to metal trays to give an even layer (*ca* 10 mm depth). [^{14}C-5-pyrazole] fipronil (0.258 kg a.s./ha) was then added in acetone to the soil surface. Trays were maintained at 75% of the 33kPa moisture holding capacity at 25°C in a chamber fitted with traps

for carbon dioxide and organic volatiles (polyurethane foam, ethylene glycol and ethanolamine/2-ethoxyethanol). Replicate chambers were either kept in the dark or exposed to a light regime which alternated between 8 hour periods of irradiation (a xenon arc lamp spectral cut-off at 290 nm; 434 W/m² at 270-400 nm at soil layer level) and 16 hours of darkness for up to 30 days. The study authors related the intensity of the lamp as 0.84 days of natural summer sunlight at 50°N (Ongar UK) and 30°N (Florida USA) over each 8 hour irradiation period. The natural irradiation duration of the study was therefore approximately 25.2 days.

Samples were taken for each treatment at day 0 and six representative time points. Radioactivity from soil samples was extracted with acetonitrile, before quantification by LSC and characterisation by TLC and HPLC. Compounds were identified by comparison of chromatographic behaviour with certified standards and for day 30 samples, identity was confirmed by GC-MS. Unextracted radioactivity was quantified by combustion/LSC. Radioactivity in polyurethane foam was extracted with acetone before quantification by LSC. The radioactivity in the other trapping solutions was also quantified by LSC.

Practically all soil radioactivity was extractable at time 0, this declined to 94 and 79 % AR for dark and irradiated samples by day 30 respectively. By day 30, CO_2 accounted for 0.2 and 2.5% AR for dark and irradiated samples respectively. Total recovery was 90-101 % AR. After 30 days, fipronil accounted for 52% and 63% AR from illuminated and dark control samples respectively. In dark control samples all extractable metabolites accounted for <10% AR except MB 45950 which accounted for 13% AR at day 30. No metabolites represented > 10% AR from illuminated samples. The metabolites MB 46513 and RPA 104615 were detected in illuminated samples but not dark control samples at up to 6.9 and 7% AR respectively by day 30. There was no indication that concentrations of these photoproducts would not have increased further if the experiment had continued longer.

The first order DT_{50} for photolytic degradation of fipronil was calculated as 34 days (r^2=0.97) under test conditions with that for the dark control 49 days (r^2=0.92). The UK equivalent natural summer sunlight DT 50 (50°N) was therefore estimated as 29 days under UK mid summer conditions (8 hour days).

(DP 71132)

7.1.3 Field studies

7.1.3.1 Field studies, soil dissipation

Results (average of 4 analyses) of a field trial relevant to UK conditions, designed to assess soil dissipation rates are summarised in Table 7.1. Fipronil formulated as a 2% w/w granule containing 0.01 MB 45940, 0.05 MB 46136 and 0.007 RPA 200766 all % w/w granule as impurities, was soil-incorporated at a rate of 0.187 kg a.s./ha at the time of drilling with maize seed on 21 May 1992 in the seed furrow (0.7g a.s./m of furrow). Twenty, 5.1-7.6 cm diameter (depth-dependent) soil cores down to a depth of 60cm were taken at each sampling time from the drilling line. Samples from either side of the drilling line were also taken. The results from these lateral samples are not tabulated in Table 7.1. Lateral movement of residues (including metabolites) was limited, with less than 9.4% of the drilling line concentration being detected in cores

collected outside the drilling line. Appropriate weather data were submitted. Cores from each sample were homogenised within depth segments (0-10, 10-20, 20-30 and 30-60cm), before analysis. Information on the methods of analysis used, which analysed for fipronil, MB 46513, MB 45950, MB 46136, RPA 200766 RPA 104615 and RPA 200761 (selected samples only) are outlined in Section 4.3.1 b) and c). Procedural recoveries for all analytes are summarised in Table 4.3. Although the range of recovery values is quite wide, the recoveries are considered acceptable for experiments used to determine soil dissipation rates. The limit of quantification was 0.002 mg/kg for all the analytes except RPA 200761, where it was 0.005 mg/kg. This represents *ca.* 0.1%-0.2 % of the highest fipronil residue measured shortly after application. Data demonstrated that residues (all analytes) in soil samples stored frozen (-18°C) for up to 12 months (15 months for RPA 104615) were stable. This data encompasses the storage times utilised in the study.

Data from trials carried out in Southern France, Spain, Italy, the USA (June-October in North Carolina and Florida), Eastern Canada (Prince Edward Island, Ontario) and Central Canada (Manitoba) are not tabulated. These trials were not evaluated in full as the climatic conditions are considered unrepresentative of conditions in the UK. In the Southern European trials application rates were comparable to those used in Northern France (ca 200 g a.s./ha),. In the US and Canadian trials application rates were lower (ca 50 and 100 g a.s./ha respectively). The Canadian trials were not GLP-compliant. To summarise:

The results from the more southern European trials indicated dissipation DT_{50} in the top 10 cm layer of 12 days (√first order kinetics r^2=0.85) and *ca.* 140 days (first order kinetics, r^2=0.79 and 0.96) but reduced downward soil movement, compared to the trial from Northern France, as might be expected in hotter generally drier climates. The pattern of metabolites present was similar to that tabulated in Table 7.1 for the Northern French trial, except concentrations of MB 46513 and RPA 104615 (photolysis products) were present in the 0-10 cm layer shortly after application in the Spanish and Southern French (but not Italian) trials. The short duration (4 months) of the US trials means they do not provide any very helpful information on the fate of metabolites in soil (soil concentrations of metabolites still increasing at study termination). In these US trials, dissipation DT_{50} in the top 15 cm layer were *ca.*10 days (√first order kinetics r^2=0.97) in Florida and *ca.* 31 days (first order kinetics, r^2=0.93) in North Carolina, in bare soil plots. In plots with grass turf the respective DT_{50} were *ca.* 3 days (√first order kinetics r^2=1.) and *ca.* 2 days (√ second order kinetics, r^2=0.99). In the Canadian trials, dissipation DT_{50} in the top 15 cm layer were *ca.*6 days (√ second order kinetics r^2=0.96) in Prince Edward Island, *ca.* 2 days (√ first order kinetics, r^2=0.92) in Ontario and *ca.* 28 days (second order kinetics, r^2=0.97) in Manitoba (in bare soil plots). With regard to metabolites, whilst the trial durations were a year, the lower application rate used resulted in fewer metabolite determinations. Generally only MB 46136 was present at 0.005-0163 mg/kg. It was unclear from the data if metabolite levels were still increasing or had reached a plateau. There was no evidence of any decline.

(DP 71134, DP71257, DP 83375)

Table 7.1 Summary of field studies, soil dissipation

Location/ soil properties/ plot size	DAA (days)	Residue (mg/kg wet weight) Soil segment depth (cm)													DT50/DT 90 (days) data fit used in estimation	Remarks:
		fipronil			MB 46136			RPA 200766			MB 45950		RPA 200761			
		0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	20-30	0-10	10-20	0-10	10-20		
Mereville Essonne N France/ loam pH 8.1 OC 1.4 % CEC 25 meq/100g 20m x 80m	0	2.08	n.a.	n.a.	0.055	n.a.	n.a.	0.008	n.a.	n.a.	0.019	n.a.	n.a.	n.a.	fipronil 15 / 160 √ first order (r^2=0.8)	No residues >0.002 mg/kg were determined in the 30-60 cm soil layer except for MB46513 which was detected at 0.003mg/kg at 244 days and RPA 200766 which was at 0.004 mg/kg at 427 and 720 days. Residues of MB 46513 were generally <0.002 mg/kg except after 60 and 184 days when residues in the 0-10cm layer were up to 0.005 mg/kg and in 20-30cm layer at 244 days when residues were 0.005 mg/kg. Residues of RPA 104615 were generally <0.002 mg/kg except after 427 days when residues in the 0-10cm layer were 0.003 mg/kg.
	7	2.13	0.012	0.004	0.077	<0.002	<0.002	0.016	<0.002	<0.002	0.025	<0.002	n.a.	n.a.		
	14	2.17	0.017	0.005	0.052	<0.002	<0.002	0.015	0.004	<0.002	0.020	<0.002	n.a.	n.a.		
	27	1.27	0.067	0.008	0.057	0.003	<0.002	0.028	0.004	<0.002	0.022	<0.002	n.a.	n.a.		
	60	0.56	0.018	0.005	0.074	0.003	<0.002	0.031	0.005	0.002	0.022	<0.002	n.a.	n.a.		
	126	0.36	0.036	0.004	0.065	0.028	0.002	0.035	0.033	0.007	0.027	0.007	<0.005	0.012		
	184	0.30	0.042	0.011	0.072	0.037	0.006	0.069	0.066	0.020	0.029	0.010	n.a.	n.a.		
	244	0.12	0.015	0.004	0.060	0.010	0.003	0.026	0.025	0.011	0.020	0.004	n.a.	n.a.		
	305	0.14	0.012	0.004	0.048	0.011	0.002	0.018	0.024	0.008	0.018	0.003	n.a.	n.a.		
	365	0.13	0.035	0.002	0.074	0.048	00003	0.052	0.061	0.014	0.021	0.015	0.023	0.007		
	427	0.61	0.017	0.004	0.306	0.045	0.010	0.092	0.041	0.018	0.093	0.040	n.a.	n.a.		
	488	0.04	0.007	<0.002	0.057	0.019	<0.002	0.023	0.027	0.006	0.016	0.005	n.a.	n.a.		
	549	0.07	0.004	<0.002	0.070	0.020	0.003	0.028	0.029	0.018	0.024	0.007	n.a.	n.a.		
	640	0.005	0.004	<0.002	0.028	0.016	<0.002	0.004	0.019	0.008	0.007	0.003	n.a.	n.a.		
	730	0.03	0.02	0.003	0.043	0.032	0.004	0.010	0.023	0.010	0.013	0.009	0.005	0.011		

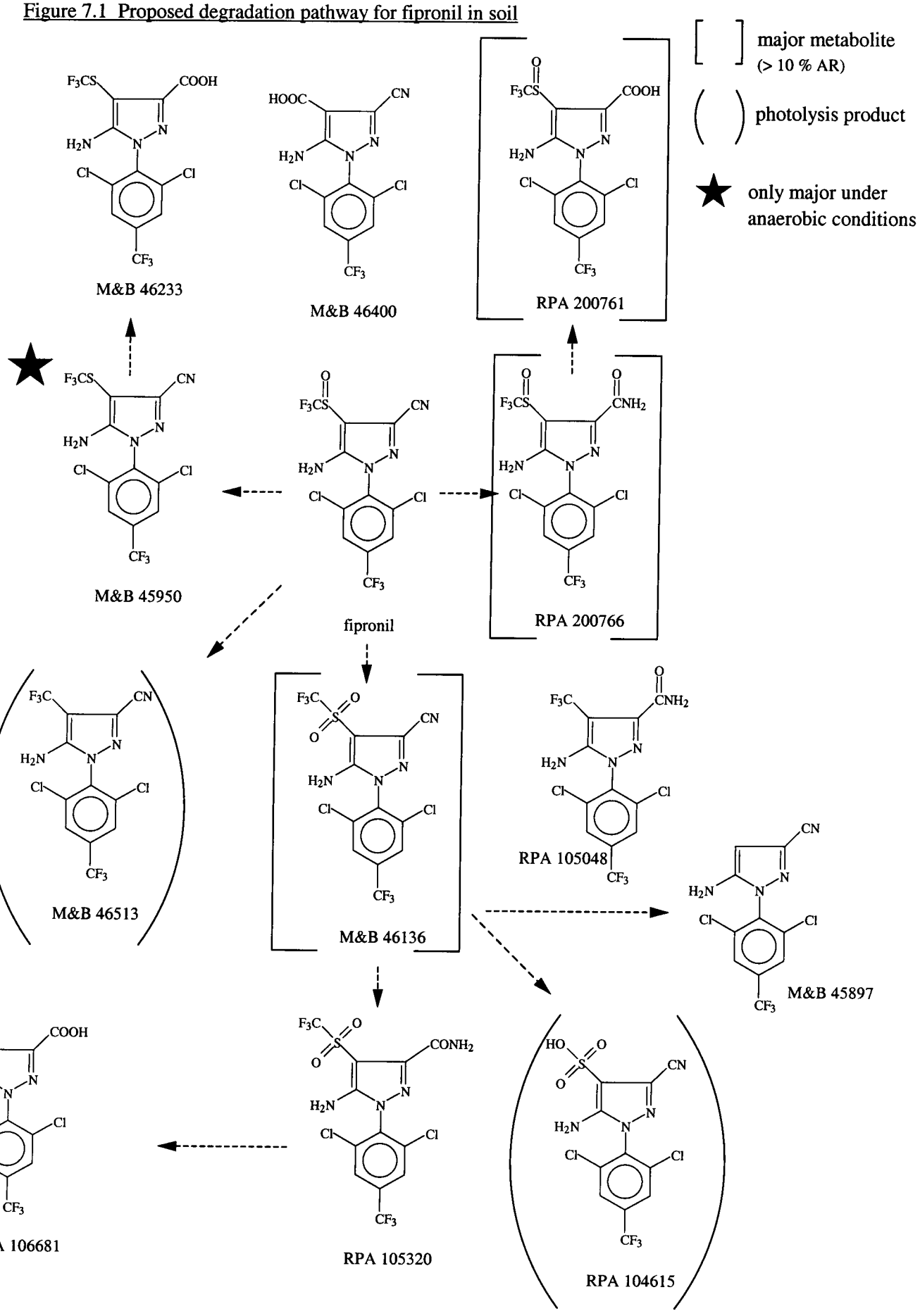
Residues of MB 45950 were < 0.002mg/kg in the 20-30cm soil layer at all sampling times. n.a.= not analysed. No analysis of RPA 200761 was carried out in soil layers deeper than 20 cm.

(DP71134)

7.1.4 Summary

Under aerobic conditions, in the dark (evidence from laboratory studies on 4 soils), it is proposed that fipronil degrades in soil, predominantly through oxidation of the sulfoxide to the sulfone, MB 46136, and through hydrolysis of nitrile group to form the amide, RPA 200766. Amide hydrolysis can also occur in high moisture content soils to further degrade RPA 200766 to its carboxylic acid, RPA 200761. (All the metabolites above are considered major as they exceeded > 10% AR in at least two soils in these studies). A minor (<10 % AR) reductive reaction of the sulfoxide to form the sulphide, MB 45950 also occurs, although under aerobic conditions in the dark control of the photolysis test, this sulfide represented 13% AR at study termination. The nitrile groups of sulfide MB 45950 and sulfone MB 46136 have also been shown to be subject to hydrolysis. Under anaerobic conditions (laboratory data not submitted with this application but reviewed previously in ACP 70 (260/98)), fipronil degradation proceeds mainly through reduction to sulfide MB 45950. The transformation of fipronil on soil in the presence of light (laboratory evidence) favours different degradation pathways. Photolysis of fipronil results in removal of SO from the sulfoxide to form MB 46513; in addition, either the sulfoxide or sulfone group undergoes CF_3 cleavage to produce the sulfonic acid, RPA 104615. However, these photolysis product are considered minor in the 30 day experiment (represented <10 % AR). Mineralisation to carbon dioxide was not a major route of dissipation in these laboratory studies (< 3 % AR). Some dissipation was accounted for by the formation of unextracted residues (up to 15 % AR in the dark and up to 21 % AR in the light). The proposed degradation pathway is outlined in Figure 7.1.

Figure 7.1 Proposed degradation pathway for fipronil in soil



In the laboratory studies discussed above the dissipation kinetics of fipronil were as summarised in Table 7.2.

Table 7.2 Experimental conditions and degradation rates of fipronil in soil under laboratory conditions

Origin/Type Texture	pH	oc (%)	Bio-mass[a]	Application Rate	Temperature/ Soil Moisture (°C)/(% WHC)	Dissipation kinetics Order / r^2	fipronil DT 50 (days)	fipronil DT 90 (days)
In darkness								
Manningtree sandy loam	7.8	1.0	57/53	200 g a.s./ha	21 / 75	first order / 0.97	130	433*
Speyer 2.2 loamy sand	6.1	1.9	414/154	200 g a.s./ha	21 / 75	first order / 0.88	310	1033*
Manningtree sandy loam	6.4	0.43	66/49	200 g a.s./ha	10 / 100	first order / 0.97	190	633
Speyer 2.2 loamy sand	6.3	3.3	471/382	200 g a.s./ha	10 / 100	first order / 0.99	250	833*
French 1 sandy clay loam	6.2	0.7	145/134	200 g a.s./ha	10 / 100	√first order / 0.94	62	>500*
French 2 sandy clay loam	6.2	1.3	277/202	200 g a.s./ha	10 / 100	first order / 0.96	120	400*
Manningtree sandy loam	6.4	0.43	66/49	200 g a.s./ha	22 / 100	first order / 0.99	120	400*
Speyer 2.2 loamy sand	6.3	3.3	471/382	200 g a.s./ha	22 / 100	first order / 0.97	120	400*
French 1 sandy clay loam	6.2	0.7	145/134	200 g a.s./ha	22 / 100	√first order / 0.96	8	85
French 2 sandy clay loam	6.2	1.3	277/202	200 g a.s./ha	22 / 100	first order / 0.91	30	100
Manningtree clay loam	6.7	1.0	-/-	258 g a.s./ha	25 / 75	first order / 0.92	49*	163*
Light exposed								
Manningtree clay loam	6.7	1.0	-/-	258 g a.s./ha	25 / 75	first order / 0.97	29*	97*

* Extrapolated beyond the duration of the study.

(a) mg C / kg dry soil at the beginning and end of incubation

oc = Organic Carbon
n.d. = not determined
WHC = Water holding capacity at 33 kPa

No dissipation of the major soil metabolites was evident in these laboratory studies, their concentrations were still increasing at study termination.

Field dissipation studies carried out in Southern France, Italy, Spain, USA and Canada (not evaluated in full) indicate fipronil dissipation DT_{50} of 2-140 days. Metabolites (most commonly MB 46136) were present in samples at most sampling times, but the metabolite composition was different in the different trials. It was unclear from the data if metabolite concentrations were increasing or had reached steady state over the duration of the trials. There was no clear evidence of a decline of any one metabolite.

In the most pertinent Northern French trial the DT_{50} and DT_{90} of fipronil were 15 and 160 days (√ first order, r^2=0.8) respectively. No decline of any metabolites was evident

from the data. Residues of MB 46513 and RPA 104615 (photolysis products) were generally not present above the limit of quantification (0.002 mg/kg).

Residues of MB 46136 were present in the 0-20 cm soil layers at plateau like concentrations generally in the range 0.01-0.08 mg/kg (0.5-3.8 % of initial fipronil concentration). A single result of 0.3 mg/kg (14 % of initial fipronil concentration) was determined 14 months after application. Residues in the 20-30 cm soil layer were lower with generally no detection in the 30-60cm soil layer.

Residues of RPA 200766 were present in the 0-30 cm soil layers at plateau like concentrations generally in the range 0.01-0.09 mg/kg (0.5-4.3 % of initial fipronil concentration). There were generally no detections in the 30-60cm soil layer.

Residues of MB 45950 were present in the 0-10 cm soil layers at plateau like concentrations generally in the range 0.02-0.09 mg/kg (1-4.3 % of initial fipronil concentration). Residues in the 10-20 cm soil layer were lower with no detections in deeper soil layers.

Residues of RPA 200761 were only sought in three samples from the 0-20 cm soil layer concentrations were <0.005-0.023 mg/kg (0.2-1.1 % of initial fipronil concentration).

7.1.5 Assessment

No data have been submitted where container growing media have been used, it is however considered reasonable to assume that behaviour will be comparable to that in field soils. Sufficient laboratory data are available to assess the route of fipronil soil dissipation. Dissipation occurred primarily from the production of breakdown products (13 identified, three of which represented >10% AR, see Figure 7.1) which did not dissipate further within the time scales of the laboratory studies. Some dissipation (15-20 % AR) was accounted for by the formation of unextractable residues. There was little mineralisation to carbon dioxide (<3 % AR). Whilst a proportion of these studies were carried out at high moisture contents (100% soil moisture holding capacity at 33kPa), this is considered appropriate for a proposed use on container-grown plants which will be watered regularly. The fipronil soil half lives from the laboratory studies are quite variable (see Table 7.2) and result in classification under the SSLRC scheme of slightly persistent – very persistent. If the results for the Speyer 2.2 loamy sand are excluded (container-grown plants are unlikely to be grown in such media) a realistic worst case laboratory DT_{50} for fipronil growing media in the containers would be 190 days (first order, 10°C 75% WHC).

In a compost heap /dump temperatures are likely to be higher due to the microbial fermentation process. Under these conditions the realistic worst case laboratory DT_{50} for fipronil of 130 days (first order, 21°C 75% WHC) is considered more representative.

One field study considered relevant to UK conditions was available (carried out in Northern France). In this study fipronil and pertinent metabolites were analysed for. These metabolites were MB 46136, RPA 200766, RPA 200761 (major metabolites (>10% AR) from aerobic laboratory studies), MB 46513 and RPA 104615 (laboratory

photolysis products, < 10% AR) and MB 45950 (low levels under aerobic conditions but major (> 10% AR) under anaerobic, laboratory conditions). The estimated field DT_{50} for fipronil in this study was 15 days, DT_{90} 160 days (√first order).

The relatively rapid dissipation of fipronil in this trial means it is likely to represent a realistic worst case for metabolite concentrations. No dissipation for the metabolites was evident, however measured concentrations had stopped increasing. It is considered appropriate to use estimated plateau concentrations from this field trial to estimate worst case plateau metabolite concentrations in container growing media and the subsequent compost and mulching material derived from these media. On this basis it is proposed that MB 46513, RPA 104615 will only be present at very low undetectable concentrations and it is estimated that MB 46136, MB 45950 and RPA 200766 will be present at 4% of the initial fipronil concentration. The estimate for RPA 200761 is that it will be present at 1% of the initial fipronil concentration.

If this were a more typical field use of the active substance the available relevant field trial database would be considered insufficient to provide an exposure assessment. However for the requested use on pot grown plants, it is considered the whole database is sufficient to estimate worst case soil residue levels. For fipronil it is proposed that the realistic worst case DT_{50} should be 190 days (first order, 10°C 75% WHC) for estimating predicted environmental concentrations in various soil based media. This figure is taken from the laboratory database. Whilst it is significantly longer than the DT_{50} from the single relevant field trial, as the complete database is fairly variable it is possible that dissipation outdoors under UK conditions could be slow.
In a compost heap / dump temperatures are likely to be higher due to the microbial fermentation process. Under these conditions the realistic worst case laboratory DT_{50} for fipronil of 130 days (first order, 21°C 75% WHC) is considered representative.

7.2 Adsorption, desorption and mobility in soil (IIA 7.1.2, 7.1.3, IIIA 9.1.2)

7.2.1 Adsorption and desorption

a) A batch equilibrium adsorption/desorption study used [^{14}C-phenyl] fipronil at 20°C was conducted to US EPA (subdivision N 163-1, 1982) guidelines. This study was evaluated previously in ACP 70 (260/98), p. 58. The results of the study are reproduced below in Table 7.3.

Table 7.3. Adsorption and desorption coefficients fipronil to five soils.

Soil type	pH	% oc	Adsorption Kf (ml/g)	1/n	Koc (ml/g)	Desorption (5th cycle) Kf (ml/g)	1/n	Koc (ml/g)
Manningtree sandy loam	6.1	0.34	4.19	0.95	1248	24.5	0.991	7307
Manningtree loam	6.9	4.25	20.69	0.938	486	23.33	0.892	549
Speyer 2.2 Loamy sand	6.3	3.35	14.32	0.947	427	22.85	0.925	681
French sandy clay loam 1	6.2	1.16	9.32	0.969	800	19.45	0.952	1670
French sandy clay loam 2	6.3	1.59	10.73	0.949	673	15.94	0.926	1000

(DP 71135)

b) A batch equilibrium adsorption/desorption study was conducted for MB 45950 according to SETAC (1995) and EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982).

[^{14}C-U-phenyl]- MB 45950 (>99%pure) in acetonitrile/0.01M calcium chloride (80 ml) was added to duplicate samples (4 g) of four soils and a sediment, at concentrations of 0.02, 0.01, 0.005 and 0.002 mg/litre for each soil. Treated slurries were shaken in tubes at 20°C for 72 hours in the dark (equilibrium confirmed in pre-test). After equilibration, the supernatant was removed by centrifugation and radioactivity quantified by LSC and characterised by HPLC. Compounds were identified by comparison of chromatographic behaviour with certified standards. Adsorbed MB 45950 was calculated by difference. After centrifugation, soil pellets were resuspended in 0.01M calcium chloride (equal to decanted volume) and again equilibrated for an hour, then analysed as above.

The adsorption and desorption isotherms for each concentration were used to calculate Freundlich coefficients (K_f) and K_{oc} values for each soil, which are given in Table 7.4 for adsorption. Desorption K_{oc} values were 1996-5574 (ml/g). HPLC analysis showed 100% of the radioactivity in the supernatant was MB 45950 after both adsorption and desorption equilibrium periods.

Table 7.4. Adsorption coefficients MB 45950 to four soils and a sediment.

Soil type	% oc	pH	1/n	K_f (ml/g)	K_{oc} (ml/g)
Bosket silt loam	0.5	6.2	1.046	28.10	5621
Rosholt sandy loam	1.2	6.7	0.950	42.36	3530
Faulkbourne loam	2.2	7.0	0.997	95.97	4362
Panholes silt loam	1.9	8.1	0.932	32.20	1695
Sediment sandy clay loam	2.3	8.2	0.970	100.02	4349

(DP 82833)

c) A batch equilibrium adsorption/desorption study was conducted for MB 46136 according to SETAC (1995) and EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982).

[^{14}C-U-phenyl]- MB 46136 (>99%pure) in 0.01M calcium chloride (75 ml) was added to duplicate samples (3.75 g) of four soils and a sediment, at concentrations of 0.1, 0.05, 0.01 and 0.005 mg / litre for each soil. The remaining experimental procedures were as described at 7.2.1 b) above. The adsorption equilibrium time (confirmed in pre-test) was 72 hours. For desorption the equilibrium time was 1.5 hours. Adsorption Freundlich coefficients (K_f) and K_{oc} values for each soil, are given in Table 7.5. For Desorption K_{oc} values were 1777-131815 (ml/g). HPLC analysis showed 100% of the radioactivity in the supernatant was MB 46136 after both adsorption and desorption equilibrium periods.

Table 7.5. Adsorption coefficients MB 46136 to four soils and a sediment.

Soil type	% oc	pH	1/n	K_f (ml/g)	K_{oc} (ml/g)
Bosket silt loam	0.5	6.2	1.141	26.55	5310
Rosholt sandy loam	1.2	6.7	0.996	48.64	4054
Faulkbourne loam	2.2	7.0	1.054	148.4	6745
Panholes silt loam	1.9	8.1	0.947	27.51	1448
Sediment sandy clay loam	2.3	8.2	0.970	80.18	3486

(DP 82835)

d) A batch equilibrium adsorption/desorption study was conducted for RPA 200766 according to SETAC (1995) and EPA guidelines (Pesticide Assessment Guidelines Subdivision N, Series 163-1, 1982).

[^{14}C-U-phenyl]- RPA 200766 (>99%pure) in 0.01M calcium chloride (60 ml) was added to duplicate samples (20 g) of four soils and a sediment, at concentrations of 5, 1, 0.2 and 0.04 mg / litre for each soil. The remaining experimental procedures were as described at 7.2.1 b) above. The adsorption equilibrium time (confirmed in pre-test) was 72 hours. For desorption the equilibrium time was 2 hours. Adsorption Freundlich coefficients (K_f) and K_{oc} values for each soil, are given in Table 7.6. For Desorption K_{oc} values were 134-629 (ml/g). HPLC analysis showed 100% of the radioactivity in the supernatant was RPA 200766 after both adsorption and desorption equilibrium periods.

Table 7.6. Adsorption coefficients RPA 200766 to four soils and a sediment.

Soil type	% oc	pH	1/n	K_f (ml/g)	K_{oc} (ml/g)
Bosket silt loam	0.5	6.2	0.892	0.86	173
Rosholt sandy loam	1.2	6.7	0.909	2.25	188
Faulkbourne loam	2.2	7.0	0.937	3.9	177
Panholes silt loam	1.9	8.1	0.912	1.83	96
Sediment sandy clay loam	2.3	8.2	0.924	4.68	203

(DP 82838)

7.2.2 Column leaching

A column leaching/aged column leaching study with [^{14}C-phenyl] fipronil was performed to satisfy US EPA (subdivision N 163-1, 1982) guidelines. This study was evaluated previously in ACP 70 (260/98), p. 59. The results of the study are reproduced below in Tables 7.7 and 7.8.

Table 7.7. Recovery of radioactivity after leaching of un-aged soil samples with 998 ml 0.005M aqueous calcium chloride.

Soil type	% Applied radioactivity recovered in soil sections and leachate							
	Soil section (cm)						leachate	total
	0-6	6-12	12-18	18-24	24-30	30-36		
Manningtree sandy loam	52.21	34.04	7.42	0.99	1.09	1.00	4.26	101.0
Manningtree loam	96.18	1.31	0.34	0.27	0.21	0.17	0.32	98.8
Speyer 2.2 Loamy sand	98.47	0.91	1.14	0.04	0.13	0.13	0.28	100.1
French sandy clay loam 1	81.76	6.77	1.86	0.87	0.54	06	0.29	92.7
French sandy clay loam 2	87.63	6.11	0.7	0.31	0.2	0.01	0.98	95.9

Table 7.8. Recovery of radioactivity after leaching of aged soil samples (35 days at 22°C) with 998 ml 0.005M aqueous calcium chloride.

Soil type	% Applied radioactivity recovered in soil sections and leachate							
	Soil section (cm)						leachate	total
	0-6	6-12	12-18	18-24	24-30	30-36		
Manningtree sandy loam	56.12	27.72	6.87	1.47	1.48	1.19	3.28	98.1
Manningtree loam	90.6	5.42	0.87	0.68	0.67	0.53	0.59	99.4
Speyer 2.2 Loamy sand	92.95	3.56	0.95	0.3	0.04	0.05	0.35	98.2
French sandy clay loam 1	81.63	4.32	0.76	0.22	0.03	0.12	2.49	89.6
French sandy clay loam 2	84.31	6.53	3.94	0.7	0.17	0.26	0.99	95.5

It should be noted that characterisation of the acetonitrile extractable radioactivity in the 35-day aged soils prior to leaching identified that fipronil represented 94% of this extractable radioactivity, except for the Speyer 2.2 loamy sand, where it represented 100%. The aged column leaching study therefore provides little useful information on the leaching potential of soil metabolites. Quantitative analysis of the radioactivity in the leachate was not possible due to the low levels present. However GC-MS identified that fipronil, MB 46136, RPA 200766 and MB 45950 were present.

(DP 71137)

7.2.3 Summary

The properties of fipronil and its metabolites are summarised below:
Batch adsorption experiments on 5 soils (for each moiety) were carried out with a good range of soil pHs.

	octanol water partition coefficient (log $P_{o/w}$) at 20°C	water solubility (mg/l) at 20°C	Koc (ml/g)
Fipronil	4.00	1.9 (pH 5.7-6.1).	427-1248
MB45950	3.7	1.1 mg/l (pH independent)	1695-5621
MB 46136	3.8	0.16 mg/l (pH independent)	1448-5310
RPA 200766	-	>20mg/l[1] with 0.01% dimethyl formamide present (pH dependence unknown)	96-203
RPA 200761	-	>100 mg/l[2] (pH dependence unknown)	-

Column leaching and aged column leaching experiments (ageing period 35 days) provided similar results with 0.3-4.3 % AR being present in leachate. Quantification of the different components in the leachate was not possible due to the relatively low levels of radioactivity present. However the leachate was characterised as containing fipronil, MB 46136, RPA 200766 and MB 45950.

7.2.4 Assessment

Under the SSLRC mobility classification scheme, from the batch adsorption data submitted, fipronil would be classified as slightly mobile, with MB 45950 and MB 46136 classed as slightly mobile - non mobile. RPA 200766 would be classed as moderately mobile. No batch sorption data were submitted for the metabolite RPA 200761. However as this moiety is a carboxylic acid derivative of RPA 200766, it would be expected to be more mobile than RPA 200766. The laboratory column leaching data provide reassurance that under relatively extreme leaching conditions, fipronil exhibited low mobility. However the aged column leaching study does not provide any useful information on the leaching potential of soil metabolites as the soil applied to the top of the column contained 94 % of the radioactivity as fipronil. The level of metabolites present was therefore minimal. The Northern French soil dissipation study (Section 7.1.3.1) provides some additional evidence that fipronil and the major soil metabolite MB 46136 were retained in the top 30 cm of a field soil profile. The metabolite MB 45950 was retained in the top 20 cm. Some positive determinations were made for RPA 200766 in the 30-60 cm deep soil layer. This study does not provide any useful information on the mobility of RPA 200761, as this moiety was analysed for in a limited number of samples, none of which originated from a depth below 20cm. It should also be noted that the design of field dissipation studies is not ideal for assessing mobility. However, as the analytical methodologies used were very sensitive (the limit of quantification represents *ca.* 0.1-0.2% of the highest fipronil level measured shortly after application) some assurance is provided by these data.

[1] Evidence for solubility from ecotoxicological studies (DP114883, 114885, 114888)

[2] Evidence for solubility from ecotoxicological studies (DP112748, 112750, 112751)

For the proposed use on container-grown plants there is a potential concern for the exposure of surface water resulting from leaching of fipronil and its metabolites when containers are watered and from the leaching of composted spent growing media used as a mulch on horticultural land. The available data are considered sufficient to provide reassurance that fipronil and the potential soil metabolites MB 46136 and MB 45950 are unlikely to be present in water that drains from treated containers or leached from mulch. The position for the metabolites RPA 200766 and RPA 200761 is less clear. It is considered that there is a potential for these two metabolites to reach surface water from the proposed use. Concentrations would be expected to be low. Crude worst case estimates of PEC are presented in Section 7.5.

7.3 Predicted environmental concentrations in soil (PECs)

Fipronil

With an application rate of 1 g fipronil / m^3 compost, assuming a media density of 1.5 g / cm^3 the initial PEC in the growing media would be 0.67 mg fipronil /kg growing media.

Assuming a first order DT_{50} of 190 days (10°C 75% WHC) the PEC in treated media would be as set out below in Table 7.9

Table 7.9 PEC growing media for fipronil before adding to compost dump

Months after appl'n	PEC growing media (mg/kg)
0	0.67
3	0.48
18	0.11

The initial PEC in the compost dump (using the reasoning outlined at the beginning of Section 7) assuming mixing and 0.1% contains residues of 0.67 mg/kg (no ageing), 5% contains residues of 0.48 mg/kg (3 months ageing), 57.9% contains residues of 0.11mg/kg (18 months ageing) and 37% contains no residue, is 0.088 mg/kg. After a further 12 months ageing in the compost dump before use as a mulch, the predicted fipronil residue would be 0.013 mg/kg assuming a first order DT_{50} of 130 days (21°C 75% WHC).

Metabolites

Following the argumentation outlined in Section 7.1.5 using data from a field trial, assuming all material in a compost dump was treated, both the initial and long term PEC for MB 46136, MB 45950, and RPA 200766 would be 0.027 mg/kg for each metabolite. The level for RPA 200761 would be 0.007 mg/kg. Following addition of 37% untreated material to the dump the PEC would be 0.017 and 0.0044mg/kg respectively.

Conclusions

It is therefore predicted that soil organisms required for the composting process could be exposed to:

fipronil at a concentration of 0.088 mg/kg;
MB 46136, MB 45950, and RPA 200766 at a concentration of 0.017 mg/kg;
RPA 200761 at a concentration of 0.0044 mg/kg.

The predicted concentrations in the soil surface mulch after 12 months in a compost dump are:
fipronil at a concentration of 0.013 mg/kg
MB 46136, MB 45950, and RPA 200766 at a concentration of 0.017 mg/kg
RPA 200761 at a concentration of 0.0044 mg/kg

Soil would be cultivated before the planting of any subsequent crops. Assuming this cultivation mixed a 5cm depth of mulch with an underlying 15cm of soil (cultivation depth 20cm) the compost would be mixed and diluted with the soil in a ratio of 4.

The resulting predicted concentrations in the top 20cm of soil are:
fipronil at a concentration of 0.00325 mg/kg
MB 46136, MB 45950, and RPA 200766 at a concentration of 0.00425 mg/kg
RPA 200761 at a concentration of 0.0011 mg/kg.
The total fipronil derived residue potentially available for uptake by following edible crops is therefore 0.0171 mg/kg[3].

It is acknowledged that the volume of compost and mulch produced from the requested use is likely to be relatively small. However within individual holdings reasonably significant areas of land could be exposed.

The predicted initial concentrations in the media associated with a plant root ball if this was planted out intact into gardens after three months could be:

fipronil at a concentration of 0.48 mg/kg
MB 46136, MB 45950, and RPA 200766 at a concentration of 0.027 mg/kg
RPA 200761 at a concentration of 0.007 mg/kg

It is important to note that these concentrations would only be present in a very small area immediately surrounding the plant. As such this is not considered a significant source of environmental exposure.

7.4 Fate and behaviour in water (IIA 7.2.1, IIIA 9.2)

7.4.1 Aqueous hydrolysis

The hydrolytic stability of fipronil was studied according to EPA guidelines (Pesticide Assessment Guidelines, Subdivision N, Series 161-1, 1982).

[3] If the growing media was peat with a density of 0.402 g/cm^3 with the underlying soil having a density 1.5 g/cm^3 this concentration is calculated as the slightly higher value of 0.021 mg/kg. The use of this lower density for peat in risk assessments for container grown plants will be used by PSD for applications received after February 2002. This application predates this development to refine environmental exposure assessments for such horticultural uses.

Sterile aqueous buffer solutions (pH 5, 7 and 9) were prepared containing [^{14}C-phenyl]-fipronil (0.89 mg a.s./litre). Samples (100 ml) of each treated buffer were then incubated at 25°C in flasks fitted with traps for carbon dioxide and organic volatiles (containing Sodium hydroxide and ethylene glycol monomethyl ether). At 0, 5, 9, 14, 20, 26 and 30 days after application, single samples of each buffer were extracted with dichloromethane and analysed for fipronil and breakdown products by TLC and HPLC. The quantity and identity of the breakdown product was confirmed by NMR. Radioactivity in trapping solutions was quantified by LSC.

At pH 5 and 7, fipronil was stable to hydrolysis. At pH 9, the measured concentrations of fipronil were used to estimate the DT_{50} of 28 days (first order, r^2= 1). At this pH, RPA 200766 (amide analogue) was the only breakdown product accounting for 52% AR by day 30. Total recovery was >99% AR with this being accounted for by fipronil and RPA 200766. No volatile radioactivity was detected. RPA 200766 was therefore stable to further hydrolysis under the test conditions at pH 9.

(DP 70991)

7.4.2 Aqueous photolysis

a) The aqueous photolysis of fipronil was studied according to EPA guidelines. (Pesticide Assessment Guidelines, Subdivision N, Series 161-2, 1982).

A sterile aqueous buffer solution (pH5) containing 1% acetonitrile was prepared containing [^{14}C-phenyl]-fipronil at a concentration of 0.9 mg a.s./litre. Aliquots (10 ml) were incubated at 25°C in flasks fitted with traps for organic volatiles (ethylene glycol monomethyl ether) and carbon dioxide (KOH). Replicate flasks were either kept in the dark or exposed to continuous irradiation (a xenon arc lamp spectral cut-off at 290 nm; 464 W/m2 at 250-780 nm at the buffer surface) for up to 6 hours. The study authors related the intensity of the lamp as 11 hours ≡ 1 day of natural summer sunlight at 30°N (Florida USA). The natural summer Florida irradiation duration of the study was therefore approximately 0.545 days. Samples were taken at day 0 and at 4 representative time points. Radioactivity was quantified directly by LSC and characterised by direct HPLC measurement and HPLC and TLC following extraction with dichloromethane.

Total recovery was 99-103 % AR. After 6 hours, fipronil accounted for 32 and 100 % AR from illuminated and dark control samples respectively. The DT_{50} for photolytic degradation of fipronil was calculated as 3.6 hours (first order, r^2=0.99) under test conditions. This was stated by the study authors as equivalent to a DT_{50} of 0.33 days Florida summer sunlight. The major breakdown product MB 46513 was identified in irradiated samples at up to 43 % AR by 6 hours. The minor metabolite RPA 104615 was also identified (8.2 % AR by 6 hours). Two minor unidentified resolved components not extracted by dichloromethane accounted for 6 and 2 % AR. Volatile radioactivity accounted for less than 0.06 % AR.

(DP 71138)

b) The direct photochemical degradation of fipronil and its quantum yield was studied according to UBA test guideline (Phototransformation of chemicals in water PART A, Direct phototransformation, 1990).

Fipronil (99.4% pure) at a concentration of 1.32 x 10^{-5} mol/l in water was tested. The spectral absorption of fipronil was characterised by an absorption band at 292 nm ($\varepsilon \approx 5433$) in the overlap with the spectrum of sunlight. The study was performed with monochromatic light at 300 nm of a xenon arc lamp. The mercury lamp power was determined by chemical actinometry to be 2.02 x 10^{-9} Einsteins at this wavelength. The exposure time was 600 seconds. Fipronil concentrations before and after irradiation were determined by HPLC.

The photolysis assuming first order kinetics results in quantum yield of 0.199 at 300nm. The study authors equated this to a theoretical environmental half-life in the range 3 hours (June) - 99 hours (December) in the top mm of natural aquatic systems in central Europe at 52°N.

(DP 82840)

7.4.3 Ready biodegradation

Ready biodegradation of fipronil was studied according to OECD guidelines (No. 301/B, 1992). This study was evaluated previously in ACP 70 (260/98), p. 52.

The study showed that the toxicity control attained 42 % degradation after 28 d, confirming that under the conditions of the test fipronil was not toxic to the sewage micro-organisms. Fipronil attained 47 % mineralisation to carbon dioxide after 28 d. However, this falls below the OECD guideline threshold, as a result fipronil would be classified as not readily biodegradable.

(DP71140)

7.4.4 Water/sediment studies

Aerobic sediment/water studies were conducted according to SETAC (1995) guidelines.

Samples of untreated Roding river and Manningtree stream water (11 cm depth) and associated sandy clay loam and sandy loam sediments (5mm sieved, 4cm depth) were equilibrated in flasks (7.5 cm i.d.) for 28 days. Following equilibration, [^{14}C-phenyl] fipronil was added in acetonitrile to separate flasks (91.85 µg fipronil/flask ≡ 200 g a.s./ha). Treated flasks were purged with oxygen and fitted with traps for carbon dioxide (sodium hydroxide). All flasks were then incubated at 20°C in the dark for up to 121 days.

Duplicate flasks were analysed at day 0 and nine representative time points. Radioactivity in the water was either quantified directly by LSC or following concentration on a solid phase extraction cartridge and characterised by TLC and HPLC. Compounds were identified by comparison of chromatographic behaviour with certified standards with some further confirmation with LC-MS. Dissolved oxygen, redox potential and pH were also measured. Sediment was extracted sequentially with acetonitrile, then acetonitrile/water. Radioactivity in the extracts was quantified by LSC and characterised by TLC and HPLC. Compounds were identified by comparison of chromatographic behaviour with certified standards, with some further confirmation with LC-MS. Radioactivity in the extracted sediment was quantified by combustion/LSC.

For the Roding river and Manningtree stream the dissolved oxygen remained above ca 50% throughout the study and pH remained relatively constant (*ca.*8 and 7.5 respectively). Mean redox potential in water and sediment were 341 and 19.5 mV respectively.

Recoveries were 91 - 100% AR. The following dissipation rate estimates were made utilising HPLC analysis results. Decline in total extracted fipronil (water and sediment) had DT_{50} of 16 and 36 days (first order $r^2 = 0.99$ and 0.96) for Roding river and Manningtree stream respectively. Dissipation from the water phase gave fipronil DT_{50} of 16 and 14 days (first order $r^2 = 1$ and 0.96) respectively. In sediment, extracted fipronil peaked at 16 and 41 % AR after 7 and 14 days with the subsequent DT_{50} being 46 and 49 days (first order, $r^2 = 0.96$ and 0.88) respectively.

In the water phase the metabolites MB 45950, RPA 200766 and MB 46126 were identified, however none are considered major as each individually only represented up to 8.8% AR at all sampling times. Unidentified radioactivity in the water phase accounted for a maximum of <2% AR at all sampling times.

In sediment, MB 45950 accounted for *ca.* 80 % AR by the end of the experiment in both systems. RPA 200766 and MB 46126 were identified, however neither are considered major as each individually only represented up to 6.6% AR at all sampling times. None of the extracted sediment radioactivity was unidentified.

Radioactivity unextracted from the sediment reached a maximum of 2.3 and 3.3.% AR at study termination and accumulated [^{14}C]carbon dioxide was negligible (0.17 and 0.05 % AR) for Roding river and Manningtree stream respectively.

(DP 71143)

7.4.5 Aquatic dissipation in the field

No data was submitted on dissipation within aquatic systems in the field, but the applicant did provide information on irrigation practices on horticultural holdings to support the aquatic exposure assessment. This identified that with the irrigation systems commonly used in the UK, water runoff losses range from 7% of applied irrigation volume (ebb and flow irrigation systems) to 55-79% of applied irrigation volume (overhead irrigation systems). In these irrigation systems a proportion of this runoff water does not pass through the growing media in the pots and is therefore a source of dilution for the media leachate. It was also apparent that whilst water is a cost to ornamental horticultural growers and some growers recycle irrigation water for reuse, a high proportion of growers run the runoff water to waste, (the use of water recycling is far more prevalent in edible horticultural production systems). This runoff water is often received by soil, but in some operations the runoff water moves through drainage systems that can discharge directly to natural surface waters (usually ditches). This worst case situation does not occur on all holdings, but such a scenario is considered realistic.

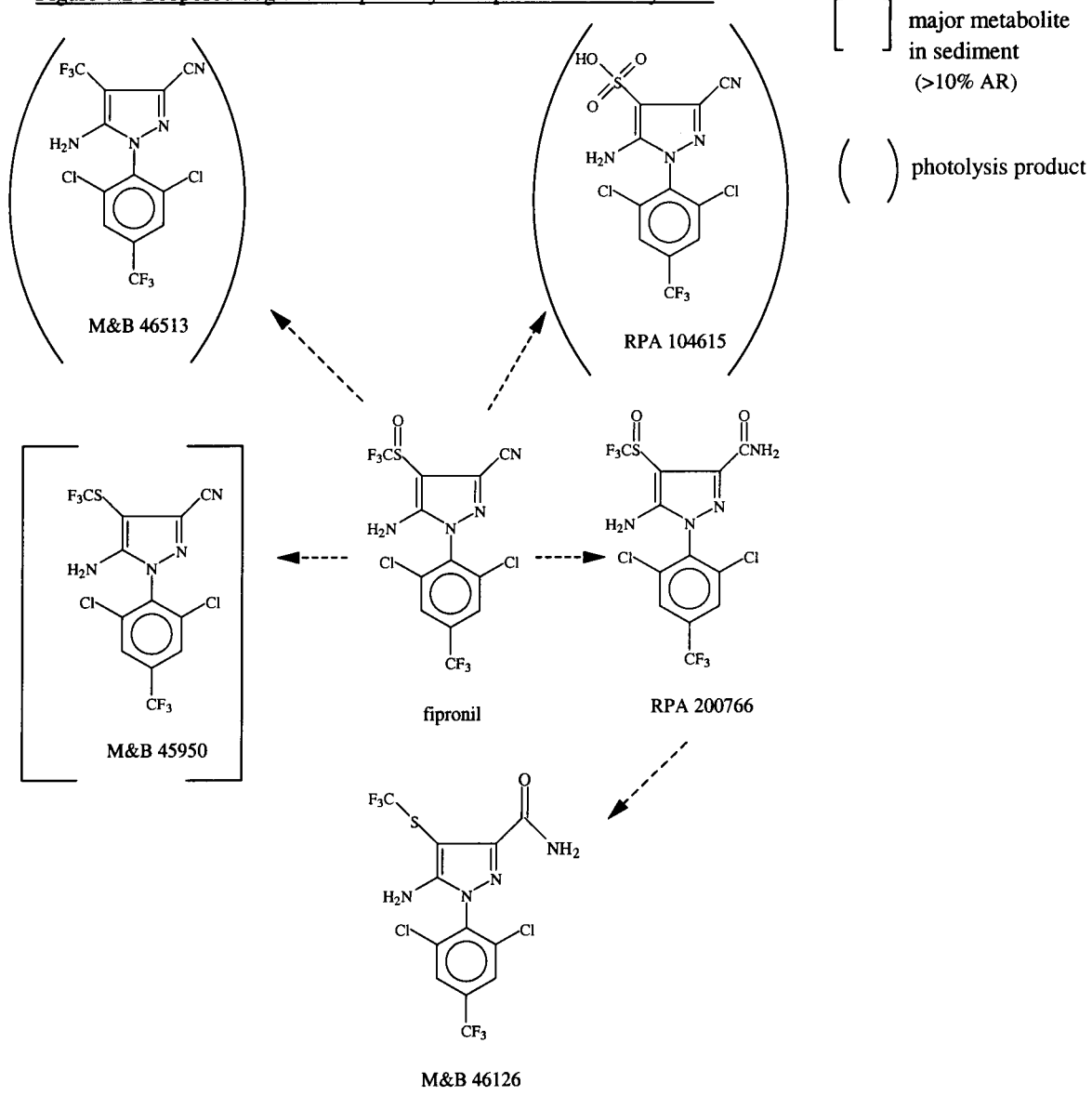
(DP112741, DP112742 and DP112744)

7.4.6 Summary

Fipronil was stable to hydrolysis (25°C) at pH 5 and 7. At pH 9 the DT_{50} (first order, r^2=1) was 28 days producing the amide moiety RPA 200766. Fipronil degraded in light-exposed water (short light path length) with at DT50 of 0.33 days Florida summer sunlight. The major photodegradate MB 46513 represented 43% AR at study termination. RPA 104615 was identified but represented 8.2% AR at study termination and is considered a minor photodegradate. The quantum yield of fipronil was estimated as 0.199. This was equated to a DT50 in the top mm of natural surface water of 3 hours (June)- 99 hours (December) in Northern Europe at 52°N.

In two natural sediment/water systems (dark laboratory measurement) DT_{50} for fipronil were up to 16 days (first order, r^2=0.99) for dissipation from the water phase. Following a peak concentration of 41% AR at 14 days a DT_{50} of 49 days (first order, r^2=0.88) for dissipation of extractable fipronil from the sediment was estimated. The metabolites MB 45950, RPA 200766 and MB 46126 were identified in water but none was considered major (all <8.8% AR at all sampling times). In sediment the metabolite MB 45950 had increased to represent 80% AR by the end of the experiment. Minor amounts (< 7% AR) of RPA 200766 and MB 46126 were also identified in the sediment. Dissipation via mineralisation to carbon dioxide and the formation of unextractable residues was minimal (<3.5% AR). The proposed degradation pathway for fipronil in water systems is outlined in Figure 7.2. From a ready biodegradation test with a sewage sludge inoculum fipronil was classified as 'not readily biodegradable'.

Figure 7.2 Proposed degradation pathway for fipronil in water systems



Information on irrigation and production practices on UK ornamental, horticultural holdings indicated that irrigation water leached through pots could in some situations find a fairly direct route through drainage systems to surface waters (ditches). The pot / media leachate would however be diluted by applied irrigation water that did not pass through the treated media.

7.4.7 Assessment

The fate and behaviour of the parent compound fipronil in water and sediment/water systems has been adequately addressed. The main potential route of exposure from the proposed use would be leaching from the containers when they are watered or there is heavy rainfall. There is, theoretically also some potential for leaching from the mulch after it is spread following composting. It is concluded in Section 7.2.4 that for fipronil and two of the main soil metabolites that leaching is unlikely as a result of their relatively high sorption to soil (results from batch soil adsorption studies, column leaching studies and field dissipation studies). However Section 7.2.4 concludes that the potential soil metabolite RPA 200766 is less well adsorbed. Also, whilst no data are available on RPA 200761 since it is an acid derivative of RPA 200766, it would be

expected to be more mobile than RPA 200766, though levels in growing media / soil would be low. The fate and behaviour in natural water systems of these two metabolites should they reach such systems cannot be elucidated from the available data where the parent compound has been studied.

7.5 Predicted environmental concentrations in groundwater and surface water (PECgw, PECsw) and sediment (PECsed)

Groundwater

Evidence from batch sorption studies, column leaching studies (fipronil only) and field dissipation studies indicate that the potential for leaching of fipronil and its metabolites MB 45950 and MB 46136 is low. Whilst the soil metabolite RPA 200766 appears more mobile (lower Koc and determined occasionally below 30cm soil depth in the field dissipation study) and RPA 200761 would be expected to be more mobile than RPA 200766, because of the limited extent of the proposed use (horticultural holdings growing containerised plants), it is considered that the potential for groundwater contamination is low.

Surface Water

It is considered that there is the potential for RPA 200766 and RPA 200761 to leach from the containers, through permeable membranes or from surface-mulched compost through underlying soil to drainage systems and then to surface waters such as ditches. Runoff from hard surfaces on which pots are standing with runoff water entering drainage systems is also possible. The applicant provided information, (see section 7.4.5) that confirmed these as potential routes of exposure to surface water. However it is accepted that surface water exposure would not occur on all holdings. The applicant proposed a framework for a PEC calculation to represent a worst case for the potential contamination of surface water using the following assumptions:

Taking a nominal area of treated compost of 100m x 50m x 20cm depth and assuming an irrigation of 5cm (any size area may be taken since the volume of water used will be proportional to the treated area). This gives a compost volume of $1x10^6$ litres with $1x10^6$ mg fipronil added. The water volume added would be 250,000 litres (equates to ca 8 irrigation events from an overhead spray line boom in September for glasshouse grown Poinsettia / bulrush. Actual irrigation volumes vary with evapotranspiration rates that are season / crop dependent).

(DP112741, DP112744)

If 0.7 % loss is assumed[4] this results in calculated worst case pot leachate concentrations of 21µg/l for RPA 200766 and 7.9µg/l for RPA 200761 if it is assumed 197,500 litres of water (79%) percolates out of the growing media

This calculation assumes RPA 200766 and RPA 200761 were present at a maximum of 57 and 21 % fipronil equivalents of the initial fipronil soil load ($1x10^6$ mg), of

[4] value in standard UK worst case drainflow calculation for compounds with a Koc range 75-499ml/g.

$5.97x10^5$ mg and $2.24x10^5$ mg respectively (includes a relative molecular weight factor). These percentages originate from soil laboratory studies (see Section 7.1.1.1). It is considered less appropriate to use data from the field dissipation study (as used for soil PEC) as the dissipation observed in this study already includes potential leaching losses, so using this approach theoretically results in an underestimate of the amount available for leaching. However, it should be noted that the pot leachate concentrations estimated are gross over-estimates, as the regular watering of pots will mean that the peak soil concentrations observed in the laboratory studies would never be reached within the pots.

Even if all pot leachate arrived directly to a surface water (from a hard surface / directly connected drainage system) there would be further dilution in any receiving water body. If a dilution factor of 1.3 is applied, (in line with standard UK 1st tier drainage assessments), to the pot leachate estimate, overestimated PECsw of values 16µg/l for RPA 200766 and 6µg/l for RPA 200761 are calculated.

This leaching assessment from irrigating pots is considered to represent a very worst case and is considered to encompass the situation of heavy rain falling on pots and the potential for leaching from composted growing media that has been disposed of by spreading on land followed by soil incorporation.

Sediment

As RPA 200766 and RPA 200761 will have water solubility's >20mg/litre and >100mg/l respectively (see 7.2.3), significant partitioning to sediment would not be expected.

7.6 Fate and behaviour in air (IIIA 7.2.2, IIIA 9.3)

No specific studies other than the physical/chemical properties measurements summarised in Section 2.1 have been submitted.

Summary

As identified in Section 2.1 fipronil has a distilled water solubility of 1.9 mg/l at 20°C (pH 5.7-6.1), an estimated vapour pressure of 3.7×10^{-7} Pa at 25°C or 1.6×10^{-7} Pa at 20°C and a Henry's Law constant of 3.7×10^{-5} $Pa.m^3.mol^{-1}$. The dimensionless Henry's Law coefficient of 1.49×10^{-8} has been calculated at 20°C. This indicates that fipronil has very low volatility and has the potential for only very slight volatility from aqueous solutions/soil water.

MB45950 has a pH independent water solubility of 1.1 mg/l at 20°C, an estimated vapour pressure of 2.3×10^{-6} Pa at 25°C or 9.3×10^{-7} Pa at 20°C and a Henry's Law constant of 3.56×10^{-4} $Pa.m^3.mol^{-1}$. The dimensionless Henry's Law coefficient of 1.5×10^{-10} has been calculated at 20°C. This indicates that MB45950 has very low volatility and has the potential for only very slight volatility from aqueous solutions/soil water.

MB46136 has a pH independent water solubility of 0.16 mg/l at 20°C, an estimated vapour pressure of 7.6×10^{-7} Pa at 25°C or 5.5×10^{-7} Pa at 20°C and a Henry's Law

constant of 1.56 x 10^{-3} Pa.m^3.mol^{-1}. The dimensionless Henry's Law coefficient of 6.4 x 10^{-10} has been calculated at 20°C. This indicates that MB46136 has very low volatility and has the potential for only very slight volatility from aqueous solutions/soil water.

Assessment

Sufficient information has been submitted to conclude that fipronil is unlikely to be present in air at any significant concentration.

7.7 Predicted environmental concentrations in air (PECa)

Concentrations in air are expected to be negligible.

7.8 Definition of the residue (IIA 7.3)

In soil under UK conditions where fipronil is incorporated, the major metabolites (>10% AR) should be regarded as, MB 46136, RPA 200766, RPA 200761 and MB 45950. Parent fipronil is also a major residue.

In regions with higher light intensities or for uses where granules were not incorporated into growing media, the photodegradation products MB 46513 and RPA 105615 may also be present.

Where fipronil is applied as a soil incorporated granule to pot grown plants the residues to which organisms could be exposed in water are RPA 200766 and RPA 200761.

For the proposed use in the UK (incorporation into media in which container-grown ornamentals will be planted) it is considered that the significant residues in growing media / soil are fipronil, MB 46136 and MB 45950. Whilst RPA 200766 and RPA 200761 will be present in media/soil and could theoretically be present in surface water, ecotoxicological (see section 8) and mammalian toxicological (see section 5.8.2.3.1 and 5.8.2.3.2) effects data indicate that these moieties should be considered not significant

7.9 Conclusions

The information submitted on environmental fate and behaviour is sufficient to support approval of 'Vi-Nil GR' for use on container-grown ornamentals. No further data are required.

8. ECOTOXICOLOGY

Background information and assumptions

'Vi-Nil GR' is a micro-granule insecticide containing fipronil (0.1% w/w) for incorporation into compost used for growing hardy ornamental nursery stock and non-edible ornamentals. The rate of use is 1 kg product/m^3 compost i.e. 1 g fipronil/m^3 compost. Assuming a media density of 1.5 g/cm^3 the initial concentration in the growing media would be 0.67 mg a.s./kg. When pots are planted out after 3 months, the concentration of fipronil is predicted to be 0.48 mg a.s./kg. Material may also be composted and then used as a mulch and incorporated into soil. The predicted concentration in soil after incorporation to a depth of 20 cm is 0.00325 mg a.s./kg. Full details of the assumptions behind these predicted environmental concentrations (PECs) are given in Section 7.3.

The granules are between 150 and 850 μm in size with a specific gravity of 1.79 g/ml. Assuming that the largest granule of 850 μm in diameter is spherical, it is likely to weigh 0.5755 mg (0.000 575 5 mg a.s./granule). The granule base is 94% cellulose complex. No information has been provided on whether or not the granules retain their integrity over time in the environment.

8.1 Effects on birds (IIA 8.1, IIIA 10.1)

8.1.1 Active substance

8.1.1.1 Acute oral toxicity (IIA 8.1.1)

The acute oral toxicity data for fipronil are summarised in Table 8.1. These data were previously evaluated by HSE in ACP 70 (260/98).

Table 8.1 Summary of acute oral avian toxicity data for fipronil

Species	Test substance, test duration (includes observation period)	Acute oral LD_{50} (mg a.s./kg)	Test guideline #	Year of study and DP number ACP 70 (260/98) reference is in brackets
Bobwhite quail *Colinus virginianus*	fipronil (96% purity) 21 day	11.3	EPA 71-1	1990 DP 71146 (p 74/75)
Mallard duck *Anas platyrhynchos*	fipronil (96% purity) 21 day	>2150	EPA 71-1	1990 DP 71147 (p 74/75)
Pheasant *Phasianus colchicus*	fipronil [1] 31 day	31	EPA 71-1	1991 (p 74/75)
Pigeon *Columba livia*	fipronil [1, 2]	Study report stated LD_{50} > 2000 mg/kg but non-emetic dose was 500 mg, so LD_{50} was >500 mg/kg	EPA 71-1	1991 (p 74/75)
House sparrow *Passer domestica*	fipronil [1] 14 day	1120	EPA 71-1	1991 (p 74/75)
Red legged partridge *Alectoris rufa*	fipronil [1] 34 day	34	EPA 71-1	1991 (p 74/75)

Tests conducted in accordance with GLP.
[1] Details of purity were not given in previous evaluation.
[2] Details of study length not given in previous evaluation.

8.1.1.2 Short-term dietary toxicity (IIA 8.1.2)

a) In a 22 day feeding study 14-day old bobwhite quail, *Colinus virginianus* were fed nominal concentrations of 4.9, 9.8, 19.5, 39, 156, 312 and 625 ppm active substance (purity >95%) for a period of 5 days followed by a 17 day recovery period. There were five vehicle control groups fed a diet containing pre-mix only and one group at each test concentration. Each group contained 10 birds undifferentiated by sex. Analysis of the diet confirmed the test concentrations to be within 71-91% of nominals with testing being undertaken at day 0.

Thirty-two mortalities occurred in the study. All ten subjects in the 156, 312 and 625 ppm treatment groups and 2 of 10 subjects in the 39 ppm treatment group died as a result of treatment. Clinical signs of toxicity were observed only in the 39 ppm test group and above. Signs observed included, lethargy, diarrhoea and anorexia. Total remission of all clinical signs was observed in the 8 survivors in the 39 ppm group by the end of day 6, one day into the recovery period. Gross pathological examinations of the thirty two birds that died and of twenty selected survivors at termination revealed

no abnormal pathological findings. The average bodyweight at test day 22 in the 39 ppm group was depressed (86 g) relative to the vehicle control (104 g). Average body weights on test day 22 for the 4.9, 9.8 and 19.5 ppm groups was comparable with the control. Anorexia was noted in the 39 and 156 ppm groups during the test period. Food consumption at concentrations below 39 ppm was comparable with the vehicle control.

The dietary LC50 of fipronil technical to the bobwhite quail was 48 ppm in the diet. The NOEC was 19.5 ppm in the diet.

The study was conducted in accordance with EPA 71-2, and in accordance with GLP.

(DP 71147)

b) In a 22 day feeding study 8-day old mallard duck *Anas platyrhynchos* were fed nominal concentrations of 39, 156, 312, 625, 1250, 2500 and 5000 ppm active substance (purity >95%) for a period of 5 days followed by a 17 day recovery period. There were five vehicle control groups fed a diet containing pre-mix only and one group at each test concentration. Each group contained 10 birds undifferentiated by sex. Analysis of the diet confirmed the test concentrations to be within 82.4 – 94.3% of nominals with testing being undertaken one day prior to the test period.

Two mortalities occurred in the study, one from the control group and the other from the top dose group. Clinical signs of toxicity noted in the 2500 and 5000 ppm treatment groups were: lethargy, anorexia and smallness of size in comparison with the controls. Total remission of clinical signs was achieved in all surviving animals by the end of the last treatment day (day 5). Gross pathological examination of the two birds that died and of thirty two selected survivors revealed a cloudy, pale yellow fluid in the yolk sac in the abdominal cavity and slightly pale kidneys in the bird which died after treatment. On test day 22 the body weights at 312 and 625 ppm was significantly lower than the vehicle control. However, this difference was not considered to be treatment related as no differences were observed at 1250, 2500 or 5000 ppm.

The dietary LC50 of fipronil technical to the mallard duck was >5000 ppm in the diet. The NOEC level was 1250 ppm in the diet.

The study was conducted in accordance with EPA 71-2, and in accordance with GLP.

(DP 71155)

8.1.1.3 Sub-chronic and reproductive toxicity (IIA 8.1.3)

a) A 142 day reproductive study was undertaken with the bobwhite quail (*Colinus virginianus*) using test concentrations of 0.2, 2 and 10 ppm of fipronil in acetone. This was evaluated in ACP 70 (268/98) p 75-76. No statistically significant effects on body weight or feed consumption were noted in the adult birds through out the treatment period. Small differences (increases or decreases) in mean body weights were considered to be random occurrences unrelated to fipronil. No statistical effects on egg shell thickness or egg quality were noted during the study. There was a significant

increase in the number of cracked eggs at 0.2 and 2 ppm, however there was no effect at 10 ppm and it was concluded that this effect was not treatment related. No statistically significant effects were noted on hatchlings to fertile eggs set, 14 day old survivors of chicks hatched during the study or feed consumption. Statistically significant effects in hatchling weight at day 1 and day 14 were noted and results are summarised in Table 8.2.

Table 8.2 Mean chick group weight at day 1 and day 14 for the different concentrations of fipronil

Parameter	Treatment regime			
	control	0.2 ppm	2 ppm	10 ppm
Mean group hatchling weight (g) at day 1, ±SD	6.9 ±0.8	7.3** ±0.9	6.7** ±0.8	6.8* ±0.7
Mean group hatchling weight (g) at day 14, ±SD	29.3 ±5.7	30.4* ±5.1	28.6 ±5.7	31.5** ±4.8

SD: standard deviation
*: statistically significant at 95% confidence level
**: statistically significant at 99% confidence level

At day 1 there was a significant increase in hatchling weight at 0.2 ppm and a significant reduction at both 2 and 10 ppm. The reduction in weight was 2.8% at 2 ppm and 1.4% at 10 ppm. At 14 days there was again a significant increase in weight at 2 ppm and a significant increase in weight at 10 ppm. There was no significant effect on weight at 2 ppm. The reductions in weight at day 1 were very small and were no longer apparent at day 14. It was therefore concluded that they were not of biological relevance and the NOEC for the study was concluded to be 10 ppm, the highest concentration tested. This is in line with that proposed in the study report where the variation in weight seen was attributed to normal biological variation.

The study was undertaken in accordance with GLP and EPA 71-4.

(DP 71156)

b) A 61 day reproductive study was undertaken with the mallard duck *Anas platyrhynchos* and evaluated in ACP 70 (260/98) p 76-77. There was no statistically significant treatment related effects on body weight or feed consumption in the parent birds (F_0) generation. Small differences (increases or decreases) in mean body weights were considered to be random occurrences unrelated to fipronil. There was no statistically significant effect on egg production, egg shell thickness, hatchability, or in hatchling body weight at day 1 and day 14. The NOEC was considered to be 1000 ppm, the highest concentration tested.

The study was undertaken in accordance with GLP and EPA 71-4.

(DP 71159)

8.1.2 Plant protection products

8.1.2.1 Palatability tests (IIIA 10.1.3)

The palatability of EXP 60166 (fipronil 2% w/w granular) was examined in a laboratory trial with bobwhite quail (*Colinus virginianus*). Adult birds with a body weight of 186-232 g were held for 14 days prior to exposure during which they received basal diet (HRC chick diet). This consisted of chick crumb which closely resembled test granules in particle size.

Birds were housed in pens with a floor space of 1.8 x 1.4 m with 10 birds (5 male and 5 female) per treatment group. Each pen contained three feed hoppers and a drinker. Ventilation fans were adjusted as necessary and controlled artificial lighting provided 10 hours continuous light and 14 hours darkness. The mean daily minimum and maximum temperature was 16 and 19 °C respectively with the mean relative humidity 78%. Water was available at all times.

Two replicates of 10 birds were given a choice of basal diet, diet containing 10% fipronil granules mixed in with the basal diet (i.e. 10% fipronil granules and 90% basal diet) and 100% fipronil granules. These different diets were placed separately in one of each of the three hoppers in the pens. The control group, also consisting of two replicates, were fed basal diet only. Birds were exposed for 14 days and were observed hourly during the first 7 hours of day 1 and then twice daily until the end of the study (day 21).

There were no mortalities and all birds remained in good health throughout the study. Feeding was observed from the basal diet food hoppers but was not observed from hoppers containing either 10 or 100% granules. Body weights of the birds remained fairly constant throughout the study with no treatment –related effects observed. Birds in the test treatment group consumed the same quantity of basal diet as the control group (105 g and 115 g/day respectively). An average consumption of 2 g/day was measured during the treatment period from the 10% fipronil granule food hopper and 1 g/day from the 100% fipronil granule hopper. These consumption values were approximate as there was the possibility of scattering of food and fipronil into wood shavings on the floor.

This study was undertaken in accordance with GLP using a protocol previously used by the testing organisation.

8.1.3 Information on the commercial nursery environment

8.1.3 1 Commercial nurseries

A case has been made by the applicant that commercial nurseries are unlikely to be environments which are 'bird or mammal friendly.' To maintain the necessary quality of plants required by the industry, programmes of weed and pest control are routinely undertaken. Therefore, there is unlikely to be food available to attract birds. In addition, there is likely to be regular activity going on, reducing the attractiveness to

birds. For these reasons the applicant argues that there are unlikely to be birds in the nursery and horticultural environment. The same argument is also made for mammals.

A case is also made that the nature of the horticultural environment is such that a number of other sources of grit are likely to be available. Sand, fertiliser granules and grit may be mixed with the compost. Therefore there are likely to a range of grit sources available and that birds are unlikely to be reliant solely on fipronil granules. Information has also been provided that when plants are transferred into larger pots e.g. 18 cm, then the plant core itself accounts for a minimum of 1/3 of the pot.

(ACP168/4 (274/00))

8.1.3.2 Amount of granules on the pot surface

Information has been provided indicating that the average number of granules on the surface of an 18 cm pot surface was 41 (equivalent to 1611 granules/m^2).

(ACP168/4 (274/00))

8.1.4 Risk to birds

Acute and short-term dietary risk

The acute and dietary toxicity data for fipronil are summarised in Table 8.3. These studies were undertaken to GLP and were considered to be acceptable for use in the risk assessment.

Table 8.3 Summary of the acute and dietary effects of technical fipronil on birds

Species	Acute oral LD_{50} (mg/kg)	Dietary LC_{50} (ppm)	Dietary NOEC (ppm)
Bobwhite quail	**11.3**	**48**	**19.5**
Mallard duck	>2150	>5000	1250
Pheasant	31	-	-
Pigeon	>500*	-	-
House sparrow	1120	-	-
Red legged partridge	34	-	-

End points in bold are used in the risk assessment.
*Based on the non-emetic dose.

These data show fipronil to be of high acute and dietary toxicity to certain birds, with a large amount of variation between bird species. Where appropriate the risk assessment has been based on a consideration of the risk to both galliform and non-galliform birds.

Fipronil is used in compost for growing ornamental shrubs and non-edible ornamental plants. Birds may be exposed to fipronil via the following routes of exposure;

i) Consumption of granules (as food or grit)
ii) Consumption of contaminated insects
iii) Consumption of contaminated earthworms

iv) Consumption of contaminated foliage

i) Consumption of granules

Quantities of grit the size of 'Vi-Nil GR' can be found in the gizzards of birds (de Leeuw et al., 1995). The authors of this report state that it would be impossible for birds to selectively pick up granules smaller than 0.5 mm. However, approximately 87% of the granules were 0.5 mm or above in size (Section 2.2.14) and so it is possible that 'Vi-Nil GR' may be taken by birds as grit. Therefore it is necessary to assess the risk from this source of exposure. Granules are 0.15 to 0.85 mm in diameter (Section 2.1.14); the weight of the largest granule is 0.5755 mg product (0.0005755 mg a.s./granule).

No actual information has been provided to indicate whether birds may actually use granules composed of cellulose as a grit or food source. In the absence of such information the risk from this possible source of exposure has been considered. It has also been assumed, as a worse case, that birds may peck in flower pots to obtain food or grit and that it is therefore necessary to consider this source of exposure. The worst-case scenario for birds is exposure to the initial PEC of 0.67 mg a.s./kg in pots. This is the concentration of active substance immediately after mixing with the compost (Section 7.3).

Information has been provided which indicates that on average 41 granules were found on the surface of an 18 cm pot. This equates to 1611 granules/m^2. In addition, the applicant highlighted that when plants are potted up, at least one third of the pot will consist of the plant itself and associated core.

It is usual to base the risk assessment on the worst-case scenario, using the lowest toxicity end point from the bird studies. However, in view of the distinct difference in toxicological end points between galliform (an order of heavy bodied land birds, e.g., partridges, grouse, quail) and non-galliform birds, the applicant has argued that this approach is inappropriate in this instance. They consider that non-galliform birds are likely to be exposed in nurseries and therefore the risk assessment should be undertaken using this toxicity end point. However, it was considered appropriate for the risk assessment to actually be undertaken using both these end points i.e. for both galliform and non-galliform birds.

An example of a small bird which may consume granules is the tree sparrow (*Passer montanus*) weighing 22 g (Buxton & Crocker 1996). Calculating the LD50 for the house sparrow using the lowest LD50 of >500 mg a.s./kg for the pigeon (a non-galliform bird) and assuming a weight of 22 g (see previous assessment), it would have to consume >11 mg a.s. to obtain a median lethal dose. Each granule contains a maximum of 0.0005755 mg a.s. and so >19 114 granules are needed to obtain a median lethal dose. When an uncertainty factor of 10 is included, to cover variability between the laboratory and field, this equates to >1911 granules. Information on granule distribution indicates that there are 1611 granules/m^2. Therefore, a bird would need to eat all the granules from an area >1.2 m^2 to obtain a median lethal dose. Assuming an 18 cm pot, this equates to a total of >47 pots. Less than two-thirds of the pot will consist of fresh compost (see above) and therefore the number is further increased to

>70 pots. This illustrates that consumption from a very large area would be necessary to obtain a median lethal dose.

If, as a worst-case, this calculation is undertaken using the lowest acute LD50 of 11.3 mg a.s./kg for the bobwhite quail (a galliform bird) a 22 g bird would have to consume approximately 0.2846 mg fipronil in order to receive a median lethal dose. Using the above information on the number of granules required to provide the median lethal dose for a sparrow can be estimated as 431. Including a 10 fold uncertainty factor, 43 granules are required for a sparrow to obtain a median lethal dose. This equates to an area of 0.027 m^2 which is just over one 18 cm pot. Since less than two thirds of the pot will consist of fresh compost the number of pots is further increased to approximately 1.6 pots.

The applicant claims that the use pattern and application method of incorporation of 'Vi-Nil GR' granules into ornamentals, means that the granules will not be readily available and will not constitute a primary source of grit for birds. Information has been provided which indicate that only a low number of granules will be present on the pot surface. Also a case is made that other sources of grit will be available in this environment.

The palatability study submitted was not considered to be appropriate for use in any refinement of the risk assessment (see below). However, as indicated above the risk to birds has aleady been addressed and these data were not necessary. For completeness the deficiencies in this study are outlined below. *Birds were given three choices; untreated feed, feed containing 10% fipronil granules and 100% fipronil granules. This study showed that when offered a choice birds ate only relatively low amounts of fipronil. However, there are a number of problems with this study. The method of presentation of the granules was not comparable with the field situation. Also during actual use all the pots in an area may have been treated and so birds may not have the opportunity to select food from treated and untreated sources. In addition the concentration tested was 10% of the basal diet, which is approximately equivalent to 0.2 g a.s./kg. However, use of fipronil is recommended at a concentration of 1 g a.s./m^3 compost. Assuming a compost density of 1.5 g/cm^3 this is equivalent to 0.00067 g a.s./kg (Section 7.3). This is the initial figure after compost incorporation. Therefore the dose tested was greatly in excess of the recommended use and any avoidance effects may have been greater. In addition the tests were not undertaken with the actual granule formulation for which approval is sought.*

Conclusion

Birds would have to seek granules from a number of pots in order to obtain a median lethal dose. Even when the worst-case data are used, granules would need to be taken from more than one pot. The nature of the horticultural environment, the fact that alternative grit sources are available and the activity going on, indicate that exposure is likely to be limited. Combining the information on exposure and the number of pots required, it is considered that the risk to birds is acceptable. To support this case, the full report on granule distribution must be submitted to PSD for full approval.

Data requirement

For full approval, the full report containing information on the average distribution of granules on the surface of an 18 cm pot must be submitted to PSD.

ii) Consumption of contaminated insects

Insects may be contaminated via exposure to granules on the soil surface. However, it is considered that the risk to birds from this source of exposure is likely to be considerably less than from the direct consumption of granules or earthworms. This is because insects are most likely to be exposed to granules on the surface of the pot or through the translocation of fipronil in the plant. Therefore this source of exposure is not considered to require further assessment.

iii) Consumption of contaminated earthworms

There is no recognised method for estimating the amount of residue in earthworms following the application of granules. As an extreme worse case, in order to undertake an assessment of the risk, it is assumed that earthworms will, on average, be exposed to the initial PEC of 0.67 mg a.s./kg i.e. this is the PEC in the growing media before addition to the compost. This is considered to be an extreme worst-case as it is unlikely that earthworms will be present in the actual growing media itself. Earthworms in agricultural fields are likely to be exposed to a much lower concentrations of fipronil i.e. 0.00325 mg a.s./kg (Section 7.3).

An example of an earthworm eating bird is an 89 g song thrush (*Turdus philomelus*) with a total daily food intake of 22 g of food per day (Kenaga 1973). The compost PEC is 0.67 mg a.s./kg. Assuming per 100 mg of earthworm there is 30 mg of contaminated soil, the concentration is 0.02 µg a.s./100 mg of worm (0.2 mg a.s./kg worm).

The toxicity exposure ratios (TERs) for the acute and dietary risk via this route of exposure are shown in Table 8.4. The TERs for both the acute and dietary risk from the consumption of contaminated earthworms are greater than the relevant Annex VI trigger of 10 and so the risk is acceptable even with the worse case exposure scenario having been used.

Table 8.4 Acute and dietary avian TERs for fipronil

Category	Time scale	Toxicity end point[1]	ETE* a.s.	TER	Annex VI trigger
Earthworm-eating bird (89 g)	Acute	LD_{50}: 1.0 mg/bird	0.0044 mg/bird	227	10
	Dietary	LC_{50}: 48 ppm	0.2 ppm	240	10

LD_{50} based on the estimated median lethal dose for the bobwhite quail of 11.3 mg a.s./kg and LC_{50} of 48 ppm.
* Estimated Theoretical Exposure

iv) Consumption of contaminated foliage

It is considered unlikely that birds will eat ornamental foliage. In addition it is considered that the risk from the consumption of foliage is likely to be less than from the direct consumption of granules or earthworms. It is therefore not considered necessary to address the risk from the consumption of foliage further.

Reproductive risk

In the two reproductive studies submitted the NOECs obtained were 10 ppm for the bobwhite quail and 1000 ppm for the mallard duck. In both instances the NOEC was the highest concentration tested and was based on parental toxicity. The high toxicity of fipronil to the bobwhite quail meant that it was not possible to test a higher concentration, since the LC50 was 48 ppm and the NOEC was 19.5 ppm. This indicates that the main concern is parental survival rather than reproduction. The worst case scenario of exposure of birds from the direct consumption of granules has already been considered (see above).

The ETE for contaminated earthworms was 0.2 ppm a.s. and the reproductive NOEC for bobwhite quail was 10 ppm. Therefore the long term TER is 50 which is above the Annex VI trigger of 5. Hence the reproductive risk from the consumption of earthworms is acceptable. Therefore the reproductive risk to birds has been addressed and is acceptable.

Risk to birds from metabolites of fipronil

The main risk is from exposure to the active substance in the granules and this has been addressed. The risk assessment for earthworms also indicates that the TERs for the active substance are considerably greater than the trigger values. It is therefore considered that the risk from any metabolites is covered by the risk assessment for the active substance.

Conclusion

The risk to birds from both fipronil and its metabolites is considered to be acceptable.

Data requirements

i) For full approval, the full report containing information on the average distribution of granules on the surface of an 18 cm pot must be submitted to PSD.

8.2 Effects on aquatic organisms (IIA 8.2, IIIA 10.2)

8.2.1 Acute toxicity of active substance and its metabolites

The acute toxicity data for fipronil are summarised in Table 8.5 (overleaf).

Table 8.5 The acute toxicity of fipronil to aquatic life

Species	Test substance, test duration	Actual conc'n (as % of nominal)	LC/EC50 µg/l (nominal conc'ns)	NOEC µg/l	Test guideline #	Reference: DP number ACP 70 (260/98) reference is in brackets
a) Fish (IIA 8.2.1)						
Oncorhynchus mykiss (rainbow trout)	Fipronil (purity 100%) 96 h flow through	81-91%	248[1]	33.8[1]	EPA 72-1	DP 71164 (p 70)
Lepomis macrochirus (bluegill sunfish)	Fipronil (purity 100%) 96 h flow through	94-112%	85.2[1]	43.2[1]	EPA 72-1	DP 71166 (p70-71)
Cyprinus carpio (common carp)	Fipronil [2] 96 h flow through	[3]	430	73	OECD 203	(p 71-72)
Cyprinodon variegatus (sheepshead minnow) [4]	Fipronil [2] 96 h flow through	[3]	130	Not established	EPA 72-3	(p 73)
b) Invertebrates (IIA 8.2.4)						
Daphnia magna	Fipronil (purity 100%) 48 h flow through	74-104%	190[1]	52[1]	EPA 72-2	DP 71184 (p67-68)
Daphnia magna	Fipronil (purity 100%) 96 h flow through	80-90%	12.9	3.98	EPA 72-2	DP101878
Crassostrea virginica (eastern oyster) [4]	Fipronil [2] 96 h flow through	[3]	770[5]	590	EPA 72-3	(p 68-69)
Mysidopsis bahia (mysid shrimp) [4]	Fipronil (purity 96.1%) 96 h static	82-101%	0.14[1]	0.062[1]	EPA 72-3	(p69)
c) Algae (IIA 8.2.6)						
Scenedesmus subspicatus	Fipronil (purity 97%) 96 h static	89-141%	E_bC_{50} : 68 E_rC_{50} : 74[4]	40	OECD 201	DP 71197 (p63-64)

All tests undertaken in accordance with GLP.
[1] Results expressed as mean measured concentrations.
[2] Details of purity were not given in previous evaluation.
[3] Details of actual or measured concentrations were not given in previous evaluation.
[4] Marine/estuarine species.
[5] End point based on reduction in shell growth compared with the untreated control.

8.2.2 Metabolites and degradation products

Acute toxicity data were provided for the metabolites RPA 200761 and RPA 200766 and the results are summarised in Table 8.5.1 and Table 8.5.2.

Table 8.5.1 Acute toxicity data for the metabolites RPA 200761

Species	Test substance, test duration	Actual conc'n (as % of nominal)	LC/EC50 µg/l (nominal conc'ns)	NOEC µg/l	Test guideline #	Reference: DP number
a) Fish (IIA 8.2.1)						
Oncorhynchus mykiss (rainbow trout)	RPA 200761 (94.5%) 96hr semi-static	102-105%	96hr: >100 000	100 000	OECD 203	DP112751
b) Invertebrates (IIA 8.2.4)						
Daphnia magna	RPA 200761 (94.5%) 48hr static	97-103%	48hr: >100 000	100 000	OECD 202	DP112750
c) Algae (IIA 8.2.6)						
Scenedesmus subspicatus	RPA 200761 (94.5%) 72 hr static	89-95.2%	72 hr EbC50: >100 000 0-72hr ErC50: >100 000	$	OECD 201	DP112748

All tests undertaken in accordance with GLP.
$ Statistical analysis of the area under the curve showed significant differences between the control and 100 mg/l treatment. However, in terms of growth rate (0-72hr) there were no effects and cell densities were similar for the control and 100 mg/l treatment. Due to the effect on the area under the growth curve a clear NOEC could not be defined.

Table 8.5.2 Acute toxicity data for the metabolites RPA 200766

Species	Test substance, test duration	Actual conc'n (as % of nominal)	LC/EC50 µg/l (nominal conc'ns)	NOEC µg/l	Test guideline #	Reference: DP number
a) Fish (IIA 8.2.1)						
Oncorhynchus mykiss (rainbow trout	RPA 200766 (purity 99.8%) in dimethyl formamide. 96 hr static renewal	0-48hr: 77-100% 96hr: 61-69%	>17 000 [1]	7 900 [1]	OECD203	DP114883
Oncorhynchus mykiss (rainbow trout)	RPA 200766 (purity not stated – dimethyl sulfoxide solvent) 96 hr static	Not stated	96hr: >20 000	<0.00019	Not to GLP. No guideline given.	DP112752
b) Invertebrates (IIA 8.2.4)						

Daphnia magna	RPA 200766 (purity 99.8%) in dimethyl formamide. 48 hr static	87-100%	48hr: > 20 000	2 400	OECD 202	DP114885
Daphnia magna	RPA 200766 (purity not stated – dimethyl sulfoxide solvent) 48 hr static	Not stated	48hr: >20 000	2 000	Not to GLP. No guideline given.	DP112754
c) Algae (IIA 8.2.6)						
Scenedesmus subspicatus	RPA 200766 (purity 99.8%) in dimethyl formamide. 96 hr static	37-52%	96hr EC50: >7 500[1,2] 72hr EbC50: > 7 500[1] 72hr ErC50: > 7 500[1]	7 500[1] for cell density and biomass. 72hr growth rate NOEC: 2 300[1]*	OECD 201	DP114888

All tests undertaken in accordance with GLP unless otherwise stated.

[1] Results expressed as mean measured concentrations.

[2] It should be noted that the maximum nominal concentration was 20 000 µg/l at which the mean measured concentration was 7 500 µg/l. The 0 hour measured concentration of 20 mg/l solution , 10 mg/l was believed to represent the functional solubility limit of RPA 200766 in the algal medium.

* It should be noted that at the higher concentrations the percentage inhibition was <10% which was considered not to be biologically relevant.

8.2.3 Effects on algal growth (IIA 8.2.6)

In addition to the data summarised in Table 8.5, three separate 120 h toxicity tests on algae were evaluated in ACP 70 (260/98) p 64-65. Studies were undertaken using EPA guidelines 122-2, 123-2 and in accordance with GLP. There was no significant effect on growth at the concentrations tested, the results obtained are summarised in Table 8.6.

Table 8.6 Algal cell counts at termination of tests with fipronil (120 h) compared to the control.

Algal species	Measured concentration of fipronil (mg a.s./l)	Cell counts (x 10^4)		
		Control	Solvent control	Treatment
Navicula pelliculosa	0.12	88	70	77
Anabaena flos-aquae	0.17	112	112	120
Selenastrum capricornatum	0.14	147	141	148

8.2.4 Effects on higher aquatic plants (IIA 8.2.8)

Effects of fipronil on *Lemna gibba* were evaluated in a 14 day study in ACP 70 (260/98) p 66. At a mean measured concentration of 0.16 mg a.s./l there was an average of 382 fronds/replicate compared with 414 fronds/replicate in the control. This represented a 7.7% decrease in growth. There was no statistically significant effect on frond biomass.

Hazard classification

Classification of the active substance for environmental effects according to 67/548/EEC (included for information):

The lowest acute toxicity value for a fresh water species was an LC50 of 0.012 mg a.s./l for *Daphnia magna*. Fipronil was not readily biodegradable and has a log Kow of >3.0. Therefore the active substance should be classified as 'Very toxic to aquatic organisms' (R50) and 'May cause long term adverse effects in the aquatic environment' (R53). On the basis of being allocated the R50/R53 phrases, fipronil is considered 'Dangerous for the environment' and should carry the 'N' symbol on the active substance label. It should also carry the following safety phrase:

S61: Avoid release into the environment. Refer to special instructions/Safety data sheet.

Plant protection products:

Currently there is no guidance in the EC as to the classification of pesticides in terms of their acute hazard to aquatic life. Therefore the acute hazard classification for the product has been based on current UK guidance. No data were provided on the actual product itself. 'Vi-Nil GR' is a granular formulation containing 0.1% w/w fipronil. The formulation would not be expected to be of a greater proportional toxicity than the active substance itself. On this basis that the LC50 for *Daphnia magna* 12 mg formulation/l i.e. between 1 and 100 mg/l and 'Vi-Nil GR' should be labelled as 'Harmful to fish and other aquatic life. Do not contaminate surface waters or ditches...'

8.2.5 Chronic toxicity

8.2.5.1 Chronic toxicity to fish (IIA 8.2.2, IIIA 10.2.4)

A 60-day post hatch study was undertaken under flow-through conditions with the rainbow trout (*Oncorhynchus mykiss*) and evaluated in ACP 70 (260/98) pp 72-73. Duplicate samples of 100 fertilised eggs were exposed to mean measured concentrations of fipronil in acetone of 2.6, 6.6, 15, 26 and 60 µg a.s./l. Embryo viability (92-97%) and survival (97-100%) at the completion of hatch was comparable with the pooled control (94 and 98% respectively) for all concentrations of fipronil.

At test termination 100% mortality was observed in the 60 µg a.s./l group and 78% mortality in the 26 µg/l group. These values were statistically significant from the

pooled control (2% mortality). Survival ranging from 93-98% was recorded among larvae exposed to the remaining test concentrations (15-2.6 µg a.s./l). Several fish at the 26 µg a.s./l level were observed to exhibit sub-lethal effects (loss of equilibrium) during the exposure period. No sub-lethal effects were observed in any of the other surviving fish.

Growth data for the two highest concentrations were excluded from the analysis for growth effects. The mean total length of larvae at both the 15 and 2.6 µg a.s./l was 58 mm and was significantly different from the pooled control (60 mm). There was no statistical difference in total length at 6.6 µg a.s./l (total length 60mm). There was no statistically significant effect on mean wet weight at the concentrations analysed i.e. 2.6 to 15 µg a.s./l. The mean wet weights at 6.6 and 15 µg a.s./l were both 2.1 g. The report argued that the effect on larval length was not considered to be biologically significant due to the absence of a dose-response relationship and since there was no statistically significant effect on larval weight. It was therefore concluded that the NOEC was 15 µg a.s./l. However, the applicant's own evaluation of these data concluded that the NOEC was in fact 6.6 µg a.s./l based on effects on larval length at higher concentrations. It was concluded that due to effects on larval length at concentrations above 6.6 µg a.s./l that this should be used as the NOEC for this study.

8.2.5.2 Bioconcentration in fish (IIA 8.2.3, IIIA 10.2.3)

a) A dynamic 49-day bio-accumulation study in bluegill sunfish (*Lepomis macrochirus*) with radiolabelled [^{14}C] fipronil was evaluated in ACP 70 (260/98) p 61-62. The 158 test fish were exposed to radiolabelled fipronil at a mean measured concentration of 765 ng a.s./l (nominal concentration 850 ng a.s./l) under flow through conditions for 35 days followed by transfer to clean water for 14 days. The results are summarised in Table 8.7. An apparent steady state period was observed in the whole fish within 14 days after initial exposure. Highest concentrations were observed at 35 days after initial exposure.

Table 8.7 Summary of results for fipronil from bio-accumulation study

Parameter	Edible	Non-edible	Whole fish
Apparent steady-state BCF (14-35 days)	164	575	321
Concentration in fish tissue at steady state (ng/g fresh weight)	139	489	273
Concentration in tissue at 35 days (ng/g fresh weight)	132	524	315
Depuration phase at 7 days (ng/g fresh weight)	22	32	32
Depuration phase at 14 days (ng/g fresh weight)	4	15	1

During the depuration phase the concentration of total radioactivity in edible, non-edible and whole fish tissue was decreased by 83, 94 and 90% during 7 days. After 14 days depuration the values were 96, 97 and 99% respectively in these tissues.

This study was undertaken in accordance with GLP and EPA 165-4.

(DP 71179)

b) The various fractions from the above bluegill sunfish bio-accumulation study were subject to extraction procedures to isolate the radiolabelled components in them. These analyses showed that absorbed fipronil was metabolised to MB 46136, MB 45950 and MB 45897. The percentages of each proposed fish metabolite are summarised in Table 8.8.

Table 8.8 Percentages of the proposed metabolites in the fish fractions (% total radioactivity recovered)

	Pooled uptake fraction			Pooled depuration fraction		
Proposed metabolite	Edible (%)	Inedible (%)	Whole (%)	Edible (%)	Inedible (%)	Whole (%)
MB 45950	9.04	9.03	8.55	15.49	16.33 (8.33)	11.21
MB 46030 (fipronil)	17.49	16.34	23.75	10.98	24.50 (12.50)	11.35
MB 45897	14.16	22.92	24.28	31.97	47.87 (24.43)	26.14
MB 46136	54.90	59.07	27.98	47.96	101.9 (52.01)	43.80

Depuration values for the inedible fraction are presented as found and as normalised (brackets) to take into account an inexplicable over-accountability.

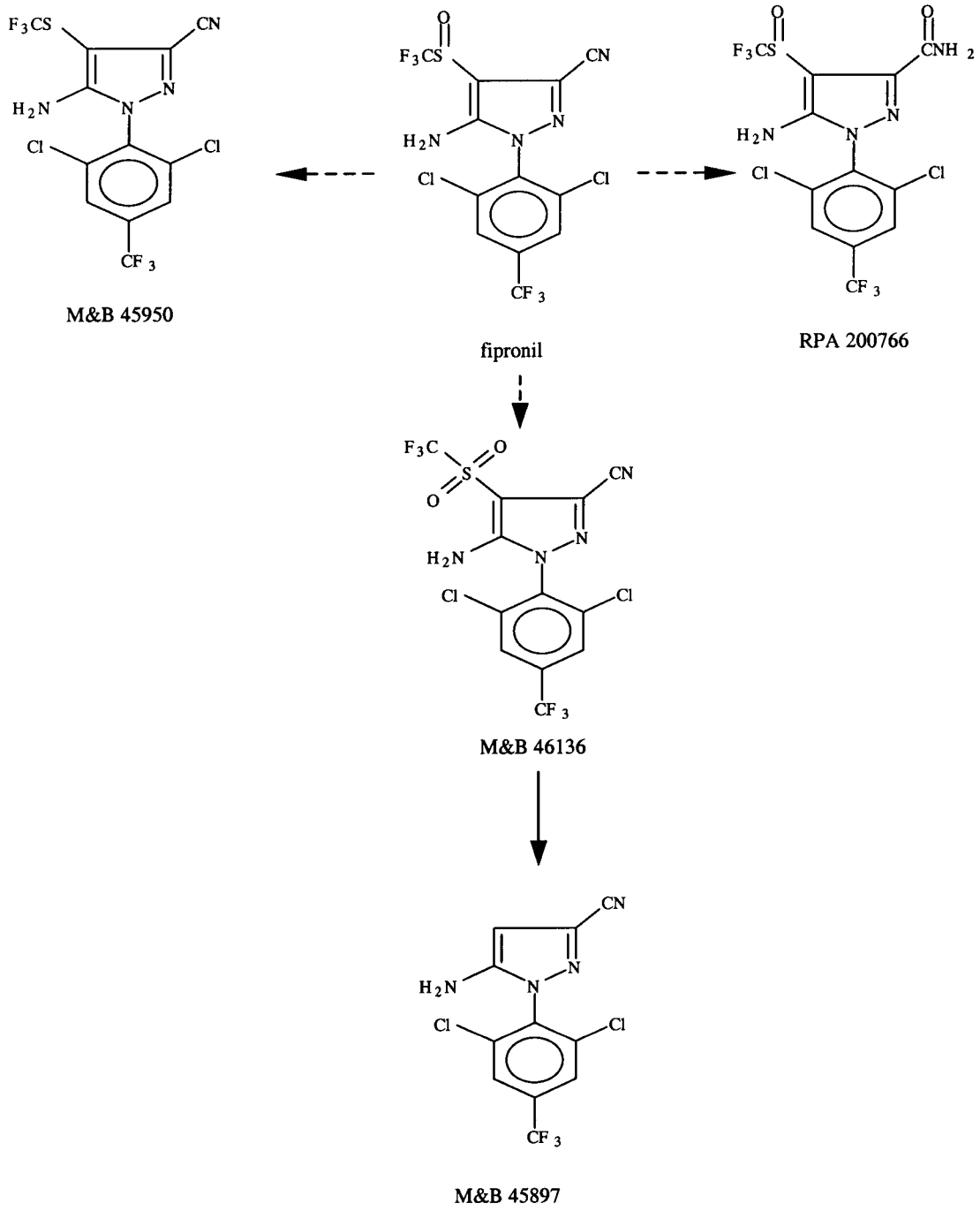
The major metabolite during the steady-state was MB 46136 with the amide RPA 200766 present in much smaller amounts (actual amount not stated) . The amounts of these metabolites in the inedible (lipophilic) fraction exceeded that in the edible fraction by a factor of 3 to 4. These finding were stated to be consistent with the octanol/water partition coefficient of the compounds, although the partition coefficients were not presented. Analysis of the samples from the early stage (days 1-3) of the depuration phase showed the same compounds to be present as when approximately 90% of the total radioactivity had been eliminated i.e. at day 7. The results suggest that no one metabolite was preferentially eliminated. The proportion of the metabolites in the edible and inedible fractions were found, in the initial stages of the depuration phase, to be approximately the same as that in the uptake phase but these ratios decreased to 1.5 by day 7. This was consistent with the reasonably rapid elimination of the compounds from both the edible and inedible fractions.

The compounds MB 46513 and RPA 104615, generally accepted as being photodegradates were not detected in any of the samples analysed. A metabolic pathway for fipronil was proposed on the basis of this information and is shown in Figure 8.1.

This study was undertaken in accordance with GLP.

(DP 71181)

Figure 8.1 Proposed metabolic pathway in the Bluegill Sunfish



8.2.5.3 Chronic toxicity to aquatic invertebrates (IIA 8.2.5, IIIA 10.2.4)

In a 21-day reproduction test under flow-through conditions *Daphnia magna* were exposed to nominal concentrations of 6.3, 13, 25, 50 and 100 µg/l fipronil (100% purity). There were 10 replicates (1 daphnid/vessel) for the control, solvent control and each test concentration. Adult survival and offspring production was measured on

days 1, 2, 4 and three times per week from day 7 to 21. Mean measured concentrations were 5.0, 9.8, 20, 34 and 79 µg a.s./l and results were based on these.

During the final six days of the test there was 50% mortality in the water control. Survival was 98% in the solvent control and the decrease in survival was determined to be due to a condition which affected the water control only. Statistical comparisons were therefore made to the solvent control.

At day 21 survival at 79 and 34 µg a.s./l was 0 and 63% respectively. Survival at 20 µg a.s./l and below was between 90-98% and was not significantly different from the control. At 20 µg a.s./l and below reproduction was comparable with the control (no statistical difference). At 20 µg a.s./l there was a significant reduction ($P \leq 0.05$) in body length, this was 4.4 mm compared with the control at 4.7 mm. There was no significant effect on growth at the concentrations below 20 µg a.s./l. An EC50 for adult mortality and overall NOEC were not presented but on the basis of the data provided the EC_{50} would be between 20-34 µg a.s./l and the NOEC 9.8 µg a.s./l. The NOEC is based on a statistically significant reduction in body length compared with the solvent control at 20 µg/l.

This study was undertaken in accordance with GLP and OECD 202.

(DP 71193)

8.2.5.4 Effects on sediment-dwelling organisms (IIA 8.2.7)

No data were provided for effects on sediment-dwellers.

8.2.5.5 Microcosm and mesocosm studies (IIIA 10.2.2)

No mesocosm data were provided.

8.2.5.6 Information on relevance of the metabolites RPA 200761 and RPA 200766

The acute oral LD50 value for RPA 200766 in the rat is >2000 mg/kg compared with 97 mg a.s./kg for fipronil. In addition, it was concluded that there was a lack of genotoxic potential and neurotoxicity. On this basis RPA 200766 was not considered to be of mammalian toxicological relevance. Due to its molecular similarity it was also concluded that RPA 200761 was also not of mammalian toxicological relevance (Section 5.8.2.3.1/2).

(DP112746, DP112747)

8.2.6 Risk to aquatic organisms

Acute and chronic risk from fipronil

Fipronil and the two main soil metabolites, MB 46136 and MB 45950 have relatively high sorption to soil and so it is concluded that there is little risk of them being present in surface water. However, there is potential for RPA 200766 and RPA 200761 to

leach from containers, through permeable membranes or from surface mulched compost through underlying soil to drainage systems and then to surface water such as ditches. This worst case situation does not occur on all holdings but such a scenario is considered realistic (Section 7.4.5 provides further details). Runoff from hard surfaces on which pots are standing with runoff water entering drainage systems is also possible. Information has been provided (Section 7.5 and 7.4.5) which confirms that potential exposure of surface water is possible. A worse case estimate for the Predicted Environmental Concentrations in surface water (PECsw) of 6 µg/l for RPA 200761 and 16 µg/l for RPA 200766 have been calculated.

It important to note that the pot leachate concentrations estimated are **gross over-estimates**, as the regular watering of pots will mean that the peak soil concentrations observed in the laboratory studies would never be reached within the pots. This leaching assessment from irrigating pots is considered to represent **a very worst case** and is considered to encompass the situation of heavy rain falling on pots and the potential for leaching from composted growing media that has been disposed of by spreading on land followed by soil incorporation. The basis of these exposure estimates is given in Section 7.5.

Acute and chronic risk from metabolites/degradation products

Acute risk

The acute toxicity end points used are those obtained from studies undertaken to Good Laboratory Practice. Toxicity data for the metabolites RPA 200761 and RPA 200766 are summarised in Tables 8.5.1 and 8.5.2. These resulting Toxicity Exposure Estimates (TERs) from comparing these toxicity values with the PECsw are shown in Table 8.9.

Table 8.9 Acute Toxicity Exposure Estimates for the metabolites RPA 200761 and RPA 200766

Species	RPA 200761			RPA 200766			Annex VI trigger value
	LC/ EC50 µg/l	PEC µg/l	TER	LC/EC50 µg/l	PEC µg/l	TER	
a) Fish (IIA 8.2.1)							
Oncorhynchus mykiss (rainbow trout)	>100 000	6	>16667	>17 000	16	>1063	100
b) Invertebrates (IIA 8.2.4)							
Daphnia magna	>100 000	6	>16667	>20 000	16	>1250	100
c) Algae (IIA 8.2.6)							
Scenedesmus subspicatus	>100 000	6	>16667	>7 500	16	>469	10

The acute TERs for the metabolites RPA 200761 and RPA 200766 are all above the relevant Annex VI trigger values. The risk to aquatic life from these metabolites is

therefore considered to be acceptable. Since fipronil is an insecticide it is not necessary to consider the risk to aquatic plants further.

Chronic risk to fish and aquatic invertebrates

It should be noted that 'The Guidance Document on Aquatic Ecotoxicology' (8.7.2000) states that only if the metabolite is more acutely toxic than the active substance should long term/chronic testing be required. The acute toxicity data provided for the metabolites RPA 200761 and RPA 200766 indicate that these metabolites are considerably less toxic to aquatic life than fipronil itself. Table 8.9.1 shows the comparative toxicity of these metabolites with fipronil. It should be noted that the highest toxicity end point for a species with fipronil has been chosen as this results in the worst case comparison with the metabolites. Details of all the aquatic toxicity end points for fipronil are given in Table 8.5. These data clearly show that fipronil is significantly more toxic than the metabolites RPA 200761 and RPA 200766. It should be noted that the testing of higher concentrations of RPA 200766 was limited by its solubility. The data indicate that fipronil is at least about 70 times (exactly 68.5 times for fish; 110 times for algae) more toxic than RPA 200766 and over 400 times (403 times for fish; 1470 times for daphnia) more toxic than RPA 200761. These figures are based on the results from a comparison of the greatest toxicity values for a species for fipronil with those for the metabolites. Fish and algae were considered in this comparison as they result in the closest comparison with fipronil.

Table 8.9.1 Acute toxicity data for fipronil, RPA 200761 and RPA 200766

Species	LC/EC50 Fipronil µg a.s./l	LC/EC50 RPA 200761 µg/l	LC/EC50 RPA 200766 µg/l[1]
Fish *Oncorhynchus mykiss*	248	>100 000	>17 000
Aquatic invertebrate *Daphnia magna*	190	>100 000	>20 000
Algae *Scenedesmus subcapitatus*	EbC50: 68 ErC50: 74	EbC50: >100 000 ErC50: >100 000	EbC50: > 7 500 ErC50: > 7 500

[1]It should be noted that the maximum concentration which could be tested was limited by the solubility of RPA 200766 (see Table 8.5.1).

In addition it was concluded that both RPA 200766 and RPA 200761 were not of mammalian toxicological relevance (Section 5.8.2.3.1/2).

Therefore in line with 'The Guidance Document on Aquatic Ecotoxicology' it is considered that the chronic risk from these metabolites is acceptable.

Effects on sediment-dwelling organisms (IIA 8.2.7)

RPA 200766 and RPA 200761 have a water solubility of >20 mg/l and >100 mg/l respectively and so significant partitioning to sediment would not be expected (Section 7.5). On this basis the risk to sediment dwelling organisms is considered to be acceptable.

Bioconcentration risk

The log P_{ow} for fipronil is 4.0 at 20 ^{0}C, the effect of pH was not investigated (Section 2.1.13). This is above the Annex VI trigger of 3 and hence the applicant has addressed the potential to bio-accumulate. The bio-accumulation study showed that in fish exposed to 765 ng a.s./l the steady state concentration was 139, 489 and 273 ng a.s./g fresh weight in the edible, non-edible and whole fish. An apparent steady state was achieved within the whole fish after 14 days. The bioconcentration factor in the whole fish was 321. The data showed that after 7 days' depuration only low levels were present in fish tissue (32 ng a.s./g fresh weight in the whole fish).

According to Annex VI, no authorisation can be granted where the BCF is >100 for products which are not readily biodegradable, unless it can be established by a risk assessment that under field conditions there is no unacceptable impact on the viability of exposed species. Fipronil itself does not occur in surface water and the metabolites RPA 200766 and 200761 have been shown to have water solubilities of >20 and >100 mg/l respectively. In view of the high water solubility of these metabolites the issue of bioaccumulation is not considered of relevance.

Conclusions and labelling

Fipronil itself is not considered to occur in surface water, however it is considered that there is potential for the metabolites RPA 200766 and RPA 200761 to leach from containers. It is important to note that the Predicted Environmental Concentrations in surface water (PECsw) calculated for these metabolites are considered to be **gross over-estimates** (see above and Section 7.5). Using these values the acute TERs for aquatic organisms are all acceptable. These metabolites are less acutely toxic than fipronil and so the chronic risk from these metabolites is also acceptable in line with 'The Guidance Document on Aquatic Ecotoxicology'. On the basis of the high water solubility of these metabolites bioaccumulation is not considered to be relevant. Due to the high water solubility of these metabolites there is not considered to be a risk to sediment dwelling organisms. On the basis of the information provided the product 'Vi-Nil GR' would carry the following aquatic hazard classification:

'Harmful to fish or other aquatic life. Do not contaminate surface waters or ditches with chemical or used container.'

8.3 Effects on terrestrial vertebrates other than birds (IIIA 10.3)

8.3.1.1 Toxicity

For an assessment of the data on the mammalian toxicity of fipronil see Section 5. The acute oral LD_{50} in the rat was 92 mg a.s./kg bw (Section 5.2.1). In the multi-generation study in rats (Section 5.6.1) the NOEL was 1.7-2.0 mg a.s./kg (30 ppm). This was based on the following effects at 17-23 mg a.s./kg/d (300 ppm) in F1/F2 pups; convulsions, low birth weight, decreases in body weight gain, decreases in both the live birth index and viability index. In addition there were decreases in the post-implantation survival index (F2 litters) and a delay in tooth eruption (F1 pups).

The acute toxicity of the soil metabolites in rats is as follows; MB 45950: 69 mg /kg (Section 5.8.2.1) MB 46136: 184 mg/kg (Section 5.8.2.2) RPA 200766: >2000 mg/kg (Section 5.8.2.3).

8.3.1.2 The Commercial nursery environment

A case has been made that the commercial nursery environments are unlikely to be mammal 'friendly' (Section 8.1.3).

8.3.2 Risk to terrestrial vertebrates other than birds

Acute risk and long term risk

Mammals, unlike birds, do not have a requirement for grit. For this reason, and due to the small size of the granules, it is considered unlikely that mammals will actively seek out the granules as a source of food. It is considered that if the risk from initial exposure to fipronil is addressed this will also cover the risk from the composted mulch. It is considered that wild mammals may be exposed to fipronil by the following routes of exposure:

i) by secondary ingestion of granules

ii) by the inadvertent consumption of granules on the soil surface

iii) by the consumption of contaminated foliage

iv) by the consumption of contaminated insects.

The assumptions made are as follows:

A small mammal which eats earthworms is the common shrew (*Sorex araneus*) weighing 13 g with a daily food intake of 13 g (Churchfield, 1986). Earthworms which have consumed compost contaminated at the highest initial PEC of 0.67 mg a.s./kg will contain residues of 0.2 mg a.s./kg (EPPO, see Section 8.1.4 for assumptions).

A small mammal which might inadvertently consume granules is the wood mouse (*Apodemus sylvaticus*) weighing 18 g (Gurney et al. 1997) consuming 5.4 g of food (EPPO). The assumptions regarding granule weight and content are the same as in Section 8.1.4. It is also assumed that the entire diet is obtained as granules.

A mammal which may consume ornamental plants is the rabbit (*Oryctolagus cuniculus*) weighing 1200 g (Gurney et al. 1997) consuming 288 g of food (EPPO). Information presented in Section 8.4.3 showed the maximum residue in foliage to be 1.5 ppm.

i) Secondary ingestion of granules

It is possible that mammals may consume granules adhering to or contained within food items e.g. earthworms. As there is no recognised method for estimating the amount of residues in earthworms exposed to granules it has been assumed that they are exposed to the initial compost PEC of 0.67 mg a.s./kg. It should be noted that this is an extreme worst-case assumption as it is unlikely that earthworms will be present initially in the growing media (see Section 8.1.4). Earthworms in agricultural fields are likely to be exposed to a much lower concentrations of fipronil i.e. 0.00325 mg a.s./kg (Section 7.3). The resulting TERs are shown in Table 8.11 and are all acceptable.

ii) Inadvertent consumption of granules on the soil surface

It is possible that a mammal may inadvertently consume granules of 'Vi-Nil GR' which occur on the pot surface. One thousandth of the granule weight consists of fipronil. For further details of the assumptions see Section 8.4. The resulting acute and long term TERs are shown in Table 8.11 and are all acceptable.

iii) Consumption of contaminated foliage

It is possible that mammals may graze the foliage of ornamental plants. In calculating the TERs it has been assumed that all the diet is obtained from a source contaminated with fipronil and there is 100% translocation of the active substance to the foliage. These are obviously worst-case assumptions. The resulting TERs are shown in Table 8.11 and are all acceptable.

iv) Consumption of contaminated insects.

Insects may be contaminated via exposure to granules on the soil surface. However, it is considered that the risk to mammals from this source of exposure is likely to be considerably less than from the inadvertent consumption of granules or earthworms (Section 8.1.3). Therefore this source of exposure is not considered to require further assessment.

The TERs for the acute and long term risk from the above routes of exposure are shown in Table 8.11.

Table 8.11 The acute and long term TERs for fipronil

Category	Time scale	Toxicity end point	ETE	TER	Annex VI trigger
Mammal (13 g) eating earthworms	Acute	LD50: 1.196 mg/animal	0.0026 mg/animal	460	10
	Long term	NOEL: 30 ppm	0.2 ppm	150	5
Mammal (18g) eating granules	Acute	LD50: 1.656 mg/animal	0.0054 mg/animal	307	10
	Long term	NOEL: 30 ppm	0.67 ppm	45	5
Mammal (1200g) grazing foliage	Acute	LD50: 110 mg/animal	0.46 mg/animal	239	10
	Long term	NOEL: 30 ppm	1.5 ppm	20	5

The acute and long term TERs from exposure via the consumption of contaminated earthworms, inadvertent consumption of granules and from the eating contaminated foliage are all above the relevant Annex VI triggers and are acceptable. The risk from the exposure to agricultural soil into which mulch has been incorporated will also be acceptable since the PEC (0.0035 mg a.s./kg) is lower than the initial PEC in compost (0.67 mg a.s./kg) (Section 7.3).

It should also be noted that the applicant has made a case that the commercial nature of the nursery environment is likely to make it an unattractive habitat for mammals. In addition, protection measures are likely to be in place to minimise any damage by grazing mammals. Also the total area of commercial usage is likely to be limited in nature.

The information provided indicates that exposure of mammals is likely to be limited. In addition, the assessment undertaken shows that even based on worst case assumptions the risk is acceptable.

Metabolites

The metabolites MB 46136, MB 45950, RPA 200766 and RPA 200761 have been identified as major soil metabolites. A number of metabolites may also occur in plants (Appendix B).

The acute toxicity of the soil metabolites in rats is as follows; MB 45950: 69 mg/kg, MB 46136: 184 mg/kg, RPA 200766: >2000 mg/kg. It was concluded that RPA 200766 and RPA 200766 were not of mammalian toxicological relevance (Section 5.8.2.3.1/2).

Exposure to these soil metabolites was shown to have occurred in the mammalian metabolism study (Section 5.1.2) and their toxicity will have been accounted for in the mammalian toxicity studies in Section 5. The most toxic metabolite is MB 45950; the acute toxicity of this metabolite is 69 mg/kg compared with 92 mg/kg for fipronil. The lowest TER for fipronil is from the long term consumption of foliage. Multiplying this TER by 0.75 to take account of the relative toxicity of MB 45960, the resulting TER for MB 45960 is 15. This is above the Annex VI trigger of 5 and is acceptable. Since this was the lowest TER, by extrapolation all the other TERs for MB 45960 are also above the appropriate Annex VI triggers and acceptable. The other soil metabolites were of equivalent toxicity or less toxic than fipronil and therefore will also be acceptable. It should also be noted that the soil PECs for these metabolites are lower than for fipronil (initially at incorporation being 0.67 mg a.s./kg) being a maximum of 0.027 mg /kg soil (for each of MB 46136, MB 45950 and RPA 200766) and only 0.007 mg/kg for RPA 200761). Full details of the calculation of these levels are given in Section 7.3. This again indicates that the TERs for the other routes of exposure would be greater for the metabolites than for fipronil.

Exposure of mammals is likely to be limited. In addition, the metabolites would need to be considerably more toxic than fipronil to pose a risk to grazing mammals. It is also unlikely that mammals would obtain all of their food from treated plants/pots and the plants are likely to have some protection from grazing damage. It is therefore

considered that metabolites in plants will pose a low risk to grazing mammals. Since the lowest TER was chosen for assessment this also shows that exposure of mammals to metabolites by other routes is also acceptable.

Conclusions and labelling

The acute and long term risk to mammals via the various routes of exposure is considered to be acceptable. The risk from soil and foliage metabolites is also considered to be acceptable.

8.4 Effects on bees (IIA 8.3.1, IIIA 10.4.1)

8.4.1 Active substance

8.4.1.1 Acute toxicity to bees (IIA 8.3.1.1)

The data for the toxicity of fipronil to bees are summarised in Table 8.12.

Table 8.12 The acute oral and contact toxicity of fipronil to bees.

Test type	Test substance	LD_{50} (µg a.s./bee)	Test guideline*	Reference
48h acute oral	Fipronil (purity 95.4%)	0.00417	MAFF UK Guideline COPR 1986	DP 71208
48h acute contact	Fipronil (purity 95.4%)	0.00593	MAFF UK Guideline COPR 1986	DP 71208

* All tests conducted without deviation and in accordance with GLP.

The following information has been provided. Based on models developed to describe transport within the plant, the applicant states that fipronil (with a log Kow of 4.0) and its toxicologically relevant metabolites are predicted to be transported in the xylem rather than in the phloem. Fipronil is therefore stated to be primarily transported to older plant parts in the xylem with very limited potential for movement in the phloem. The applicant states that transport in the xylem is associated with movement to leaves with little movement to newly developing leaves, flowers or seeds. Detectable residues from the application of granules have resulted in detectable residues in older parts of the plant but not in the new developing parts. On the basis of this information the applicant considered that exposure of bees was unlikely.

A summary was provided of a semi-field study was undertaken in sunflowers grown from seed treated with fipronil at equivalent to a rate of 50 to 70 g a.s./ha. Honeybees were tested inside tunnels when the sunflowers were at full flower. Bees were kept in the tunnels for 9 days and periodic assessments for mortality were made before and after exposure. No treatment related effects were stated to have been observed and this was considered to support the lack of translocation of fipronil to aerial plant parts attractive to bees.

(ACP168/1 (274/2000) summary)

Radiolabelled studies are stated to have shown that less than 5% of fipronil applied to soil is taken up by plants. A summary was provided from a study in maize where a 1.5% granule of fipronil was applied at 420 g a.s./ha (equivalent to 0.56 mg a.s./kg). This resulted in residues in crop parts as follows; forage 0.1 ppm, fodder 1.53 ppm and grain <0.01.

(DP 82792)

8.4.3 Risk to bees

Fipronil is highly toxic to bees. It is therefore necessary to consider the potential risk to bees visiting flowering ornamentals grown in the treated compost. Based on models developed to describe transport within the plant, the applicant states that fipronil (with a log Kow of 4.0) and its toxicologically relevant metabolites are predicted to be transported in the xylem rather than in the phloem. The applicant states that transport in the xylem is associated with movement to leaves with little movement to newly developing leaves, flowers or seeds.

The initial PEC from the proposed use is 0.67 mg a.s./kg i.e. similar to the radiolabel study. These data indicate that some low levels of residues may occur in plant parts from this sort of application rate. As the acute oral toxicity value is so low (0.00417 μg a.s./bee) there was considered to be a potential risk to bees from flowering ornamentals. Further information was provided in support of the assessment of the risk to bees. These indicated that translocation was likely to be low. In addition a semi-field study using sunflower seeds treated at 50-70 g a.s./ha indicated that the risk to bees was acceptable. On this basis provisional approval is proposed. However, since only summary details were provided for some of the information, the complete reports are required for full approval.

It should be noted that any proposed change of use of fipronil, for instance to use as a spray on flowering crops, would need to be carefully considered.

Data requirement

To address the risk to bees it is necessary that the reports on the concentration of residues in flower parts of plants grown in treated compost is provided. In addition, the full report for the semi-field study with sunflower seeds is required.

8.5 Effects on arthropods other than bees (IIA 8.3.2, IIIA10.5)

8.5.1 Plant protection products

8.5.1.1 Toxicity to terrestrial arthropods (IIIA 10.5.1, 10.5.2)

The laboratory data for the toxicity of 'EXP 60720 (80% WG)' to arthropods are summarised in Table 8.13. It should be noted that these data were generated using a wettable powder diluted in water and applied as a spray. 'Vi-Nil GR' is in fact recommended for incorporation as a granule in compost.

Table 8.13 The effects of formulated fipronil (80% WG) on non-target arthropods

Species	Test substance (% purity/ formltn type)	Test type, substrate	Application dose g a.s./ha	Effect(s)	Test guideline*	Reference
Aphidius rhopalosiphi	EXP 60720	Laboratory, glass	25 100	60% mortality 87% mortality	Mead Briggs 1992	DP 71213
Typhlodromus pyri	EXP 60720	Laboratory, glass	25 100	98% mortality 100% mortality E = 100%	Overmeer 1988	DP 71210
Pardosa spp.	EXP 60720	Laboratory, sand	25 100	60% mortality 74% mortality Control 12% mortality	Draft BBA VI.23-2.1.9	DP 71228
Coccinella septempunctata	EXP 60720	Laboratory, glass	25 100	100% mortality 100% mortality Control 7% mortality	BBA VI.23.1.5	DP 71230

*All tests conducted without deviation and in accordance with GLP unless stated otherwise.

8.5.2 Risk to non-target terrestrial arthropods other than bees

The data generated showed fipronil to be toxic to non-target arthropods. However, data were generated from the use of an 80% wettable powder formulation of fipronil as a spray at 100 and 25 g a.s./ha, rather than from the exposure to granules in compost. The initial PEC in the growing media is 0.67 mg a.s./kg (Section 7.3) which would be equivalent to a field application rate of a spray at 500 g a.s./ha (assuming distribution within the top 5 cm of soil and a soil density of 1.5 g/cm^3). However, the PEC for fipronil in agricultural soil after incorporation of composted material is much lower i.e. 0.00325 mg a.s./kg which is equivalent to a field application rate of 9.75 g a.s./ha (with the assumption of incorporation into the top 20 cm of soil, Section 7.3).

It is considered that the initial use of fipronil in compost is likely to cause minimal exposure of non-target arthropods as the medium is likely to contain few non-target arthropods. Non-target arthropods are most likely to be exposed to fipronil from the use of composted media as a mulch and when pots are planted out. At planting out there will be dilution of compost with surrounding soil (Section 7.3). In addition, it is only likely that relatively small areas will be planted out with treated plants. It is therefore considered that it is unlikely that this scale of use will be sufficient to have an impact on the arthropod population. Similarly the scale of use of composted media as a mulch is considered unlikely to have an impact on the arthropod population. It is therefore considered that the risk to non-target arthropods from the use of fipronil in compost is acceptable.

Conclusions and labelling

It is considered that the risk to non-target arthropods from the use of fipronil in compost is acceptable.

8.6 Effects on earthworms (IIA 8.4, IIIA 10.6)

8.6.1 Active substance

A 14-day LC_{50} study in artificial soil was performed on *Eisenia foetida* using fipronil (purity 97%) at a concentration of 1000 g a.s./kg dry weight of soil. There were 10 earthworms per replicate with four replicates in the control and for the toxic standard (chloroacetate) and six at 1000 g fipronil/kg.

There was no significant difference in mortality in the control and at 1000 g a.s./kg (3% and 2% mortality respectively). There was also no significant difference in mean worm weight between the control and 1000 g a.s./kg. The LC_{50} was >1000 mg a.s./kg and the NOEC was 1000 g a.s./kg.

The study was undertaken in accordance with OECD guideline 207 and GLP.

(DP 71231)

8.6.2 Risk to earthworms

Acute risk

Fipronil is not highly toxic to earthworms, its LC_{50} was >1000 mg a.s./kg. The log Pow for fipronil is >2 (Section B 2.1.13) and therefore account needs to be taken of the higher organic content of the test soil compared with normal agricultural soil. The LC_{50} is therefore divided by two and is >500 mg a.s./kg.

The risk to earthworms from use in pots is considered to be low. However, exposure is likely to occur at planting out. As a very worse case scenario the initial PEC in the actual growing media of 0.67 mg a.s./ha is compared with the LC_{50} was >500 mg a.s./kg. The resulting acute TER is >746 which is above the Annex VI trigger of 10 and is acceptable. It should be noted that this is based on very worst case assumptions; in reality earthworms are most likely to be exposed to fipronil in agricultural soil after the incorporation of composted material, when the concentration is predicted to be only 0.00325 mg a.s./kg (Section 7.3). Even organisms involved in the composting process itself are predicted to be exposed to lower concentrations than in the initial growing media i.e. 0.088 mg a.s./kg initially and 0.013 mg a.s./kg after 12 months composting.

Chronic risk

Fipronil has a DT_{50} of 190 days and a single application is made to compost. The acute toxicity of fipronil is low (LC_{50} was >500 mg a.s./kg) and no sub-lethal effects occurred at the concentration tested. In addition, there will be a dilution factor (at planting out or from use as a composted mulch) and, it is therefore considered that the long term-risk to earthworms is acceptable.

Metabolites

The DT90s of the soil metabolites MB 46136, MB 45950, RPA 200766 and RPA 200761 are not known. From the data provided in Section 7 these metabolites may be present in compost or mulch. However levels are predicted to be lower than for fipronil itself (section 7.3). Earthworms are only likely to be exposed to metabolites at planting out and via composted media used as a mulch. As for arthropods, the extent of exposure is likely to be limited by the scale of use. It is therefore not considered necessary for the risk from the metabolites to be addressed further. In addition, aquatic toxicity data for the metabolites RPA 200766 and RPA 200761 showed these to be of lower toxicity than fipronil itself (Section 8.2).

Conclusions

The acute and chronic risks to earthworms from fipronil, and its metabolites, were considered to be acceptable.

8.7 Effects on soil macro-organisms other than earthworms (IIIA 10.6.2)

No studies were submitted on soil macro-organism other than earthworms and soil dwelling beetles.

8.7.2 Risk to non-target, soil macro-organisms other than earthworms

Conclusions

On the basis of the proposed use it was considered that the risk to earthworms and arthropods was acceptable. Therefore the risk to soil macro-organisms is also considered to be acceptable.

8.8 Effects on soil micro-organisms (IIIA 8.5, IIIA 10.7)

8.8.1 Active substance

8.8.1.1 Impact on soil microbial activity (IIA 8.5)

In a 28 day study the effect of fipronil (96.7% purity) at 200 and 1000 g a.s./ha on carbon and nitrogen mineralisation was assessed in two soils amended with alfalfa. The soils used were a clay loam and a sandy loam. There was no significant difference in carbon mineralisation between the two treatments and the control after 28 days. There was no significant difference in nitrogen mineralisation between the treatments and the control in the sandy loam. In the clay loam the nitrate nitrogen level at 28 days was significantly different ($P \leq 0.05$) from the control at 28 days. The values for the control, 200 and 1000 g a.s./ha treatments were 48.7, 68.5, 65.4 mg nitrogen/kg soil. The report stated that the level of nitrate in the control were anomalously low, as normally in a soil of this type nitrate levels of between 60-70 mg/kg would be expected.

(DP 71232)

8.8.2 Risk to non-target soil micro-organisms

The study on the effects of fipronil on soil microbial processes showed there to be no significant effect on carbon mineralisation in two soils. In one of the two soils there was an effect on the level of nitrate. However, the report states that the level of nitrate in the control was lower than would normally be expected.

It is considered that soil microbes in the wider horticultural environment are most likely to be exposed to fipronil from the application of a mulch of composted material to the soil surface. The level of fipronil in agricultural soils has been predicted to be 0.00325 mg a.s./kg (Section 7.3) equivalent to 9.75 g a.s./ha. It is likely that composted material will only be used in limited areas and that there is only likely to be a fairly limited amount of such material. Therefore, based on scale of the proposed use, it is considered that the risk to soil microbial organisms is acceptable.

Metabolites

Again based on the scale of the proposed use it is considered that any risk arising from the metabolites of fipronil is acceptable.

Conclusions

There is only likely to be limited exposure of soil microbes to composted mulch. Therefore it is considered that the risk to soil microbial organisms is acceptable.

8.9 Effects on other non-target organisms (flora and fauna) believed to be at risk (IIA 8.6, IIIA 10.8)

8.9.1 Toxicity of active substance or plant protection products (IIA 8.6, IIIA 10.8)

Data were provided from trials in which 'Vi-Nil GR' was used at twice the recommended rate and the compost used to grow a range of ornamental plants (Section 3.6). No phytotoxic effects were observed although for a number of plants a slight reduction in height was noted. In some plant species an inconsistent effect on root growth was also observed.

8.9.2 Risk to other non-target flora and fauna

Only relatively minor effects were seen in the ornamental species tested. Exposure of non-target flora and fauna is most likely to occur from the use of composted mulch. In view of the scale of use it is considered that the exposure of non-target flora and fauna is likely to be limited. It is therefore considered that this risk is acceptable.

Conclusions

It is considered that the risk to non-target flora and fauna is acceptable.

8.10 Effects on biological methods of sewage treatment (IIA 8.7)

No data have been provided on the toxicity of the active substance or the product to micro-organisms used to treat sewage.

8.10.2 Risk to biological methods of sewage treatment

It is not considered likely that use of fipronil would result in the contamination of sewage treatment works. It is therefore considered that no further data are required on effects on micro-organisms used in sewage treatment.

8.11 Conclusions

In summary, the data and information were considered to show that the risk to birds and mammals was acceptable. To support the case made, the new information on granule distribution must be submitted to PSD for full approval. The risk to aquatic life, earthworms, non-target arthropods and soil micro and macro-organisms was considered to be acceptable. The risk to bees was also considered acceptable. For full approval, the complete reports are required for the supporting information submitted i.e. for the information on the low systemicity of fipronil in plants and semi-field trials for bees. Provisional approval is proposed, with these data requirements.

Data requirements for Full Approval:

i) The full report containing information on the average distribution of granules on the surface of an 18 cm pot must be submitted to PSD. This data requirement is from the bird section of the document.

ii) To address the risk to bees it is necessary that the information on the concentration of residues in flower parts of plants grown in treated compost is provided. In addition, the full report for the semi-field study with sunflower seeds is required.

References relied on

Best, B.L. and Gionfriddo, J.P. 1991 'Characterization of grit use by cornfield birds', Wilson Bull.; 103(1) pp 68-82.

Buxton, J.M. and Crocker, D.M. 1996, 'Birds and Farming: Information for Risk Assessment', MAFF Internal Contract Milestone Report

Churchfield, 1986 'Shrews', The Mammal Society, ISBN 0 904614 15 8

de Leeuw J, Gorree M, de Snoo G. R, Tamis W.L M, van der Poll, R.J and Luttick, R 'Risk of granules and treated seeds to birds on arable fields.' CML Report 118, 1995

Fischer, D.L. and Best, L.B, 1995 'Avian consumption of blank pesticide granules applied at planting to Iowa cornfields.' Environmental Toxicology and Chemistry, vol 14, No. 9 pp 1543-1549.

Gurney, J.E., Perrett, J and Crocker, D.R. 1997, 'Mammals and Farming: Information for Risk Assessment', MAFF Internal Contract Milestone Report

'Guidance Document on Aquatic Ecotoxicology in the frame of Directive 91/414/EEC' Draft working document (08.07.2000; 8075/VI/97 rev 7).

REFERENCES

Reports relating to the active substance

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
		Documents A to I	Anon.	Oct 1998	No	82760
		Revised Document J Confidential information	Anon.	June 1999	No	82751
		Document L-II (Annex II tier 1)	Anon.	Oct 1998		82763
		Revised Document M-II – Sections 1 - 6	Anon	June 1999	No	82764
		Document N (tier 3) overall summary	Anon.	Oct 1998		82766
		Revised Document O	Anon	June 1999	No	82768
		Classification and labelling	Anon	June 1999	No	82769
		Chemical names etc	Anon	June 1999	No	82770
		Batch details for tox samples	Anon	June 1999	No	82771
		Sift response letter	Williams, J.	23/6/99	No	82772
		Second sift response letter	Williams, J	27/7/1999	No	82773
		Third sift response	Williams, J	2/8/1999	No	83590
		Letter concerning PC properties, ecotoxicology and fate	Williams, J	3/9/99	No	85251
		Letter concerning PC properties, analytical methods and ecotoxicity	Williams, J	24/9/99	No	86151
		Letter concerning analytical methods and ecotoxicity	Williams, J	28/9/99	No	86390

1. IDENTITY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
1.8, 1.9 &1.10	R.P.IND./CRIT/ D/C&P/MMA/G UN No. 215. Study No. 94/04	Fipronil (MB 46030). Product Identity and composition.	Guillochon, D.	12/8/94	Yes	70943
1.11/01	R&D/CRLD/AN 9415791. Study No. 92-37	Fipronil Technical Grade Active Ingredient Analysis and Certification of Product Ingredients.	Gomez, F.	10/6/94	Yes	70950
1.11/02	R&D/CRLD/AN/ 9716758. Study No. 97-163	Technical fipronil Analysis and Certification.	Cousin, J.	10/12/97	Yes	70960
1.11/03	-	Impurity profiles of batches used for toxicity testing	Anon.	June 1999	No	82774
	RP/IND/CRIT/D/ CP/MMA/PCR No 322/97/418	Fipronil (MB 46030) Product identity and composition	Charreau, Ph.	6/4/98	Yes	86156

2. CHEMISTRY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
2.1.1/01, 2.1.3/01, 2.2, 2.4.1/01 & 2.4.2	AG/CRLD/AN 9116304. Study No. 91-21	MB 46030 Technical Grade: Physical Properties.	Chabassol, Y. & Hunt, G.	28/10/91	Yes	70963
2.1.1/02, 2.1.3/02, 2.4.1/02 & 2.5.1/01	R&D/CRLD/AN/ 9616101 Study No. 95-1012	Fipronil - Active ingredient: Batch AJK232 - Analytical Log Number EA232SD1: Suitability for use as an Analytical Standard Reference Material.	Chabert, M.S. & Lecourt, N.C.	11/9/96	Yes	82778
2.3.1	AG/CRLD/AN 9116670. Study No. 91-16	MB 46030 TECHNICAL GRADE: Vapour Pressure Curve.	Chabassol, Y. & Reynaud, R.	22/11/91	Yes	70969
		Estimation of the vapour pressure of pure fipronil	Bossy, A	9/4/99	No	85254
2.5.1/02	AG/CRLD/AN 9215955. Study No. 92-21	Fipronil - NMR, IR and MS Spectra	Garnier, C., Ott, M. & Guesnet, J-L	19/5/92	Yes	70974
2.5.1/03	A403/192-193	UV Spectra	No author specified	No date given	No	70978
2.5.2/01	Lab Project ID: P 92/164	Insecticides: Fipronil: Impurity/Metabolite: 5-Amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethyl-thio-pyrazole: (M&B 45,950), Batch JJW 2120 Suitability for Continued Use as an Analytical Standard.	Buddle, G., Mills, E. & Mountain, L.	6/92	Yes	70984
2.5.2/02	Lab Project ID: P 92/165	Insecticides: Fipronil: Impurity/Metabolite: 5-Amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-3-cyano-4-trifluoromethyl-sulphonyl-pyrazole: (M&B 46,136), Batch AJK 165/1 Suitability for Continued Use as an Analytical Standard.	Buddle, G., Mills, E. & Mountain, L.	6/92	Yes	70987
2.5.2/03	Doc No. 44320 Raw Data File: 93020RJS	RPA 200766: Analytical Standard Reference Material Characterization of Batch 57TDS62.	Seymour, R.J. & Viola, M.D.	18/3/94	No	82780
2.5.2/04	R&D/CRLD/AN/ 9816705 Study No. 98-208	RPA 200766 - IR Spectrum.	Vidal, J. & Guesnet, J-L.	22/10/98	Yes	82782
2.5.2/05	R&D/CRLD/AN/ 9816706 Study No. 98-209	RPA 100344: NMR, IR, MS and UV-Visible Spectra.	Just, D. *et al*	22/10/98	Yes	82785

2.5.2/06	Project Report File No. 40985 Study No. AC-91-001	Appendix D: Thiodicarb Spectral Analysis. Pages 51 to 81 of report only.	Seymour, R.J.	21/3/91	Yes	82787
2.6	AG/CRLD/AN 9115826. Study No. 91-06	MB 46030 TECHNICAL GRADE: Water Solubility at 20°C.	Chabassol, Y. & Reynaud, R.	13/8/91	Yes	70988
2.7	AG/CRLD/AN 9115835. Study No. 92-12	MB 46030 TECHNICAL GRADE: Solubility in Organic Solvents.	Chabassol, Y. & Reynaud, R.	9/8/91	Yes	70989
2.8	AG/CRLD/AN 9116710. Study No. 91-22	MB 46030 Octanol/Water Partition Coefficient at 20°C.	Chabassol, Y. & Reynaud, R.	5/12/91	Yes	70990
2.9.1	AG/CRLD/AN 9215072. Study No. 91-25	^{14}C-MB 46030 Hydrolysis at 25°C.	Corgier, M. & Plew, A.	16/3/92	Yes	70991
2.10/01	AG/CRLD/AN 9216106. Study No. 91-11	Fipronil Technical Grade Stability Study.	Chabassol, Y., Hunt, G. & Yslan F.	3/7/92	Yes	70992
2.10/02	-	Statement on comparison of artificial, UK and US sunlight.	Lowden	23/6/99	No	82791
2.11	96-130-SEC. Study No. 96-124	Determination of Flammability and Ability for Self Heating of Fipronil Technique.	Fillion, J.	9/12/96	Yes	70993
2.13	CID/P/SE/Sec 352/92/1704 PV	Fipronil (M&B 46030) Minimum Ignition Energy, Lower Explosive Limit (Dust Cloud) and Auto Ignition (Layer).	Vandermarliere, P.	10/1/92	No	70994
	99-290-SEC Study no. 99-123	Fipronil – explosion and oxidising properties	Tran Thanh Phong, J	13/7/99	Yes	86153
2.14	R&D/CRLD/AN 9616626. Study No. 96-125	Fipronil - Surface tension and particle size distribution.	Cousin, J.	16/12/96	Yes	70998
2.15	AG/CRLD/AN 9116118. Study No. 91- 24	MB 46030 TECHNICAL GRADE: Oxidising or Reducing Action.	Chabassol, Y & Hunt, G.	6/9/91	Yes	70999
	Agredoc R01082	MB 46136 - Water solubility	Cousin, J.	25/3/97	Yes	112725
	Agredoc R01089	MB 45950 - Water solubility	Cousin, J.	23/3/95	Yes	112727
	Agredoc R01084	MB 46136 - n-octanol /water coefficient.	Cousin, J.	27/3/97	Yes	112728
	Agredoc R010191	MB 45950 - n-octanol /water coefficient.	Cousin, J.	23/8/98	Yes	112730
	Agredoc R010165	MB 46136 - Vapour pressure	Cousin, J.	3/6/96	Yes	112731
	Agredoc R010169	MB 45950 - Vapour pressure	Cousin, J.	26/8/96	Yes	112732
	Agredoc C014924	MB 46136 - Henry's law constant calculation	Bascou, J.P.	26/7/2001	No	112734
	Agredoc C014925	MB 45950 - Henry's law constant calculation	Bascou, J.P.	26/7/2001	No	112735

3. FURTHER INFORMATION

Point	**CO. REF.**	**Study Title**	**Author**	**Study Date**	**GLP**	**DP No.**

3.2.2	No reference specified	The Systemicity of Fipronil.	Holmsen, J.D.	6/99	No	82792
3.5.1	No reference specified	Fipronil - Mode of Action	Bushey, D.	8/4/93	No	71001
3.7	Sheet No. 1049	Fipronil - Safety Data Sheet	No author specified	20/2/95	No	71003 Public Domain
3.8.1	AG/CRLD/AN 9216225	Pyrolysis Test on Active Ingredient: MB 46030.	Lagoutte, E. & Robieux, M.	18/5/92	No	71004
3.9	No reference specified	Examination of methods of treatment to remove pesticides from water intended for human consumption.	Grohmann, A. & Dizer, H.	April 1991	No	71007

4. ANALYTICAL METHODS

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
4.1/01	F-735-09-93 (E)	Fipronil: Technical Grade Active Ingredient HPLC Determination of Active Ingredient Content.	Robles, J. M. & Gomez, F.	10/2/94	No	71011
4.1/02	B-658-10-91 (E)	Technical Fipronil: HPLC Determination of MB45950, MB46136, RPA 200766 and Toluene.	Hunt, G. & Chabassol, Y.	3/12/91	No	71013
4.1/03	F-799-03-95 (E)	Fipronil: Technical Grade Active Ingredient HPLC Determination of Sulphur.	Robles, J. M. & Gomez, F.	8/3/95	No	71016
4.2.1	AGR/MOA/FIP 11A	M&B46030: Analytical Method for the Determination of Fipronil and its Metabolites (M&B45950, M&B46136, M&B46513 and RPA 200766) in Cereal, Vegetable and Fruit.	Communal, P. Y.	1/12/94	Yes	71020
4.2.2	SOP - 90231	Fipronil: Method of Analysis for Possible Residues of Fipronil and its Nonpolar Metabolites, MB 45950, MB 46136, MB 46513 and RPA 200766, in Soil.	Ibrahim, A. S.	31/8/92	No	71024
4.2.3	P91/143	Insecticides: Fipronil: Analytical Method for the Determination of Residues in Ground Water.	Manley, J. D.	June 1992	No	71026
4.2.4	R&D/CRLD/AN/ 9615839. Study No. 96-21	Fipronil: Method for the Determination in Air.	Corgier, M. M. & Turier, G. P.	1/7/96	Yes	71028

4.2.5	Fipronil-Animal-GC/ECD 12/95	Method of Analysis for the Determination of Fipronil (MB46030) and its Metabolites (MB45950 and MB46136) in Milk, Eggs, Liver, Kidney, Muscle and Fat Tissues.	Robinson, T. W.	13/12/95	No	71032
	P92/122	Analytical method for the determination of residues of the metabolite RPA 104615 in soil	Manley, J D	Jan 1993	Yes	86154
	98-44 (method AR 163-98)	Fipronil and its metabolites (MB 45950, MB 46136 and MB 46513): Analytical method for the determination of residues in drinking water	Bourgade, C, Jendrzejczak, F & Yslan, F	2/4/98	Yes	86157
	Docmap 441371 (study code 96-109)	Validation of the assay method (AGR/MOA/FIP12) of fipronil and its metabolites (MB 46513, MB 45950 & MB46136) in human plasma samples.	Communal, P. Y.	February 1997	Yes	112737
	Docmap 444228 (study code 98-67)	Validation of the assay method (AGR/MOA/FIP12) of fipronil and its metabolites (MB 46513, MB 45950 & MB46136) in human plasma samples.	Laneury, J.P.	July 1998	Yes	112738
	Agredoc B003366	Fipronil - method of analysis for the determination of fipronil (MB46030) and its metabolites (MB 45950 & MB 46136) in milk, eggs, liver, kidney, muscle and fat tissues.	Robinson, T.W., Tew, E.L. & Hudson, J.R.	26/7/2001	Yes	112739

5. TOXICOLOGY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
5.1	Hazleton UK., Report No 7040-68/117. Hazleton UK., Report No 7040-68/117-add	Final Report. (14C)-M&B 46,030: Absorption, distribution, metabolism and excretion in the rat. Addendum to Report.	Powles, P Powles, P.	26/6/92 23/5/94	Yes Yes	71035 71037
5.2.1	HRC: Report No 881300D/M&B 290/AC.	Acute oral toxicity to rats of M&B 46,030.	Gardner, J.	17/10/88	Yes	71039
5.2.2	HRC: Report No 881113D/M&B 291/AC.	Acute dermal toxicity to rats of M&B 46,030.	Gardner, J.	11/10/88	Yes	71040

5.2.3	LSR: Report No 90/RHA358/0791	M&B 46030: Acute inhalation toxicity study in the rat.	Cracknell, S.	10/1/91	Yes	71042
5.2.4	HRC: Report No 881031D/M&B 292/SE	Irritant effects on rabbit skin of M&B 46,030.	Liggett, M. P.	8/8/88	Yes	71044
5.2.5	HRC: Report No 881032D/M&B 293/SE	Irritant effects on the rabbit eye of M&B 46,030.	Liggett, M. P.	8/8/88	Yes	71046
5.2.6/01	Pharmaco-LSR: Report No 93/RHA503/0167	M&B 46030: Delayed Contact Hypersensitivity Study in Guinea-Pigs.	Johnson, I. R.	13/2/93	Yes	71069
5.2.6/02	Pharmaco-LSR: Report No 90/RHA357/0602	M&B 46030: Dermal sensitization study in guinea pigs.	Smith, K. D.	2/11/90	Yes	71070
5.3.1	M&B 327/891321	M&B 46030 Toxicity to Rats by Dietary Administration for 4 weeks.	Peters, D. H. et al.	21/5/90	Yes	71076
5.3.2/01	LSR: Report No 90/RHA298/0781	M&B 46030: Toxicity Study by Dietary Administration to CD Rats for 13 Weeks.	Holmes, P.	9/4/91	Yes	71079
5.3.2/02	LSR: Report No 90/RHA310/0842	M&B 46030: Toxicity Study by Oral (Capsule) Administration to Beagle Dogs for 13 Weeks.	Holmes, P.	21/11/91	Yes	71081
5.3.3	UC Bushy Run: 92N1165	M&B 46030: Twenty-One Day Repeated Cutaneous Dose Toxicity Study in New Zealand White Rabbits #2.	Hermansky, S.J. & Wagner, C.L.	23/6/93	Yes	71083
5.4.1/01	Microtest Research Limited, Report No. MAB 20/S.	Study to determine the ability of M&B 46030 to induce mutation in four histidine-requiring strains of Salmonella typhimiurim.	Clare, C. B.	5/10/88	Yes	71084
5.4.1/02	Microtest Research Limited, Report No. MAB 20/HLC	Study to evaluate the chromosome damaging potential of M&B-46030 by its effects on cultured human lymphocytes using an in vitro cytogenetics assay.	Marshall, R. R.	20 July 1988	Yes	71087
5.4.1/03	LSR: Report No 90/RHA304/0418	M&B 46030: Investigation of mutagenic activity at the HGPRT locus in a Chinese Hamster V/79 Cell Mutation System.	Lloyd, J. M.	5/12/90	Yes	71088
5.4.2	LSR: Report No 90/RHA305/1377	M&B 46030: Assessment of clastogenic action on bone marrow erythrocytes in the micronucleus test.	Edwards, C. N.	8/3/91	Yes	71091
5.5/01	Pharmaco-LSR Ltd, Report No 93/RHA432/0166	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.	Aughton, P.	11/6/92	Yes	71094

5.5/02	LSR: Report No 92/RHA313/0971	M&B 46030: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks.	Broadmeadow, A.	9/3/93	Yes	71096
5.6.1	LSR: Report No 92/RHA425/0309	M&B 46030: Reproductive performance study in rats treated continuously through two successive generations.	King, V. C.	26/6/92	Yes	71100
5.6.2/01	HRC: Report No M&B 335 + 326/90582	The effect of M&B 46,030 on pregnancy of the rat.	Brooker, A. J. & John, D. M.	13/8/91	Yes	71104
5.6.2/02	LSR: Report No 90/RHA321/0772	M&B 46030: Teratology study in the rabbit.	King, V. C.	29/11/90	Yes	71106
5.7	UC Bushy Run 91N0099	M&B 46030: Single exposure peroral (gavage) neurotoxicity study in Sprague Dawley rats.	Gill, M. W., Wagner, C. L. & Driscoll, C. D.	27/4/93	Yes	71111
5.8.1/01	SA 93272	MB 45950: Acute Oral LD50 in the Rat.	Dange, M.	31/5/94	Yes	82798
5.8.1/02	A/D/1856	MB 45950: Acute Dermal Toxicity Study in the Rat.	Haynes, G.	3/88	Yes	82801
5.8.1/03	A/S/1858	MB 45950: Acute Dermal Irritation/Corrosion Study.	Haynes, G.	9/87	Yes	82802
5.8.1/04	A/E/1857	MB 45950: Eye Irritation Study in the Rabbit.	Haynes, G.	9/87	Yes	82804
5.8.1/05	SA 93305	MB 45950: Salmonella typhimurium Reverse Mutation Assay (Ames Test).	Percy, A.	17/2/94	Yes	82809
5.8.1/06	MAB 18/HLC	Study to Evaluate the Chromosome Damaging Potential of M&B 45950 by its Effects on Cultured Human Lymphocytes using a In-Vitro Cytogenetics Assay.	Marshall, R.R.	3/2/88	Yes	82812
5.8.1/07	881364D/M&B 286/AC	Acute Oral Toxicity to Rats of MB 46136.	Gardner, J.R.	14/10/88	Yes	82814
5.8.1/08	88961D/M&B 287/AC	Acute Dermal Toxicity to Rats of MB 46136.	Gardner, J.R.	2/9/88	Yes	82815
5.8.1/09	88833D/ M&B 288/SE	Irritant Effects on Rabbit Skin of MB 46136.	Liggett, M.P.	27/7/88	Yes	82816
5.8.1/10	MAB 21/S	Study to Determine to Ability of MB 46136 to Induce Mutation in Four Histidine-Requiring Strains of Salmonella typhimurium.	Clare, C.B.	18/10/88	Yes	82818
5.8.1/11	MAB 21/HLC	Study to Evaluate the Chromosome Damaging Potential of M&B 46136 by its Effects on Cultured Human Lymphocytes using an In-Vitro Cytogenetics Assay.	Marshall, R.R.	15/11/89	Yes	82820
5.8.1/12	SA 93016	RPA 200766: Acute Oral LD50 in the Rat.	Dange, M.	20/12/93	Yes	82826

5.8.1/13	SA 93174	RPA 200766: Salmonella typhimurium Reverse Mutation Assay (Ames Test).	Percy, A.	23/9/93	Yes	82828
5.8.1/14	SA 95273 Document No. 601437	RPA 200766: 28-Day Toxicity Study in the Rat by Dietary Administration.	Berthe, P.	10/5/96	Yes	82830
5.8.1/15	-	Summary paper on toxicity of fipronil metabolites.	Anon.	June 1999	No	82831
5.8.1/16	881022D/M&B 289/SE	Irritant effects on the rabbit eye of M&B 46,136	Liggett, P	11 Aug 1988	Yes	84610
5.8.2/01	HRC Report No. M&B 352/90958	M&B 46030: An investigation into the potential effects of thyroid function in male rats by studying thyroxine clearance.	Peters, D. H., et al.	12/4/91	Yes	71115
5.8.2/02	HRC Report No. M&B 353/90920	M&B 46030: An investigation into the potential effects of thyroid function in male rats by using the Perchlorate Discharge Test.	Peters, D. H., et al.	12/4/91	Yes	71119
5.8.2/03	HRC Report No. M&B 360/901275	M&B 46030: 4-week dietary study to investigate thyroid hormone levels in the rat.	Peters, D. H., et al.	20/5/91	Yes	71122
5.8.2/04	HRC/ITT 2/ 930645	The effect of single and repeated oral doses of M&B 46030 (1mg/kg/day and 10mg/kg/day) on the biliary excretion of intravenously administered 125I-Thyroxine (T4) from bile duct cannulated rats.	Taylor, T.	7/7/93	Yes	71124
5.8.2/05	UC Bushy Run 92N1074	MB 46030: Ninety day dietary neurotoxicity study in Sprague Dawley rats.	Driscoll, C. D. and Hurley, J. M.	15/9/93	Yes	71125
5.8.2/06	LSR Report No. 90/RHA371/0790	M&B 46030: Neurotoxicity Study by oral (capsule) administration to female beagle dogs for up to 14 days followed by a 28 day reversibility period.	Holmes, P.	21/11/91	Yes	71126
5.8.2/07	RNP 536/973345	Fipronil Neurotoxicity to Rats by Acute Oral Administration (Including a Time to Peak Effect Study).	Hughes, E.W.	6/11/97	Yes	82832
	92/RHA311/0464	M&B 46030: Toxicity by oral (capsule) administration to beagle dogs for 52 weeks.	Holmes, P.	1992	Yes	112755
	93/RHA465/0243	M&B 46030: Toxicity by oral (capsule) administration to beagle dogs for 52 weeks.	Holmes, P.	1993	Yes	112756
	282/465	Fipronil: Chromosomal aberration test in CHL cells *in vitro*.	Wright, N.P.	1995	Yes	112757

		Fipronil: Comment on the findings of the chromosomal aberration test in CHL cells *in vitro*.	Percy, A.	9/7/2001	No	112758
		Fipronil: Rebuttal to the UK PSD definition of the short term systemic acceptable operator exposure level.	Percy, A.	9/7/2001	No	112759
		Fipronil: Absorption of fipronil in the rat following oral administration at 4 mg/kg.	Fisher, P.J.	7/7/2001	No	112760
	HWI-6224-210	Dermal absorption of 14C-fipronil Regent 80WDG in male rats (preliminary and definitive phases).	Cheng, T.	10/2/95	Yes	116914
	93/RHA305/0571	M&B 406030: Assessment of clastogenic action on bone marrow erthrocytes in the micronucleus test	Edwards, C.N.	8/3/91	Yes	116915
	R010246	Fipronil: Bile excretion study in the rat. Rhone Poulenc report no. 600849, study no. SA 95020	Totis, M.	Sept 1995	?	114688
		Justification for a dermal adsorption factor of 1% for use in human risk assessments. (Position paper)	Bars, R.G.	29/6/99	No	117141
	WHO/PCS/98.6	JMPR (1998) Pesticide residues in food - 1 Part II. Toxicology and Environmental. World Health Organisation, Geneva 997 evaluations.		1998.	No	Public domain
	WHO/PCS/01.3	JMPR (2001) Pesticide residues in food - 2000 evaluations. Part II. Toxicological . World Health Organisation, WHO/PCS/01.3, Geneva		2001.	No	Public domain
	FAO Plant Production and Protection Paper 167	JMPR (2002) Pesticide residues in food - 2001. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group		2001	No	Public domain

6. RESIDUES

This application is for use on non-edible ornamentals only.

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
	Agredoc R010589	Fipronil (14C-phenyl ring labelled) subsurface soil treatment: accumulation study on confined rotational crops.	Jesudason, P.A. & Mackie, S.J.W	14/12/95	Yes	112745

7. ENVIRONMENTAL FATE AND BEHAVIOUR

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
7.1.1.1.1	Hazelton UK, Project No. 68/109-1015	(14C)-M&B 46030: Aerobic soil metabolism.	Waring, A.R.	25/2/93	Yes	71129
7.1.1.1.2	D. Ag. 1743	M&B 46030 - 14C Soil Photolysis Study.	Burr, C.M. & Austin, D.J.	4/6/92	Yes	71132
7.1.1.2.1	Study No. P 91/087; Doc No. 200514	Insecticides: Fipronil: Rate of Degradation in Four Soils under Aerobic Conditions at 10°C and 22°C.	Humphreys, S.P., Lowden, P. & Oliver, R.G.	17/6/94	Yes	71133
7.1.1.2.2	Doc No. 200795	Fipronil: Terrestrial Field Soil Dissipation Study in Europe.	Boussemart, M.J. & Wicks, R.J.	Feb 1995	Yes	71134
7.1.2/01	Report No. P91/084	M&B 46030-14C: Adsorption/desorption on five soils.	Godward, P.J., Quarmby, D.L. & Austin, D.J.	21/5/92 & Amend't 19/4/96	Yes	71135
7.1.2/02	RPA Doc: 201594 RPAL Study: 13510	[^{14}C}-M&B 45950: Adsorption/Desorption to and from Four Soils and One Sediment.	Burr, C.M.	19/11/97	Yes	82833
7.1.2/03	RPA Doc: 201555 RPAL Study: 13509	[14C]-M&B 46136: Adsorption/Desorption to and from Four Soils and One Sediment.	McMillan-Staff, S.L.	14/11/97	Yes	8835
7.1.2/04	RPA Doc: 201554 RPAL Study: 13447	[^{14}C]-RPA 200766: Absorption/Desorption to and from Four Soils and One Sediment.	McMillan-Staff, S.L.	24/11/97	Yes	82838
7.1.3.1 & 2	D. Ag. 1744	Insecticides: M&B 46030 - 14C: Leaching Study with Five Soils.	Godward, P.J., Quarmby, D.L., & Austin, D.J.	8/6/92	Yes	71137
7.2.1.1 = 2.9.1	AG/CRLD/AN 9215072. Study No. 91-25	14C-MB 46030 Hydrolysis at 25°C.	Corgier, M. & Plewa, A.	16/3/92	Yes	70991
7.2.1.2/01	AG/CRLD/AN/9 215873. Study No. 91-55	14C-MB 46030 Aqueous Photolysis.	Corgier, M. & Plewa, A.	15/5/92	Yes	71138

7.2.1.2/02	Study No. 97/176 ENH/ENV Doc No. CP/MAN/ENH/338/97/299	Determination of the Direct Phototransformation of Fipronil in Water.	Boinay, P.	25/8/97	Yes	82840
7.2.1.3.1	SPL Project No. 238/042	Fipronil: Assessment of Ready Biodegradability; CO2 Evolution Test.	Mead, C.	6/1/97	Yes	71140
7.2.1.3.2	RPA Document 201604; RPA Study 13333	[^{14}C]-Fipronil Degradation and Retention in Two Water/Sediment Systems.	Ayliffe, J. M.	Feb 1998	Yes	71143
	CRL/SB/TXT1189	The use of the product 'Vi-Nil GR' on non-edible ornamentals in horticulture formulated as a granule containing 0.1% w/w fipronil.	Leake, C.R.	27/7/2001	No	112741
		Spent peat compost survey conducted by Hortichem on behalf of Aventis.		May 2001	No	112742
	HDC no. C166	Protected ornamentals: the efficiency of water use in different production systems.	Briercliffe, T.	Dec 2000	No	112744
	TXT561.doc	The non-relevance of the metabolite RPA 200761 Rhone- Poulenc Environmental Sciences overview Paper	Leake, C.R.	21/7/98	No	112746
	TXT331.doc	The non-relevance of the metabolite RPA 200766 Rhone- Poulenc Environmental Chemistry overview Paper	Leake, C.R. & Blacker, A.M.	12/12/97	No	112747

8. ECOTOXICOLOGY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
8.1.1/01	BLAL# 89-QD-133	M&B 46030 Technical: 21 day acute oral LD50 study in Bobwhite Quail	Pederson, C. A.	21/5/90	Yes	71146
8.1.1/02	BLAL# 89-DD-70	M&B 46030 Technical: 21 day acute oral LD50 study in Mallard Ducks.	Pederson, C. A.	7/8/90	Yes	71147
8.1.2/01	BLAL# 89 QC 135	M&B 46030 Technical: 22-Day Acute Dietary LC50 Study in Bobwhite Quail.	Pederson, C.A.	7/8/90	Yes	71154
8.1.2/02	BLAL# 89 DC 132	M&B 46030 Technical: 22-Day Acute Dietary LC50 Study in Mallard Ducklings.	Pederson, C.A.	7/8/90	Yes	71155
8.1.3/01	BLAL No. 108-005-07	MB 46030 Technical: Toxicity and Reproduction Study in the Bobwhite Quail.	Pederson, C. A. & DuCharme, D. R.	19/6/92	Yes	71156

8.1.3/02	BLAL No. 108-013-08	MB 46030 Technical: Toxicity and reproduction study in Mallard Ducks.	Pederson, C. A. & Lesar, C.L.	8/6/93	Yes	71159
8.2.1/01	TES Report No. J9005012a	M&B 46030: Acute toxicity to Rainbow Trout, Oncorhynchus mykiss, under flow-through test conditions.	Scott Ward, G.	5/8/91	Yes	71164
8.2.1/02	TES Report No. J9005012b	M&B 46030: Acute toxicity to Bluegill, Lepomis macrochirus, under flow-through test conditions.	Scott Ward, G.	14/3/91	Yes	71166
8.2.2.1& 8.2.2.2	SLI Report No. 92-1-4084	(M&B 46030) - The toxicity to Rainbow Trout (Oncorhynchus mykiss) during an early-life stage exposure.	Machado, M. W.	22/6/92	Yes	71169
8.2.3/01	IRI Report No. 8892; Project No. 381457	[14C]-M+B 46,030: Bioaccumulation Test in Bluegill Sunfish.	Chapleo, S. & Hall, B.E.	21/10/92	Yes	71179
8.2.3/02	P92/302	[^{14}C] M&B 46,030: Investigation into the Nature and Possible Structures of Metabolites in Fish used in a Bioaccumulation Study at Inveresk Research International (Study No. IRI/381457).	Roohi, A. *et al.*	26/2/93 (Amend: 25/5/94)	Yes	71181
8.2.4	SLI Report No. 89-11-3161	(M&B 46030) - Acute toxicity to Daphnids (Daphnia magna) during a 48-hour flow-through exposure.	McNamara, P. C.	30/3/90	Yes	71184
8.2.5	SLI Report No. 90-01-3210	The Chronic Toxicity of M&B 46030 to Daphnia magna Under Flow-through Conditions.	McNamara, P. C.	5/6/90	Yes	71193
8.2.6	SPL Project No. 282/95	The Algistatic Activity of M&B 46030.	Handley, J. W. et al.	18/6/91	Yes	71197
	SLI report no. 94-4-5224	MB 46030 – acute toxicity to mysids (Mysidopsis bahia) under static conditions	Machado M W	28/4/94	Yes	85260
8.3.1.1	HRC Report No. RNP 391/911047	The Acute Contact and Oral Toxicity to Honey Bees of M&B 46030.	Cole, J.H.	8/10/91	Yes	71208
8.3.2/01	AEU Report No. RP-96-6	A laboratory evaluation of the side-effects of the insecticide EXP 60720 (an 80% wettable granule formulation of fipronil) on the predatory mite Typhlodromus pyri.	Vinall, S.	23/1/97		71210
8.3.2/02	AEU Report No. RP-96-4	A laboratory evaluation of the side-effects of the insecticide EXP 60720 (an 80% wettable granule formulation of fipronil) on the parasitic wasp Aphidius rhopalosiphi.	Longley, M	3/1/97		71213

8.3.2/03	AEU Report No. RP-96-3	A laboratory evaluation of the side-effects of the insecticide EXP 60720 (an 80% wettable granule formulation of fipronil) on the lycosid spiders of the Pardosa genus.	Mead-Briggs, M.	22/11/96		71228
8.3.2/04	AEU Report No. RP-96-5.	A laboratory evaluation of the side-effects of the insecticide EXP 60720 (an 80% wettable granule formulation of fipronil) on the ladybird Coccinella septempunctata.	Vinall, S. & Mead-Briggs, M.	23/1/97		71230
8.4.1	Safepharm Project No: 282/94	The Acute Toxicity of M&B 46030 to Earthworms (Eisenia foetida).	Handley, J.W. & Wetton, P.M.	15/5/91	Yes	71231
		Bioaccumulation in the soil to earthworm system	Connell D W & Markwell R D	1990	No	85257 Public domain
8.5	ELL Report No. ELL/687A/1	A Laboratory Assessment of the Effects of M&B 46030 on Soil Microflora Respiration and Nitrogen Turnover.	Alred, D. & Seal, K.J.	3/2/92	Yes	71232
	Docmap 604557	RPA 200761: Algal inhibition test.	Mead, C. & Mullee, D.M.	14/9/99	Yes	112748
	Docmap 604558	RPA 200761: Acute toxicity to *Daphnia magna*	Wetton, P.M. & Mullee, D.M.	10/9/99	Yes	112750
	Docmap 604559	RPA 200761: Acute toxicity to Rainbow Trout (*Oncorhynchus mykiss*)	Wetton, P.M. & Mullee, D.M.	10/9/99	Yes	112751
	Fipronil IV.46	RPA 200766: Acute toxicity (96h) to Rainbow Trout (*Oncorhynchus mykiss*) - Static screening data.	Suteau, P.	17/7/92	No	112752
	Fipronil IV.45	RPA 200766: Acute toxicity (48h) to *Daphnia magna* - Static screening data.	Suteau, P.	16/10/92	No	112754
	C015729	RPA 200766 - Acute toxicity to rainbow trout (*Oncorhynchus mykiss*) under static renewal conditions	Machado, M.W.	2001a	Yes	114883
	C014939	RPA 200766 - Acute toxicity to Daphnids (*Daphnia magna)* under static conditions	Machado, M.W.	2001b	Yes	114885
	C015726	RPA 200766 - Toxicity to freshwater green alga, *Scendesmus subspicatus.*	Hoberg, J.R.	2001	Yes	114888
	C-ECBI.5/ M&BDM.3	M&B 46030: acute toxicity to Dapnia magna under flow through conditions.	Scott Ward, G. Rabe, B	1989	Yes	101878

Additional reports relating to the formulation

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
		Document L-III (Annex III tier 1)	Anon	Oct 1998	No	82841
	-	Revised Document M-III – Sections 1 - 7	Anon	June 1999	No	82842

2. CHEMISTRY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
2.2.2, 2.3, 2.4.2 & 2.6.2	RP AG/3658	Fipronil 0.1 Granular Product Chemistry	Siemann, L.	3/2/95	Yes	71235
2.7.1/01, 2.8.6.1 & 2.8.6.2	DOC No. 201782 RPAL Study No. 14042	Fipronil GR: Assessment of Storage Stability of the Formulation in the Proposed Marketed Pack at Ambient Temperature and at 54°C for 14 days.	Patel, P. T	2/7/98	Yes	71238
2.7.1/02	Doc No. 201817 RPAL Study No. 14059	Fipronil GR: Assessment of the Loading and Stability in Composts.	Lingwood, A & Patel, P.T	15/10/98	Yes	71240
	Study no. 14058	Fipronil GR: Assessment of the storage stability of the formulation in the porposed marketed pack at ambient temperature for up to 2 years.	Patel, P.T.	3/5/2000	Yes	112736

7. TOXICOLOGY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
7.1.1	UC Bushy Run: 93N1366A	EXP 60819A: Acute Peroral Toxicity Study in the Rat.	Myers, R. C.	26/4/94	Yes	71241
7.1.2	UC Bushy Run: 93N1366B	EXP 60819A: Acute Percutaneous Toxicity Study in the Rabbit.	Myers, R. C.	26/4/94	Yes	71242
7.1.3	UC Bushy Run: 93N1359	EXP 60819A: Acute Dust Inhalation Study in Rats.	Nachreiner, D. J.	30/3/94	Yes	71243
7.1.4	UC Bushy Run: 93N1366C	EXP 60819A: Cutaneous Irritancy Study in the Rabbit.	Myers, R. C.	26/4/94	Yes	71245
7.1.5	UC Bushy Run: 93N1366D	EXP 60819A: Ocular Irritancy Study in the Rabbit.	Myers, R. C.	26/4/94	Yes	71246
7.1.6	UC Bushy Run: 94N1370	EXP 60819A: Dermal Sensitization Study in the Guinea Pig Using the Buehler Technique.	Myers, R. C. & Nachreiner, D. J.	26/4/94	Yes	71249
7.2.1.2	Study No. 94/136	Fipronil Worker Exposure Study During Application of Regent 20GR in Banana	Pontal, P.G., Carmichael, N.G. & Mondot, S.	20/5/96	Yes	71250

		Plantation.				
7.3	No reference specified	In Vitro Skin Permeability of M&B 46030.	Walters, K.A. & Brain, K.R.	No date specified	No	71251
7.4/01	MSDS # NCFH-1	Safety Data Sheet for Biodac.	None specified	17/4/89	No	71252 Public Domain
7.4/02	F Number: B0376A	Safety Data Sheet for Propylene Glycol.	None specified	15/8/89	No	71253 Public Domain
7.4/03	012621	Safety Data Sheet for 1-Methyl-2-Pyrrolidone.	None specified	4/9/84	No	71255 Public Domain

9. ENVIRONMENTAL FATE AND BEHAVIOUR

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
9.1.3	US93V02R; File No. 44324	A Terrestrial Field Soil Dissipation Study with Fipronil (MB-46030) Applied into Slits in Bare Soil and Soil with Established Turf.	Chancey, E.L & Norris, F.A.	23/5/94	Yes	71257

10. ECOTOXICOLOGY

Point	CO. REF.	Study Title	Author	Study Date	GLP	DP No.
10.1.3	RNP 397/920551	EXP 60166 (Fipronil 2% Granular) Palatability to the Bobwhite Quail.	Hakin, B. & Rodgers, M.	7/7/92	Yes	71260

Other references relied on:

Uniform Principles for Safeguarding the Health of Applicators of Plant Protection Products. Biologische Bundesanstalt für Land- und Forstwirtschaft (BBA), Bundesgesundheitsamt, und Industrieverband Agrar e.V. (1992) ISBN 3489-27700-7.

Kissel J.C., Richter K.Y. and Fenske R.A. (1996) Field measurement of dermal soil loading attributable to various activities : Implications for exposure assessment. *Risk Analysis* 16 : 115-125.

Driver J. H., Konz J. J., Whitmyre G. K. (1989). Soil adherence to human skin. *Bull. Environ. Contam. Toxicol.* 43: 814-820.

Best, B.L. and Gionfriddo, J.P. 1991 'Characterization of grit use by cornfield birds', Wilson Bull.; 103(1) pp 68-82.

Buxton, J.M. and Crocker, D.M. 1996, 'Birds and Farming: Information for Risk Assessment', MAFF Internal Contract Milestone Report

Churchfield, 1986 'Shrews', The Mammal Society, ISBN 0 904614 15 8

de Leeuw J, Gorree M, de Snoo G. R, Tamis W.L M, van der Poll, R.J and Luttick, R 'Risk of granules and treated seeds to birds on arable fields.' CML Report 118, 1995

Fischer, D.L. and Best, L.B, 1995 'Avian consumption of blank pesticide granules applied at planting to Iowa cornfields.' Environmental Toxicology and Chemistry, vol 14, No. 9 pp 1543-1549.

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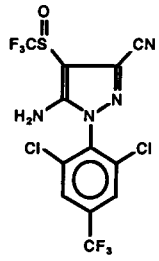
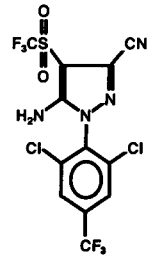
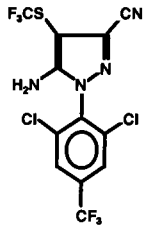
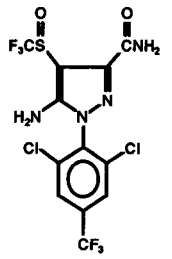
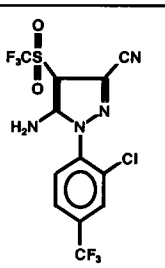
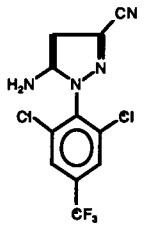
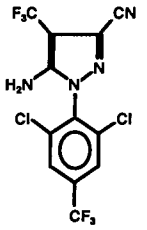
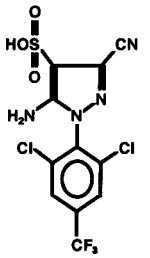
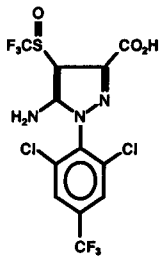
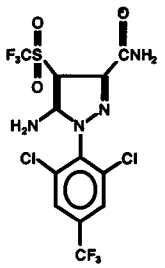
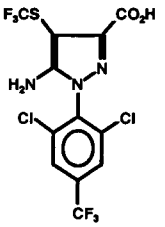
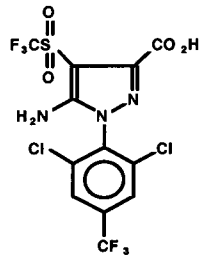
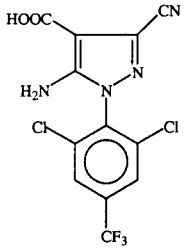
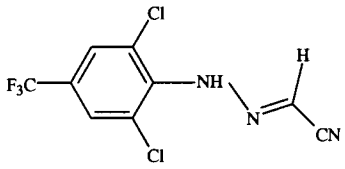
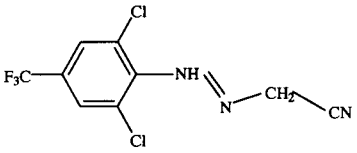
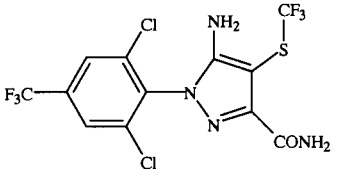
'Guidance Document on Aquatic Ecotoxicology in the frame of Directive 91/414/EEC' Draft working document (08.07.2000; 8075/VI/97 rev 7).

NAMES AND STRUCTURES OF DEGRADATION PRODUCTS/METABOLITES

Code(s) or name(s)	Structure	Occurrence
Fipronil (MB46030): 5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1RS)-(trifluoromethyl) sulfinyl]-1H-pyrazole-3-carbonitrile		Active Substance
MB 46136: 5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carbonitrile		Technical Impurity Mouse - Tissues & Faeces Rat - Tissues & Faeces Rabbit - Tissues & Faeces Sunflower - Soil incorporation Sugar beet - Soil incorporation Corn - Soil incorporation Cotton - Soil incorporation & Foliar spray Rice - Foliar spray & Granules Rotational crop - carrot leaf/ root; sorghum stover/forage; wheat straw/forage. Soil
MB 45950: 5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)thio]-1H-pyrazole-3-carbonitrile		Technical Impurity Mouse - Tissues Rat - Tissues & Faeces Rabbit - Tissues & Faeces Rice - Foliar spray & Granules Rotation crop - carrot root Soil
RPA 200766: 5-Amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(1RS)-(trifluoromethyl)-sulfinyl]-1H-pyrazole-3-carboxamide		Technical Impurity Rabbit - Liver Corn - Soil incorporation Cabbage - Foliar spray Rotational crop - carrot leaf/root; sorghum forage/stover; wheat grain/forage/straw Soil Water
RPA 100344: 5-Amino-1-[2-chloro-4-(trifluoromethyl)phenyl]-4-[(1RS)-(trifluoromethyl) sulfinyl] -1H-pyrazole-3-carbonitrile		Technical Impurity

Code(s) or name(s)	Structure	Occurrence
MB 45897:		Rat - Urine Rabbit - Urine Soil
MB 46513: (Fipronil-desulfinyl)		Cabbage - Foliar spray Potato - Foliar spray Rice - Foliar spray & Granules Rotational crop - wheat straw; sorghum stover Photolysis
RPA 104615:		Cabbage - Foliar spray Rotational crop - carrot root/ leaf; sorghum stover/ forage Photolysis
RPA 200761:		Corn - Soil incorporation Cotton - Soil incorporation & Foliar spray Rotational crop - carrot root/ leaf; sorghum stover/ forage; wheat grain/ straw Soil
RPA 105320:		Sugar beet - Soil incorporation Rotational crop - carrot leaf; wheat straw Soil
MB 46233:		Soil

Code(s) or name(s)	Structure	Occurrence
RPA 106681:		Soil
M & B 46400		Soil
RO/1		Rat - urine
RO/2		Rat - urine
RPA 105058		Rat - urine Rotational crop - wheat straw

(DP 82770, DP 83590)

SOIL CHARACTERISTICS

Study Ref. (DP No.)	Soil	Classification USDA	pH	OC (%)	Sand (%)	Silt (%)	clay (%)	microbial biomass (mg C/kg soil) day 0	microbial biomass (mg C/kg soil) *ca.* day 336±30	CEC (meq/ 100g soil)	MHC at 33 kPa (%)
DP71129	Manningtree	sandy loam	7.8	1.0	56	35	9	56.7	52.9	6.4	13.8
DP71129	Speyer 2.2	Loamy sand	6.1	1.9	88	9	3	414.4	153.7	3.3	14.2
DP71133	Manningtree	sandy loam	6.4	0.43	80	11	9	65.7	48.8/25	6.7	10.1
DP71133	Speyer 2.2	loamy sand	6.3	3.3	83	9	8	471.4	382/255	10.8	10.6
DP71133	French 1	sandy clay loam	6.2	0.7	47	27	26	145.4	134/77	14.1	17.9
DP71133	French 2	sandy clay loam	6.2	1.3	50	26	24	298.7	277/202	19.1	19.8
DP71132	Manningtree	clay loam	6.7	1.0	46	31	23	-	-	8.9	17.3
DP71134	Mereville	loam	8.1	1.4	30	46	21	192	-	24.8	33.8
DP71135	Manningtree	sandy loam	6.1	0.34	77	11	12	-	-	7.2	9.2
DP71135	Manningtree	loam	6.9	4.25	46	29	25	-	-	36.5	31.3
DP71135	Speyer 2.2	loamy sand	6.3	3.4	83	9	8	-	-	10.8	10.7
DP71135	French 1	sandy clay loam	6.2	1.2	58	18	24	-	-	12.6	21.0
DP71135	French 2	sandy clay loam	6.3	1.6	47	19	34	-	-	20.4	23.6
DP82833/5/8	Bosket	silt loam	6.2	0.5	36	56	8	-	-	5.7	25.4
DP82833/5/8	Rosholt	sandy loam	6.7	1.2	64	29	7	-	-	6.5	20.7
DP82833/5/8	Faulkbourne	loam	7.0	2.2	34	43	24	-	-	15.0	22.8
DP82833/5/8	Panholes	silt loam	8.1	1.9	21	55	24	-	-	65.7	25.9
DP82833/5/8	Sediment	sandy clay loam	8.2	2.3	52	23	25	-	-	63.6	30.0
DP71137	Manningtree	sandy loam	6.1	0.34	77	11	12	58	-	7.2	9.2
DP71137	Manningtree	loam	6.9	4.25	46	29	25	1420	-	36.5	31.3
DP71137	Speyer 2.2	loamy sand	6.3	3.4	83	9	8	471	-	10.8	10.7
DP71137	French 1	sandy clay loam	6.2	1.2	58	18	24	268	-	12.6	21.0
DP71137	French 2	sandy clay loam	6.3	1.6	47	19	34	700	-	20.4	23.6

Sediment	Organic matter (%)	Sand (%)	Silt (%)	clay (%)	Texture	pH	CEC (meq/100g)	P (mg/kg)	N (mg/kg)
Roding river	2.3	52.3	22.7	25.0	Sandy clay loam	8.2	63.6	953.7	1911.1
Manningtree stream	2.7	61.1	31.4	7.4	Sandy loam	6.8	8.1	615.6	2086.0

Water	pH	hardness (mg eq $CaCO_3$/l)	Organic C (mg/l)	P (mg/l)	N (mg/l)
Roding river	8.2	465	5.0	0.5	3.6
Manningtree stream	6.8	463	3.2	0.8	2.0

ND = not detected
CEC Cation Exchange Capacity

REVISED PROPOSED PRODUCT LABEL:

VI-NIL® GR

Contains 1.0 g/kg (0.1% w/w) fipronil

For the control of vine weevil in container grown ornamentals, both outdoors and in glasshouses.

STATUTORY CONDITIONS RELATING TO USE

FOR USE ONLY AS AN HORTICULTURAL INSECTICIDE

For use on:	Hardy ornamental nursery stock and non-edible ornamentals
Maximum individual dose:	1 kg product in 1 m^3 compost
Maximum number of treatments:	1 per batch of compost
Latest time of application:	Before planting in treated compost

Engineering control of operator exposure must be used where reasonably practicable in addition to the following personal protective equipment:
WEAR SUITABLE PROTECTIVE GLOVES when handling the product, admixing with compost, or handling treated compost.
However, engineering controls may replace personal protective equipment if a COSHH assessment show they provide an equal or higher standard of protection.

NOT TO BE USED ON FOOD CROPS.

READ ALL PRECAUTIONS BEFORE USE (MAFF XXXXX)

Marketing Company:
Hortichem Ltd
1b Mills Way
Boscombe Down Business Park
Amesbury
Wiltshire
SP4 7RX
Tel: 01980 676500
Fax: 01980 626555
e mail: hortichem@hortichem.co.uk

Size (Kg)
Batch No.

DIRECTIONS FOR USE

General Information

VI-NIL GR is a compost-incorporated insecticide from the control of vine weevil larvae. It is a specially formulated micro-granule containing fipronil. Fipronil is the first insecticide from a new chemical group, the phenyl pyrazoles. It has a unique mode of action compared to other insecticides on the market. It can be used at any stage of plant propagation to potting on. Compost treated with VI-NIL GR will give up to two years protection against vine weevil.

VI-NIL GR should be evenly mixed into the compost by hand or by mixing machine. This thorough mixing is essential to give satisfactory pest control. The white micro-granules can easily be seen and are a help in checking even mixing. Treated compost should be used within 30 days of mixing. Store treated compost in a clean, dry area.

VI-NIL GR can be used on all compost types including, peat, peat/bark and other mixed composts. If more than 20% inert material (e.g. Rockwool) is incorporated, reduce the rate of VI-NIL GR in proportion to the reduction in amount of compost in the mix.

VI-NIL GR should not be used in compost for aquatic plants or marginals.

Rate

1 kg of VI-NIL GR per cubic metre of compost.

Treatment at potting up and potting on

VI-NIL GR must be incorporated into fresh compost each time the plant is re-potted. If untreated liners/plugs are potted into treated compost, control of vine weevil grubs can not be guaranteed in the untreated portion.

Crop safety

A wide range of ornamental species have been safely treated with VI-NIL GR. Species not listed below should be grown in small numbers to check for crop safety, before using VI-NIL GR on a large scale. VI-NIL GR can be used in plug trays, liners and in the final pot.

VI-NIL GR has been successfully used in the following ornamentals:

AZALEA
CYCLAMEN
FUSCHIA
JUNIPER
POLYANTHUS
RHODODENDRON
THUJA
and many more

PRECAUTIONS

WEAR SUITABLE PROTECTIVE GLOVES when handling the product or admixing with compost.

NOT TO BE USED ON FOOD CROPS.
AVOID ALL CONTACT BY MOUTH
WASH HANDS AND EXPOSED SKIN before meals and after work
KEEP OUT OF REACH OF CHILDREN
KEEP IN ORIGINAL CONTAINER tightly closed, in a safe place.
EMPTY CONTAINER COMPLETELY and dispose of safely.

Approval Holder: Rhône-Poulenc Agriculture Ltd
Fyfield Road
Ongar
Essex
CM5 OHW.

24 hr Emergency Telephone number: 0800 220876
or nearest National Poisons Information Centre.

VI-NIL® is a registered trademark of Rhône-Poulenc Agriculture Ltd.