AUSTRALIAN PESTICIDES AND VETERINARY MEDICINES AUTHORITY

AUSTRALIA

CHEMICAL REVIEW PROGRAM

REVIEW OF THE MAMMALIAN TOXICOLOGY

AND

METABOLISM/TOXICOKINETICS

OF

FIPRONIL

prepared by

Office of Chemical Safety & Environmental Health
Office of Health Protection

of the

Department of Health and Ageing

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ABBREVIATIONS

Time

d	Day
h	Hour
min	Minute
mo	Month
wk	Week
s	Second
yr	Year

Weight

bw	Bodyweight
g	Gram
kg	Kilogram
µg	Microgram
mg	Milligram
ng	Nanogram

Length

cm	Centimetre
m	Metre
µm	Micrometre
mm	Millimetre

Dosing

id	Intradermal
im	Intramuscular
inh	Inhalation
ip	Intraperitoneal
iv	Intravenous
po	Oral
sc	Subcutaneous
mg/kg bw/d	mg/kg bodyweight/day

Volume

L	Litre
mL	Millilitre
µL	Microlitre

Concentration

M	Molar
ppb	Parts per billion
ppm	Parts per million

Clinical chemistry and haematology

ALT	Alanine aminotransferase (SGPT)
ALP	Alkaline phosphatase
AST	Aspartate aminotransferase (SGOT)
CPK	Creatine phosphatase (phosphokinase)
GGT	Gamma-glutamyl transferase
Hb	Haemoglobin
Hct	Haematocrit
LDH	Lactate dehydrogenase
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
NTE	Neurotoxic target esterase
PCV	Packed cell volume (Haematocrit)
RBC	Red blood cell/erythrocyte
T_3	Triiodothyroxine
T_4	Thyroxine
TSH	Thyroid stimulating hormone (thyrotropin)
WBC	White blood cell/leucocyte
WBC-DC	White blood cells – differential count

Anatomy

CNS	Central nervous system
GIT	Gastro-intestinal tract

Chemistry

DMSO	Dimethyl sulfoxide
GC	Gas chromatography
GLC	Gas liquid chromatography
HPLC	High pressure liquid chromatography
MS	Mass spectrometry
TLC	Thin layer chromatography

Terminology

ADI	Acceptable Daily Intake
ARfD	Acute Reference Dose
GHS	United Nations Globally Harmonised System for Classification and labelling of Chemicals
GLP	Good Laboratory Practice
LOEL	Lowest Observed Effect Level
MOE	Margin of Exposure
MRL	Maximum Residue Limit or Level
NOEL	No Observed Effect Level
NOAEL	No Observed Adverse Effect Level
PHED	Pesticide Handlers' Exposure Database
PPE	Personal protective Equipment

Organisations & publications

ACPH	Advisory Committee on Pesticides and Health
AERP	Adverse Experience Reporting Program (through APVMA)
AFSSA	Agence Française de Sécurité Sanitaire des Aliments (French Food Health Safety Agency)
AFSSE	Agence Française de Sécurité Sanitaire Environmentale (French Environmental Health Safety Agency)
AGCS	Advisory Group on Chemical Safety
APVMA	Australian Pesticides and Veterinary Medicines Authority
ECETOC	European Chemical Industry Ecology and Toxicology Centre
EFSA	European Food Safety Authority
EMEA	European Medicines Agency
FAO	Food and Agriculture Organisation of the UN
FAISD	First Aid Instructions & Safety Directions
IARC	International Agency for Research on Cancer
JMPR	Joint Meeting on Pesticide Residues
NDPSC	National Drugs and Poisons Scheduling Committee
NHMRC	National Health and Medical Research Council
NOHSC	National Occupational Health & Safety Commission
NRA	National Registration Authority for Agricultural and Veterinary Chemicals
US EPA	United States Environmental Protection Agency
WHO	World Health Organisation

EXECUTIVE SUMMARY

The major reason for this review is that adverse experience reports in humans, particularly skin reactions, were referred to the Australian Pesticides & Veterinary Medicines Authority (APVMA) for home veterinary products containing fipronil. Following the data call-in process, a number of additional data submissions on the toxicology of fipronil were received from industry and the public. This information, together with all previously submitted registration data and relevant published data, were assessed in detail.

Fipronil is a broad spectrum insecticide belonging to the phenylpyrazole family of chemicals. Its primary site of action is at the gamma-aminobutyric acid (GABA) receptor in the central nervous system. Fipronil was first used in Australia as an agricultural chemical in 1994. Its current uses are wide-ranging and include insecticidal seed dressings, sprays to control various insect pests in pasture and crops, granules for use on turf, cockroach and ant baits, and topical veterinary products.

Fipronil has an ADI of 0.0002 mg/kg bw/d, based on a NOEL of 0.02 mg/kg bw/d in a chronic/carcinogenicity study in rats. This is a group value to cover fipronil, desulfinyl fipronil and fipronil sulfide and is affirmed in this review. The ARfD was 0.003 mg/kg bw, based on a NOEL of 0.3 mg/kg bw/d in a 3-month neurotoxicity study in rats. The ARfD was revised in 2006 to 0.02 mg/kg bw, based on a NOEL of 2.5 mg/kg bw/d for reduced landing footsplay in an acute oral neurotoxicity study in rats and the application of a 100-fold safety factor. This is the current listing in the ARfD list and is also a group value. Following a review of all the toxicity data it was concluded that there should be no change to the approval status of fipronil or to the ongoing registration of existing fipronil products in Australia.

Fipronil is in S6 of the SUSDP with a cut-off to S5 at 10%, and is unscheduled at 0.05% fipronil or less. As no new information provided for this review indicates that these levels are inappropriate, it is recommended that the current scheduling of fipronil is maintained.

Some amendments have also been made to the Safety Directions (SD) for products available in Australia.

TOXICOLOGY HAZARD PROFILE OF FIPRONIL

Absorption, distribution, metabolism and excretion in mammals

Rate and extent of oral absorption	T_{max}= 4-6 h; 80-90% absorbed.
Distribution	Wide distribution in the tissues. Highest tissue concentrations in the fat and adrenals.
Potential for accumulation	Slow release of radiolabel from tissues, indicating potential for fipronil metabolites to accumulate therein.
Rate and extent of excretion	Long elimination half-life (150-200 h). Most in the faeces and bile, relatively little in the urine.
Metabolism	Extensive; numerous metabolites in faeces, bile and urine.
Toxicologically significant compounds (animals, plants and environment)	Fipronil, the photodegradate desulfinyl fipronil, fipronil sulfone and fipronil sulfide.

Acute toxicity

Rat oral LD_{50} (mg/kg bw)	97
Worst oral LD_{50} in other species	91 (mice)
Rat dermal LD_{50} (mg/kg bw)	>2000 (no deaths)
Worst dermal LD_{50} in other species	354 (rabbits)
Rat inhalation LC_{50} (mg/m^3)	360
Worst inhalation LC_{50} in other species	No data
Skin irritation	Slight irritant
Eye irritation	Slight irritant
Skin sensitization	Non-sensitiser (Buehler; Magnusson & Kligman)

Short-term toxicity

Target/critical effect	Increased liver weight; thyroid hypertrophy; neurobehavioural effects; reduced bodyweight gain
Lowest relevant oral NOEL (mg/kg bw/d)	0.5 (90-day study in dogs)
Lowest relevant dermal NOEL (mg/kg bw/d)	5 (21-day, rabbit)
Lowest relevant inhalation NOEC (mg/m^3)	No data

Genotoxicity	Non-genotoxic

Long-term toxicity and carcinogenicity

Target/critical effect	Clinical signs of neurotoxicity; increased thyroid weight and decreased T4 levels; increased liver weight with microscopic abnormalities; increased severity of progressive senile nephropathy
Lowest relevant NOEL (mg/kg bw/d)	0.02 (rat chronic dietary, 89-91 weeks)

Carcinogenicity	No evidence of carcinogenicity

Reproductive toxicity

Reproduction target/critical effect	Parental: Reproductive effects of reduced litter size and pup viability were observed at ≥27 mg/kg bw/d, but increased thyroid and liver weights, decreased pituitary weight, increased incidence of follicular epithelial hypertrophy occurred at ≥2.5 mg/kg bw/d. Offspring: Reduction in mating performance and fertility index occurred at 27 mg/kg bw/d. Also at this dose, F1 pups showed clinical signs of neurotoxicity, reduced survival, reduced bodyweight gain and developmental delays
Lowest relevant reproductive NOEL (mg/kg bw/d)	Parental: 2.5 Offspring: 2.5
Developmental target/critical effect	Maternal: No evidence of developmental toxicity at the highest dose tested (1 mg/kg bw/d in rabbits, 20 mg/kg bw/d in rats), but decreased food consumption and bodyweight gain was seen in dams at these doses. Offspring: No evidence of teratogenicity at the highest dose tested.
Lowest relevant developmental NOEL (mg/kg bw/d)	Maternal: 1 Offspring: 1
Delayed neurotoxicity	No evidence of delayed neurotoxicity
Immunotoxicity	No data
Dermal absorption	Less than 1%

Summary

ADI (mg/kg bw/d) 0.0002
[clinical signs of neurotoxicity, increased thyroid weight, decreased T4 levels, increased severity of progressive senile nephropathy]
ARfD (mg/kg bw) 0.02
[decreased landing footsplay]

NOEL (mg/kg bw/d)	Study	Safety factor
0.02	Rat chronic/carcinogenicity	100
2.5	Rat acute neurotoxicity	100

Health Value in drinking water

Current: None
Proposed: 0.0007 mg/L

SUMMARY TOXICOLOGY REPORT

Introduction

In Australia, fipronil is the active constituent in 41 registered products used for the control of a wide range of insect pests in a variety of crops and turf; in ant, cockroach and fruit fly baits; and in veterinary products for tick and flea control in cats and dogs. Only those products registered prior to the commencement of the review (29 in all) are included in this review. Fipronil is included in Schedule 6 of the SUSDP, with cut-offs to Schedule 5 when in preparations containing 10% or less of fipronil, and is unscheduled in preparations containing 0.05% or less of fipronil. The current ADI for fipronil is 0.0002 mg/kg bw/d based on a NOEL of 0.02 mg/kg bw/d in a 2-year study in rats and including a safety factor of 100. The ARfD is 0.02 mg/kg bw based on a combined NOEL of 2.5 mg/kg bw/d from two acute oral neurotoxicity studies in rats and including a safety factor of 100. Both the ADI and ARfD are group values (fipronil, desulfinyl fipronil and fipronil sulfide). There is no National Health and Medical Research Council Health Value for fipronil in drinking water. Maximum Residue Limits (MRLs) have been set for fipronil in a range of vegetables, fruit, grain, herbs, spices, seeds, nuts, and oils, and in sugar cane, eggs, honey, milks, mushrooms, edible offal (mammalian and poultry) and meat (mammalian and poultry). The MRL's range from 0.005 to 0.2 mg/kg.

Absorption, Distribution, Metabolism and Excretion

Percutaneous absorption

Regent 80 WDG (~80% fipronil), spiked with [^{14}C]-fipronil, was applied to the skin of male rats at 0.07, 0.67 or 3.88 mg/cm^2 (volume 100 µL, area of application 12.5 cm^2) for various periods of up to 24 h. At 24 h post-application, the percentage of the applied dose that was systemically absorbed was very low (0.37, 0.40 and 0.07% in increasing order of dose). If the radioactivity in and on the skin is included, these amounts increase to 2.5, 4.1 and 0.6% respectively. Fipronil was poorly absorbed across rat skin *in vivo* (Cheng 1995).

Absorption rates of [^{14}C]-radiolabelled fipronil (200 g/L fipronil, formulation EXP60145A, equivalent to the Australian product Regent 200 SC), testosterone and hydrocortisone (20% w/w suspension of each in EXP60145A), and these suspensions diluted with distilled water, were measured through human, rat or rabbit epidermal membranes *in vitro*. Fipronil generally permeated rat and rabbit skin at an approximately 10-fold greater rate than human skin when applied at 200 g/L, 4 g/L or 0.2 mg/L, though at 0.2 mg/L permeation through rat and human epidermis was similar. The permeation rate of fipronil through human skin was less than the permeation rate for hydrocortisone and testosterone, and on this basis fipronil is a slow penetrant when applied in formulation EXP60145A (Walters & Brain 1990).

In vitro absorption studies through human and rat epidermis were conducted with ultra-low volume (ULV, 2.5 and 26 g/L), soluble concentrate (SC, 0.5 and 50 g/L), and emulsifiable concentrate (EC, 6 and 312 g/L) formulations of radiolabelled fipronil. The 26 g/L ULV product used in this study is similar to the current Australian product Regent 25 UL. In all cases, absorption was not proportional to the fipronil concentration applied, and the absorption rate was considerably higher through rat epidermis than through human epidermis (usually 10-fold). Radioactivity retained in the epidermis was high relative to the amount that penetrated, generally more so for rat epidermis than human. The amount absorbed was greater for the higher concentrations, but the percentage absorbed was lower (Ward 1997a, 1997b, 1998).

Oral absorption/ distribution/ excretion

The levels of radioactivity were analysed over time in the blood and tissues of rats after administration of single oral doses of 4 or 40 mg/kg bw/d [^{14}C]-fipronil. At 4 mg/kg bw, peak blood concentrations were achieved at 5-6 h post-dosing, but absorption was slower at 40 mg/kg bw, with maximal blood concentrations occurring at approximately 36 h. Elimination was slow at both doses, more so in females. Radioactivity was distributed widely in the tissues, with a similar distribution at either dose. Apart from the stomach and GIT and their contents, the highest levels of radioactivity were in the abdominal fat, followed by the adrenals, pancreas, thyroids, skin and fur, ovaries, uterus and the liver (Totis & Fisher 1994).

The tissue distribution of radioactivity was studied in the rat, mouse and rabbit by whole-body autoradiography at 12 and 72 h following a single oral dose of 5 mg/kg bw [^{14}C] fipronil. Radioactivity was widely distributed in the tissues, being highest in brown fat, fat and the Harderian gland. Elimination was slow, as tissue concentrations were only marginally reduced at 72 h post-dosing (Whitby 1991).

Bile duct-cannulated male and female rats were given single doses of 4 or 40 mg/kg bw [^{14}C]-fipronil by gavage. The major route of excretion was the faeces. The bile was also an important excretory route, and in male rats that received 40 mg/kg bw/d, the amount of radioactivity excreted in the bile slightly exceeded the amount excreted directly in the faeces. Excretion via the urine was relatively low in all groups. Elimination was slow, with ~80% and ~60% of the administered dose remaining in the tissues 72 h after dosing at the low and high doses, respectively. Systemic absorption was estimated to be ~90% at the low dose and 80% at the high dose. The vast majority of the radioactivity in the bile comprised numerous metabolites of fipronil, with little present as the parent compound. The metabolite profiles of bile from both dose groups and either sex were qualitatively similar, though most components were not chemically defined (Totis 1995).

Distribution/ metabolism

In an unpublished paper that compared the results of metabolism studies in mice, rats, dogs, rabbits, goats, hens and fish, it was concluded that the fate of fipronil in all of these species was qualitatively similar. The quantitative differences were greatest when metabolism of fipronil in the rabbit was compared with the rat, mouse and dog (Savage 1993).

Brain and blood samples were analysed for fipronil, MB 4590 (fipronil sulfide) and MB 46136 (fipronil sulfide) from male mice fed 100 ppm (approximately equal to 15 mg/kg bw/d) fipronil in the diet for 14 days, or 75 and 150 ppm over 28 days (approximately equal to 11 and 22 mg/kg bw/d), or until mortality reached 50%. Fipronil was detected in the brain of one mouse in the 100 ppm group that died prematurely, but otherwise only MB 46136 (fipronil sulfone) was detected in the blood and brain tissue of treated mice. Relative to survivors, mice that died did not have higher levels of fipronil or MB 46136 (fipronil sulfone) in the brain (Fisher 1991, 1992).

Rats were given a single oral dose of [^{14}C]fipronil at 4 or 150 mg/kg bw, or repeat doses of 4 mg/kg bw/d (unlabelled) for 14 days, followed by a radiolabelled dose on day 15. The major route of excretion was the faeces, from which up to 45% of the radiolabel was recovered at the single low dose, 75% at the high dose, and 60% after repeated dosing for the period up to 168 h post-treatment. Relatively low levels were found in the urine (up to 6, 16 and 29% after single low, single high and repeat dosing, respectively). Much of the radioactivity was recovered from the tissues (up to 45% at the single low dose, with the highest concentration detected in the fat) and

~20% after repeated dosing, but only 3-5% at the high dose. Fipronil was metabolised rapidly, with numerous metabolites found in both the faeces and urine. The major metabolite detected in the tissues was MB 46136 (fipronil sulfone), with unchanged fipronil found only in the faeces. After the single low dose, blood radioactivity peaked at 4-6 h, but elimination was slow (blood half-life up to 200 h after the single low dose), indicating a potential for bioaccumulation. Peak blood concentration was approximately in proportion to dose, but at 150 mg/kg bw the absorption phase was longer, and the elimination phase shorter than at the low dose of 4 mg/kg bw (Powles 1992).

Bile was collected from a group of rats given single oral doses of 4 mg/kg bw [^{14}C]-fipronil for 72 h post-dosing. The collected bile was then infused over a 24 h period into the duodenum of a second group of rats. For both groups, at least 70% of the radioactivity was absorbed. In the orally dosed group, 13% of administered radioactivity was recovered in the bile, 16% in the faeces, and <3% in the urine, with 59% in the tissues. The latter comprised 34% in the residual carcass, with the liver, skin and GIT contents accounting for most of the remainder. In comparison to the orally dosed group, the infused group had a similar amount of administered radioactivity in the faeces, slightly more in the urine, considerably more in the bile (38%), but only ~26% in the tissues. This study demonstrated that fipronil metabolites excreted in the bile may be reabsorbed and redistributed into the tissues (Kemp 1999).

Preliminary to a comparative metabolism study, mice (Broadmeadow 1991a), rats (Broadmeadow 1991b) and rabbits (Cummins 1991) were treated with 0.4 or 4 mg/kg bw/d [^{14}C]-fipronil for up to 14 days, followed by a reversibility period of up to 7 days. There were no deaths, no clinical signs of toxicity, or effects on bodyweight gain or food consumption. In the main study, in all species the principal metabolite in the tissues was MB 46136 (fipronil sulfone). At 0.4 mg/kg bw/d, MB 46136 (fipronil sulfone) increased over the dosing period, with levels greatest in fat, followed by thyroid, liver, brain and blood. At 4 mg/kg bw/d, maximum levels of radioactivity were achieved earlier in rats and mice than in rabbits, the latter also showing relatively long elimination times. The parent compound was detected in tissues, particularly during the first 24 h of dosing, and remained longest in the fat, particularly in rabbits. Fipronil sulfone (MB 46136) was found in the tissues in trace amounts, with RPA 200766 (fipronil amide) below the limit of quantification (Brockelsby 1991).

In a comparative metabolism study in rabbits, rats and mice, elimination of radiolabel was slow following single oral doses of 5 mg/kg bw [^{14}C]-fipronil. A large percentage of the dose had not been excreted at 7 days post dosing (80% in rabbits, 40% in rats, and 60% in mice). Calculated elimination half-lives were approximately 14 days for rabbits, and 3 days for rats and mice. The highest concentrations of fipronil/metabolites were in the fat (rabbit>rat>mouse). For liver, kidney, muscle, brain and thyroid, little difference in radioactivity levels was seen between rat and mouse, but radioactivity was generally higher in rabbits. No significant levels of radiolabelled carbon dioxide were found in trapped air. The principal tissue metabolite was MB 46136 (fipronil sulfone), with traces of fipronil and MB 45950 (fipronil sulfide). Fipronil, fipronil sulfide and fipronil sulfone were found in the faeces (Lowden & Savage 1991).

Radiolabelled fipronil was incubated with rat or rabbit hepatocytes for 0, 1, 3, 5 or 24 hours to compare the metabolic pathways. TLC showed that fipronil was metabolised completely by rat hepatocytes, and partially by rabbit hepatocytes. HPLC analysis showed that both rat and rabbit hepatocytes metabolised fipronil to MB 46136 (fipronil sulfone, the principal metabolite). RPA104615 (fipronil detrifluoromethyl sulfonate) was also detected in the 24 hour incubation samples for both species, but this was considered likely to represent a product of the photolytic degradation of fipronil sulfone. Results indicated that rat hepatocytes metabolised fipronil faster than rabbit hepatocytes (Guyomard 1993; Fisher 1992).

When fipronil was incubated with rat or human liver microsomes, fipronil sulfone was the only metabolite detected. The Km values for fipronil metabolism were similar in rat and human microsomes, but the Vmax in the rat was approximately 3 times that in humans. There was a 40-fold variation in the rate of fipronil metabolism across human microsomes from 19 individuals, which correlated with levels of CYP3A4 and CYP2C19 (Tang *et al*. 2004).

Acute Studies

Active constituent

When administered by the oral route, fipronil was moderately toxic to mice, (LD_{50} ~91 mg/kg bw) (Mondot & Dange 1995) and in rats (LD_{50} = 97 mg/kg bw) (Gardner 1988a). Dermal toxicity was low in rats (LD_{50} >2000 mg/kg bw; aqueous suspension) and moderate in rabbits (LD_{50} = 354 mg/kg bw; corn oil) (Gardner 1988b, Myers & Christopher 1992). Inhalation toxicity was moderate in rats (LC_{50} = 682 mg/m^3; or 360/420 mg/m^3 in males/females) (Cracknell 1991; Nachreiner 1995). Toxicological signs were consistent with the known mode of action for fipronil as a GABA antagonist, and included hyperactivity, abnormalities of gait and posture, tremors and convulsions. Necropsy findings were limited to dermally exposed rabbits in which there were effects on the lungs, kidneys and spleen (e.g. discolouration/ enlargement/blood in urine, kidneys).

Fipronil moistened with water was not a skin irritant in rabbits (Liggett 1988a), but it was a slight skin irritant in another study which used corn oil as the vehicle (Myers and Christopher 1993a). Two eye irritancy studies in rabbits reported low levels of irritation, but differed in the time for which symptoms persisted. Overall, fipronil was a slight eye irritant (Liggett 1988b, Myers and Christopher 1993b). Fipronil was not a skin sensitiser in guinea pigs, with no evidence of a skin sensitisation potential seen in a Buehler assay (Smith 1990) and maximisation test of Magnusson & Kligman (Johnson 1993).).

In rats, MB 46513 (desulfinyl fipronil) showed high acute oral toxicity (LD_{50} = 18/15 mg/kg bw, males/females, Dange 1993a) and low acute dermal toxicity (LD_{50} >2000 mg/kg bw, Dange 1993b). In the oral study, deaths occurred on days 2 to 4, preceded by convulsions. Clinical signs in both studies were consistent with neurotoxic effects. The livers of decedents were enlarged, with various pathological changes including pale colour, foci of necrosis, and early fibrosis.

The acute oral toxicity of MB 45950 (fipronil sulfide) in rats was moderate when administered in corn oil (LD_{50} = 83 mg/kg bw, Dange 1994a), but in another study that used water as the vehicle, it had low acute oral toxicity (LD_{50} = 580 mg/kg bw, Haynes 1988a). Toxicity was characterised by piloerection, red staining of the fur, hypoactivity and convulsions. In both studies, males were more susceptible than females. When applied undiluted to rat skin, the dermal LD_{50} was >500 mg/kg bw (Haynes 1988b). In rabbits, MB 45950 (fipronil sulfide) was a slight eye irritant, but was not a skin irritant (Haynes 1987 a,b).

Fipronil sulfone (MB 46136) had moderate acute oral toxicity when administered to rats in corn oil (LD_{50} = 218 mg/kg bw, Gardner 1988c). Clinical signs included abnormal gait, lethargy, pallor of the extremities, diarrhoea, increased respiratory rate, ataxia, increased salivation and convulsions. In aqueous suspension, the dermal LD_{50} in rats was >2000 mg/kg bw (Gardner 1988d). When applied undiluted to rabbits, MB 46136 (fipronil sulfone) was not a skin irritant, but it was a slight eye irritant (Liggett 1988 c,d).

The acute oral toxicity of RPA 105048 (fipronil desulfinyl amide) was moderate in rats (LD_{50} = 467 mg/kg bw), with clinical signs of neurotoxicity similar to MB 46513 (desulfinyl fipronil) (Dange 1994f). The other metabolites of fipronil that were tested [RPA 200766 (fipronil amide), RPA 200761 (fipronil carboxylic acid), RPA 105320 (fipronil sulfonyl amide), and RPA 104615 (fipronil detrifluoromethyl sulfonate)] all had low acute oral toxicity in rats. The LD_{50} values for these compounds were >2000 mg/kg bw, with no deaths (Katchadourian 1995, Dange 1994e & 1993 c,d).

Products/Formulations (Australian)

The acute oral toxicity of Regent 200SC Insecticide (suspension concentrate, 200 g/L) in aqueous vehicle was low in rats (LD_{50} = 1099 mg/kg bw) and moderate in mice (LD_{50} = 324 mg/kg bw) (Dreher 1990 a,b). The dermal toxicity was low (LD_{50} > 4192 mg/kg bw and LD_{50} = 2493 mg/kg bw in rats and rabbits respectively) (Dreher 1990 c,d). Inhalation toxicity in rats was moderate (LC_{50} = 1070 mg/m^3) (Blagden 1993a). The product was a slight skin irritant and a moderate eye irritant in rabbits, and did not cause skin sensitisation in guinea pigs in a modified Buehler assay (Dreher 1993a, Glaza 1997, Dreher 1993b).

Acute studies were conducted with a 1 g/kg GR product (Code EXP60819A), similar to the product marketed in Australia, Chipco Choice Insecticide (1 g/kg GR), but differing in the carrier. This product had low acute oral (LD_{50} > 5000 mg/kg bw), dermal (LD_{50} > 2000 mg/kg bw), and inhalation (LC_{50} > 5160 mg/m^3) toxicity in rats (Myers 1994 a,b, Nachreiner 1994). No deaths were seen in any of these studies. The product was a slight eye irritant, but not a skin irritant in rabbits, and was not a skin sensitiser in guinea pigs in a Buehler assay (Myers 1994 c,d, Myers and Nachreiner 1994).

Regent 500 FS Seed Dressing Insecticide (500 g/L SC, EXP80415, equivalent to Cosmos Insecticidal Seed Treatment) had moderate oral toxicity (LD_{50} = 290 mg/kg bw), low dermal toxicity (LD_{50}>2000 mg/kg bw) and moderate inhalation toxicity (LC_{50} = 260 mg/m^3) in rats (Allen 1993a,b; Blagden 1993b). It was a slight skin irritant, but not an eye irritant in rabbits, and was not a skin sensitiser in guinea pigs using the modified Buehler assay (Allen 1993 c,d,e).

Frontline Spray (suspension concentrate, 2.5 g/L) had low acute oral toxicity (LD_{50} > 5000 mg/kg bw) and low dermal toxicity (LD_{50} > 2000 mg/kg bw) in rats (Clouzeau 1993a,b). Inhalation toxicity in the rat was also low (LC_{50} > 5060 mg/m^3) (Robinson 1993). The formulation caused moderate eye irritation, but no skin irritation in rabbits (Clouzeau 1993d,c). It was not a skin sensitiser in guinea pigs in a Buehler assay (Clouzeau 1993e).

Frontline Spot On for Cats and Frontline Spot On for Dogs containing 100g/L of fipronil, had low oral (LD_{50}=3000 mg/kg bw), dermal (LD_{50}>5000 mg/kg bw) and inhalation (LC_{50}>6320 mg/m^3) toxicity in rats (De Jouffrey 1994a & 1995, Kieran 1995). The formulation was a slight skin irritant and a moderate eye irritant in rabbits (De Jouffrey 1994bc). It was not a skin sensitiser in guinea pigs using the Buehler assay (De Jouffrey 1994d).

Frontline Plus for Cats was a slight skin irritant and a slight eye irritant in rabbits (Findlay 1999a,b). It was not a skin sensitiser in guinea pigs using the modified Buehler assay (Findlay 1999c).

Regent 25 ULV Insecticide, a product of similar formulation and toxicity profile to current Australian registered fipronil UL products, had low acute oral toxicity (3208 mg/kg bw), low acute dermal toxicity (> 4000 mg/kg bw) and low acute inhalation toxicity (LC_{50} > 5000 mg/m^3) in rats

(Warshawsky 1995a,b; Hilaski 1995). It was a slight eye irritant but not a skin irritant in rabbits, and was not a skin sensitiser in guinea pigs using the Buehler assay (Warshawsky 1995 c,d,e).

Goliath Cockroach Bait (0.5% fipronil) had low acute oral toxicity in rats (LD_{50}>2000 mg/kg bw) and low dermal toxicity in rabbits (LD_{50}>2000 mg/kg bw) (Mercier 1996a, 1995). It was a slight eye irritant, but not a skin irritant in rabbits, and was non-sensitising to the skin of guinea pigs using the modified Buehler assay (Mercier 1996 b,c,d).

Goliath^{R} Gel Cockroach Bait had low acute oral toxicity in male rats (LD_{50} = 4400 mg/kg bw), and low acute dermal toxicity in rats (LD_{50}> 5000 mg/kg bw). The formulation was not a skin or an eye irritant in rabbits, and was non-sensitising to the skin of guinea pigs using the maximisation test of Magnusson and Kligman (Grunert 1996 a-e).

Regent 800 WDG Insecticide (containing 800 g/kg fipronil) had moderate acute oral toxicity (LD_{50} = 177 mg/kg bw) and moderate acute inhalation toxicity (LC_{50} = 630 mg/m^3) in rats, and moderate dermal toxicity (LD_{50} = 569 mg/kg bw) in rabbits (Allen 1994a, Blagden 1994, Allen 1994b). It was a moderate eye irritant and a slight skin irritant in rabbits, and did not cause skin sensitisation in guinea pigs using the maximisation test of Magnusson and Kligman (Allen 1994 c,d,e).

Short-Term Repeat-Dose Studies

In a 6-week study in mice, fipronil was administered in the diet at 0, 15, 40, 300 or 800 ppm, equal to (M/F) 0/0, 2.4/2.9, 6.5/8.2, 20/22 and 37/43 mg/kg bw/d respectively. All mice at 300 and 800 ppm, and 11 males and 4 females at 110 ppm, died or were killed *in extremis* during the first 2 weeks, and 2 males at 40 ppm died during week 5. Clinical signs present at ≥300 ppm comprised thin build, hunched appearance, piloerection, respiratory abnormalities, pallor, body tremors and abnormal gait and posture. Some mice at ≥300 ppm had convulsions, with irritability and/or overactivity observed at ≥40 ppm from weeks 2 or 3. Food consumption was decreased in males at ≥15 ppm and females at ≥110 ppm, with associated bodyweight loss or reduced bodyweight gain. Liver weights were increased in a dose-related manner in all treated groups, with histopathological findings in the liver (mainly fatty vacuolation) increased in incidence and severity at all doses relative to controls. A NOEL was not achieved in this study due to increased liver weights and accompanying histopathology in both sexes, and reduced bodyweight gain in males at all doses. The LOEL was 2.4 mg/kg bw/d (Holmes 1990).

In a 4 week dietary study, fipronil was administered to rats at 0, 25, 50, 100, 200 or 400 ppm, equal to 0/0, 3.4/3.5, 6.9/6.7, 12.6/12.9, 24.5/24.9 and 45.3/54.9 mg/kg bw/d respectively. One 400 ppm female died during week 1. Bodyweight loss or reduced bodyweight gain occurred at ≥100 ppm and was related to decreased food consumption. Platelet numbers were increased in males at ≥200 ppm. Liver weights were increased in all groups, accompanied by minimal hepatocyte enlargement at ≥100 ppm. Thyroid weights were increased, particularly in females, and minimal thyroid follicular hypertrophy was noted in all treated groups. A NOEL was not established due to increased liver weights and thyroid follicular hypertrophy at all dose levels tested (Peters *et al*. 1990).

Dogs were dosed orally with capsules containing fipronil at 1 or 20 mg/kg bw/d for 4 weeks, or 0 or 10 mg/kg bw/d for 6 weeks. No animals died, but clinical signs were observed at 20 mg/kg bw/d, including underactivity, hunched posture, thin appearance, head nodding, facial twitching, continuous swallowing and abnormal posture or jerking of forelimbs, with a possible convulsive episode in one female on day 9. Head nodding was also reported for one female at 10 mg/kg bw/d. Reduced food consumption and associated weight loss occurred in dogs at 20 mg/kg bw/d. At the 3-week neurological examination, exaggerated flexor reflexes, head jerks or head nodding were noted

in animals at ≥10 mg/kg bw/d, with Hb and RBC also increased in some animals in these groups, and slightly elevated albumin and total protein at 20 mg/kg bw/d. The NOEL was 1 mg/kg bw/d based on neurological signs and increased Hb and RBC at ≥10 mg/kg bw/d (Holmes 1991a).

Rabbits were exposed dermally to fipronil over a 21-day period (5 d/week) at 0, 1, 5 or 10 mg/kg bw/d, under occluded dressings. Near the end of the study, two rabbits at 10 mg/kg bw/d had an episode of extreme hyperactivity. Also at 10 mg/kg bw/d, rabbits exhibited decreased food intake and decreased bodyweight gain. There were no observable effects at 5 mg/kg bw/d (Hermansky & Wagner 1993).

Subchronic Studies

Rats were fed fipronil in the diet for 13 weeks at concentrations of 0, 1, 5, 30 or 300 ppm, respectively equivalent to 0/0, 0.07/0.07, 0.33/0.37, 1.93/2.28 and 19.9/24.0 mg/kg bw/d in males/females. No deaths or clinical signs were observed, but food intake and bodyweight gains were initially depressed at 300 ppm. Total plasma proteins (globulins) were increased at 300 ppm. At ≥30 ppm, liver and thyroid weights were increased in both sexes, with an associated increased incidence of follicular cell hypertrophy and hyperplasia of the thyroid in both sexes at the top dose, and in 30 ppm males. The NOEL was 0.3 mg/kg bw/d, due to increased liver and thyroid weights at 2 mg/kg bw/d (Holmes 1991b).

Fipronil was administered to dogs in capsules at 0, 0.5, 2 or 10 mg/kg bw/d for 13 weeks. Dogs treated at 10 mg/kg bw/d showed convulsions, tremors, head-nodding, hunched posture, underactivity, inappetence, emaciation, disorientation, ataxia, apparent blindness, irregular heart rate, limb jerks and constricted pupils. Four dogs in this group were sacrificed *in extremis* in week 2. In dogs receiving 2 mg/kg bw/d or less, there were no deaths, and apart from inappetence, no treatment-related signs. Bodyweight loss, or decreased bodyweight gain occurred at ≥2 mg/kg bw/d. Alkaline phosphatase was increased and cholesterol levels decreased in males at 10 mg/kg bw/d. The NOEL was 0.5 mg/kg bw/d due to clinical signs (inappetence) and decreased bw gain at 2 mg/kg bw/d (Holmes 1991c).

Chronic/Carcinogenicity Studies

Groups of mice were fed fipronil in the diet at levels of 0, 0.1, 0.5, 10 or 30 ppm, equal to 0/0, 0.01/0.01, 0.05/0.06, 1.2/1.2, 3.4/3.6 mg/kg bw/d (males/females) for 53 (toxicity phase) or 78 weeks (oncogenicity phase). There were no clinical signs attributable to treatment. Mortality was not related to treatment, but unscheduled deaths were high (40-52/104 in the oncogenicity phase). An additional group of 72 males and 72 females fed 60 ppm fipronil had low weight gains and a high death rate (14 males, 7 females) by week 9, preceded by convulsions in 3 cases. Weight gains at ≥10 ppm were generally decreased. Necropsies were unremarkable. Both sexes treated with 30 ppm and males receiving 10 ppm had higher liver weights than controls and an increased incidence of hepatocellular hyperplasia and chronic degenerative changes in the liver. Male mice (oncogenicity phase) at 30 ppm showed a slightly higher (but not statistically significant) incidence of malignant hepatocellular tumours. The incidence was well within the historical control range. The study provided no evidence that the administration of fipronil in the diet at levels of up to 30 ppm was carcinogenic in mice. The NOEL was 0.5 ppm, equal to 0.05 mg/kg bw/d, based on increased liver weights and microscopic changes to the liver at 1.2 mg/kg bw/d (Broadmeadow, 1993).

Fipronil was fed to rats at dietary levels of 0, 0.5, 1.5, 30 or 300 ppm, equal to (males/females) 0/0, 0.02/0.03, 0.06/0.08, 1.3/1.6, 13/17 mg/kg bw/d respectively. After 52 weeks, some rats were

killed, some received normal diets for a 13-week reversibility period, and others were maintained on treated diets for 89-91 weeks. The 2-year study duration was shortened because of poor survival. Several rats in groups treated at ≥1.5 ppm showed neurological signs, including convulsions. These signs disappeared following cessation of treatment. Mortality was not related to treatment, except in the high-dose group during the early part of the study. Bodyweight gains of the 300 ppm group were reduced during treatment. Prothrombin time was slightly decreased in females at ≥30 ppm and in 300 ppm males. Also at 300 ppm, cholesterol, calcium, total protein and globulins were increased, and albumin was decreased. Circulating thyroxin (T4) levels were consistently lower than control values in all treated groups, and this was reversible. Thyroid-stimulating hormone (TSH) levels were sometimes higher at ≥30 ppm, and at least partially reversible during the recovery period. The incidence and severity of progressive senile nephropathy was increased in rats treated at ≥30 ppm. Liver and thyroid weights were significantly increased at ≥30 ppm at 52 weeks, with thyroid weights returning to normal after the recovery period. After 89-91 weeks of treatment, thyroid weights were increased in males at ≥1.5 ppm and females at ≥30 ppm. In rats treated for one year and assigned to the reversibility period, six (4/15 from the 300 ppm group) had follicular cell tumours. In the oncogenicity phase there was a significant increase in benign follicular cell adenomas for females receiving 300 ppm and for males at ≥1.5 ppm. Follicular cell carcinomas were also increased in male and female rats receiving 300 ppm compared with controls. These tumours are not considered relevant to humans (see Discussion). The NOEL was 0.5 ppm, equal to 0.02 mg/kg bw/d, based on clinical signs of neurotoxicity, increased thyroid weight, decreased T4 levels, and increased severity of progressive senile nephropathy at 1.3 mg/kg bw/d (Aughton 1993).

Dogs were orally dosed with fipronil in capsules at 0, 0.2, 2 or 5 mg/kg bw/d for 52 weeks. One dog receiving 2 mg/kg bw/d and two receiving 5 mg/kg bw/d were killed (in weeks 11, 31 or 34) after exhibiting convulsions, tremors, stiff limbs, gait abnormalities, lack of coordination, nervous behaviour, inappetence and bodyweight loss. All dogs receiving 5 mg/kg bw/d and 8/12 receiving 2 mg/kg bw/d showed intermittent signs of neurological disturbance (tenseness, nervous behaviour, hyperaesthesia, stiffness, abnormal gait and twitching of the facial muscles). There were no clear effects of treatment at 0.2 mg/kg bw/d. Food consumption and growth of treated survivors were similar to that of controls, as were haematology, blood chemistry and urinalysis findings. There were no ophthalmoscopic abnormalities, and organ weights were not affected. Pathology was unremarkable. The NOEL was 0.2 mg/kg bw/d based on neurological signs and bodyweight loss at 2 mg/kg bw/d (Holmes 1992).

Dogs were dosed with fipronil at 0, 0.075, 0.3, 1 or 3/2 mg/kg bw/d via the diet for 52 weeks, the top dose being reduced from 3 to 2 mg/kg bw/d after 32 days due to compound-related toxicity. One female at 3 mg/kg bw/d was killed on day 32, having displayed signs of neurological disturbance from day 10, including convulsive episodes. Elevated Hb, Hct, RBC, ALP, total protein and cholesterol were observed in this dog, and liver weight was slightly increased. Neurotoxic signs were also observed in another 3 males and 1 female at 3/2 mg/kg bw/d, commencing in week 1, and in 2 females at 1 mg/kg bw/d in weeks in weeks 13 or 20. Some dogs at 1 and 2/3 mg/kg bw/d had periods of inappetence, but bodyweight gains were not significantly affected. Plasma analyses revealed dose-related concentrations of the parent compound and the metabolite MB 46136 (fipronil sulfone), and these did not change markedly over the treatment period. Fipronil and MB 46136 (fipronil sulfone) levels were essentially similar in both sexes, with the metabolite levels exceeding the corresponding levels for fipronil. Spleen weights were increased (24-26%) in males at 3/2 mg/kg bw/d, with a higher incidence of swollen or large spleens, and hyperplasia of the splenic red pulp. The NOEL was 0.3 mg/kg bw/d based on clinical signs of neurotoxicity at 1 mg/kg bw/d (Holmes 1993).

Reproduction Study

Fipronil was fed to rats for two generations at dietary levels of 0, 3, 30 or 300 ppm, respectively equal to (males/females) 0/0, 0.25/0.27, 2.5/2.7 and 26/28 mg/kg bw/d. Mortality (spontaneous or humane sacrifice) was increased at ≥30 ppm in both parental generations, with deaths preceded by convulsions or other signs of neurotoxicity. At 300 ppm, reduced bodyweight gain was observed in both parental generations, and this was associated with decreased food consumption in F_0 animals. At ≥30 ppm, thyroid and liver weights were increased in F_0 and F_1 adults, ovarian weights were decreased in the F_0, and pituitary weights were reduced in the F_1. In both generations, follicular epithelial hypertrophy of the thyroid was seen in males at 30 ppm, and in both sexes at 300 ppm. At 300 ppm in the F_0 generation, there were reductions in mean litter size at day 1 *post partum*, and in pup viability up to day 4. Convulsions were observed in 13 offspring from 9 litters. In the F_1, mating performance was slightly reduced, with a consequent reduction in fertility index. The bodyweight of 300 ppm F_1 offspring at day 1 and subsequent weight gain to weaning were reduced, and tooth eruption was delayed. Also at 300 ppm, the F_2 offspring had a reduced post-implantation survival index, a reduced viability index at day 4 *post partum*; mean bodyweight at day 1 *post partum* and weight gain to weaning were lower; there was a slight delay in unfolding of the pinna, and convulsions were seen in 4 offspring from 3 litters. The parental NOEL was 3 ppm (equal to 0.25 mg/kg bw/d) due to increased thyroid and liver weights, decreased pituitary weight, and an increased incidence of follicular epithelial hypertrophy of the thyroid at 2.5 mg/kg bw/d. The NOEL for effects on the offspring was 30 ppm (equal to 2.5 mg/kg bw/d) due to clinical signs of neurotoxicity, reduced pup viability and bodyweight gain, and developmental delays at 27 mg/kg bw/d. The reproductive NOEL was 2.5 mg/kg bw/d, based on reduced litter size and pup viability in the F_0 and a reduction in mating performance and fertility index in the F_1 at 27 mg/kg bw/d (King 1992).

Developmental Studies

Pregnant rats were orally dosed with 0, 1, 4 or 20 mg/kg bw/d fipronil on gestation days 6-15. Maternal toxicity (decreased food consumption and bodyweight gain) was observed in 20 mg/kg bw/d dams only. There were no treatment-related effects on litter values or the incidence of abnormal offspring. The NOEL for maternotoxicity was 4 mg/kg bw/d, due to decreased bodyweight gain at 20 mg/kg bw/d. The developmental NOEL was 20 mg/kg bw/d, the highest dose tested (Brooker & John, 1991).

Fipronil was orally administered to pregnant rabbits on gestation days 6-19 at 0, 0.1, 0.2, 0.5 or 1 mg/kg bw/d. There was a dose-related decrease in bodyweight gain in dams at ≥0.5 mg/kg bw/d. Embryo and foetal survival, growth, and morphological development *in utero* were unaffected by treatment. The maternal NOEL was 0.2 mg/kg bw/d due to reduced bodyweight gain at 0.5 mg/kg bw/d. The developmental NOEL was 1 mg/kg bw/d, the highest dose tested (King 1990).

Genotoxicity Studies

Fipronil produced negative results in *in vitro* assays for chromosome aberration in human lymphocytes, in reverse mutation tests in bacteria, and in a gene mutation test in Chinese hamster ovary cells (Marshall 1988a, Clare *et al*. 1988a, Lloyd 1990). Fipronil was also negative *in vivo* in a mouse micronucleus test (bone marrow) (Edwards 1991, 1995). The only positive finding was in a chromosome aberration study in Chinese hamster lung cells *in vitro* after an incubation period of 6 h (with and without an exogenous source of metabolic activation). As clastogenicity was not observed after longer incubations (24-48 h), this was not considered a toxicologically significant effect (Wright 1995). On the weight of evidence, fipronil was not considered to be genotoxic.

In vitro genotoxicity studies were also conducted on various metabolites of fipronil. The metabolites MB 46513 (desulfinyl fipronil), MB 45950 (fipronil sulfide) and MB 46136 (fipronil sulfone) were not genotoxic in reverse mutation tests in *Salmonella typhimurium* or in chromosomal aberration studies in human lymphocytes (Adams 1996 a,b, Asquith 1987, Clare 1988b, Marshall 1988b & 1989, Percy 1993b & 1994a). Various other metabolites (RPA 200766 (fipronil carboxylic acid), RPA 104615, RPA 105320, RPA 105048, RPA 097920, RPA 200766 (fipronil sulfone amide)) were also not genotoxic in *in vitro* tests (Percy 1993 a,b,c,d, 1995, 1996; Allais 2002a,b). The metabolite RPA 097920 produced positive results in a chromosome aberration study using human lymphocytes, but this occurred at concentrations that caused marked cytotoxicity (Johnson 1995). Genotoxicity studies of fipronil metabolites, MB 46513 (fipronil desulfinyl) and RPA 200766 (fipronil carboxylic acid) produced negative results in rat micronucleus studies (bone marrow) *in vivo* (Proud 1996; Mehmood 2002).

Neurotoxicity Studies

Rats were dosed once by oral gavage with fipronil in corn oil at doses of 0, 0.5, 5 or 50 mg/kg bw, and killed 16-19 days later. Six deaths (5 males, 1 female) occurred in the high dose group, and survivors showed a variety of changes in nervous system function, including convulsions, tremors, head bobbing and myoclonic movements. At 7-8 h post-treatment decreases in open field activity, various reflexes, muscle tone and/or body temperature and motor activity were seen at 50 mg/kg bw, and decreased hind leg splay was observed at ≥5 mg/kg bw. The NOEL was 0.5 mg/kg bw, based on decreased hind leg splay at 5 mg/kg bw (Gill *et al*., 1993).

Rats were given single gavage doses of 0, 2.5, 7.5 or 25 mg/kg bw fipronil as a suspension in corn oil. At 25 mg/kg bw, staining/soiling of the head and anogenital regions was observed on day 2. During week 1, reduced bodyweight gains associated with decreased food consumption occurred in both sexes at 25 mg/kg bw, and in females at 7.5 mg/kg bw. Unusual behaviour/posture, increased grip strength, stationary position following positioning for tail pinch, reduced body temperature and decreased locomotor activity were noted at 25 mg/kg bw. At ≥7.5 mg/kg bw, landing footsplay and/or the frequency of grooming was decreased, and/or the incidence of vocalisation was increased. The NOEL was 2.5 mg/kg bw, based on reduced bodyweight gain and decreased landing footsplay at 7.5 mg/kg bw (Hughes 1997).

Fipronil was administered to rats in the diet at 0, 0.5, 5 or 150 ppm for 13 weeks (equal to 0/0, 0.02/0/03, 0.3/0.3, and 7.2/8.6 mg/kg bw/d for males/females). Early in the treatment period, food consumption and bodyweight gain were reduced at 150 ppm. No effects on nervous system structure or function were observed at any dose. In week 4 there was an increased incidence of exaggerated startle and tail pinch response in 150 ppm males, and in week 13 forelimb strength was increased in 150 ppm females. The NOEL was 5 ppm (equal to 0.3 mg/kg bw/d) due to neurobehavioural abnormalities at 150 ppm (Driscoll & Hurley, 1993).

Fipronil (20 mg/kg bw/d) was administered by capsule to dogs for 5, 7 or 13 days (until signs of neurotoxicity were apparent). All dogs were then observed for at least 28 days. Signs of neurotoxicity, which were evident in all treated dogs and continued for 2-10 days post treatment, included abnormal gait, tremors, stiffening of limbs or body, convulsions, head-nodding and facial twitches. One dog appeared to lose vision. Other signs included underactivity, inappetence, bodyweight loss, abnormal gait, behavioural abnormalities, hunched posture and peripheral vasodilatation. Neurological examination revealed a range of abnormalities and there was evidence of slow recovery during the reversibility period. Pathology was unremarkable (Holmes 1991d).

In a developmental neurotoxicity study, pregnant female rats were treated with fipronil in the diet at 0, 0.5, 10 or 200 ppm, from gestation day 6 to lactation day 10 inclusive. The doses were respectively equal to 0, 0.05, 0.9, and 8.7 mg/kg bw/d. Two 200 ppm dams died during the treatment period. Bodyweight loss associated with reduced food consumption was observed in 200 ppm dams on gestation days 6-10. Pup live birth index, viability index and weaning index were reduced at this dose, with reduced pup weight at ≥10 ppm throughout lactation. Delayed lower incisor eruption was noted at 200 ppm, and delays in sexual development were apparent in both sexes at 200 ppm. At 200 ppm, auditory response time was decreased, and swimming development was delayed. The maternal NOEL was 10 ppm, equal to 0.9 mg/kg bw/d, due to bodyweight loss at 8.7 mg/kg bw/d. The NOEL for pup toxicity was 0.5 ppm, equal to 0.05 mg/kg bw/d, due to reduced pup weight during lactation at 0.9 mg/kg bw/d (Mandella 1995).

Neurochemical effects

In a published study that examined the effects of fipronil on the levels of serotonin and its metabolite 5-hydroxy-3-indole acetic acid in the brains of rats dosed orally with 5 or 10 mg/kg bw/d fipronil for 6 days, levels of serotonin and its metabolite in the hypothalamus, hippocampus and striatum were decreased by 26-45% relative to controls (Anadon *et al*., 2004)

Metabolite studies

Studies have been conducted on a range of fipronil metabolites. The summaries of the acute and genotoxicity studies for these metabolites are presented with the corresponding studies for the active (Chapters 3 and 9 respectively). Other studies for metabolites are summarised below.

MB 46513 (desulfinyl fipronil)

Metabolism and Toxicokinetics

Single doses of [^{14}C]-MB 46513 (desulfinyl fipronil) were administered to rats by gavage at 1 mg/kg bw or 10 mg/kg bw. A repeat oral dose experiment was also conducted at 1 mg/kg bw/d over 15 days, with radiolabelled material administered on the final day only. The rats were sacrificed 7 days after treatment. The major route of excretion was the faeces, accounting for approximately 45-70% of the radioactivity administered. Unchanged desulfinyl fipronil accounted for up to 44% of radioactivity in the faeces, along with numerous other metabolites, the latter suggestive of a role for biliary excretion, though this was not studied directly. Urine accounted for up to 11% of the administered radioactivity, comprising up to 17 metabolites, but only a trace of the parent compound. The tissues accounted for 20-41% of the administered radioactivity, which was higher in females than males. Radioactive residues were highest in the fat and the residual carcass, with unchanged desulfinyl fipronil the only radioactive compound found in tissue extracts. The high levels remaining in the tissues were consistent with the long elimination half-life in whole blood of approximately 150-170 hours for males and 210-221 hours for females. The large number of metabolites identified in the excreta showed that desulfinyl fipronil was metabolised extensively by oxidation and hydrolysis, as well as sulfate, glucuronide and glutathione conjugation (Totis 1996).

Percutaneous absorption

In a dermal absorption study in rats, [^{14}C]-MB 46513 (desulfinyl fipronil) was applied to shaved intact skin under non-occlusive dressings at concentrations of 0.8, 8.1 or 80.3% (w/v) in 1% carboxymethylcellulose for intervals of up to 24 h. At 0.8% and 8.1% MB 46513 (fipronil

desulfinyl) respectively, 2.6% and 0.35% of the radioactivity was absorbed systemically over 24 h, with a total absorption of 9.3% and 1.7% respectively when radioactivity retained at the application site was also included. For the 80% group, the application site appeared to become saturated early in the experiment, reaching a maximum total absorption (systemic absorption + radioactivity at the application site) of ~0.7% of the applied dose at 4 h post-application (Cheng 1996).

Short-term repeat-dose studies

In a preliminary 28-day study, mice received MB 46513 (desulfinyl fipronil) in the diet at 0, 0.5, 3, 30 or 60 ppm, equal to (M/F) 0/0, 0.08/0.10, 0.49/0.61, 5.02/5.65 and 7.05/12.1 mg/kg bw/d respectively. Deaths occurred at 30 and 60 ppm, more so in males, along with clinical signs of increased motor activity, excessive jumps, irritability to touch and compulsive biting. Body weightBodyweight gain was reduced in these groups, associated with reduced food consumption, particularly in males. Liver weight relative to bodyweight was increased in 30 ppm males and 60 ppm females, along with microscopic changes (centrilobular hypertrophy) in some instances. The no-effect level was 3 ppm (equal to 0.5 mg/kg bw/day), based on treatment-induced clinical signs and deaths, reduced bodyweight gain, as well as increased relative liver weight and microscopic changes to the liver at 5 mg/kg bw/d (Dange 1994b).

In a 14-day gavage study, 5 rats/group were dosed with MB 46513 (fipronil desulfinyl) at 0, 0.3, 1, 3, or 10 mg/kg bw/d. One 3 mg/kg bw/d female died, and all rats at 10 mg/kg bw/d died or were killed moribund on days 5-8. Some animals had convulsions prior to death, and clinical signs (piloerection, chromodachryorrhea, prostration, excessive reaction to noise, curled up at handling, hunched posture, nasal discharge and few faeces) were noted at ≥3 mg/kg bw/d. Also at these doses, food consumption and bodyweight gain were reduced, and at 3 mg/kg bw/d, total bilirubin was decreased. No effects were observed at 1 mg/kg bw/day (Dange 1994c).

Rats received MB 46513 (desulfinyl fipronil) in the diet for 28 days at 0, 0.5, 3, 30 or 100 ppm, equal to (M/F) 0/0, 0.04/0.04, 0.23/0.24, 2.20/2.32 and 3.74/3.80 mg/kg bw/d respectively. One 30 ppm male died on day 6, and all 100 ppm rats died (days 5-15). One 100 ppm rat had tonic/clonic convulsions prior to death, and other clinical signs (piloerection, appeared thin, emaciation, excessive vocalisation and curled up at handling) occurred at ≥30 ppm. Also at these doses, food consumption and bodyweight were decreased, T3 and T4 levels were reduced (changes in T3 only in 100 ppm females), and total bilirubin was lower than controls at 30 ppm. No effects were observed at 3 ppm (equal to 0.23 mg/kg bw/d) (Dange 1995a).

In a 28-day study, dogs were administered MB 46513 (desulfinyl fipronil) in the diet at 0, 27, 80 or 270 ppm. At 270 ppm, the dogs were sacrificed on day 10 due to lack of food consumption. The 80 ppm animals were sacrificed moribund on days 10 or 15. There were no deaths at 27 ppm. Food consumption at 27 ppm was equal to 1 mg/kg bw/d, and at 80 and 270 ppm during week 1, dogs consumed (M/F) 1.9/1.7 and 2.3/2.3 mg/kg bw/d respectively, with much lower consumption in week 2, associated with weight loss. One animal at 27 ppm had clonic convulsions just prior to scheduled sacrifice. Neurotoxic effects were also seen at higher doses, with reduced motor activity, staggering step, irritability, increased salivation, absence of/few faeces and emaciation observed at 80 ppm, and emaciation and few faeces at 270 ppm. At 80 ppm, inflammatory and degenerative changes were seen in the liver, and thymic atrophy, red foci on the lungs and black spots on the gastric mucosa were also observed. A no-effect level was not established in this study due to clonic convulsions at the lowest dose, equal to 1 mg/kg bw/d (Dange 1995b).

Subchronic studies

Mice were fed 0, 0.5, 2 or 10 ppm MB 46513 (desulfinyl fipronil) in the diet for 90 days, equal to (M/F) 0/0, 0.08/0.11, 0.32/0.43 and 1.74/2.15 mg/kg bw/d respectively. At 10 ppm, one female died on day 5, 9 males died on days 20-62, and one male was killed moribund on day 84. Aggression and irritability to touch were observed in 2 male mice at 2 ppm, and 1 at 10 ppm, but due to the high death rate, and similar signs in other studies, the effects at 2 ppm could not be dismissed. The livers of some decedent males showed enlargement and/or mild centrilobular hypertrophy, or hepatocellular mitotic figures. The NOEL was 0.5 ppm (equal to 0.08 mg/kg bw/d), based on clinical signs of aggression and irritability to touch at 0.3 mg/kg bw/d (Bigot 1996).

In a 90-day study, rats received MB 46513 (desulfinyl fipronil) in the diet at 0, 0.5, 3, 10 or 30 ppm, equal to (M/F) 0/0, 0.03/0.04, 0.18/0.21, 0.59/0.71 and 1.8/2.1 mg/kg bw/d, respectively. One moribund male was killed on day 45, and 3 females died (days 11, 13 or 64). Clinical signs of aggression, irritability to touch and excessive vocalisation were observed in the 10 and 30 ppm groups, mainly in weeks 3-5, and occasionally in one male at 3 ppm. Bodyweight gain was reduced in 10 and 30 ppm rats of both sexes, though only slightly in the 10 ppm females. Food consumption was reduced at 30 ppm early in the study. Bilirubin and cholesterol levels were lower than controls in 30 ppm females, with slight reductions in T3 and/or T4 levels in both sexes at this dose. At macroscopic examination of the rats that died prior to scheduled sacrifice, adrenals were enlarged in all animals, necrotic areas were present in the livers of two females, plus focal gastric ulcerations/erosions in the male and one of the females. The NOEL was 3 ppm (equal to 0.18 mg/kg bw/d), based on treatment related clinical signs and reduced bodyweight gain at 0.6 mg/kg bw/d (Dange 1994d).

Dogs were fed diets containing MB 46513 (desulfinyl fipronil) at 0, 3.5, 9.5 or 35 ppm for 90 days, equal to (M/F) 0/0, 0.1/0.1, 0.27/0.29 and 0.95/1.05 mg/kg bw/d respectively. One 35 ppm female was killed on day 28, exhibiting increased salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing and dyspnoea. Necropsy revealed marked coronary arteritis and myocardial necrosis. Another female at this dose displayed excessive barking and aggressiveness on day 28 and salivation, irritability and tremors on day 86. Mean bodyweights and food consumption were comparable between groups. Gross and microscopic examination of various tissues revealed no changes considered to be treatment related. Based on clinical signs seen in females at 35 ppm (equal to 1.05 mg/kg bw/d), the NOEL was 9.5 ppm (equal to 0.27 mg/kg bw/d) (Dange 1996).

Chronic study

In a 2-year combined chronic/carcinogenicity study, MB 46513 (desulfinyl fipronil) was administered to rats in the diet at 0, 0.5, 2 or 10 ppm. As a result of increased mortality rates being observed by study week 26, the dose for 10 ppm females was reduced to 6 ppm. In ascending order, doses were equal to (M/F) 0/0, 0.025/0/032, 0.098/0.127 and 0.497/0.546 mg/kg bw/d. Overall, the mortality rate was increased in all treated groups of both sexes, but was considered possibly related to treatment only in females at ≥2 ppm. An increased incidence in the clinical signs of aggressiveness and irritability was considered treatment-related in males at ≥2 ppm. Convulsions were seen in all groups, though was considered related to treatment only in females at ≥2 ppm. The incidence of spongiosis hepaticus was increased in males at ≥2 ppm, but as this was similar to the control incidence in a separate concurrent study, it was considered incidental to treatment. There were no neoplastic findings that could be attributed to treatment. The NOEL was 0.5 ppm (equal to 0.03 mg/kg bw/d), based on increased mortality and convulsions in females at 2 ppm (equal to 0.1 mg/kg bw/d), and an increased incidence of aggressiveness and irritability to touch in males at the same dose (equal to 0.12 mg/kg b/w/d) (Bigot 1998).

Developmental

In a developmental study, MB 46513 (desulfinyl fipronil) was administered to rats by gavage at 0, 0.2, 1.0 or 2.5 mg/kg bw/d during gestation days 6 to 15. Maternal bodyweight gain was reduced at 2.5 mg/kg bw/d in conjunction with reduced food consumption. The incidence of maternal hair loss, sometimes severe, was also increased at this dose. Fused placentas were noted for one dam at 1 mg/kg bw/d and 3 dams at 2.5 mg/kg bw/d, but no associated adverse effects on the foetuses were apparent. Foetal bodyweight was reduced at 2.5 mg/kg bw/d, and this was associated with slightly delayed ossification of various bones, but no foetal abnormalities were observed. The NOEL for maternal toxicity was 1 mg/kg bw/d, based on reduced bodyweight gain and severe hair loss at 2.5 mg/kg bw/d. The NOEL for embryo-foetal developmental toxicity was 1 mg/kg bw/d, based on delayed skeletal ossification at doses of 2.5 mg/kg bw/d (Foulon 1997).

Neurotoxicity

In an acute neurotoxicity test, rats were given single doses of MB 46513 (desulfinyl fipronil) at 0, 0.5, 2 or 12 mg/kg bw by gavage, then observed for 14 days. A functional observation battery (FOB) and motor activity assessment were performed on all animals before treatment, at 6 h post dosing, and on days 7 and 14. In a dose range-finding study, the time of peak effect was determined to be 4 to 6 hours after dosing. In the main study, no unscheduled deaths occurred. A significantly lower bodyweight gain was reported for both sexes at 12 mg/kg bw over the first week, and this was a consequence of significantly decreased food consumption for both sexes at this dose. Bodyweight gains recovered by study week 2, and in some animals the rate slightly exceeded controls at this time. Bodyweight gains were unaffected at lower doses, and final mean bodyweights were comparable for all groups. In the FOBs, both sexes receiving 12 mg/kg bw/d showed treatment related decreased footsplay, decreased locomotor activity and lower rectal temperature at 6 hours, and increased incidences of slow righting reflex (males only) on days 7 and 14. There were no significant differences in brain weights or measurements, and slight increases in axonal degeneration at 12 mg/kg bw were not considered toxicologically significant. The no-effect level in this study was 2 mg/kg bw, based on decreased bodyweight gain in the first week after treatment, and findings in the FOB tests (decreased footsplay, drop in rectal temperature, slow righting reflex, decreased locomotor activity) at 12 mg/kg bw (Hughes 1996).

<u>MB 45950 (fipronil sulfide)</u>

In a preliminary study, dogs were treated orally with 0, 1, 5 or 15 mg/kg bw/d of MB 45950 (fipronil sulfide) in gelatin capsules for 28 days. There were no deaths or treatment-related clinical signs. At 15 mg/kg bw/d, female bodyweight gain was reduced, associated with reduced food consumption in 1/2 of these animals. Also at this dose, females had slightly increased Hct, Hb and RBC numbers, while ALP activity was increased in males. The No Observed Effect Level was 5 mg/kg bw/d (Broadmeadow 1991a).

MB 45950 (fipronil sulfide) was administered to rats in the diet at 0, 10, 25, 50 or 300 ppm, equal to 0, 0.7/0.8, 1.8/2.2, 3.5/4.1, 21.5/24.6 mg/kg bw/d for males/females respectively, for 13 weeks. There were no deaths. At 300 ppm, damaged vibrissae and nasal (discharge) staining were noted. Food consumption was reduced in week 1, and overall bodyweight gain was reduced in males. Absolute and relative liver and thyroid weights were increased at 50 and 300 ppm in both sexes, with liver weights also increased in 25 ppm females. In both sexes the incidences of thyroid follicular cell hypertrophy and hyperplasia were increased at 300 ppm, with findings of hypertrophy in 1 or 2 animals at 50 ppm, and 1 male at 25 ppm. The NOEL was 10 ppm, (equal to 0.7 mg/kg

bw/d) based on increased liver weight in females and thyroid follicular cell hypertrophy in one male at 25 ppm (equal to 1.8 mg/kg bw/d) (Broadmeadow, 1991b).

RPA 200766 (fipronil amide)

The fipronil metabolite RPA 200766 (fipronil amide) was administered to rats in the diet for 28 days at 0, 50, 500, 5000 or 15000 ppm, equal to 0, 3.8/4.4, 38/44, 385/387 or 1087/1063 mg/kg bw/d, respectively, for males/females. One 15000 ppm female died. Bodyweight gain was reduced at ≥5000 ppm, and this was associated with decreased food consumption. At ≥5000 ppm, urea was increased in females and creatinine was increased in males, along with an increase in urine volume in males at ≥5000 ppm and in females at 15000 ppm indicating renal toxicity. In both sexes, cholesterol was increased at ≥500 ppm and triglycerides were increased at ≥5000 ppm, while total protein was increased in males at 15000 ppm, indicative of toxic effects on the liver. Liver weights were increased at ≥500 ppm, and hepatocellular hypertrophy was seen at 5000 ppm. Thyroid and prostate weights were increased in males at ≥500 ppm. Adrenal weights were increased in all treated male groups, accompanied by extra-medullary haemopoiesis at 5000 ppm, and fine/coarse vacuolation of the zona fasciculata at all doses in males and in 5000 ppm females. Because of increased adrenal weights and microscopic changes to this organ in all male groups, a NOEL was not established in this study. The LOEL was 4 mg/kg bw/d (Berthe, 1996).

RPA 104615 (fipronil detrifluoromethyl sulfonate)

Rats were treated with RPA 104615 (fipronil detrifluoromethyl sulfonate) in the diet for 28 days at 0, 50, 500, 5000 or 10000 ppm (equal to 0, 4.5/4.7, 45.7/50.4, 458/487 and 916/950 mg/kg bw/d for males/females, respectively). At ≥5000 ppm, prothrombin times were increased in males, triglycerides were increased in females, and plasma ALP was increased in both sexes. Total cholesterol was increased in females at 10000 ppm, and urinary pH was increased in all treated male groups, though in the absence of related findings, the latter was not considered toxicologically significant. Liver weights were increased at ≥5000 ppm, and there was an increased incidence of apparent hypertrophy of the follicular epithelium in the thyroid of males at 10000 ppm. The NOEL was 500 ppm (equal to 45 mg/kg bw/d) based on liver toxicity (increases in liver weights, plasma ALP and triglyceride levels, and prothrombin times) at 5000 ppm, equal to 460 mg/kg bw/d (Dange 1998).

MB 45897/RPA 097920 (fipronil detrifluoromethylsulfinyl)

Rats were dosed with RPA 097920 (fipronil detrifluoromethylsulfinyl) at 0, 50, 200 or 1000 mg/kg bw/d in maize oil by gavage for 4 weeks. Hunched posture and hypoactivity were observed at ≥200 mg/kg bw/d, with fur loss at 1000 mg/kg bw/d. Bodyweight gain was reduced in males at 1000 mg/kg bw/d. In females at 1000 mg/kg bw/d, RBC, Hb and Hct were slightly lower than controls. Total protein was increased in both sexes at 1000 mg/kg bw/d, along with α2-globulins, β-globulins and/or albumin. Lower levels of K and Cl and higher urea levels were observed in males of this group. Absolute and relative liver weights were increased at 1000 mg/kg bw/d in both sexes, associated with periacinar hepatic hypertrophy in some animals. The NOEL was 200 mg/kg bw/d, based on changes in haematology, clinical chemistry, and increased liver weight with associated pathological changes at 1000 mg/kg bw/d (Johnson 1995).

Human studies

Case studies

In a case study involving a worker who had sprayed Regent 200 SC (dilution not provided) for 5 hours without wearing any personal protection equipment, headache, nausea, vertigo and weakness were reported, commencing 2 h after completion of the spraying operation, and resolving spontaneously after 5 h. Physical and biochemical examinations were normal, but interpretation of symptoms was confounded by the patient's history of heart disease (Chodorowski & Anand 2004).

Mohamed *et al.* (2004) reviewed 7 prospectively recorded cases of self-poisoning with the fipronil product Regent 50 SC, sometimes ingested with other pesticides and/or alcohol. One patient who had ingested 100 mL of the fipronil product was admitted to hospital unconscious, whereupon he experienced several episodes of epileptic fits, subsequently developed pneumonia, and after 17 days without regaining consciousness, died. For the other cases overall, fipronil poisoning was characterised by vomiting, agitation and seizures, and normally had a favourable outcome if resuscitation and supportive care were provided. This study indicated that in humans, fipronil is rapidly absorbed, with clinical toxicity peaking in the first few hours and correlating with blood levels of fipronil and its metabolites. In two patients there was sufficient information to estimate an elimination half-life of 36-47 h.

Exposure studies – Dog and cat stroking studies

Studies were conducted to quantify the fipronil residues that can be dislodged by stroking the coat of a cat or dog treated with either Frontline Top Spot (10% fipronil) or Frontline Spray (0.25% fipronil) when used according to label instructions. Samples were collected from cotton gloves worn by the experimenter. Sampling occurred on 3 occasions on the day of treatment, then on a number of days up to day 29 post-treatment, from cats and medium dogs treated with the spot-on formulation (0.5 mL or 1.34 mL), or with the spray (6 mL/kg bw). Where detectable, fipronil sulfone and fipronil sulfide were found at very low levels relative to the parent compound, but desulfinyl fipronil was not detected. In nearly all cases, fipronil dislodgeable residues were detected throughout the study period, with maximum levels on the day of application, except for the spot-on treatment of dogs, where the time of maximum residue levels varied between days 1 to 3. The mean maximum amounts of fipronil residues dislodged after spray application were approximately 300 µg and up to 3 mg for cats and dogs respectively. For the spot-on, mean levels were up to ~7 mg on cats stroked at 1 h post-application, or 1 mg if left for 4 h, and for dogs up to ~4 mg was dislodged (Hughes 1997 b,c,d,e; de Fontenay 1997 a,b,c,d).

The amount of fipronil residues that could be dislodged when human hands vigorously contacted a dog treated with Frontline Spot On (1.34 mL; 10% fipronil) was measured. Maximum dislodgeable residues were detected at 1 day post-application, approximately equivalent to 2.4 mg fipronil, decreasing to about half this level after 3 weeks, with little remaining at day 29, and none detectable on day 36 (Jennings 2002).

In a study to evaluate the presence of desulfinyl fipronil on dog hair after treatment of the animal with Frontline spot-on or spray formulations, dogs were allowed access to outside natural light for approximately 6 hours/day on weekdays and 3 h/d on weekends/holidays for a period of 4 weeks. The outermost section of hairs removed from the backs of dogs treated with the spot-on had desulfinyl fipronil levels equivalent to 3.7-6.2% of the total fipronil residues present, increasing over the period from day 3 to day 14 post-application, but below the LOQ thereafter. Following treatment with the spray, desulfinyl fipronil residues were maximal on day 3, and detectable up to day 21, representing ~4.5-2.4% of total fipronil over this interval (Astruc *et al.* 1998).

Occupational exposure and indoor air levels following termiticide treatment

A total of 16 houses were treated by pest control operators with 0.07-0.08% Termidor 80WG in water at 4.968 L/m on outer and interior walls and 2.484 L/m injected into foundation walls. Exposures in 16 of the workers ranged from 0.03 to 5.5 µg/kg/h, with a mean of 0.95 µg/kg/h, following application to houses with crawl-spaces. Airborne fipronil residues in treated houses ranged from 0.006 - 0.081 ng/L in slab-construction houses, 0.005 - 0.042 ng/L in houses with cellars and 0.004 - 0.011 ng/L in houses with crawl-spaces. Airborne fipronil residues were detected most frequently on the day of application, with residues still detectable in three houses 7 days after application. Airborne fipronil residues were below the limit of detection in 4 of the 16 treated houses (Honeycutt 2001).

Other Studies

Mechanism of action

In a series of *in vitro* experiments, Bushey (1993) demonstrated that fipronil reversed the effect of gamma-aminobutyric acid (GABA) in the CNS of housefly maggots, inhibited GABA-activated transport of chloride ions across the cell membrane in rat brain microsomes, and inhibited the GABA-induced electrical response in oocytes expressing the GABA receptor. It was also demonstrated that the fipronil binding site co-localised with the GABA-gated chloride channel in rat brain and insect tissue.

Fipronil was shown to bind strongly *in vitro* to GABA-gated chloride channels in rat and mouse brain. In contrast, the metabolites RPA 200766 (fipronil amide) and RPA 105048 (desulfinyl fipronil amide) showed no activity in binding assays. It was stated that the major fipronil metabolites MB 45950 (fipronil sulfide) and MB 46136 (fipronil sulfone) bind well to the GABA receptor *in vitro*, but data were not provided. These results are consistent with results of assays for GABA-induced chloride ion flux in rat brain membranes, in which fipronil was inhibitory but RPA 200766 (fipronil amide) and RPA 105048 (desulfinyl fipronil amide) were not (Fitzgerald 1993).

Blood pressure, heart rat and electrocardiographic studies

Rabbits received oral doses of 0 or 4 mg/kg bw fipronil. Arterial blood pressure, heart rate and ECG were recorded before and 1, 24, 48, 72, 96 and 120 hours after dosing. Under the conditions of the study, fipronil did not influence any of these functions, nor were there signs of toxicity (Richard & Camperoux 1990).

Electroencephalogram studies

The cortical EEG was recorded prior to dosing and at 24 h intervals post dosing for 1 week, for 30 min duration, in two groups of rabbits receiving either a single gavage dose of the vehicle, or 4 mg/kg bw fipronil. Fipronil induced a significant 'right shift' (towards higher frequencies) in the mean and median frequency in the EEG at 72 h, with percent total electrical activity also increased at 72, 144 and 168 h. The EEG waveforms were not considered 'pathological', and the possible slight CNS activation was not accompanied by behavioural changes (Algate *et al*. 1991a).

Two groups of rabbits received daily gavage doses of 4 or 8 mg/kg bw fipronil. The cortical EEG was recorded prior to the first treatment and at 1 h post dosing for the next 4 days. The study was terminated on day 5, following the death of three rabbits (two in the 4 mg/kg bw/d group) from toxic effects. Neurotoxic clinical signs, predominantly tremors, were observed throughout the study period, and both groups of rabbits lost weight. Detailed spectral analysis revealed evidence of

possible slight CNS (EEG) activation, which was more notable in the 4 mg/kg bw/d group. The peak effect was at 72 h (Algate *et al*. 1991b).

Enzyme induction

The ability of fipronil to induce hepatic microsomal cytochrome P-450 proteins and phase II conjugation enzymes in the mouse, rat and rabbit was investigated following oral gavage administration daily for 4 or 14 days. Doses of 1.2 or 5.0 mg/kg bw/d were administered to rats and mice, and 0.3 or 1.2 mg/kg bw/d to rabbits. A number of mice in each group died (more females than males), and this was preceded by substantial weight loss. Relative liver weight was increased in female rats at doses of 5 mg/kg bw/d for both treatment periods. An associated finding was increased total levels of cytochrome P450 in both sexes after the 4-day treatment, but levels were similar to controls after the corresponding treatment for 14 days. No inductive response was apparent in rabbits, but mice showed an increase in microsomal protein content and associated mixed function oxidase activities after 14 days' dosing. Effects on Phase II enzymes were limited to decreased cytosolic glutathione S-transferase in rats (14 days treatment only), decreased methylumbelliferone glucuronyl transferase activity in male rabbits (after 4 days) and mice (both sexes and both treatment periods), and decreased microsomal glucuronidation of 1-naphthol in mice (after 14 days) (Shavila *et al*. 1990).

Thyroid function tests

The effect of fipronil on thyroid function was investigated in rats by comparing it with the effect of propylthiouracil (PTU), a known inhibitor of iodide organification in many species, and noxyflex, another thiourea compound that lowers serum thyroxine levels and reduces iodide organification in cultured porcine thyrocytes *in vitro*. A large reduction in the ^{125}I content of thyroids, and relatively less ^{125}I radioactivity in the thyroid than in the blood, was observed in PTU-treated animals given perchlorate. Neither noxyflex nor fipronil inhibited iodide organification. Potassium perchlorate did not change blood and thyroid ^{125}I levels. It was concluded that fipronil and noxyflex enhanced the accumulation of radiolabelled iodide in the thyroid and triggered a stimulation of thyroid activity by a mechanism not involving direct inhibition of iodide organification (Peters 1991).

Rats received fipronil at 0, 0.1, 1, 5 or 30 ppm (equal to 0, 0.01, 0.1, 0.5 and 2.9 mg/kg bw/d) in the diet for 4 weeks. At 5 ppm, decreases in T3, T4 and/or TSH levels and a marginal increase in thyroid weights were recorded for males only. At 30 ppm, T3 levels fluctuated, T4 levels were reduced and TSH levels were increased. Findings were attributed to an increase in T4 clearance from the blood, resulting in a reduction of the feedback inhibitory control of T4 on thyroid function. At 1 or 0.1 ppm, an effect on T3, T4 or TSH was not established, and there were no compound-related organ weight differences or microscopic findings. The no observed effect level was 1 ppm, equivalent to 0.1 mg/kg bw/d (Peters *et al*. 1991).

Rats were given a single oral dose, or daily doses for 14 days, of fipronil at 0, 1 or 10 mg/kg bw/d, followed by sodium iodide immediately after the last dose, and ^{125}I-thyroxine (T4) 4 h later (iv). Phenobarbitone was employed as the positive control. Two weeks treatment with either fipronil or phenobarbitone enhanced biliary clearance of radiolabelled T4. Excretion of T4-conjugated products during 0 to 5 hours increased about 3-fold with 1 mg/kg bw fipronil, 4-fold for 10 mg/kg bw fipronil, and 5-fold for phenobarbital. Single doses of fipronil at both levels, and phenobarbital, raised biliary excretion of conjugated ^{125}I during 0-5 hours by 48-74%. Bile output increased following phenobarbital or 10 mg/kg bw fipronil treatment for 14 days (Chasseaud *et al*. 1993).

Rats were dosed for 1 or 14 days with fipronil (10 mg/kg bw/d, orally), or phenobarbital (80 mg/kg bw/d, ip). Fipronil at 10 mg/kg bw/d for 14 days increased T4 clearance from blood. This was paralleled by a decrease in T4 terminal half-life in blood. After only 1 day of dosing with fipronil (10 mg/kg bw), similar, but less marked effects were noted. Phenobarbital produced changes similar to those affected by fipronil at 1 or 14 days post-dosing (Peters 1991).

HAZARD ASSESSMENT

Discussion

Reasons for the review

Concerns over human health and animal safety have prompted the decision by the APVMA to review fipronil and reconsider the safety of products containing this active constituent to persons using them in both agricultural and veterinary situations, and the adequacy of label instructions. These concerns have arisen from a number of adverse experience reports associated with veterinary products containing fipronil. In these reports, symptoms reported included skin reactions in animals and humans, neurological signs and deaths in target animals (though concurrent infestations with paralysis ticks have been a confounding factor in the latter) and deaths following off-label use in domesticated rabbits. Dermal effects were also reported in humans handling animals with fipronil products and, potentially, constitute the major health concern arising from these adverse experience reports. A critical evaluation of these adverse experience reports is included in this review. The potential toxicity of photodegradation products of fipronil is also addressed in this report.

Adequacy of database

A range of studies on fipronil and its metabolites were submitted for the review. Taking into account data that have been evaluated previously by the OCSEH, the toxicology package for fipronil and its metabolites/degradates is comprehensive.

However, given the potential concerns that the dermal effects reported in the human adverse experience reports may be associated with the use of veterinary products containing fipronil, and the possibility that exposure to fipronil photodegradates may be the causative agent for these skin reactions, the inclusion of a sensitisation study for the photodegradate desulfinyl fipronil would have been informative.

Mode of action

Fipronil belongs to the phenylpyrazole class of insecticides/acaricides, which also contains acetoprole, ethiprole, pyrofluprole, pyriprole and vaniliprole, none of which are registered in Australia. Ethiprole is registered in Japan and an evaluation is available.[1] This compound differs from fipronil in chemical structure by replacement of the trifluoromethylsulfinyl group at the 4th carbon on the pyrazole ring with an ethylsulfinyl moiety. It is much less lipophilic than fipronil, and according to the Japanese evaluation, appears to be relatively less toxic to mammals. In common with fipronil, ethiprole produced thyroid and liver tumours in rats and mice respectively, but though

[1] Evaluation Report Ethiprole. Food Safety Commission. Pesticides Expert Committee. 21 July 2004. (www.fsc.go.jp/english/ethiprole_fullreport.pdf)

its mode of action in insects and its potency has been described as similar to fipronil [2], neurotoxicity was not reported in mammalian studies[1].

Fipronil acts at the γ-aminobutyric acid (GABA) receptor in the CNS as a non-competitive blocker of the GABA-gated chloride channel, GABA being the chief inhibitory neurotransmitter in the mammalian brain. The binding of GABA to its receptor allows the passage of chloride ions through the central pore, the consequence of which is hyperpolarization of the neuron, which decreases the probability that it will propagate an action potential. Blockage of the GABA-gated chloride channel by fipronil reduces neuronal inhibition, which may lead to hyper-excitation of the CNS, convulsions and death.

In vitro assays have demonstrated that the GABA receptors of insects are more sensitive to the action of fipronil than the analogous receptors in mammals (Bushey 1993; Hainzl 1998). It has also been shown that fipronil may act at the insect L-glutamate-gated chloride channel, which has no known counterpart in the vertebrate nervous system, thereby providing an additional explanation for the greater toxicity of fipronil in insects relative to mammals.[3] It has been reported that two fipronil metabolites of mammals and plants (fipronil sulfone and fipronil sulfide), and the major photodegradation product (desulfinyl fipronil), act in a similar way to the parent compound at the GABA receptor (Fitzgerald 1993)[4]. The toxicity of these fipronil derivatives is discussed in detail below.

Neurotoxicity

In keeping with the mode of action of fipronil outlined above, clinical signs such as tremors, hyperactivity, irritability, aggression, twitching, jerking, gait abnormalities and convulsions were observed in mice, rats, rabbits and/or dogs across a range of oral studies with fipronil, and in an acute dermal toxicity study in rabbits. Results obtained in an EEG performed on rabbits treated with fipronil were indicative of slight CNS activation (Algate *et al*. 1991a). However, neurotoxicity studies did not reveal any changes to the histopathology, weight, or dimensions of the brain in these animals (Hughes 1997a; Holmes 1991d).

Convulsions and other clinical signs of neurotoxicity also occurred in mice, rats and dogs treated with fipronil desulfinyl via the oral route. The possible differences in neurotoxicity between desulfinyl fipronil and the parent compound are discussed below. Convulsions were not observed in the limited number of repeat dose (dietary) studies performed with other fipronil metabolites, but were reported in acute toxicity studies using fipronil sulfone, fipronil sulfide and desulfinyl fipronil amide (but not fipronil carboxylic acid, fipronil sulfonyl amide and fipronil detrifluoromethyl sulfonate).

Toxicokinetics and Metabolism

Following acute oral dosing in rats, the rate at which fipronil appeared in the blood was dependent on dose. At a relatively low dose (4 mg/kg bw), the maximum concentration of radiolabel in the blood was achieved at 4-6 h after dosing, and was distributed widely in the tissues. At doses of 40 mg/kg bw, absorption was slower, with blood levels peaking at 34-38 h (Totis & Fisher 1994).

[2] Caboni P, Sammelson RE, Casida JE (2003) Phenylpyrazole insecticide photochemistry, metabolism and GABAergic action: ethiprole compared with fipronil. Journal of Agricultural and Food Chemistry 51: 7055-7061.
[3] Zhao X, Yeh JZ, Salgardo VL, Narahashi T (2004) Fipronil is a potent open channel blocker of glutamate-activated chloride channels in cockroach neurons. Journal of Pharmacology and Experimental Therapeutics. 310:192-201.
[4] Hainzl D, Casida JE (1996) Fipronil insecticide: Novel photochemical desulfinylation with retention of neurotoxicity. Proceedings of the National Academy of Sciences, USA. 93: 12764-12767.

Elimination was slow (half-life 135-241 h) at both 4 and 40 mg/kg bw, and occurred mainly via the faecal (including biliary) route (Totis & Fisher 1994). The results of biliary excretion experiments indicated that fipronil and/or its metabolites in the tissues were released slowly, and mainly via the bile (Totis 1995). Re-uptake of ~25% of biliary metabolites was demonstrated, with redistribution of a significant proportion of the re-absorbed metabolites into the tissues, contributing to the extended elimination phase (Kemp 1999). The slow rate of elimination and considerable enterohepatic recirculation indicate the potential for accumulation of fipronil and/or its metabolites in tissues. In support of this, the autoradiographic study shows stores of radioactivity in the fat in rats, rabbits, and mice (Whitby 1991). The urine was a minor route of excretion at a dose of 4 mg/kg bw (~5% of the dose) but accounted for up to 25% of administered radioactivity at 150 mg/kg bw (Powles 1992). Taking biliary excretion into account, absorption of a single oral dose of fipronil was 80-90% of the administered dose.

Fipronil was readily metabolised, with little or no parent compound detected in the tissues and urine. The highest tissue levels of fipronil and/or its metabolites were found in the fat, with significant levels in the adrenal gland, pancreas, skin, liver, ovaries, uterus, thyroids, kidney, muscle and brain (Totis & Fisher 1994; Kemp 1999). The main metabolite detected in the tissues and excreta was fipronil sulfone, though in the faeces the parent compound and fipronil sulfide were also prominent (Brockelsby *et al*. 1991; Powles 1992). Fipronil and fipronil sulfone were detected in the brains of mice after dietary fipronil administration for periods of 2-4 weeks at ~10-20 mg/kg bw/d, demonstrating that the parent compound and its major metabolite crossed the blood brain barrier (Fisher 1991, 1992). The range and type of metabolites in the bile was indicative of extensive metabolism prior to excretion via this route (Totis 1995).

No sex differences were detected in the toxicokinetics of fipronil or its metabolism, but interspecies differences were apparent. *In vitro* studies showed that rabbit hepatocytes metabolised fipronil at approximately half the rate of rat hepatocytes, but both produced the same metabolite, fipronil sulfone (Fisher 1992, Guyomard 1993). Relative to rats and mice, *in vivo* studies found that fipronil sulfone was eliminated more slowly in rabbits, with relatively high levels of this metabolite persisting in rabbit tissues, particularly the fat (Brockelsby 1991). In an *in vitro* study, human microsomes also metabolised fipronil to fipronil sulfone, though the metabolism rates varied widely (~40-fold) within the pool of donors (Tang *et al*. 2004). Overall, the available evidence suggests that metabolism of fipronil is qualitatively similar across laboratory mammalian species. The slow metabolism and elimination of fipronil in rabbits may at least partially explain why this species appears to be more sensitive to the effects of fipronil than other species (e.g. King 1990, Myers & Christopher 1992).

Percutaneous absorption

In rats, dermal absorption of fipronil was low (<1%), but greater amounts (up to ~6%) were found in the skin at the application site (Cheng 1995). There was insufficient information in this study to determine whether the fipronil reservoir in the skin subsequently entered the systemic circulation. As fipronil and/or its metabolites is expected to accumulate in the tissues following repeated exposure, it is reasonable to consider that fipronil in the skin was available for systemic absorption. In that case, the maximum amount of the applied dose that could be absorbed is approximately 7% (Cheng 1995). *In vitro* studies under the same laboratory conditions indicated that rat skin was slightly over 10-times more permeable to fipronil relative to human skin (Walters & Brain 1990, Ward 1997a,b & 1998). Therefore, taking into account the 10-fold difference in permeation between rat and human skin, and allowing for the fipronil found in the skin to be absorbed systemically, it is reasonable to apply a dermal absorption factor of 1% to exposure scenarios for which an oral to dermal extrapolation is required for human risk assessment. If a single dermal

exposure was being considered, it would be acceptable to take into account only the fipronil that entered the systemic circulation.

Like the parent, dermal absorption of the photodegradate desulfinyl fipronil *in vivo* in rats was also low Cheng 1996). As for fipronil, amounts of desulfinyl fipronil that remained associated with the application site following washing were greater than those absorbed, and were of a similar magnitude. No *in vitro* studies were provided to facilitate the calculation of the likely dermal permeation of desulfinyl fipronil in humans relative to rats. However, given the similarity in molecular structure of fipronil and desulfinyl fipronil, and their similar behaviour in rat *in vivo* dermal absorption studies, it is considered reasonable to assume that they will be dermally absorbed in humans to a similar extent.

Acute toxicity of the technical active

Acute oral exposure to fipronil resulted in a rapid onset of clinical signs of neurotoxicity, with deaths occurring in rats and mice at ≥80 mg/kg bw and ≥50 mg/kg bw respectively from 4 hours to within a week of dosing (Gardner 1988a; Mondot & Dange 1995). The oral LD_{50} values were similar in rats and mice (~90-100 mg/kg bw), though different vehicles were used, and it is unclear whether vehicle plays a role in fipronil toxicity. In the toxicokinetic study of Powles (1992), rats were dosed at 150 mg/kg bw using an aqueous carrier, with no deaths reported, so it is possible that fipronil is more toxic when administered in corn oil, as was the case in the rat acute oral study. It is also possible that the choice of vehicle influenced the relative acute dermal toxicity of fipronil in rats and rabbits, though the differences in metabolism of fipronil between these species, as discussed above, may also play a role. When administered in water via the dermal route, fipronil showed low toxicity in rats, with no toxic effects observed after 24 hours' exposure at 2000 mg/kg bw (Gardner 1988b). Given the poor solubility of fipronil in water (~2 mg/L), little of this dose would be expected to have been available for absorption. On the other hand, fipronil in corn oil had moderate dermal toxicity in rabbits (LD_{50} values of 445/354 mg/kg bw for males/females), with severe clinical signs of neurotoxicity, including convulsions, at 250 mg/kg bw and above (Myers & Christopher 1992). The flux penetrance of fipronil through rat and rabbit skin *in vitro* was similar when measured under the same experimental conditions (Walters & Brain 1990). Inhalation of fipronil dust resulted in moderate acute toxicity in rats (LD_{50}'s of 360-680 mg/m^3), again with clinical signs mainly consistent with neurotoxicity (Cracknell 1991; Nachreiner 1995).

The skin irritation potential of fipronil was tested in two separate rabbit studies, one in which the fipronil was applied semi-occluded under a gauze pad moistened with water, and the other in corn oil under an occlusive dressing (Liggett 1988b, Myers & Christopher 1993). The latter study indicated that fipronil was a slight skin irritant, whereas the former reported no reactions. The effect of the different protocols on the outcomes of these studies is not known, but given that skin irritation was observed in 6/6 rabbits in the Myers & Christopher (1993) study, compared to the 3/3 showing no irritation in the other study, it is concluded that fipronil is a slight skin irritant. Results of two eye irritation studies were also conflicting. Conjunctival irritation persisted beyond 72 h for half the animals in one study (resolving by 14 days), along with 'minor transient corneal opacity' and iritis (Myers & Christopher 1993b), but reactions in the other study were limited to slight conjunctival effects that had all resolved by 72 hours, with no effects on the cornea or iris (Liggett 1988). As the term 'corneal opacity' was used to describe a change that was apparent at 1 hour and not thereafter, this is not considered to represent the type of change that would normally be interpreted as moderate irritation. Taking this into account, on the basis of these two studies, fipronil is a slight eye irritant.

When tested for skin sensitisation in guinea pigs by the maximisation test of Magnusson and Kligman (Johnson 1993), although skin reactions were seen in test animals they were considered irritant in nature. Therefore, this study provided no reliable evidence that fipronil is a skin sensitiser in guinea pigs. Furthermore, a negative result was also obtained in another dermal skin sensitisation study in guinea pigs, this time using the Buehler method (Smith 1990). Although some adverse experience reports have reported dermal reactions occurring in humans following exposure to veterinary products containing fipronil, which may potentially be allergic in nature, expert opinion is that this data does not reliably demonstrate that fipronil is a skin sensitiser. This issue is discussed in detail in Chapter 15.

Toxicity of metabolites and photodegradates

There are several metabolites or photodegradation products of fipronil that are of toxicological concern (see Toxicology Hazard Profile on page 10, and Appendix VII for structures). An extensive database exists for the major photodegradate, desulfinyl fipronil, which, along with another photodegradation product, fipronil detrifluoromethyl sulfonate, has not been identified as a metabolite in mammals. For this reason, and in conjunction with its relatively high toxicity (oral LD_{50} = 18/15 mg/kg bw in male/female rats, Dange 1993a),
and its presence as a residue in crops, desulfinyl fipronil is of particular interest for this review. On the other hand, fipronil detrifluoromethyl sulfonate has been shown to have low acute toxicity (oral LD_{50} >2000 mg/kg bw in rats, Dange 1993c), and therefore is not of toxicological concern.

The toxicity of metabolites and photodegradates of fipronil is summarised in the Table below. There is evidence that desulfinyl fipronil may be more acutely toxic than fipronil at their respective LD_{50}s, and in a short-term and a subchronic dietary study. However, effects occurred at similar doses of fipronil and desulfinyl fipronil following chronic treatment, in developmental studies, and in acute neurotoxicity studies. Overall, it is not clear why desulfinyl fipronil was relatively more toxic than parent compound in some studies but not in others. Like fipronil, desulfinyl fipronil was widely distributed in the tissues following oral dosing, with the highest concentrations in the fat, and elimination was slow, with faeces the preferred excretory route (though the contribution of biliary secretion is not known for the desulfinyl derivative). The following is a detailed comparison of the toxicity of fipronil and desulfinyl fipronil.

Desulfinyl fipronil had a lower oral LD_{50} in rats than did fipronil (18/15 mg/kg bw vs. 92/103 mg/kg bw for males/females; Dange 1993a, Gardner 1988a). However, in acute neurotoxicity studies performed at doses below their respective LD_{50} values, the NOELs were similar for these two compounds (~2 mg/kg bw; neurobehavioural effects) (Hughes 1996 & 1997a). NOELs were not achieved for fipronil in the rat and mouse short term oral dosing studies, and the LOELs in these studies were well above the NOELs in the corresponding studies with desulfinyl fipronil (Holmes 1990, Peters *et al*. 1990, Dange 1994b & 1995a). In the mouse short term studies, effects on the liver and reduced bodyweight gain were seen at similar doses for both compounds (2.4 or 5 mg/kg bw/d), but neurobehavioural changes that were seen from 5 mg/kg bw/d for desulfinyl fipronil occurred in one fipronil-treated mouse at ~6 mg/kg bw/d, but mainly at ≥20 mg/kg bw/d, suggesting that desulfinyl fipronil was relatively more neurotoxic in this instance. This is supported by results from *in vitro* binding studies using mouse brain membranes, in which the GABA receptor IC_{50} values were 97±4 nM for desulfinyl fipronil, 1010 ± 20 nM for fipronil, and >10,000 nM for detrifluoromethylsulfinyl fipronil.[4] Short-term dosing with desulfinyl fipronil proved to be lethal for one male rat at 2.2 mg/kg bw/d and all rats at 3.7 mg/kg bw/d, whereas in comparison only one female rat died at the highest dose tested of ~55 mg/kg bw/d fipronil in the short-term study. Given the lack of accompanying clinical signs or abnormal pathology findings, this death may not have been treatment-related. In the 4 week dog study with desulfinyl fipronil, clonic convulsions were

seen at 1 mg/kg bw/d, but no effects were noted at this dose in the corresponding fipronil study, with a possible convulsive episode in one dog at 10 mg/kg bw/d (Dange 1995b, Holmes 1991a).

In the 13-week rat dietary study with desulfinyl fipronil, clinical signs were seen occasionally in one male at 0.18 mg/kg bw/d (Dange 1994d). As the same signs (aggression, irritability to touch and excessive vocalisation) were seen also at the higher doses, this could not be dismissed as unrelated to treatment. In the chronic study performed with desulfinyl fipronil, similar signs were not seen in animals at a comparable dose level during, and for some time after, the treatment period corresponding to the subchronic study, but these rats were a different strain and from a different source, which may account for this (Bigot 1998). Taking dose choices into account, and not placing undue weight on the one finding at the LOEL in the desulfinyl fipronil study which may represent a threshold effect, it is reasonable to conclude that the fipronil and desulfinyl fipronil are of similar toxicity when administered subchronically in the diet. Fipronil and desulfinyl fipronil were also similarly toxic in the respective chronic rat studies (Aughton 1993, Bigot 1998).

A comparison of the rat developmental studies suggests that desulfinyl fipronil was more toxic than fipronil, as foetal developmental delays and reduced bodyweight were apparent at 2.5 mg/kg bw/d (NOEL 1 mg/kg bw/d) desulfinyl fipronil, but no foetal effects were seen at 20 mg/kg bw/d fipronil (Foulon 1997; Brooker & John 1991). Also, maternal bodyweight loss occurred at 2.5 mg/kg bw/d desulfinyl fipronil, below the maternal NOEL of 4 mg/kg bw/d for fipronil. However, results are not consistent between the fipronil developmental and developmental neurotoxicity studies (Mandella 1995). Though dosing was over a longer period in the latter, maternal bodyweight gain was reduced during gestation, with a NOEL of 0.9 mg/kg bw/d, equivalent to the same endpoint in the desulfinyl fipronil developmental study. The pup data in the fipronil developmental neurotoxicity study are not directly comparable with results for foetuses in the desulfinyl fipronil developmental study, but if it is accepted that 0.9 mg/kg bw/d represents a threshold dose for reduced pup weight in the neurotoxicity study, then at least this is consistent with the NOEL for foetal effects for desulfinyl fipronil. Therefore, when these three studies are considered together, there is sufficient evidence that desulfinyl fipronil is not more toxic than fipronil in this context.

The primary metabolites of fipronil identified in mammals are fipronil sulfide, fipronil sulfone (the major metabolite found in mammals) and fipronil amide. The oral LD_{50} values for these compounds were respectively 69/100, 184/257 and >2000 mg/kg bw for males/females, when administered to rats in corn oil. For fipronil sulfone, the LD_{50} was increased to 464/732 mg/kg bw when administered in an aqueous carrier. As well as being the most acutely toxic of the derivatives of fipronil for which there is data, the desulfinyl, sulfide and sulfone products also show relatively high affinity for a specific site on the GABA receptor, as assessed by binding assays in competition with radiolabelled EBOB (4'-ethynyl-4-*n*-[2,3-$^3H_2$]propylbicycloorthobenzoate) and TBPS (tert-butylbicyclo-phosphoro-thionate), both of which are known to bind within the GABA-gated chloride channel. Fipronil and its sulfone and desulfinyl derivatives have been shown to inhibit EBOB binding *in vitro* in human brain tissue at IC_{50} values of approximately 942, 155 and 64 nM respectively.[5] It has been reported elsewhere that the sulfone and sulfide metabolites of fipronil exhibit good binding activity, but fipronil amide and desulfinyl fipronil amide do not (Fitzgerald 1993). Except for fipronil sulfone, a correlation generally exists between the ability of the compound to compete with EBOB for binding to the mammalian GABA receptor *in vitro*, and its *in vivo* toxicity in mammals. Fipronil sulfone has greater affinity for the GABA receptor than does fipronil, yet its oral LD_{50} in rats is about twice that of fipronil. Other selected

[5] Hainzl D, Cole LM, Casida JE (1998) Mechanisms for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chemical Research in Toxicology 11: 1529-1535.

metabolites/degradates tested had oral LD_{50}s of >2000 mg/kg bw, with the exception of desulfinyl fipronil amide, a metabolite of fipronil desulfinyl in rats, which had an oral LD_{50} of 467 mg/kg bw.

Many of the clinical signs observed after acute dosing with the sulfone or sulfide metabolites were similar to those for fipronil, which is consistent with their affinity for the GABA receptor as described above. Most metabolites/degradates tested had low acute dermal toxicity in rats (LD_{50}s>2000 mg/kg bw), though fipronil sulfide was only tested at 500 mg/kg bw, at which dose no effects were observed. In rabbits, fipronil sulfone and fipronil sulfide were slight eye irritants, but were not skin irritants.

Table 1: Comparison of the toxicity of fipronil and its metabolites/degradative products

ACUTE LD_{50} STUDIES (RAT)			
Compound	**LD_{50} po (mg/kg bw) males/females (combined)**	**LD_{50} dermal (mg/kg bw)**	**Reference**
Fipronil	(97)	>2000	Gardner (1988a)
MB 46513 (desulfinyl fipronil)	18/15	>2000	Dange (1993A)
MB 45950 (fipronil sulfide)	69/100 (83)	>500	Dange (1994a)
MB 46136 (fipronil sulfone)	184/257 (218)	>2000	Gardner (1988c)
RPA 200766 (fipronil amide)	>2000	ND	Dange (1993d)
RPA 104615 (fipronil detrifluoromethyl sulfonate)	>2000	ND	Dange (1993c)
RPA 105048 (desulfinyl fipronil amide)	467	ND	Dange (1994f)
RPA 200761 (fipronil carboxylic acid)	>2000	ND	Katchadourian (1995)
RPA 105320 (fipronil sulfonyl amide)	>2000	ND	Dange (1994c)

ACUTE NEUROTOXICITY STUDIES (RAT)				
Compound	**NOEL (mg/kg bw)**	**LOEL (mg/kg bw)**	**Endpoint**	**Reference**
Fipronil	2.5	7.5	Reduced footsplay	Hughes (1997); Gill (1993)
MB 46513 (desulfinyl fipronil)	2	12	Reduced footsplay, slow righting reflex, decreased locomotor activity, drop in rectal temperature	Hughes 1996

SHORT-TERM REPEAT-DOSE STUDIES (MOUSE)				
Fipronil	-	2.4	Decreased bodyweight (males); increased liver weight + pathology	Holmes (1990)
MB 46513 (desulfinyl fipronil)	0.5	5	Clinical signs, death, reduced bw gain; increased liver weight + microscopic changes	Dange (1994b)

Table 1: Comparison of the toxicity of fipronil and its metabolites/degradative products, cont...

SHORT-TERM REPEAT-DOSE STUDIES (RAT)				
Fipronil	-	3.4	Increased liver weights; thyroid follicular hypertrophy	Peters *et al*. (1990)
MB 46513 (desulfinyl fipronil)	0.23	2.2	Decreased bw; decreased T3 & T4 and total bilirubin	Dange (1995a)
RPA 200766 (fipronil amide)	-	3.8	Clinical signs; reduced bw	Dange (1994d)
RPA 104615 (fipronil detrifluoromethyl sulfonate)	45	460	Increased liver weight; increased ALP, triglycerides, prothrombin time	Dange (1998)
MB 45897 (detrifluoromethyl sulfinyl fipronil)	200	1000	Increased liver weight + microscopic changes	Johnson (1995)

SHORT-TERM REPEAT-DOSE STUDIES (DOG)				
Fipronil	1	10	Neurological signs; increased Hb & RBC	Holmes (1991a)
MB 46513 (desulfinyl fipronil)	-	1	Clonic convulsions	Dange (1995b)
MB 45950 (fipronil sulfide)	5	15	Increased ALP, Hct, Hb, RBC	Broadmeadow (1991a)

SUBCHRONIC STUDIES (RAT)				
Fipronil	0.3	2	Increased liver and thyroid weights	Holmes (1991b)
Fipronil (neurotoxicity)	0.3	7.5	Neurobehavioural changes	Driscoll & Hurley (1993)
MB 46513 (desulfinyl fipronil)	0.03	0.2	Clinical signs	Dange (1994b)
MB 45950 (fipronil sulfide)	0.7	1.8	Increased liver weight; thyroid follicular cell hypertrophy	Broadmeadow (1991b)

SUBCHRONIC STUDIES (DOG)				
Fipronil	0.5	2	Neurobehavioural changes	Driscoll & Hurley (1993)
MB 46513 (desulfinyl fipronil)	0.27	1	Clinical signs	Dange (1996)

CHRONIC STUDIES (RAT)				
Fipronil	0.02	0.06	Clinical signs of neurotoxicity; increased thyroid weight; decreased T4 levels; increased severity of progressive senile nephropathy	Aughton (1993)
MB 46513 (desulfinyl fipronil)	0.025	0.1	Increased mortality, increased incidence of convulsions	Bigot (1998)

Table 1: Comparison of the toxicity of fipronil and its metabolites/degradative products, cont...

DEVELOPMENTAL STUDIES (RAT)				
Fipronil	Maternal: 4 Foetal: 20	Maternal: 20 Foetal: -	Maternal: decreased bodyweight gain Foetal: no effects at the highest dose tested	Brooker & John (1991)
Fipronil (neurotoxicity)	Maternal: 0.9 Offspring: 0.05	Maternal: 8.7 Offspring: 0.9	Maternal: bodyweight loss Offspring: reduced pup weight during lactation	Mandella (1995)
MB 46513 (desulfinyl fipronil)	Maternal: 0.2 Foetal: 1	Maternal: 0.5 Foetal: -	Maternal: decreased bodyweight gain Foetal: no effects at highest dose tested	King (1990)

Effects on the liver and thyroid

Following repeated dosing with fipronil, liver weights were increased in mice and rats, with an isolated finding in one animal in the 12-month dietary study in dogs, with no associated pathology. Increased liver weight generally occurred at dose levels exceeding 1 mg/kg bw/d, though a marginal increase was seen in male rats treated at 0.5 mg/kg bw/d fipronil in the diet for 4 weeks (Peters *et al*. 1990). Microscopic changes to the liver were generally minimal, comprising mainly peri-, centri- or pan-acinar fatty vacuolation and hepatocyte enlargement in rats and mice, with focal necrosis and chronic degenerative changes also in mice. Hepatocytic carcinomas were also reported in mice (Broadmeadow 1993), but their incidence was well within the historical control range, and they were not considered to be relevant to human health. In contrast to fipronil, liver weights were unaffected by desulfinyl fipronil, but there were observations of pale liver, with centrilobular hypertrophy in mice the main microscopic finding (Bigot 1996).

Effects of fipronil on the thyroid were seen in rats, but in the one study that measured T3/T4 levels in dogs, no changes were seen. Thyroid weights were increased in rats, and this was usually associated with thyroid follicular cell hypertrophy and/or hyperplasia at the microscopic level (eg Aughton 1993). Plasma levels of T4 were decreased, and TSH was increased, but reductions in T3 levels were inconsistent (Peters *et al*. 1991). Increased levels of T4-conjugated products were found in the bile, and a study using ^{125}I-thyroxine demonstrated that treatment with fipronil increased T4 clearance from blood (Peters *et al*. 1991). Fipronil did not affect iodine organification in the thyroid (Peters 1991a). These findings are consistent with fipronil acting indirectly on the thyroid by increasing the loss of thyroid hormone through the liver. As a result of this increased excretion, modulation of the hypothalamus-pituitary-thyroid axis is expected to occur, leading to a compensatory increase in TSH secretion and consequent stimulation of the thyroid gland, manifested as hypertrophy and possible progression to follicular cell neoplasms[6]. This proposed mechanism of disruption of thyroid homeostasis in rats has been demonstrated for a range of xenobiotic chemicals, and because of the differences between rats and humans with respect to thyroid biochemistry and physiology, the effects on the thyroid in rats are not considered to be relevant to human health risk assessment. In the chronic/carcinogenicity study in rats, an increase in both benign follicular cell adenomas and follicular cell carcinomas of the thyroid was seen, but these neoplastic changes were considered to be related to treatment only at a relatively high level of

[6] IARC Scientific Publications No.147. Species differences in thyroid, kidney and urinary bladder carcinogenesis. CC Capen, Dybing E, Rice JM, Wilburn JD (Eds) http://www-cie.iarc.fr/htdocs/iarcpubs/pub147/pub147consensus.html

exposure (13 mg/kg bw/d), in the presence of clear NOELs. Taking into account the lack of relevance of this mechanism of toxicity to humans, these changes are not of concern to human health.

No changes in thyroid weight were detected in the studies with desulfinyl fipronil, and though some changes in T3/T4 levels were reported in the short-term and subchronic rat studies, these were very inconsistent and the assay appeared unreliable. Also in contrast to fipronil, there was no change in TSH levels. Overall, it appears that desulfinyl fipronil does not share the thyroid stimulating effects of the parent compound. With the exception of fipronil detrifluoromethyl sulfone for which thyroid hypertrophy was noted in a single study (28-day dietary study in rats) at a very high dose in a single sex (males, 916 mg/kg bw/d), no thyroid effects were observed for the other metabolites tested, but all had the effect of increasing liver weight.

Genotoxicity

Fipronil produced negative results in several genotoxicity tests *in vitro* (Clare 1988a, Lloyd 1990, Marshall 1988a) and one *in vivo* (Edwards 1995*)*, but positive results were obtained in an *in vitro* chromosomal aberration test using Chinese hamster lung cells (Wright 1995). However, a similar study using human lymphocytes with higher concentrations of fipronil was negative for this same genotoxic endpoint. Also taking into account the negative results in the other genotoxicity studies, and the lack of evidence for carcinogenicity being the result of a genotoxic mechanism in chronic rodent studies (as opposed to reactive hyperplasia, *i.e.* a threshold mechanism), fipronil is not a genotoxic hazard.

Of the fipronil metabolites/degradates tested, there was only one positive finding from 19 studies. This occurred with RPA 097920 (detrifluoromethylsulfinyl fipronil), a rat metabolite of fipronil sulfone, in an *in vitro* chromosomal aberration test using human lymphocytes, and only in the absence of metabolic activation, with a high level of cytotoxicity (Johnson 1995). No other genotoxicity studies were performed on RPA 097920 (detrifluoromethylsulfinyl fipronil), but as no carcinogenicity concerns have arisen from the fipronil rat chronic study in which rats would be expected to be exposed to this metabolite, the positive finding in the genotoxicity study is not of particular concern. The weight-of-evidence indicates that fipronil and its metabolites do not present genotoxic hazards to humans.

Reproduction and developmental effects

No developmental abnormalities were reported for fipronil administered to rats and rabbits at oral doses up to 20 mg/kg bw/d and 1 mg/kg bw/d respectively. The reproduction study (King 1992) in rats showed reduced litter size and pup viability in the F_0, and a slight reduction in mating performance and fertility index in F_1 animals at 27 mg/kg bw/d. In common with the other rat studies, thyroid and liver effects were observed for the parental animals, though a decrease in pituitary weight was also noted (all at 2.5 mg/kg bw/d). Convulsions were recorded for both pups and dams in the reproduction study, but these occurred only at the relatively high dose of 27 mg/kg bw/d. Fipronil does not cause reproductive toxicity at levels of exposure relevant to humans.

TOXICOLOGICAL RESULTS FOR RISK ASSESSMENT

ADI

To establish an ADI, a summary of results determined in those studies deemed adequate for regulatory purposes are shown in Tables 2 (fipronil) and 3 (desulfinyl fipronil) below.

Table 2: Toxicological results from studies using fipronil

Species	Study Type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
SUBCHRONIC					
Rat	13 weeks, dietary	0.3	2.0	Increased liver and thyroid weights	Holmes 1991b
Dog	13 weeks, capsules	0.5	2	Inappetence and reduced bodyweight gain (females)	Holmes 1991c
CHRONIC					
Mouse	78 weeks, dietary	0.05	1.2	Increased liver weight and microscopic changes to the liver	Broadmeadow 1993
Rat	89-91 weeks, dietary	0.02	0.06	Clinical signs of neurotoxicity; increased thyroid weight; decreased T4 levels; increased severity of progressive senile nephropathy	Aughton 1993
Dog	52 weeks, capsules	0.2	2	Clinical signs of neurotoxicity, bodyweight loss	Holmes 1992
Dog	52 weeks, dietary	0.3	1	Clinical signs of neurotoxicity	Holmes 1993
REPRODUCTION					
Rat	2-generation reproduction, dietary	Parental: 0.25 Offspring: 2.5	Parental: 2.5 Offspring: 27	Parental: increased thyroid and liver weights; decreased pituitary weight; increased incidence of follicular epithelial hypertrophy of the thyroid Offspring: reduced survival; reduced bodyweight gain; developmental delays	King 1992
Rat	developmental neurotoxicity, dietary	Maternal: 0.9 Offspring: 0.05	Maternal: 8.7 Offspring: 0.9	Maternal: bodyweight loss Offspring: reduced pup weight during lactation	Mandella 1995
DEVELOPMENTAL					
Rat	developmental, gavage	Maternal: 4 Foetal: 20	Maternal: 20 Foetal: -	Maternal: decreased bodyweight gain Foetal: no effects at the highest dose tested	Brooker & John 1991
Rabbit	developmental, gavage	Maternal: 0.2 Foetal: 1	Maternal: 0.5 Foetal: -	Maternal: decreased bodyweight gain Foetal: no effects at highest dose tested	King 1990

Table 3: Toxicological results from studies using desulfinyl fipronil

Species	Study Type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
Mouse	90-day, dietary	0.3	1.7	Deaths	Bigot 1996
Rat	90-day, dietary	0.03	0.2	Clinical signs	Dange 1994b
Dog	90-day, dietary	0.27	1.0	Clinical signs	Dange 1996
Rat	2-yr, dietary	0.025	0.1	Increased mortality, increased incidence of convulsions	Bigot 1998
Rat	Developmental, gavage	Maternal: 0.2 Foetal: 1	Maternal: 1 Foetal: 2.5	Maternal: reduced bw gain Foetal: retarded skeletal ossification	Foulon 1997

The current ADI for fipronil of 0.0002 mg/kg bw/d was set in 1994, based on a NOEL for neurological signs and haematological changes in a chronic dietary study in rats and a 100- fold safety factor to account for intra- and inter- species variation. Examination of the new studies (Holmes 1993, Mandella 1995; see Table above) indicates that the rat is the species most sensitive to chronic dietary exposure, and hence remains the most suitable species on which to base the ADI upon in the absence of human data. Furthermore, results in the rat are closely supported by a chronic dietary study in mice (Broadmeadow 1993). The NOEL in the chronic rat study (0.02 mg/kg bw/d) is less than the NOELs in the reproduction (0.25 mg/kg bw/d, King 1992) and developmental studies (lowest NOEL 0.05 mg/kg bw/d, Mandella 1995), and therefore is protective of these endpoints.

In repeat-dose studies, the mammalian metabolite, MB 45950 (fipronil sulfide), was of lower toxicity than the parent compound, and so does not need to be considered further here. Limited studies provided for other metabolites indicate that their toxicity is low relative to the parent compound also. As the photodegradate desulfinyl fipronil is not a mammalian metabolite, and is present as a residue in plants, its toxicity must be considered when setting the ADI. The NOELs/LOELs for fipronil and desulfinyl fipronil in their respective chronic dietary studies showed that the two compounds produce toxic effects at similar doses following long-term exposure. Therefore, basing the ADI on the NOEL from the fipronil chronic rat study will also cover potential hazards from chronic dietary exposure to desulfinyl fipronil. The results of the developmental study with desulfinyl fipronil indicate that this will also be protective of developmental effects for the photodegradate. The information available for fipronil sulfone and fipronil sulfide indicates that they have similar toxicity to the parent. Therefore, using a safety factor of 100, and the NOEL from the chronic rat study for fipronil, the ADI is maintained at 0.0002 mg/kg bw/d. This is a group value to cover fipronil, desulfinyl fipronil, fipronil sulfide and fipronil sulfone.

ARfD

The current ARfD is 0.02 mg/kg bw, based on a combined NOEL of 2.5 mg/kg bw/d from two acute neurotoxicity studies in rats and a safety factor of 100. This value is described in the ARfD list as ‘a group value for fipronil, fipronil-desulfinyl, fipronil-sulfenyl and fipronil-sulfonyl’. This value was incorporated into the ARfD List in 2006.

Table 4: Summary of results from toxicological studies for establishing an ARfD

Species	Study Type	NOEL (mg/kg bw/d)	LOEL (mg/kg bw/d)	Effect	Reference
FIPRONIL					
Rat	Acute neurotoxicity	2.5	7.5	Reduced footsplay	Hughes 1997
Rat	Acute neurotoxicity	0.5	5	Reduced footsplay	Gill 1993
Rat	Developmental	Maternal: 4 Foetal: 20	Maternal: 20 Foetal: -	Maternal: decreased bodyweight gain Foetal: no effects at the highest dose tested	Brooker & John 1991
Rat	Developmental neurotoxicity	Maternal: 0.9 Offspring: 0.05	Maternal: 8.7 Offspring: 0.9	Maternal: bodyweight loss Offspring: reduced pup weight during lactation	Mandella 1995
Rabbit	Developmental	Maternal: 0.2 Foetal: 1	Maternal: 0.5 Foetal: -	Maternal: decreased bodyweight gain Foetal: no effects at highest dose tested	King 1990
DESULFINYL FIPRONIL					
Rat	Acute neurotoxicity	2	12	Reduced footsplay, slow righting reflex, decreased locomotor activity, drop in rectal temperature	Hughes 1996
Rat	Developmental	Maternal: 0.2 Foetal: 1	Maternal: 1 Foetal: 2.5	Maternal: reduced bw gain Foetal: retarded skeletal ossification	Foulon 1997

A summary of the studies relevant to the ARfD is provided in the Table above.

The previous ARfD of 0.003 mg/kg bw, was based on a NOEL of 0.3 mg/kg bw/d for neurobehavioural effects from a 3-month neurotoxicity study in rats, and a safety factor of 100. This value was adopted from JMPR on 10 January 2001. The rationale given by the JMPR for basing the ARfD on a NOEL from a repeat dose study, was 'because of concern about the prolonged toxicokinetics of fipronil' (JMPR 1997). While it is agreed that fipronil and its metabolites are eliminated slowly, and with repeated dosing there is a likelihood of accumulation, the submitted acute neurotoxicity studies in rats have been given primary consideration in setting the ARfD. The 14-day follow-up period in the acute neurotoxicity studies was considered adequate for the observation of any delayed effects.

More recently, the no-effect level for fipronil of 2.5 mg/kg bw in the acute neurotoxicity study of Hughes (1997) was considered the most appropriate study on which to base the ARfD. A similar earlier study (Gill 1993) resulted in a no-effect level of 0.5 mg/kg bw, but the LOEL in the study of Gill exceeded the NOEL in the Hughes study. These acute neurotoxicity studies share a similar neurobehavioural endpoint of reduced footsplay. The LOELs for these studies were established at 7 h post-dosing, judged by the authors as the time to peak effect. In a preliminary acute neurotoxicity study, rats were submitted to FOB testing at 2, 4 and 7 h following a single oral dose of 25 mg/kg bw. Convulsions were seen in 1 male at 4 and 7 h and 1 female at 7 h, chewing action in 1 male at 4

and 7 h, lip licking in 2 females at 7 h, and wet anogenital regions in both sexes at 7h. However, no later test points were considered. In another preliminary study, one male (50 mg/kg bw) had convulsions at 5 and 24 h post-dosing, and another (80 mg/kg bw) at 4-7 h. In the toxicokinetic studies, Cmax in the plasma occurred at 4-6 h after a dose of 4 mg/kg bw. Therefore, as the LOELs in the main studies occurred at doses of 5-7.5 mg/kg bw, it is a reasonable estimate that effects at these doses coincided with Cmax, and therefore represent a true time of peak effect. It is unlikely that effects occurred at doses lower than the LOELs, and were overlooked due to selection of the incorrect time of peak effect.

As the NOELs in the rat developmental neurotoxicity study (Mandella 1995) and the rabbit developmental study (King 1990) are less than 2.5 mg/kg bw/d, the possibility that the effects observed in these studies may have arisen from a single dose needs to be considered here. The endpoint in common to these studies is decreased bodyweight gain. This was also seen in the acute neurotoxicity studies, so must be considered a possible acute effect. In the case of the rat developmental neurotoxicity study, maternal bodyweight loss was observed at ~8.7 mg/kg bw/d (NOEL 0.9 mg/kg bw/d). However, in the rat reproduction study, maternal bodyweight gain was reduced at 27 mg/kg bw/d, but not at 2.5 mg/kg bw/d (King 1992). Therefore, taking dose selection into account, the combined maternal NOEL is 2.5 mg/kg bw/d, equal to the NOEL in the acute neurotoxicity study. Comparison of the developmental neurotoxicity study with the reproduction study is appropriate, as in both of these studies the test material was delivered in the diet, and treatment continued into the lactation period. In the developmental neurotoxicity study, pup weights at birth and during lactation were slightly reduced at ~0.9 mg/kg bw/d (3-9%), with a NOEL of 0.05 mg/kg bw/d. This is in conflict with the results in the rat reproduction study, in which reduced pup weight at birth and pup weight gain till weaning were observed at 27 mg/kg bw/d, but not at 2.5 mg/kg bw/d. This calls into doubt whether the small reduction in pup weight at 0.9 mg/kg bw/d in the developmental neurotoxicity study constitutes a biologically significant effect. Also, taking into account that these changes are unlikely to have arisen from a single dose, this endpoint was not considered an appropriate basis for the ARfD. The maternal LOEL in the rabbit developmental study is 0.5 mg/kg bw/d, again based on reduced bodyweight gain. However, the intragroup variability was high, and reduced bodyweight gain was largely attributable to relatively low food consumption confined to the interval spanning gestation days 13 to 19. As the biological significance of the reduced weight gain is debatable, and there is a strong likelihood that the apparent reduced weight gain in this study was not due to a single treatment, the rabbit developmental study was also not considered an appropriate basis for the ARfD.

As for the ADI, the toxicity of desulfinyl fipronil should also be considered here. The effects in the developmental study were associated with reduced food consumption in the dams during treatment, with consequent reduced bodyweight gain, and delayed ossification in the foetuses is expected to be secondary to maternal toxicity. These effects are unlikely to have arisen from a single dose, so are not relevant to the ARfD. The no-effect level for desulfinyl fipronil in an acute neurotoxicity study (Hughes 1996) was 2 mg/kg bw. The doses used in the fipronil and desulfinyl fipronil studies do not allow a precise comparison of the relative toxicity of the two compounds in the acute neurotoxicity tests. For practical purposes, as similar no-effect levels were demonstrated for the two compounds, with a similar endpoint, it is appropriate to base the ARfD on the NOEL of 2.5 mg/kg bw from the acute neurotoxicity study for fipronil, and incorporating a safety factor of 100 for inter- and intra-species variability. Toxicity studies indicate that the acute toxicity of fipronil sulfone and fipronil sulfide is similar to that of fipronil. Other metabolites/degradates are less acutely toxic than fipronil, so they do not need to be considered further in this context. The recommended ARfD was therefore 0.02 mg/kg bw, and this is the value included in the current ARfD List..This is a group value to cover fipronil, desulfinyl fipronil, fipronil sulfide and fipronil sulfone.

HUMAN EXPOSURE

In Australia, fipronil is registered for a range of agricultural uses, including seed dressings, the control of locusts and a wide range of insect pests in bananas, brassicas, cotton, potatoes, sugarcane and in turf. Fipronil is also included in a number of bait products for use by householders and commercial building treatments. Home veterinary products containing fipronil are registered for use on cats and dogs.

Diet

Fipronil has not been included in the Australian Diet Surveys (formerly known as Market Basket Surveys). It was detected in 1/293 barley samples in the National Residue Survey (NRS) 2003/4 at 0.046 mg/kg of the whole grain. The traceback investigation was inconclusive and could not confirm the reason for the contravention, as there was no evidence of fipronil use. As fipronil is not registered for use on barley, it was suggested that there might have been contamination from previous fipronil treatments of canola. The NRS 2002/3 reported the detection of fipronil residues on 2/45 samples of canola, but no further details were provided. Other crops that were sampled, but in which no residues of fipronil were found in the NRS from 2002/3 to 2004/5, were sorghum, pecan nut, lupin, wheat (whole grain, bran and flour), oat, field pea and chick pea. The dietary risk assessment for fipronil will be performed by the APVMA and FSANZ.

Water

Information is lacking with respect to likely public exposure to fipronil via the drinking water. Fipronil is not included in the *WHO (2004) Guidelines for drinking-water quality* under 'Guideline values for chemicals from agricultural activities that are of health significance in drinking water' nor is it listed under 'Chemicals from agricultural activities excluded for guideline derivation', or 'Chemicals from agricultural activities for which guideline values have not been established'. The current Australian Drinking Water Guideline (NHMRC, 2004), does not include a Guideline Value or a Health Value for fipronil, however the OCSEH is in the process of recommending a health-based Guideline Value. Fipronil is not expected to be present in drinking water at detectable levels, other than as a result of spillage or through misuse. The agricultural use of fipronil may potentially lead to contamination of source waters through processes such as run-off, spray drift or entry into ground-water, however the exposure of the general population is expected to be well below levels that may cause health concerns.

Non-dietary exposure considerations

Occupational exposure to fipronil will be considered in the separate OHS Review. The non-dietary exposure to fipronil that may occur during the use of HV products for the treatment of tick and flea infestations in cats and dogs, or through subsequent contact with a treated pet is considered in this Review.

CONSIDERATION OF PUBLIC HEALTH STANDARDS

Approval Status

There are no objections on toxicological grounds to the ongoing approval of fipronil technical active sourced from Bayer CropScience Hangzhou Company Limited, China APVMA Number 59286); Gharda Chemicals Limited, India (APVMA Number 58418); BASF Agri-Production SAS, France (APVMA Approval Numbers 51985, 52547); Bayer CropScience SA, France (APVMA Approval Numbers 46789, 49120).

Impurity Limits

An integral part of the safety assessment of an active constituent is a consideration of the chemical composition of the material. Active constituents will contain measurable levels of impurities, which can arise during manufacture and/or from subsequent degradation during storage. The chemical identity of these impurities is generally well characterised. The impurities present in the active constituent are usually of no particular concern since health standards are established on the basis of toxicology studies conducted using the mixture. However, for those which have high acute toxicity, genotoxicity or teratogenic potential, concentration limits need to be set, so that the toxicological profile of the active constituent does not appreciably alter in the event of slight changes in the proportions of the impurities.

In the *APVMA Standards – Active Constituents*, the composition of fipronil is listed as 950 g/kg (on a dry weight basis). No impurity limits are stated. This review did not identify any impurities of toxicological concern, therefore this standard remains appropriate.

Residue definition

In the APVMA Standard for Maximum Residue Limits in Food and Animal Feedstuff (APVMA, November 2005, Table 3), the residue of fipronil is defined as the sum of:
fipronil and;
the sulfenyl metabolite (5-amino-1-[2,6-dichloro-4-(trifluoromethyl) phenyl]-4-[(trifluoromethyl) sulfenyl]-1*H*-pyrazole-3-carbonitrile);
the sulfonyl metabolite (5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1*H*-pyrazole-3-carbonitrile);
and the trifluoromethyl metabolite (5-amino-4-trifluoromethyl-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1*H*-pyrazole-3-carbonitrile).

In the current review, these metabolites are also referred to as MB 45950 (fipronil sulfide), MB 46136 (fipronil sulfone) and MB 46513 (desulfinyl fipronil) respectively (see Appendix VII for structures and alternative nomenclature). Desulfinyl fipronil is a photodegradative product of fipronil found in plants, but is not a metabolite in mammals. Overall, it has a similar toxicity profile to fipronil. The toxicity of the mammalian metabolites fipronil sulfone and fipronil sulfide is similar to the parent compound. Therefore, from a toxicological perspective, the residue definition is appropriate.

Acceptable Daily Intake (ADI)

The ADI for humans is the level of intake of a chemical that can be ingested daily over an entire lifetime without appreciable risk to health. It is calculated by dividing the overall NOEL for the most sensitive toxicological endpoint from a suitable study (typically an animal study) by an appropriate safety factor. The magnitude of the safety factor is selected to account for uncertainties in extrapolation of animal data to humans, intraspecies variation, the completeness of the toxicological database and the nature of the potential toxicologically significant effects.

The current Australian ADI for fipronil is 0.0002 mg/kg bw/d. It was set in June 1994 by the application of a safety factor of 100 to the NOEL of 0.02 mg/kg bw/d in a chronic/carcinogenicity rat study, based on the occurrence of neurological signs and haematological changes. This review confirmed this ADI. This is a group value to cover the parent compound and metabolites as specified by the residue definition.

Acute Reference Dose (ARfD)

The ARfD is the estimate of the amount of a substance in food or drinking water, expressed on a milligram per kilogram bodyweight basis, which can be ingested over a short period of time, usually one meal or one day, without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation. The current ARfD is 0.02 mg/kg bw, based on a NOEL of 2.5 mg/kg bw for decreased landing footsplay in an acute oral neurotoxicity study in rats, incorporating a safety factor of 100. This value was established in 2006 and is a group value to cover the parent compound and metabolites as specified in the residue definition. The ARfD was previously 0.003 mg/kg bw based on the NOEL from a 3-month neurotoxicity study in rats.

Drinking Water Quality Guidelines

Health Values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The values are derived so as to limit intake *from water alone* to about 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg at a daily water consumption of 2 L over a lifetime. There is currently no guideline or health value for fipronil in Australian drinking water. Given that the ADI for fipronil is 0.0002 mg/kg bw/d, the health-based Guideline Value may be calculated as:

$$0.0007 \text{ mg/L} = \frac{0.02 \text{ mg/kg bodyweight per day} \times 70 \text{ kg} \times 0.1}{2 \text{ L/day} \times 100}$$

where:

- 0.02 mg/kg bw/day is the NOEL based on a 2-year chronic study in rats.
- 70 kg is taken as the average weight of an adult.
- 0.1 is a proportionality factor based on the conservative assumption that at least 10% of the ADI will arise from the consumption of drinking water.
- 2 L/day is the estimated amount (maximum) of water consumed by an adult.
- 100 is a safety factor for using a NOEL from a study conducted in rats. The safety factor of 100 is comparable to that used for the ADI (10 for interspecies variations, 10 for intraspecies variations).

Resolutions of the Advisory Group on Chemical Safety

At its inaugural meeting (29th March 2006) the Advisory Group on Chemical Safety (AGCS) provided advice on whether there is sufficient evidence that fipronil is a skin sensitiser in humans. They expressed concerns about the poor quality of the human adverse reaction reports for fipronil, relative to controlled scientific experiments. The Group suggested that the sponsors should be encouraged to provide more information on human adverse reactions observed overseas, to further assist in determining the skin sensitisation potential of fipronil. They also suggested it would be beneficial if sponsors provided sensitisation data on the main photodegradate of fipronil, preferably through testing in the localized lymph node assay (LLNA).

At its 8th meeting (9th December 2008), the AGCS considered additional data provided by the companies, and the advice of an expert in dermatology on the dermal skin reactions observed in human adverse reaction reports. In the draft minutes of the meeting, it was agreed that the data provided through the APVMA AERP is of limited value in distinguishing whether the reported skin reactions were irritant or allergic in nature. Furthermore, the Group agreed that the use pattern and current reporting from widespread occupational use suggest there is insufficient evidence to class fipronil as a skin sensitiser. It was agreed that adverse reactions should continue to be monitored but ideally assessed by an expert clinician as to the true nature of these skin reactions. The meeting notes were not ratified at the time of preparation of this document (September 2009).

Poisons Scheduling

Fipronil is listed in Schedule 6 of the Standard for Uniform Scheduling of Drugs and Poisons (SUSDP), except when included in Schedule 5 (preparations containing 10 per cent or less of fipronil), or in preparations containing 0.05 per cent or less of fipronil, which are unscheduled. As no new information provided for this review indicates that these levels are inappropriate, it is recommended that the current scheduling of fipronil is maintained.

First-Aid Instructions

First Aid Instruction ‘a’ applies to fipronil products. This remains appropriate.

Safety Directions

The current safety directions for products containing fipronil are shown below.

Table 5: Existing Safety Directions for fipronil

(as listed in FAISD Handbook Edition 1/2009 current to 31 March 2009)

BA 0.03 g/station or less in propylene glycol impregnated in cardboard	
160 162 164 210 211 351 279 283 290 312 360 361	May irritate eyes and skin. Avoid contact with eyes and skin. Wash hands after use. When using the product wear rubber gloves. After each day's use wash gloves.
DU 5 g/kg or less *	
160 162 163 180 210 162 220 221 279 280 283 290 292b 294 306 (dust) 360 361 366 351	May irritate the eyes, nose and throat. Repeated exposure may cause allergic disorders. Avoid contact with eyes. Do not inhale dust. When opening the container and using the product, wear cotton overalls buttoned to the neck and wrist [or equivalent clothing], elbow-length pvc gloves,and disposable dust face mask covering mouth and nose. After each day's use, wash gloves and contaminated clothing. Wash hands after use.
EC 300g/L or less	
129 131 133 207 162 160 164 210 211 279 280 281 282 290 292b 294 297 340 343 351 360 361 363 366	Harmful if absorbed by skin contact or swallowed. Will damage eyes. May irritate the skin. Avoid contact with eyes and skin. When opening the container, preparing spray or using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) elbow-length PVC gloves and goggles. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use, wash gloves, goggles and contaminated clothing.
GB 0.2 g/kg or less *	
180 351 289 290 292b 294 360 361 366	Repeated exposure may cause allergic disorders. Wash hands after use. If applying by hand wear cotton overalls buttoned to the neck and wrist [or equivalent clothing] and elbow-length pvc gloves. After each day's use, wash gloves and contaminated clothing.
GR 1 g/kg or less	
161 162 210 162 351	Will irritate the eyes. Avoid contact with eyes. Wash hands after use.
HG BA gel 0.5 g/kg or less	
160 162 164 210 211 351	May irritate the eyes and skin. Avoid contact with eyes and skin. Wash hands after use.
HG BA 0.5 g/kg or less in plastic labyrinth	
Nil	

* Added to FAISD Handbook following initiation of review, therefore not included in full review process.

Table 5: Existing Safety Directions for fipronil, cont...

HG WP 10 g/kg or less	
Nil	
HV LD 2.5 g/L or less	
161 162 180 210 162 279 283 290 312 340 343 351 360 361	Will irritate the eyes. Repeated exposure may cause allergic disorders. Avoid contact with eyes. When using the product wear rubber gloves. If product in eyes, wash it out immediately with water. Wash hands after use. After each day's use, wash gloves.
HV SA 100 g/L or less	
161 162 164 180 210 211 340 343 351	Will irritate the eyes and skin. Repeated exposure may cause allergic disorders. Avoid contact with eyes and skin. If product in eyes, wash it out immediately with water. Wash hands after use.
SC 100 g/L or less	
161 162 164 180 210 211 351 279 280 281 282 290 291b 300 303 295 360 361 366 364	Will irritate the eyes and skin. Repeated exposure may cause allergic disorders. Avoid contact with eyes and skin. Wash hands after use. When opening the container, preparing spray and using the prepared spray, wear chemical resistant clothing buttoned to the neck and wrist and a washable hat, half facepiece respirator with combined dust and gas cartridge, elbow-length PVC or nitrile gloves. After each day's use, wash gloves and contaminated clothing and respirator, and if rubber wash with detergent and warm water.
SC 200 g/L or less, more than 100 g/L	
129 132 133 161 162 164 180 210 211 279 280 285 290 292b 294 297 279 282 290 292b 294 351 360 361 363 366	Harmful if inhaled or swallowed. Will irritate the eyes and skin. Repeated exposure may cause allergic disorders. Avoid contact with eyes and skin. When opening the container and preparing the product for use wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow-length PVC gloves. When using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow-length PVC gloves. Wash hands after each day's use. Wash gloves, goggles and contaminated clothing.
SC 500 g/L or less, more than 200 g/L	
130 132 133 210 164 279 280 285 282 290 292b 294 351 360 361 366	Poisonous if inhaled or swallowed. Avoid contact with skin. When opening the container, preparing product for use, and using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing) and elbow-length PVC gloves. Wash hands after use. After each day's use, wash gloves and contaminated clothing.

Table 5: Existing Safety Directions for fipronil, cont...

UL 25 g/L or less	
161 162 210 162 279 280 281 290 294 351 360 361	Will irritate the eyes. Avoid contact with eyes. When opening the container and preparing spray wear elbow-length PVC gloves. Wash hands after use. After each day's use, wash gloves.
WG 800 g/kg or less	
130 131 132 133 161 162 164 210 211 220 221 279 280 285 282 290 292b 294 299 340 343 350 360 361 365 366	Poisonous if absorbed by skin contact, inhaled or swallowed. Will irritate the eyes and skin. Avoid contact with eyes and skin. Do not inhale dust. When opening the container, preparing product for use, and using the prepared spray, wear cotton overalls buttoned to the neck and wrist (or equivalent clothing), elbow-length PVC gloves and face shield or goggles. If product in eyes, wash it out immediately with water. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, face shield or goggles and contaminated clothing.
WP 10 g/kg or less	
279 280 285 290 294b 360 361	When opening the container and preparing product for use wear elbow-length rubber gloves. After each day's use, wash gloves.

Table 6: Existing Safety Directions for thiodicarb product containing fipronil

SC 400 g/L or less with fipronil 80 g/L or less	
130 132 133 161 162 190 210 211 220 222 279 280 281 282 290 292 294 296 298 300 303 350 360 361 362 364 366	Poisonous if inhaled or swallowed. Will irritate the eyes. Repeated minor exposure may have a cumulative poisoning effect. Avoid contact with eyes and skin. Do not inhale vapour. When opening the container, preparing spray and using the prepared spray wear cotton overalls buttoned to the neck and wrist and a washable hat, elbow-length PVC gloves, face shield, impervious footwear and half facepiece respirator with combined dust and gas cartridge. After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water. After each day's use, wash gloves, face shield, respirator and if rubber wash with detergent and warm water, and contaminated clothing.

In Chapter 16 of this review, safety directions have been considered for some fipronil-based products, some of which are intended for home garden use or for use on companion animals. The compositional data of all products considered in this review are given in Appendix IX. Estimated acute toxicity profiles of products for which no toxicology data were provided, are included in Appendix XI. The recommended new and revised safety directions are summarised below.

NOTE: with the exception of products intended for home garden or home veterinary use, the OHS Review of Fipronil will advise of any changes that are necessary to personal protective equipment *before* amendments are included in the FAISD Handbook.

Table 7: New/revised Safety Directions for fipronil

Note. Addition/modifications are shown in italics, deletions show as strike through.

Agricultural products

~~**EC 300 g/L or less ***~~	**[Deleted entry]**

~~**WP 10 g/kg or less ***~~	**[Deleted entry]**

SC 500 g/L or less, more than 200 g/L	
Poisonous if inhaled or swallowed	130 132 133
Will irritate the skin	*161 164*
Avoid contact with skin	210 164
Wash hands after use	351

SC 200 g/L or less, more than 100 g/L	
Harmful if inhaled or swallowed	129 132 133
Will irritate the eyes and skin	161 162 164
~~*Repeated exposure may cause allergic disorders*~~	~~*180*~~
Avoid contact with eyes and skin	210 211
If product in eyes, wash it out immediately with water	*340 343*
Wash hands after use	351

SC 100 g/L or less	
Harmful if swallowed	*129 133*
May irritate the eyes	*160 162*
~~*Repeated exposure may cause allergic disorders*~~	~~*180*~~
Avoid contact with eyes	*210 162*
Wash hands after use	351

* Deleted entries explained in 'Recommendations 5. First Aid and Safety Directions'

Baits

BA gel 0.5 g/kg or less	
Wash hands after use	*351*

(Note: this is a *new entry* to replace the current entry of 'HG BA gel 0.5 g/kg or less')

Home garden

~~**HG WP 10 g/kg or less ***~~	[Deleted entry]

~~**HG BA Gel 0.5 g/kg or less ***~~	[Deleted entry] included under new entry [BA gel 0.5 g/kg or less]

* Deleted entries explained in Recommendations: 5. First Aid and Safety Directions

Veterinary products

HV LD 2.5 g/L or less	
Will irritate the eyes	161 162
~~Repeated exposure may cause allergic disorders~~	~~180~~
Avoid contact with eyes	210 162
When using the product wear rubber gloves	279 283 290 312
If product in eyes, wash it out immediately with water	340 343
Wash hands after use	351
After each day's use, wash gloves	360 361

HV SA 100 g/L or less	
Will irritate the eyes and skin	161 162 164
~~Repeated exposure may cause allergic disorders~~	~~180~~
Avoid contact with eyes and skin	210 211
If product in eyes, wash it out immediately with water	340 343
Wash hands after use	351

Table 8: Revised Safety Directions for thiodicarb/fipronil products

SC 400 g/L or less, with fipronil 80 g/L or less	
Poisonous if inhaled or swallowed	130 132 133
Will irritate the eyes	161 162
Avoid contact with eyes	*210 162*
If product in eyes, wash it out immediately with water	*340 343*
Do not inhale vapour	220 222
Repeated minor exposure may have a cumulative poisoning effect	190
After use, and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water	350

Table 9: Amended safety directions for fipronil products registered AFTER commencement of review

Note: These amendments ONLY take into account the deletion of the 180 statement. The remaining safety directions have not been re-evaluated in this review nor the accompanying OHS review, as these entries occurred after the commencement of this review.

DU 5 g/kg or less	
May irritate the eyes, nose and throat	160 162 163
~~Repeated exposure may cause allergic disorders~~	~~180~~
Avoid contact with eyes	210 162
Do not inhale dust	220 221
Wash hands after use	351

RECOMMENDATIONS

1. Approval Status

There is no objection on toxicological grounds to the continued approval of fipronil from the existing sponsors and manufacturers.

2. Acceptable Daily Intake

The current ADI for fipronil is 0.0002 mg/kg bw/d, derived by applying a 100-fold safety factor to a NOEL for neurological signs and haematological changes in a chronic/carcinogenicity study in rats. The ADI is affirmed at 0.0002 mg/kg bw/d.

3. Acute Reference Dose

The ARfD was amended from 0.003 to 0.02 mg/kg bw in 2006, This was derived by applying a 100-fold safety factor to a NOEL of 2.5 mg/kg bw for decreased landing footsplay in an acute neurotoxicity study in rats. This value is included in the current ARfD List (current as of Dec 07).

4. Poisons Scheduling

The current poisons scheduling of fipronil remains appropriate.

5. First Aid and Safety Directions

The recommended safety directions for the reviewed **fipronil** products are summarised in Table 10 below. The current entries for fipronil EC 300 g/L, WP 10 g/kg or less, HG WP 10 g/kg or less and GB 0.2 g/kg or less should be deleted as there are no registered products in these categories. The current entry for HG BA gel 0.5 g/kg or less should be deleted as it is included under the new entry BA gel 0.5 g/kg or less. The current entry for DU 5 g/kg or lesshas been amended in terms of deletion of the 180 statement only, as the product was registered after the data-call in for the review.

Table 10: Recommended Safety Directions for fipronil products assessed in the current review

Status	Formulation	Recommended changes to Safety Directions
No change	HG BA 0.5 g/kg or less in plastic labyrinth	-
No change	BA 0.03 g/station or less in propylene glycol impregnated in cardboard	-
No change	WG 800 g/L or less	-
No change	UL 25 g/L or less	-
No change	GR 1 g/kg or less	-
Amended entry	HV SA 100 g/L or less	161 162 164 210 211 340 343 351
Amended entry	HV LD 2.5 g/L or less	161 162 210 162 279 283 290 312 340 343 351 360 361
Amended entry[#]	DU 5 g/kg or less	160 162 163 210 162 220 221 351
Amended entry *	SC 500 g/L or less, more than 200 g/L	130 132 133 161 164 210 164 351
Amended entry *	SC 200 g/L or less, more than 100 g/L	129 132 133 161 162 164 210 211 340 343 351
Amended entry *	SC 100 g/L or less	129 133 160 162 210 162 351
New entry	BA gel 0.5 g/kg or less	351
Deleted entry	EC 300 g/L	
Deleted entry	WP 10 g/kg or less	
Deleted entry	HG WP 10 g/kg or less	
Deleted entry	HG BA gel 0.5 g/kg or less	

Note:
*Following the Fipronil Review of OH&S, advice on PPE will be incorporated into this entry before it is included in the FAISD Handbook.
[#] This entry has only been evaluated in terms of sensitisation and the necessity of the 180 statement. The remaining safety directions were not reviewed in either the toxicology or OHS reports as the entry occurred after the data-call in period.

The recommended safety directions for the reviewed **thiodicarb** product containing fipronil are summarised in the following table.

Table 11: Recommended Safety Directions for thiodicarb product containing fipronil

Amended entry *	SC 400 g/L or less, with fipronil 80 g/L or less	130 132 133 161 162 210 162 340 343 220 222 190 373 350

Note: * Following the Fipronil Review of OH&S, advice on PPE will be incorporated into this entry before it is included in the FAISD Handbook.

6. Product Registration

There are no objections on public health grounds to the continued registration of the products containing fipronil considered in this review.

MAIN TOXICOLOGY REPORT

1 INTRODUCTION

Fipronil is a broad spectrum insecticide belonging to the phenylpyrazole family of chemicals. The GABA (γ-aminobutyric acid) receptor/chloride channel complex is considered to be its primary site of action. In insects, blockage of the GABA-gated chloride channel reduces the neuronal inhibitory effects of GABA, leading to hyper-excitation of the central nervous system, convulsions, and death. Fipronil shows tighter binding to insect GABA-gated chloride channels relative to the corresponding channel in vertebrates. This property has been considered a major contributor to its considerable enhanced toxicity in insects over mammals. However, recent studies have shown that fipronil and its sulfone metabolite also block glutamate-activated chloride channels, which are found in insects but not in mammals, suggesting that this additional mechanism of action may also contribute to the higher toxicity of fipronil in insects.

Fipronil was first used in Australia as an agricultural chemical product in 1994. Products containing fipronil are used widely in agriculture as insecticidal seed dressings in rice, canola, sorghum and cotton, as ultra low volume (ULV) sprays to control locusts in pasture and sorghum and as sprays to control a wide range of insect pests in bananas, brassica, cotton, potatoes, sugarcane and in mushroom production where is applied to the peat moss. A granular formulation is registered for use in recreational, domestic and commercial turf. Fipronil is also included in a number of household products and commercial building treatments such as cockroach baits and gels, and in ant bait stations. Veterinary products containing fipronil are marketed for use on cats and dogs as ready-to-use spray or concentrated spot-on formulations.

The Australian Pesticides & Veterinary Medicines Authority (APVMA) received a number of human and animal adverse experience reports associated with the use of veterinary products containing fipronil. In these reports, symptoms reported include skin reactions in animals and humans, neurological signs and deaths in target animals (often involving concurrent infestations with paralysis ticks) and deaths following off-label used in domesticated rabbits. Hence the APVMA initiated the reconsideration of the approvals of the active constituent fipronil, registration of products containing fipronil and the approval of all associated labels. Following the data call-in process a number of additional data submissions on the toxicology of fipronil were received from industry and the public, and these data together with all previously submitted registration data and relevant published data, have been assessed in detail. In addition, the OCSEH sought advice from independent experts and the Advisory Group on Chemical Safety (AGCS) regarding skin sensitisation potential.

1.1 History of Public Health Considerations in Australia

Australian public health standards for agricultural and veterinary chemicals that may enter the food chain include the Poisons Schedule, First Aid and Safety Directions, and the human acceptable daily intake (ADI). A further regulatory standard called the maximum residue level (MRL) is a measure of the residues present in unprocessed food (eg. grain, meat etc.) and hence is an indicator of good agricultural practice.

From the mid 1950s until 1992, Australian public health standards were set by committee process under the auspices of the NHMRC. "Pesticide Tolerances" in food were first set in 1956 by the Food Additives Committee. Between 1962 and 1966, the Food Additives Committee maintained a

Sub-Committee on Pesticides and Agricultural Chemical Residues in or on Foods (later re-named the Pesticide Residues in Food Sub-Committee), which adopted the then Canadian scheme as a basis for establishing tolerances. From 1967 onwards, Australian MRLs and ADIs for pesticides were established by the Pesticide and Agricultural Chemicals Committee (PACC), until the Department of Health and Ageing became directly responsible for setting ADIs in November 1992. Responsibility for pesticide and veterinary chemical MRLs in food was transferred to the NRA in June 1994, after which the PACC was removed from the control of the NHMRC and re-constituted as the Advisory Committee on Pesticides and Health (ACPH). The ACPH provides the Department of Health and Ageing, the TGA and the APVMA with advice on issues of policy and practice having possible implications for public health and the proper use of chemicals in agriculture and elsewhere.

Poisons Schedules for agricultural and veterinary chemicals, drugs and some other hazardous substances are set by the National Drugs and Poisons Schedule Committee (NDPSC). Originally known as the Committee on Poisons Scheduling, the NDPSC was established in 1955 as a sub-committee of the NHMRC Public Health Committee. The NDPSC publishes its decisions in the Standard for the Uniform Scheduling of Drugs and Poisons, which recommends controls on availability, labelling, packaging and advertising. These are incorporated into and enforced by the various Australian State and Territory legislative systems. In 1994, the NDPSC was transferred from the NHMRC to the Australian Health Ministers' Advisory Council, and was re-constituted again in 1999 as a Statutory Committee of the Therapeutic Goods Administration.

A third committee formerly involved in chemicals management was the NHMRC Standing Committee on Toxicity (SCOT), which was active between 1985 and 1994. SCOT was responsible for providing specialised advice on complex toxicological matters to all the NHMRC Public Health Committee subordinate committees, including the PACC and NDPSC. In response to referrals from these committees, SCOT undertook evaluation of some drugs, pesticides, food additives, poisons, consumer products, chemicals and other hazardous substances relevant to public health.

Fipronil

Maximum Residue Limits

Maximum Residue Limits (MRL) for fipronil have been set in a wide range of vegetables, fruits, and herbs, as well as rice, sorghum, sugar cane, sunflower seeds, pecans, peanut and peanut oil (crude), cottonseed and cottonseed oil (crude), rape seed, eggs, honey, milks, poultry and mammalian meat (in the fat), edible offal (mammalian and poultry), and mushrooms.

Acceptable Daily Intake (ADI) and Acute Reference Dose (ARfD)

The current ADI for fipronil is 0.0002 mg/kg bw/d. This ADI was derived from a NOEL of 0.02 mg/kg bw/d for neurological signs and haematological changes observed in a 104-week study in rats and using a 100-fold safety factor. The ARfD of 0.02 mg/kg bw was revised from the previous figure of 0.003 mg/kg bw in 2006. The current ARfD was derived by applying a 100-fold safety factor to a NOEL of 2.5 mg/kg bw for decreased landing footsplay in an acute neurotoxicity study in rats. This amended value is included in the current ARfD List. The ADI and ARfD are group values which includes fipronil, fipronil-desulfinyl (desulfinyl fipronil), fipronil-sulfenyl (fipronil sulfide) and fipronil-sulfonyl (fipronil sulfone).

Poisons Scheduling

Fipronil is included in Schedule 6 of the SUSDP except when included in Schedule 5 (preparations containing 10 per cent or less of fipronil), or in preparations containing 0.05 per cent or less of fipronil (unscheduled). A history of the consideration of fipronil by the NDPSC is detailed in the table below:

Table 12: NDPSC regulatory activities in Australia for products containing fipronil

Date	Regulatory activity
August 1994	NDPSC: Recommended fipronil be included in Schedule 6 on the basis of its acute oral LD_{50} of 97 mg/kg bw, with a 2 per cent cut-off to Schedule 5. The S5 classification covered 2 granule formulations on the basis of acute studies submitted for the formulations.
February 1995	NDPSC: Consideration of a company request for exemption from scheduling for a spray preparation containing 0.25% fipronil, with no additional toxicology studies provided. The Committee considered that because of the eye irritancy potential of the spray and its proposed domestic use, an S5 listing for spray preparations containing 0.25% of fipronil remained appropriate.
August 1996	NDPSC: Consideration of data supporting a company request for inclusion of a product containing 10% fipronil in S5. On the basis of the submitted data, the Committee considered that S5 was suitable for the 10% product. The cut-off for S5 entry was increased to include preparations containing 10 per cent or less of fipronil.
May 1997	NDPSC: A company requested exemption from scheduling for two cockroach bait stations and one gel cockroach bait, each containing 0.05% fipronil, supported by acute studies on the formulations. The Committee decided that the gel formulation should remain in S5, based on the acute oral toxicity (LD_{50} = 4400 mg/kg bw), slight eye and skin irritation, and the greater potential for exposure. The baits in bait stations were exempted from scheduling as the potential for exposure was low due to the nature of the packaging.
August 1997	NDPSC: The company supplied additional information regarding the use pattern of the cockroach gel bait that had been included in S5 at the previous meeting. The Company advised that the proposed use pattern involved the application of tiny drops in areas typically out of sight and almost always practically out of reach. The Committee considered that in view of this additional information, and the LD_{50} of the product being close to the cut-off for exemption from scheduling, the preparation should be exempted from scheduling.

MRLs in drinking water

Where a pesticide is registered for use in water or water catchment areas, the Joint Committee of the Agricultural and Resource Management Council of Australia and New Zealand and the NHMRC set Guideline and Health Values for the chemical in drinking water. A Guideline Value is generally based on the analytical limit of determination, and is set at a level consistent with good water management practice and that would not result in any significant risk to the consumer over a lifetime of consumption. Exceeding the Guideline Value indicates undesirable contamination of drinking water and should trigger action to identify the source of contamination and prevent further contamination. However, a breach of the Guideline Value does not necessarily indicate a hazard to public health.

Health Values are intended for use by health authorities in managing the health risks associated with inadvertent exposure such as a spill or misuse of a pesticide. The values are derived so as to limit intake from water alone to about 10% of the ADI, on the assumption that (based on current knowledge) there will be no significant risk to health for an adult weighing 70 kg at a daily water consumption of 2 L over a lifetime. The current Australian Drinking Water Guideline does not include a Guideline Value or a Health Value for fipronil, however the OCSEH is in the process of recommending a health-based Guideline value to the NHMRC (see section on 'Consideration of Public Health Standards').

1.2 International Toxicology Assessments

JMPR

Fipronil has been evaluated by the Joint Meeting on Pesticide Residues (JMPR) in 1997 and 2000. The 1997 evaluation considered the toxicological profiles of fipronil and the photodegradation product desulfinylated fipronil (fipronil-desulfinyl). The latter was included on the basis that it appeared more toxic than the parent compound.

The JMPR evaluation concluded that fipronil was not a sensitiser in the guinea-pig by the Buehler method, but a mild sensitiser by Magnusson-Kligman. The ADI for fipronil was established at 0–0.0002 mg/kg bw on the basis of a NOAEL (No Observed Adverse Effect Level) of 0.019 mg/kg bw/d in a 2-year toxicity and carcinogenicity study in rats, and a safety factor of 100. A temporary ADI of 0–0.00003 mg/kg bw was set for fipronil-desulfinyl, based on a NOAEL of 0.029 mg/kg bw/d in a 90-day study in rats and a safety factor of 1000. Further studies in rats exposed to fipronil-desulfinyl in the diet were requested (short-term neurotoxicity, developmental neurotoxicity, and results of an ongoing long-term study), as without these studies, determination of an ADI was considered impractical. An ARfD of 0.003 mg/kg bw was set for both fipronil and fipronil-desulfinyl, based on a NOAEL of 0.3 mg/kg bw/d in a 13-week neurotoxicity study in rats administered fipronil in the diet, and a safety factor of 100. Because of the prolonged toxicokinetics of fipronil, the acute neurotoxicity study in rats was not considered an appropriate basis for the ARfD.

At the 2000 Meeting, it was concluded that the NOAELs in the long-term studies with fipronil and fipronil-desulfinyl, which were both based on clinical signs of neurotoxicity, were comparable. The chronic NOAEL for fipronil desulfinyl was 0.025 mg/kg bw/d. Therefore, a group ADI of 0-0.0002 mg/kg bw was established for fipronil and fipronil-desulfinyl. As the NOAEL for developmental toxicity of fipronil-desulfinyl in rats (1 mg/kg bw/d) exceeded the chronic NOAEL considerably, the requirements for the other studies previously requested were waived. The 2000 Meeting also confirmed the ARfD of 0.003 mg/kg bw as a group ARfD for fipronil and fipronil-desulfinyl, alone or in combination.

US EPA

Fipronil has been evaluated by the US EPA to establish tolerances for combined residues of fipronil and its metabolites MB 46136 (fipronil sulfone) and MB 45950 (fipronil sulfide) in or on a range of raw agricultural commodities (40 CFR Part 180, Final rule, 26 November 1997; http://www.epa.gov/fedrgstr/EPA-PEST/1997/November/Day-26/p30949.htm). The RfD (reference dose) for chronic dietary exposure was established at 0.0002 mg/kg bw/day, based on a NOEL of 0.019 mg/kg bw/day and an uncertainty factor of 100; the NOEL was from a combined chronic toxicity/carcinogenicity study in rats with a LOEL of 1.5 ppm for an increased incidence of clinical

signs (seizures and death) and alterations in clinical chemistry (protein) and thyroid parameters (increased TSH, decreased T4). The NOEL specified for acute dietary exposure was 0.5 mg/kg bw, based on decreased hind leg splay in male and female rats observed at a LOEL of 5 mg/kg bw in an acute neurotoxicity study.

In July 2004, the EPA approved requests submitted by Bayer CropScience to voluntarily cancel the registrations of certain pesticide products containing fipronil for use on rice or rice seed. Any distribution, sale or use of the products subject to this cancellation order was only permitted in accordance with the terms of the existing stocks provision of this cancellation order.

The US EPA has approved fipronil products for agricultural and domestic (veterinary and home garden) uses. Registered domestic fipronil products are approved for use on cats and dogs for flea control, and on turf to control fire ants (US EPA, 2007a). The US EPA conducted an aggregate dietary exposure estimate for fipronil and concluded that chronic dietary exposure to fipronil residues from both primary and secondary sources, as a result of its use on field corn, potatoes, rice and cotton does not represent a significant risk to any segment of the population (US EPA, 2007a). The US EPA also approved agricultural fipronil products for use on potato and sweet potato in August 2007 and for use on pine seedling in June 2007 (US EPA, 2007b).

New York State

New York State refused the use of Frontline Spray in 1996/1997 due to an unacceptable risk to workers that could be applying the product to around 20 dogs per day. Registration was subsequently granted in 1999.

European Union

The European Food Safety Authority (EFSA)
The EFSA, the lead agency in the EU's pesticide review process, completed a re-evaluation of fipronil and its products in 2006 (EFSA, 2006). An ADI of 0.0002 mg/kg bw/d and an ARfD of 0.009 mg/kg bw were referenced for fipronil. In 2007, the European Commission Health and Consumer Protection Directorate-General reviewed the EFSA report and additional data in order to ascertain if fipronil should be included in Annex I of Directive 91/414/EEC. The Directive provides for the establishment of a positive list of active substances (Annex I), that have been shown to be without unacceptable risk to people or the environment. The positive list of active substances are authorised for use in plant protection products within the community. The 2007 review concluded that the available data supported use of fipronil as a seed dressing on sunflower and maize. The overall conclusion was that fipronil be included in Annex I of Directive 91/414/EEC for use as a seed dressing (EC, 2007).

AFSSA/AFSSE: Agence Française de Sécurité Sanitaire des Aliments (French Food Health Safety Agency)/ Agence Française de Sécurité Sanitaire Environmentale (French Environmental Health Safety Agency)

In 2005 AFSSA/AFSSE conducted a human health risk assessment of fipronil. The report assessed the use of fipronil as a veterinary medicine or biocide for domestic and professional use, and as a plant protection product for farming and amateur gardening. Potential human exposure to fipronil was evaluated for workers and the general population as a whole from consumption of foods containing fipronil residues, domestic use of plant protection products and biocides and from contact with pets treated with veterinary medicinal products containing fipronil. The report cites an

ADI of 0.0002 mg/kg bw/d based on a NOEL of 0.019 from a long-term study in rats and applying a safety factor of 100.

Human toxicovigilance data was reported for exposure to both veterinary and agricultural products. Cases of both acute and repeat exposure to preparations containing fipronil were presented and effects in both cases were generally benign. Such effects included simple local irritation following dermal exposure or accidental spraying in the eye, or minor digestive disorders in the event of accidental ingestion of low quantities.

The AFSSA report concluded on the basis of modelling results for exposure scenarios compared to the most relevant toxicological reference values (i.e. acute, subacute and chronic NOEL's) and on the basis of other available data that:

- The theoretical dietary exposure of the adult population to fipronil residues remains within the set safety limits, however, for young children there are cases where exposure scenarios exceed the ADI. The report emphasised that very few analyses of fipronil residues in food stuffs were available and analyses were mainly limited to uses requested within the framework of the European re-evaluation (maize and sunflower).
- The use of domestic biocidal products and plant protection products bearing the note "approved for use in gardens" presents an acceptable risk for an adult applier (with the exception of the "Special wasp and hornet nests" product which should be reserved for professional use).
- Exposures linked to contact with animals treated with veterinary medicinal products containing fipronil present an acceptable risk. The report noted that safety margins were lowest for young children and therefore attention should be drawn to precautionary statements on domestic products addressing a possible risk to young children.
- Due to lack of available exposure data for farmers and pest control professionals, potential risk could not be evaluated.

In, 2007 the United Kingdom (UK) published a revocation notice for fipronil agricultural products that did not comply the specific condition for fipronil's inclusion in Annex I of Directive 91/414/EEC: 'only uses as insecticide for use as seed treatment may be authorised.'

1.3 Chemistry – Technical Active

Approved common name: Fipronil (ISO)

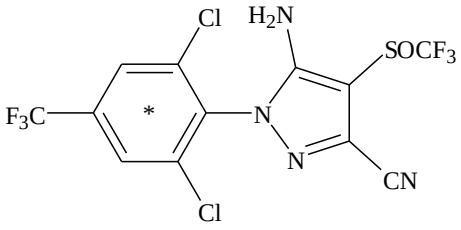
Manufacturer's Code: MB 46030; M&B46030

Chemical name: (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethyl -sulfinylpyrazole-3-carbonitrile (IUPAC)

5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile (CA)

CAS No.: 120068-37-3

Chemical Structure:



*indicates isotope label

Empirical formula: $C_{12}H_4Cl_2F_6N_4OS$

Molecular weight: 437.15

Chemical class: Phenylpyrazole

Chemical and physical properties

Colour:	White	
Odour:	Mouldy smell at 23°C	
Physical state:	Powder at room temperature	
Density (20°C):	1.477 – 1.626	
Melting point:	195.5 - 203 °C	
Partition coefficient: (log K_{ow})	4.0 at 20 °C (Octanol/water partition)	
Henry constant:	3.7×10^{-10} atm.m^{3}mol^{-1}	
Vapour pressure:	$2.8 \times 10^{--9}$ mm Hg (3.7×10^{-9} hPa) at 25°C	
Solubility:		
in water:	1.9 mg/L (distilled water at 20°C)	
	2.4 mg/L (pH 5), 2.2 mg/L (pH 9)	
	(not temperature-dependent)	
in organic solvents:	Acetone	54.59
	Dichloromethane	2.23
	Ethylacetate	26.49
	Hexane	0.003
	Methanol	13.75
	1-Octanol	1.22

	2-Propanol	3.62
	Toluene	0.30
Hydrolysis at 25°C:	Stable (pH 5)	
	Stable (pH 7)	
	DT50 = 28 days (converted to RPA200766) (pH 9)	
Oxidation stability (air):	Unreactive to tapwater, ammonium dihydrogen phosphate, metallic zinc, dilute, neutral potassium permanganate.	
Thermal stability:	No evidence of degradation at 30-150°C.	
Dangerous goods classification:	Pesticides solid, Toxic, n.o.s UN No 2588 Packaging Groups III	

Technical active - Declaration of Composition and Batch Analysis

At the initiation of the review (September 2003), there were 4 active constituent approvals for fipronil. Declarations of composition for technical grade fipronil are shown in Appendix VIII.

Impurities of Toxicological Concern

APVMA Compositional Specifications for fipronil:
950 g/kg minimum (on a dry weight basis)

The FAO specifications[7] for fipronil (FAO Specification 581/TC/S/F, 1998) state:

The fipronil content shall be declared (not less than 950 g/kg on the dry weight basis) and, when determined, the content obtained shall not differ from that declared by more than ± 25 g/kg.

Fipronil is usually manufactured as a wet material to reduce its dustiness and the resulting risk of inhalation. The only impurity listed in the FAO specifications is:
Water (MT 30.5, CIPAC I to be published); for the wet material: 60 to 90 g/kg.
FAO specifications also exist for ultra low volume liquids (FAO Specification 581/UL/S/F, 1998).

Metabolites of Toxicological Concern

The metabolite of greatest toxicological concern is desulfinyl fipronil, a photolytic metabolite of fipronil that is present on treated plants as the major residue. Desulfinyl fipronil is of particular concern as it is not produced during metabolism of fipronil in mammals. The metabolites fipronil sulfide and fipronil sulfone are also of concern as they have similar toxicity to the parent and are present as residues.

[7] http://www.fao.org/WAICENT/FAOINFO/AGRICULT/AGP/AGPP/Pesticid/

1.4 Products

At the initiation of the review (September 2003), there were 29 registered products containing fipronil. These comprise home veterinary spot-on and spray formulations, as well as baits (solids and gels), and soluble concentrate, granular, wettable granule, and ultra-low volume formulations. Registration details and formulations are shown in Appendices II and IX. Currently, there are 41 products listed on PUBCRIS, however, only those products registered at the time of the data call-in are included in this review. An additional product (DU, 5 g/kg fipronil or less) was considered for the neccessity of the safety direction 180 ('repeated exposure may cause allergic disorders') only.

2 METABOLISM AND TOXICOKINETICS

2.1 Mice

Fisher PJ (1992) Fipronil (MB46030): Preliminary determination of blood and brain levels in the mouse during dietary administration over 14 days. Report no. SA 9175. Lab: Rhône-Poulenc – Secteur Agro, Centre de Recherche, Valbonne, France. Sponsor: Rhône-Poulenc – Secteur Agro, Direction Mondiale de la Toxicologie, Lyon, France. Unpublished report date: 10 January 1992. (GLP/QA, Study Guidelines: none stated)

Six male mice (CD1 strain) were fed 100 ppm fipronil (approximately equal to 15 mg/kg bw/d) in the diet over 14 days. Brain and blood samples from one mouse that died from compound-related effects, and from those that survived until termination, were analysed for fipronil, MB 46136 (fipronil sulfone) and MB 45950 (fipronil sulfide) by HPLC.

The mouse that died before the end of dosing lost weight for the 8 successive days before its death. However, two survivors showed a similar rate of weight loss. Fipronil was not detected in samples from mice that survived the dosing period; and MB 46136 (fipronil sulfone) was the only metabolite detected in blood and brain samples from these mice. The mouse that died had both fipronil (19.9 µg/g) and fipronil sulfone (18.9 µg/g) in the brain. Surviving mice had a mean brain concentration of 12.4 µg/g fipronil sulfone. The results suggested that a higher dose level and/or a longer dosing period would provide better comparative information on fipronil concentrations in the blood and brains of mice that survive or succumb to dosing.

Fisher PJ (1991) Fipronil (MB46030): Determination of blood and brain levels in the mouse during dietary administration over 28 days. Report no. SA 9178. Lab: Rhône-Poulenc – Secteur Agro, Centre de Recherche, Valbonne, France. Sponsor: Rhône-Poulenc – Secteur Agro, Direction Mondiale de la Toxicologie, Lyon, France. Unpublished report date: 23 December 1991. (GLP/QA, Study Guidelines: none stated)

Two groups of 10 male mice (CD1 strain) were fed fipronil in the diet at nominal rates of 75 or 150 ppm (approximately equal to 11 and 22 mg/kg bw/d respectively) over 28 days, or until a mortality rate of 50% was attained. Blood and brains from mice that died from toxic effects, and from those that survived until termination, were analysed by HPLC for fipronil, MB 46136 (fipronil sulfone) and MB 45950 (fipronil sulfide).

Fipronil was not detected in any samples, and fipronil sulfone was the only metabolite detected. The concentration of MB 46136 (fipronil sulfone) in the blood of mice that died were higher than in

survivors. However, the difficulty in sampling blood from the dead mice led to few results being available to perform this comparison. Levels of fipronil sulfone in the brains of mice that died were similar to those in survivors (low dose group, 11.2 and 9.62 µg/g respectively; high-dose group 17.0 and 15.6 µg/g, respectively).

2.2 Rats

Powles P (1992) (^{14}C)-M&B 46,030: Absorption, distribution, metabolism and excretion in the rat. HUK Report no: 7040-68/117. Lab: Hazleton UK, North Yorkshire, England. Sponsor: Rhone Poulenc Agriculture, Essex. Expt. dates: 11.9.1991-26.6.1992. Unpublished report date: 26 June 1992. (GLP/QA: yes. Study Guideline: US EPA 85-1)

Materials and Methods A single oral dose of [^{14}C]-fipronil was administered by oral gavage to two groups of Charles River CD(SD)BR strain rats (5/sex/group) at 4 (group A) or 150 (group B) mg/kg bw as an aqueous suspension. Additional groups were treated similarly for measurement of the kinetics of radioactivity in the blood. Radiolabelled material retained in selected tissues, or excreted, was characterised using chromatographic and spectroscopic procedures. A similar investigation was carried out following repeated daily oral doses of 4 mg/kg bw unlabelled fipronil for 14 days, with a radiolabelled dose of 4 mg/kg bw given on day 15 (5/sex) (group C). Rats were killed after the last collection of samples, 168 h post dosing.

Results For all treatment regimes, there were no sex differences in the distribution of [^{14}C]-fipronil in the excreta and tissues, with >95% of administered radioactivity recovered from each group. For group A, 45.6% (males) and 46.0% (females) of radioactivity was voided in faeces, 5.6% (males and females) in urine and 46.0% (males) and 45.8% (females) remained in the body at termination. Following pre-treatment at the low dose (group C), faecal excretion accounted for 56%/61% (males/females), and 16.2%/13.8% (males/females) was found in the urine, with ~20% remaining in the carcass/tissues. At the high dose (group B), a larger proportion was recovered in faeces (66.9%, males; 75.1%, females), with urinary excretion accounting for 29.2% and 22.0% for males and females respectively, and 3-5% in the tissues. At 168-h post dosing, the highest radioactivity was detected in fat, with moderate levels in adrenal gland, pancreas, skin, liver, kidney, muscle, ovaries and uterus.

As estimated from the amount of radioactivity in the urine and body, the proportion of fipronil absorbed was treatment dependent. At the single low dose, approximately 50% was absorbed. This was reduced to 40% following pre-treatment, and to 25% after the single high dose. The proportion absorbed was readily metabolised, with no fipronil detected in urine or tissues. At 4 mg/kg bw, the maximum blood concentration of radioactivity was attained at 4-6 h post dosing (0.68 µg equivalent of [^{14}C] fipronil/g, males; 0.60 µg equivalent/g, females). The subsequent decline in concentration was slow: by 168 h post dosing, the level was still about 40% of the maximum level attained. The elimination half-lives were long (approximately 150 h, males; 200 h, females), which may reflect slow release from the tissues. At 150 mg/kg bw, the peak blood concentration of radioactivity increased relative to the low dose (~19.6 µg equivalent/g in both sexes). The absorption phase was longer than at the lower dose level. Radioactivity levels declined more rapidly than at the lower dose level, and the elimination half-lives were reduced (54.4 h, males; 51.2 h females). HPLC analysis of urine after glucuronide and sulfate deconjugation, revealed 14 radiolabelled components; the two major metabolites were pyrazole ring opened compounds, with fipronil and its amide, detrifluoromethylsulfinyl, sulfide, and sulfone derivatives also present. In faeces, at least 11 radiolabelled metabolites were detected. The most prominent was unchanged fipronil, with lesser amounts of MB 46136 (fipronil sulfone) and MB 45950 (fipronil sulfide). At

later time points, MB 46136 (fipronil sulfone) was the dominant metabolite, and was also the major radioactive component in fat, liver, kidney, muscle and uterus.

Totis M & Fisher PJ (1994) Fipronil: Tissue kinetic study in the rat. Study No. SA94255. Lab and Sponsor: Rhône-Poulenc – Secteur Agro, Centre de Recherche, Sophia Antipolis, France. Expt. dates: 21.7.1994-9.9.1994. Unpublished report date: 17 October 1994. (GLP/QA: yes; Study Guidelines: Safety Evaluation of Agricultural Chemicals (59, Nohsan No. 4200, Japan)

Materials and Methods Rats (5/sex/dose for each time-point) were given a single oral dose of [^{14}C]-fipronil at 4 or 40 mg/kg bw. The rats (CD strain) were from Charles River France and weighed approximately 300 g. The dosing mixture was prepared using [U-^{14}C phenyl]-fipronil (Batch: GHS 826; Radiopurity: 99%; Specific activity: 19.85 mCi/mmole), and unlabelled fipronil (Batch: AJK232/C2; Purity: 99.4%) in 0.05% w/w aqueous methyl cellulose and 0.01% w/v Tween 80 as solvent. Blood samples were collected prior to dosing, at approximately 0.5, 1, 2, 3, 4, 6, 8 and 24 h after dosing, and at 24 h intervals thereafter until approximately 14 days post-dosing. Animals were exsanguinated at the time points corresponding to ½Tmax (absorption), Tmax, ½Tmax (elimination) and 168 h, which were determined in a preliminary study (tabulated below).

Table 13: Absorption and elimination parameters in a single oral dosing study in rats (Totis & Fisher, 1994)

Time for samples	½Tmax (abs) (h)		Tmax (abs) (h)		½Tmax (elim) (h)	
	Males	Females	Males	Females	Males	Females
4 mg/kg bw	0.8	0.8	4.8	6.2	96	94
40 mg/kg bw	3.0	4.0	34	38	77	78

Organs/tissues (liver, kidneys, heart, lungs, brain, spleen, pancreas, muscle, abdominal fat, ovaries, testes, GIT plus contents, stomach plus contents, bone (femur) and marrow, adrenals, uterus, thyroid and the skin and fur) were collected. The amounts of radioactivity in the various samples were determined by liquid scintillation counting with or without combustion (pre-treatment).

Results As shown in the Table below, absorption was rapid for both sexes at 4 mg/kg bw, with the Cmax achieved at 5-6 h. However, the 40 mg/kg bw group displayed a much slower absorption/distribution (34-38 h). The rate of absorption appeared to be independent of sex. A slow elimination was observed in both dose groups, especially in females (135-245 h), possibly reflecting a slow release from a compartment such as fat.

Table 14: Absorption and elimination parameters in a single oral dosing study in rats (Totis & Fisher, 1994) - Blood parameters

	Cmax (µg equiv./g)		Tmax (abs) (h)		$t_{½}$ (elim) (h)	
	Males	Females	Males	Females	Males	Females
4 mg/kg bw	0.5	0.4	4.8	6.2	182	245
40 mg/kg bw	6.7	7.6	34	38	135	171

Radioactivity was widely distributed in the tissues, peaking in the tissues at the blood Tmax, except for those involved in the absorption process, i.e. stomach and intestinal tract (see Table below). The distribution was similar in both dose groups, with the level in each tissue 3 to 6 times higher at the high dose than the low dose. Excluding the stomach and intestinal tract, the highest tissue levels in

descending order were abdominal fat, adrenals, pancreas, thyroids, skin and fur, ovaries, uterus and the liver.

Table 15: Tissue distribution (µg equivalents/mg)

	4 mg/kg bw								40 mg/kg bw							
	Males				Females				Males				Females			
Time (h)	**0.8**	**4.8**	**96**	**168**	**0.8**	**6.2**	**94**	**168**	**3**	**34**	**77**	**168**	**4**	**38**	**78**	**168**
Blood	0.62	0.62	0.26	0.22	0.7	0.6	0.2	0.2	2.1	3.8	2.3	0.6	2.2	5.1	2.9	0.9
Stomach*	147	0.5	0.3	0.6	53	0.7	0.4	0.6	381	64	10	0.88	185	148	8.7	1.3
Intestinal tract*	46	13	3.2	2.1	46	9.4	3.6	2.9	423	73	49	4.9	360	65	56	5.6
Fat	11	31	24	16	13	31	25	22	69	229	115	32	80	201	135	38
Adrenals	9.0	11	8.3	5.2	10	9.6	5.1	3.9	34	54	20	16	39	47	29	14
Pancreas	5.2	6.7	6.5	4.4	6.1	5.3	3.2	2.6	31	38	13	6.2	26	32	20	5.6
Thyroids	3.7	5.8	2.7	2.2	4.2	4.1	2.7	2.9	25	29	16	10	16	16	23	13
Skin&fur	1.9	5.1	4.1	3.3	2.4	5.4	3.8	3.8	17	29	16	6.4	20	29	19	6.2
Gonads	1.4	1.7	1.0	1.0	5.9	5.6	5.4	4.6	6.0	9.3	4.2	1.5	20	44	20	9.8
Liver	9.2	6.8	3.4	2.4	12	7.7	3.2	2.9	31	36	17	5.8	32	32	20	6.3
Brain	1.8	2.1	1.1	0.8	2.4	2.3	1.1	1.0	8.7	9.7	4.5	1.6	8.8	9.7	5.6	2.0

*plus contents

Totis M (1995) Fipronil: Bile excretion study in the rat. Study No. SA95020. Lab: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. ROI0246. Sponsor: Rhone-Poulenc Agro KK. Minato-ku Tokyo, Japan. Expt. dates: 4.4.1995 – 30.8.1995. Unpublished report date: 8 September 1995. (GLP/QA: yes; Study Guidelines: Safety Evaluation of Agricultural Chemicals (59, Nohsan No. 4200, Japan)

Materials and Methods Bile duct-cannulated Sprague Dawley rats (4/sex/dose, CD strain from Charles River France) were given a single oral dose of [^{14}C]-fipronil at 4 or 40 mg/kg bw. The dosing mixture was prepared using [U-^{14}C phenyl]-fipronil (Batch: GXR 366A; Radiopurity: 98.8%; Specific activity: 19.85 mCi/mmole), and unlabelled fipronil (Batch: AJK232/C2; Purity: 99.4%) in 0.05% w/w aqueous methyl cellulose and 0.01% w/v Tween 80 as solvent. Urine and faeces (also cage wash) were collected at the time intervals of 0, 0-10, 10-24, 24-48 and 48-72 h, and bile was collected at 0, 0-5, 5-10, 10-24, 24-48 and 48-72 h post-dosing. At 72 h, all animals were exsanguinated under anaesthesia, and selected tissues (intestinal tract and contents, stomach and contents, cardiac blood, skin, fur and residual carcass) were collected. All samples were analysed quantitatively for radioactivity by liquid scintillation counting directly or after pre-treatment (combustion and solubilisation). The quantification of the metabolites present in bile samples was performed using HPLC and TLC (Thin Layer Chromatography) with or without pre-enzymatic hydrolysis.

Results The recoveries of radioactivity ranged from 93-116% of the administered dose. At both the low and high doses, the major route of excretion was the faeces. The bile was also an important excretory route, and in 40 mg/kg bw/d males, the amount of radioactivity excreted in the bile slightly exceeded the amount excreted directly in the faeces. A relatively small proportion of the dose was eliminated in the urine at both doses (see Table below). At 72 h post dosing, the total radioactivity remaining in the tissues was 80-83% of the administered dose at the low dose, and 56-66% at the high dose. Radioactivity in the stomach (and its contents) at 40 mg/kg bw was relatively high and variable. A finding of high radioactivity in stomach contents 72 h after dosing is unusual, and suggests that fipronil delayed gastric emptying. High levels in stomach + contents were reported in an earlier study (Totis and Fisher, 1994) at 38 h post-dosing in females dosed at

40 mg/kg bw. As estimated from the amount of radioactivity found in urine, bile, blood and tissues, approximately 80-90% of the dose was absorbed.

Table 16: Elimination and tissue distribution (n=2-4)

	4 mg/kg bw		40 mg/kg bw	
	Males	**Females**	**Males**	**Females**
Elimination (0-72 h) (% dose)				
Faeces	13.7	9.4	21.4	35.4
Bile	7.60	6.76	24.9	11.6
Urine	0.85	1.62	4.66	2.58
Tissue distribution (at 72 h) (µg equiv./g tissue)				
Blood	0.37	0.35	5.82	4.25
Plasma	0.54	0.54	9.86	7.27
Intestinal contents	0.64	0.84	32.0	30.6
Intestinal tract	4.97	5.65	19.5	23.5
Stomach contents	0.59	0.91	95.6	135.5
Stomach	3.98	3.48	81.9	101.6
Skin & fur	4.88	6.47	15.9	27.7
Residual carcass	3.51	3.39	17.6	18.6
Total in the tissue (% dose)	80.2	83.4	55.8	66.3

The profiles of [^{14}C]-fipronil metabolites in the bile were qualitatively similar in males and females, and at the low and high doses. The absorbed portion of the dose was extensively metabolised prior to secretion into the lumen of the gut, with 16 metabolite fractions and only a small amount of unchanged parent (< 0.26% over 72 h) detected in the bile. The main bile metabolite (BMET/3, polar, chemically undefined) represented 22% of the dose for males and 8.1% for females at 40 mg/kg bw, and 3.1% for males and 1.3% for females at 4 mg/kg bw. Two other undefined fractions (BMET/5 and BMET/7, less polar) represented >0.5% of the dose, with the retention time of BMET/7 corresponding to the carboxylic acid metabolite of fipronil. Other fractions corresponding to detrifluoromethylsulfinyl fipronil, fipronil and fipronil sulfone were confirmed in the bile by both HPLC and TLC techniques, but accounted for <0.5% of the dose. Following enzymatic hydrolysis, some of these fractions were found to comprise a mixture of glucuronide conjugates, and one was possibly a sulfo-conjugate.

Kemp L (1999) [^{14}C]-Fipronil: Biliary reabsorption study in the rat. Report No. RNP567/983185. Lab: Huntington Life Sciences Ltd, Eye, Suffolk, England. Sponsor: Rhone-Poulenc, Centre de Recherche de Sophia Antipolis, France. Expt. dates: 18.3.1998-24.7.1998. Unpublished report date: 22 January 1999. (GLP/QA: yes; Study Guidelines: US EPA 85-1)

Materials and Methods Male Sprague Dawley (CD) rats (from Charles River UK Ltd, Margate, Kent) were 7-9 weeks of age and 230-250 g bw. [^{14}C]-Fipronil (Lot No: GHS 932; Radiochemical purity: 99.4%; Specific activity: 979 MBq/mmol) and non-radiolabelled fipronil (Lot No: BES 1785; Purity: 99.9%) were used in the study. Three male bile duct-cannulated rats (Group 1) received a single oral dose of approximately 4 mg/kg bw (mean achieved dose 3.26 mg/kg bw) of [^{14}C]-fipronil in 0.5% aqueous carboxymethylcellulose + 0.01% Tween 80 by gavage. Bile, urine, faeces and cage wash were collected during the intervals 0-24, 24-48 and 48-72 h, and a blood sample and selected tissues/organs were collected at sacrifice (72 h). Another 3 bile-duct cannulated rats (Group 2) were dosed by infusing radioactive bile (24 h duodenal infusion of bile collected from the Group 1 rats and pooled) at a rate of 0.9 mL/h. Bile, urine, faeces and cage wash were collected at 0-24, 24-48, 48-72 and 72-96 h, and a blood sample and selected tissues/organs

were collected at sacrifice (96 h) (see Table below). Radioactivity of all samples from Groups 1 and 2 were analysed by scintillation, directly or after pre-treatment (combustion and solubilisation).

Results

Table 17: Recovery of radioactivity

		Group 1 (oral dosing)		Group 2 (bile infusion)	
Achieved dose (kBq)		1697		73.82	
(mg/kg bw)		3.26		0.13	
		%	**ng equiv/g**	**%**	**ng equiv./g**
Bile	24 h	4.62		25.51	
	48 h	4.34		8.16	
	72 h	3.77		1.45	
	96 h	-	-	1.11	
	Total	**12.73**		**37.64**	
Faeces	24 h	8.59		5.40	
	48 h	3.36		11.11	
	72 h	4.05		1.40	
	96 h	-	-	2.12	
	Sub Total	**16.00**		**17.96**	
Urine	24 h	1.86		4.02	
	48 h	0.29		1.87	
	72 h	0.46		0.27	
	96 h	-	-	0.23	
	Sub Total	**2.61**		**7.39**	
Cage wash	24 h	0.12		0.59	
	48 h	0.04		0.21	
	72 h	0.16		0.05	
	96 h	-	-	0.21	
	Sub Total	**0.33**		**1.15**	
Tissues	Liver	7.75	5986	-	-
	Skin	7.17	1424	4.51	
	GI tract	7.34	2562	-	-
	Blood		417	-	8.1
	Adrenals	0.08	11227		
	Brain	0.54	2321		
	Fat	0.62	18028		
	Heart	0.22	1883		
	Kidneys	0.75	2846		
	Muscle	0.22	1509	-	-
	Pancreas	0.61	5896		
	Thyroid	0.00	5965		
	Carcass	34.03	2043	22.12	
	Sub total	**59.33**		**26.63**	
Total		**91.00**		**90.76**	

For both groups, at least 70% of the administered radioactivity was absorbed. Approximately 13% and 38% of the dose was recovered from the bile following oral dosing and dosing with bile by duodenal infusion, respectively. About 16-18% of the dose administered by either route was excreted via the faeces, representing material that was not absorbed. Only a relatively small amount of radioactivity (2.6% for Group 1, 7.4% for Group 2) was excreted via the urine.

For Group 1, at 72 h after dosing, the majority of the absorbed radioactivity (59% of the dose) was recovered from the tissues, of which 7.8% was in the liver, 7.2% in the skin, 7.3% in the GIT and its contents, and 34% in the residual carcass. The highest concentration of radioactivity was in the

fat and adrenals. For Group 2, only 26% of the dose was found in the tissues, comprising 4.5% in the skin and 22% in the residual carcass, the only tissues investigated, indicating that fipronil metabolites excreted in the bile may be redistributed into and/or retained in the tissues following re-absorption.

2.3 Comparative studies

Whitby BR (1991) (^{14}C)-M&B 46,030: Whole body autoradiography following oral administration to the rat, mouse and rabbit. Report no. 6580-68/105. Lab: Hazleton UK, Harrogate, North Yorkshire, England. Sponsor: Rhone-Poulenc Agriculture, Ongar, Essex, UK. Expt. dates: 14.6.1990-29.6.1990. Unpublished report date: January 1992. (GLP/QA: yes; Study Guidelines: none stated)

The tissue distribution of radioactivity was studied in the rat, mouse and rabbit by whole-body autoradiography at 12 and 72 h following a single oral dose of 5 mg/kg bw [^{14}C]-fipronil. Absorption was rapid, followed by wide distribution. Radioactivity was highest in brown fat, fat and the Harderian gland. Elimination was slow, as tissue concentrations were only marginally reduced at 72 h post dosing.

Broadmeadow A (1991a) M&B 46030: Toxicokinetic study by oral (gavage) administration to female CD rats for 14 days followed by a 7 day reversibility phase. LSR report no. 90/RHA366/0772. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie Lyon, France. Expt. dates: 10.4.1990-1.5.1990. Unpublished report date: 28 March 1991. (GLP/QA: yes; Study Guidelines: none)

Broadmeadow A (1991b) M&B 46030: Toxicokinetic study by oral (gavage) administration to female CD-1 mice for 14 days followed by a 7 day reversibility phase. LSR report no. 90/RHA366/0773. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie Lyon, France. Expt. dates: 17.4.1990-8.5.1990. Unpublished report date: 28 March 1991. (GLP/QA: yes; Study Guidelines: none)

Cummins HA (1991) M&B 46030: Toxicokinetic study by oral (gavage) administration to female New Zealand White rabbits for 14 days followed by a 7 day reversibility phase. LSR report no. 90/RHA363/0961. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie Lyon, France. Expt. dates: 8.5.1990-29.5.1990. Unpublished report date: 12 Feb 1991. (GLP/QA: yes; Study Guidelines: none)

Note: These three studies, each in a different species, are presented in this section as they are preliminary to the Brockelsby *et al*. (1991) toxicokinetic study in several species evaluated below. As the methodology was the same for each study, this has been condensed into a single summary.

Thirty female CD1 strain mice, Charles River CD rats, or New Zealand White rabbits received fipronil by gavage at 0.4 mg/kg bw/d for up to 14 days. A second group of 40 of each species received 4 mg/kg bw/d for up to 14 days. Ten animals of each species were allocated to a reversibility phase of up to 7 days, and 5 of each species were killed to provide blood and tissue samples at 6, 24 or 48 h after the first dose; after completing 4, 9 or 14 days of treatment; or, in the 4 mg/kg bw/d group only, after completion of 3 or 7 days recovery. Control groups of 30 female mice, 12 female rats, or 6 female rabbits received vehicle only, and were killed at appropriate times for comparison. No mice, rats or rabbits died or showed adverse clinical signs. Food consumption, bodyweight gain and efficiency of food conversion were unaffected by treatment.

Brockelsby CH, Cooper JD, Doble ML, Godward PJ, Maycey PA, Savage EA, Tan JK (1991) Insecticides: M&B 46,030: Comparative toxicokinetic study in rabbits, rats and mice: Analysis of tissues. D. Ag. 1670. (A scientific report from the Analytical Chemistry Department of Rhone-Poulenc Agrochimie Limited). Sponsor: Rhone-Poulenc Agriculture Limited, Essex, England. Unpublished report date: August 1991. (GLP/QA: yes; Study Guidelines: none)

Materials and Methods Experimental animals and dosing regimes are described above in Broadmeadows (1991a,b) and Cummins (1991). Gas chromatography was employed to determine levels of fipronil and its sulfide, sulfone and amide metabolites in tissues (blood, fat, brain, liver and thyroid).

Results At both dose levels for each species, MB 46136 (fipronil sulfone) was the principal metabolite in each tissue. While some tissues contained up to 6.6 mg/kg fipronil, for many, significant levels of the parent compound were present only in the 6 h and 1 day samples. The parent compound remained longest in fat (particularly rabbit fat). Only trace levels of MB 45950 (fipronil sulfide) were detected in any tissue. Levels of RPA 200766 (fipronil amide) in liver from all species were below the limit of quantification.

In low dose animals, levels of fipronil sulfone increased over the dosing period to reach 12-16 mg/kg in fat, 0.13-0.20 mg/kg in blood, 0.6-0.7 mg/kg in brain, 1.6-7 mg/kg in liver, and 3-9 mg/kg in thyroid gland. In contrast, species differences emerged between the 4 mg/kg bw/d groups with respect to the rate of fipronil sulfone deposition in the tissues. In rabbits, maximum fipronil sulfone levels were found in blood, fat and brain at day 15 (0.4, 54 and 2.4 mg/kg respectively) and in liver at day 18 (19 mg/kg). In rats, maximum values were achieved much earlier, with the values for mice falling between those of rabbits and rats. The half-life values for MB 46136 (fipronil sulfone) in rabbit blood and fat were 11 and 10 days respectively; and in rodents 5 and 6-7 days respectively. The half-lives of MB 46136 (fipronil sulfone) in brain varied from 4 days (mouse) to 9 days (rat). For all species, the half-life of MB 46136 (fipronil sulfone) in liver was 3-5 days, and its half-life in the thyroid of rats and mice was 5 days, but the half-life in the thyroid was not calculable due to high variability in the data.

Lowden P & Savage EA (1991) Insecticides: M&B 46,030: Comparative metabolism study in three mammalian species: rabbit, rat and mouse (interim report). D. Ag. 1663. (A report from the Analytical Chemistry Department of Rhone-Poulenc Agrochimie Limited). Sponsor: Rhone-Poulenc Agriculture Limited, Essex, England. Unpublished report date: July 1991. (GLP/QA; Study Guidelines: none stated)

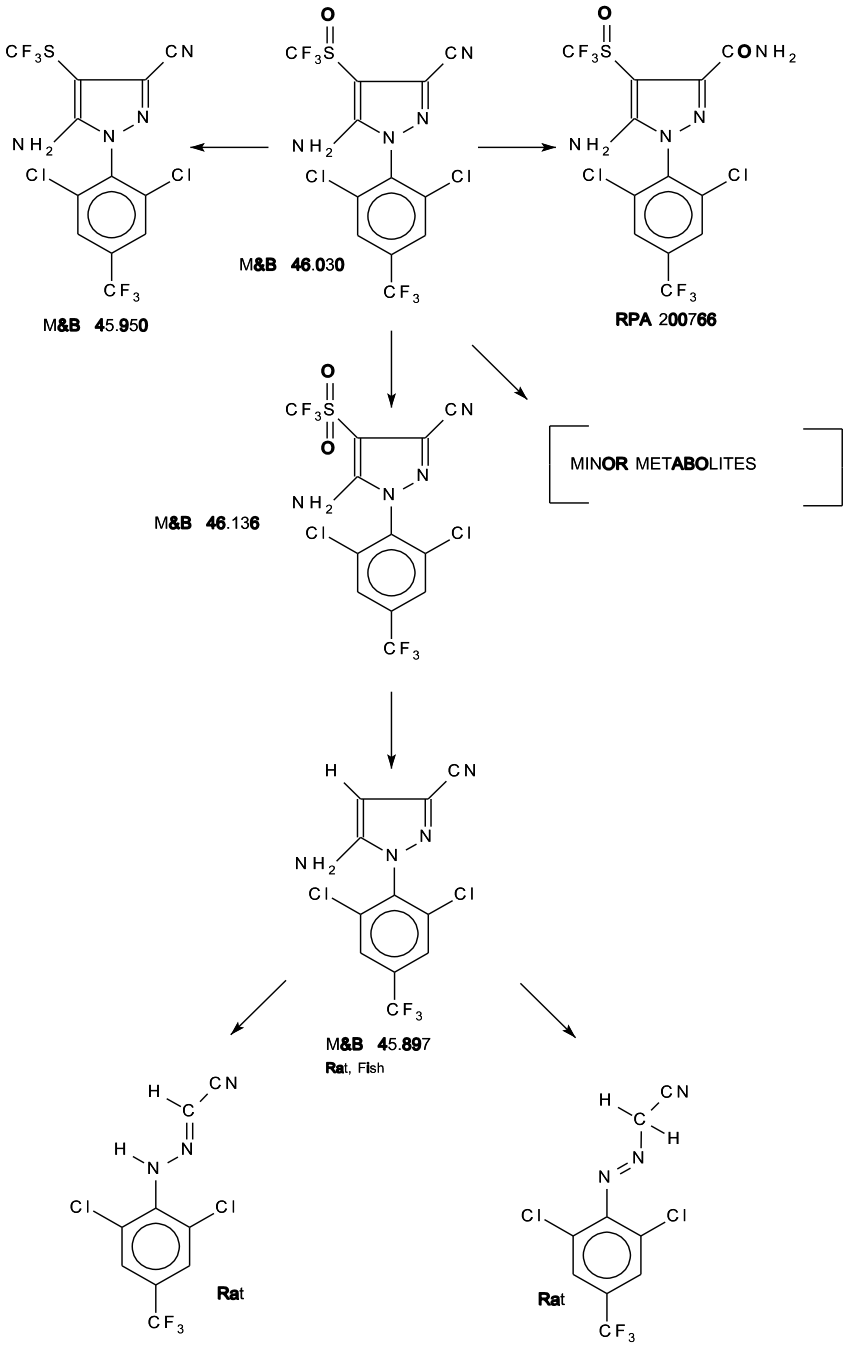
Female rabbits (2 New Zealand White), rats (5 Charles River CD strain) and mice (10 Charles River CD1 strain) were given single oral doses of 5 mg/kg bw [^{14}C]-fipronil in aqueous methyl cellulose (0.5% w/v) containing Tween 80 wetting agent (0.01% v/v). Liquid scintillation counting on various samples showed that elimination of radiolabel was slow, with approximately 80% of the dose not excreted from the rabbit 7 days post-dosing (mouse, 60%; rat, 40%). Calculated half-lives of elimination were approximately 14, 3 and 3 days for rabbit, rat and mouse respectively. The highest levels of radioactivity were in fat (rabbit: >20 µg/g; rat: 10 µg/g; mouse: 5 µg/g). For liver, kidney, muscle, brain and thyroid, little difference was seen between rat and mouse (all values 0.5 µg/g - < 2 µg/g), but rabbit values were generally higher. No significant levels of radiolabelled carbon dioxide were detected up to 48 h post dosing, so air trapping was not continued further.

HPLC, TLC and gas chromatography (GC), followed by mass spectroscopic (MS) and NMR examination, showed that the principal tissue metabolite was MB 46136 (fipronil sulfone), with traces of fipronil and MB 45950 (fipronil sulfide). Traces of RPA 200766 (fipronil amide) were detected in rabbit liver only. Fipronil, fipronil sulfide and fipronil sulfone were found in faecal extracts in variable proportions, depending on species. Major urinary metabolites did not correspond to available standards. NMR data indicated the presence in the urine of all species, of a compound in which the pyrazole CF_3 group had been removed, as was also seen in rat faecal extract. The presence of other compounds with a substitution on the amino group was also suggested by the NMR data. Finally, a compound formed by the loss of the SO_2CF_3 group from fipronil sulfone (or its analogues) was detected by NMR in rat and mouse (but not rabbit) urine.

Savage EA (1993) Fipronil: Absorption, distribution, metabolism and elimination in animals. Sponsor: Rhône-Poulenc Agriculture Limited, UK. Unpublished report date: 31 March 1993. (QA/GLP/Study Guidelines: not applicable)

This report was in the form of a paper that summarised the results of various other reports, several of which are evaluated in this review (Powles 1992, Whitby 1991, Brockelsby *et al*. 1991, Lowden & Savage 1991). Limited comment was also provided on metabolism studies in mice, rats, dogs, rabbits, goats, hens and fish, and the composite metabolic pathway shown in Figure 1 was proposed. This paper concluded that, qualitatively, the fate of fipronil in all of the above species was similar, but that there were quantitative differences. The most notable differences were seen when comparing the rabbit with the rat, mouse and dog.

Figure 1: Composite Metabolic Pathway of Fipronil in Animals



Abbreviations:
M&B 46,030 (fipronil); M&B 45950 (fipronil sulfide); RPA 200766 (fipronil amide); M&B 46,136 (fipronil sulfone); M&B 45,897 (detrifluoromethylsulfinyl fipronil)

Guyomard C (1993) Comparative study of the hepatic metabolism of ^{14}C M&B 46030 in rat and rabbit hepatocytes. Report No. 85-2RF. Lab: Biopredic, Rennes, France. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: April 1992-February 1993. Unpublished report date: 17 February 1993. (GLP/QA: no)

In a comparative metabolism assay, radiolabelled fipronil (99.3% purity) was incubated with isolated rat or rabbit hepatocytes for 0, 1, 3, 5 or 24 h. Results from thin-layer chromatography showed that fipronil was metabolised completely by rat hepatocytes, and partially by rabbit hepatocytes. After 3 hours' incubation, approximately twice as much of the principal metabolite was produced by rat hepatocytes as by rabbit hepatocytes. Hepatocytes from both species produced the same two metabolites: a major non-polar compound, and a minor polar compound. The samples were sent to Rhône-Poulenc Agrochimie for HPLC analysis (see Fisher 1992 below).

Fisher PJ (1992) Fipronil: Analysis of metabolites/degradates from hepatocyte incubations. Report no. SA92017. Lab: Rhône-Poulenc Agrochimie, Centre de Recherche, Valbonne, France. Sponsor: Rhône-Poulenc Expt. dates: 10.3.1992-13.3.1992. Unpublished report date: 29 October 1992. (GLP/QA: no)

Incubation media from isolated rat and rabbit hepatocytes treated with fipronil for up to 24 h (Guyomard 1993) were analysed by HPLC. Hepatocytes from both sources metabolised fipronil to MB 46136 (fipronil sulfone). RPA 104615 (fipronil detrifluoromethyl sulfonate) was also observed in the 24-h incubation samples for both species. This compound was not detected as a mammalian metabolite *in vivo* in rats and rabbits (Powles 1992; Lowden and Savage 1991). However, it has been shown to be a product of aqueous photolysis via MB 46136 (fipronil sulfone) and as the incubations were performed in diffused light, the study author proposed that it had formed by aqueous photolysis rather than by hepatic metabolism. Rat hepatocytes metabolised fipronil faster than did rabbit hepatocytes.

Tang J, Usmani A, Hodgson E, Rose RL (2004) In vitro metabolism of fipronil by human and rat cytochrome P450 and its interactions with testosterone and diazepam. Chemico-Biological Interactions 147: 319-329.

The metabolism of fipronil was investigated *in vitro* in rat liver microsomes (prepared from Long-Evans rats, Charles River, Raleigh, NC) and in single-donor human liver microsomes (from BD Biosciences, Woburn, MA). Fipronil sulfone was the only metabolite detected after incubations of 30 minutes. The Km values for fipronil were similar in liver microsomes from humans and rats (19.9 and 27.2 µM respectively), but the Vmax was greater in rat than in human liver microsomes (0.39 and 0.11 nmol/mg protein/min, respectively). There was a 40-fold variation in the rate of fipronil metabolism across human liver microsomes obtained from 19 individuals. Whether this variability is typical of the human population is not known, as fipronil metabolising activity may have been affected by the disease state of the donors or the livers. This may also have impacted on the comparison of fipronil metabolism in rats and humans. Further investigations showed that the rate of fipronil metabolism was most closely correlated to the levels of the cytochrome P450 isoform CYP3A4, the most abundant isoform in human liver, and to a lesser extent, CYP2C19. Overall, the metabolism of fipronil in rat and human liver appears to be at least qualitatively similar.

2.4 Dermal absorption (formulations)

Cheng T (1995) Dermal absorption of ^{14}C-fipronil REGENT 80 WDG in male rats (preliminary and definitive phases). Report No. HWI 6224-210. Lab: Hazleton Wisconsin, Inc., Madison, Wisconsin. Sponsor: Rhone Poulenc Ag Company, Research Triangle Park, North Carolina. Expt. dates: 6 July 1994 – 6 September 1994. Unpublished report date: 10 February 1995. (GLP/QA: yes. Guidelines: US EPA 40 CFR Part 158. 85-3)

Materials and Methods The extent of absorption of [^{14}C]-fipronil was analysed following application of Regent 80 WDG (Lot No. OP930794, containing 78.9% fipronil) spiked with [^{14}C]-fipronil (Lot No: GHS-826; radiopurity: 98%; specific activity 19.8 mCi/mmole) to the skin of rats. Male Charles River Crl:CD BR rats (from Portage, Michigan USA), approximately 7 weeks old and 58 to 217 g bw at the beginning of the study, were assigned to 6 groups (2 groups in the preliminary phase and 4 groups in the definitive phase). Approximately 100 µL of the dosing suspension was applied with a glass spreader onto pre-shaved and washed intact skin (approximately 12.5 cm^2, defined by plastic enclosures glued onto the skin) at the back and shoulders of each rat, under a non-occlusive cover. A preliminary phase was conducted to establish methodology and is not reported here. In the definitive phase, groups of 24 male rats received dermal applications of 0.070, 0.668, or 3.88 µg/cm^2 [^{14}C]-fipronil Regent 80 WDG, suspended in 1% aqueous carboxymethyl-cellulose (CMC). Concentrations of fipronil in the solutions applied were approximately 0.8, 8 and 40% respectively. Two male control rats received 1% CMC. Four treated rats/group were killed at ½, 1, 2, 4, 10, and 24 h after dermal application. Urine and faeces and test site skin washings were collected prior to the kills. The non-occlusive cover, enclosure, skin wash, blood, cage wash and wipe, residual urine from the bladder, skin at the application site, carcass and faeces were collected from each animal and samples were measured for radioactivity by LSC.

Results Mean total recoveries of radioactivity were 99-105%. Radioactivity in the excreta (cage wash and wipe, urine and faeces) was less than 0.02% of the applied dose in all groups. There was no detectable radioactivity in the blood at any time point. Results are shown in the Table below. Direct absorption (radioactivity in the blood, excreta, and carcass) at 24 h was 0.37, 0.40, and 0.075%, approximately equal to 0.003, 0.033, and 0.034 mg/animal, respectively. However, measuring excreta only to 24 h limits answering the question as to the extent of transfer of radioactivity from the subcutaneous fat to the systemic circulation. Indirect absorption (sum of direct absorption plus radioactivity on/in skin site) at 24 h was 2.19, 3.69, and 0.57% approximately equal to 0.019, 0.308, and 0.267 mg/animal, respectively. These results show that there was very little absorption of radioactivity across the dermis and that the absorption was approximately dose-proportional at low and intermediate concentrations but had reached saturation at the high concentration.

Table 18: Recovery (%) of the dermally administered radioactivity

Dose ($\mu g/cm^2$)	Kill time (h)	Cover	Enclosure rinse	Skin wash	Skin site	Carcass	Cage wash & wipe	Urine	Faeces	Total recovery	Absorbed	
											Indirect	Direct
0.070	**0.5**	nd	0.30	98.8	1.14	nd	nd	<0.005	nd	100	1.14	<0.005
	1	nd	0.22	98.7	1.51	0.07	nd	nd	nd	100	1.58	0.07
	2	nd	0.17	97.9	2.45	0.46	nd	nd	nd	101	2.91	0.46
	4	nd	0.11	97.8	1.86	nd	nd	<0.005	nd	99.7	1.86	<0.005
	10	nd	0.29	96.2	1.87	0.65	nd	<0.005	nd	99.0	2.52	0.65
	24	nd	0.09	96.8	1.82	0.36	nd	0.01	nd	99.1	2.19	0.36
0.668	**0.5**	0.01	0.19	101	0.60	nd	nd	nd	nd	101	0.61	nd
	1	0.09	0.27	95.4	5.75	0.06	nd	nd	nd	101	5.82	0.06
	2	<0.005	0.21	101	0.85	0.05	nd	<0.005	nd	102	0.90	0.05
	4	0.01	0.09	100	1.58	nd	nd	nd	0.10	101	1.65	0.10
	10	0.01	0.19	101	1.57	nd	0.01	<0.05	0.01	103	1.59	0.02
	24	0.01	0.18	97.1	3.29	0.38	nd	0.01	0.01	100	3.69	0.40
3.88	**0.5**	0.01	0.06	105	0.35	nd	nd	nd	nd	105	0.35	nd
	1	nd	0.15	101	0.80	0.64	nd	nd	nd	103	1.44	0.64
	2	0.01	0.07	103	0.35	0.05	nd	nd	nd	104	0.40	0.05
	4	0.01	0.11	101	0.76	0.07	nd	nd	nd	102	0.83	0.07
	10	0.01	0.16	103	0.69	0.18	nd	<0.005	<0.005	104	0.87	0.18
	24	0.01	0.11	103	0.49	0.07	nd	<0.005	nd	104	0.55	0.07

2.4.2 *In vitro*

Walters KA & Brain KR (1990) *In vitro* skin permeability of M&B 46030. Study No. RD 8. Lab: Pharmaserve Limited, Manchester, UK and An-eX Analytical Services, Ltd, University of Wales College of Cardiff, Cardiff UK. Sponsor: Rhone-Poulenc Agrochemie, Lyon, France. Unpublished report date: 31 October 1990. (GLP: yes; QA, Study Guidelines: none stated)

Materials and Methods The absorption of fipronil formulated with EXP60145A (composition not provided, but corresponds to the Australian product Regent 200 SC Insecticide) was measured through human, rabbit and rat epidermal membranes *in vitro*. Female rats and rabbits were killed by cervical dislocation, then skin on the dorsal and flank regions was trimmed and excised, and subcutaneous fat removed. Female human abdominal skin was obtained at autopsy. Female rats (CD strain), 21-28 days old, were from Charles River Ltd, Margate, UK, and female rabbits (NZW), 2.0-2.5 kg bw, were from Froxfield Farms (UK) Ltd. The test formulation was prepared using [U-^{14}C phenyl]-fipronil, Batch: GHS 634A; Radiopurity: 98.7%; Specific activity: 45.1 µCi/g), and unlabelled fipronil (Batch: CFA 252/61; Purity: 96.2%). Radiolabelled hydrocortisone and testosterone were used as reference permeants (4 g/L in an aqueous dilution of EXP60145A, presumably lacking fipronil, though this was not made clear in the report). Epidermal membranes were set up in glass diffusion cells with a receptor chamber temperature of 37°C, and an area of 0.75 – 2.75 cm^2 available for diffusion. To establish the integrity of the skin, its permeability to tritiated water was tested. Radiolabelled formulation (200 g/L fipronil, EXP 60145A), or a 1:50 (4.0 g/L) or 1:1000 (0.2 g/L) aqueous dilution of this formulation were applied to the epidermal surface at 100 µL/cm^2. Samples were taken from the receptor chambers at 1, 2, 3, 4, 6, 8 and 24 h for quantitation of radioactivity by LSC.

Results Fipronil permeated both rat and rabbit skin at an approximately 10-fold greater rate than human skin, when applied at each of the concentrations tested, except at 0.2 g/L fipronil, at which dose results for rat and human epidermis were similar (see Table below). A greater percentage of the applied amount permeated from the least concentrated solution (0.2 g/L), but flux rates were not proportional to concentration. The penetration rate of fipronil through human skin, at an equivalent concentration to the reference compounds (4.0 g/L), is an order of magnitude less than that of hydrocortisone, a relatively poor permeant. Hence, fipronil was a relatively slow penetrant when applied in formulation EXP60145A.

Table 19: *In vitro* flux and penetrance of fipronil for rat, rabbit, and human epidermal membranes

	Conc. (g/L)	Time (h)	Rat		Rabbit		Human	
			Flux ($\mu g/cm^2/h$)	% dose penetrated	Flux ($\mu g/cm^2/h$)	% dose penetrated	Flux ($\mu g/cm^2/h$)	% dose penetrated
Fipronil	200	8	1.92	0.08	1.68	0.07	0.18	0.01
		24	9.92	1.19	6.34	0.76	0.42	0.05
	4.0	8	0.07	0.14	0.33	0.67	0.03	0.07
		24	0.99	5.96	0.80	4.80	0.03	0.18
	0.2	8	0.02	0.90	0.35	13.9	0.02	0.98
		24	0.24	24.2	0.42	50.1	0.11	12.9
Testosterone	4.0	8	7.72	15.4	2.94	5.89	0.17	0.33
		24	11.2	67.2	4.90	29.4	0.37	2.24
Hydrocortisone	4.0	8	3.19	6.38	3.02	6.05	0.29	0.58
		24	6.44	38.7	2.79	16.7	0.41	2.47

Ward RJ (1997a) Fipronil: *In vitro* absorption from a 25 g/L ULV formulation through human and rat epidermis. Study No. JV1497. Lab: Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Expt. dates: 18.11.1996 – 13.12.1996. Unpublished report date: 13 October 1997. (QA/GLP/Study Guidelines: none stated)

Samples of epidermis from rats (Wistar-derived strain from Charles River UK Ltd., Margate, Kent UK) and humans (derived from whole skin samples) were prepared and mounted onto glass diffusion cells with an exposed area of 2.54 cm^2. Membrane integrity was tested prior to commencement by measuring electrical resistance. Receptor chambers were filled with fluid (50% v/v ethanol in deionised water) and placed in a water bath at 30°C. Each radiolabelled formulation (26 g/L fipronil, lot 1880 FD 96, radioactivity 4.7 MBq/2.75 g; or 2.5 g/L fipronil, lot 1897 FD 96, radioactivity 7.1 MBq/3.50 g, a 1:10 dilution in oil) was applied and spread over the surface of the epidermal samples at 10 $\mu L/cm^2$. Fresh receptor fluid was added to the chamber to replace samples removed at 6, 8, 10, and 24 h, for analysis by LSC. After 24 h, the donor chamber and epidermis samples were washed, and the washings and epidermis samples were analysed by LSC.

Mean absorption rates of 0.009 and 0.035 $\mu g/cm^2/h$ were obtained for human epidermis and rates of 0.108 and 0.467 $\mu g/cm^2/h$ were obtained for rat epidermis, at 2.5 and 26 g/L fipronil, respectively (see Table below). Rates of absorption were fairly constant throughout the 24 h exposure period for both human and rat epidermis. Absorption was not dose-proportional, with a 4-fold increase in the amount of radioactivity absorbed for a 10-fold increase in fipronil concentration. Absorption through rat epidermis was 12-13 times faster than that through human epidermis.

Table 20: Fipronil absorption through rat and human epidermis

Species	Fipronil concentration	2.5 g/L				26 g/L			
	Time (h)	6	8	10	24	6	8	10	24
Human	amount absorbed (µg/cm^2)	0.042	0.054	0.072	0.210	0.39	0.42	0.47	0.83
	% absorbed	0.17	0.22	0.29	0.86	0.15	0.16	0.18	0.32
	absorption rate (µg/cm^2/h)	0.007	0.007	0.007	0.009	0.065	0.053	0.047	0.035
Rat	amount absorbed (µg/cm^2)	0.54	0.69	0.97	2.60	3.2	4.4	5.4	11.2
	% absorbed	2.1	2.8	3.9	10.3	1.2	1.7	2.1	4.3
	absorption rate (µg/cm^2/h)	0.090	0.086	0.097	0.108	0.533	0.550	0.540	0.467

Radioactivity on the spreader and the donor cell were considered unavailable for absorption, and radioactivity in rinsings was considered unabsorbed. At 24 h, less than 1% of the administered radioactivity had penetrated the human epidermis, and 4-10% had penetrated in the rat epidermis (see Table below). Radioactivity retained in the epidermis was considerable, with 10-25% of administered radioactivity located in the rat epidermis, and 4.1-2.6% in the human samples.

Table 21: Fipronil distribution (% radioactivity recovered)

Species		Spreader	Donor cell	Skin wash	Epidermis	Absorbed	Total
Human	2.5 g/L	17.2	24.1	65.4	2.64	0.86	110
	26 g/L	7.68	18.5	87.6	4.14	0.30	118
Rat	2.5 g/L	4.19	26.1	60.4	24.7	10.3	126
	26 g/L	3.86	12.0	78.6	10.1	4.29	109

Ward RJ (1997b) Fipronil: *In vitro* absorption from 50 g/L SC formulation through human and rat epidermis. Study No. JV1495. Lab: Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Expt. dates: 18.11.1996 – 7.10.1997. Unpublished report date: 7 October 1997. (QA/GLP/Study Guidelines: none stated)

Fipronil absorption across human and rat epidermis was measured as described above (Ward 1997a). Each radiolabelled formulation (49.7 g/L fipronil, lot 1881 FD 96, radioactivity 3.0 MBq/2.47g; 0.5 g/L fipronil, lot 1882 FD 96, radioactivity 1.6 MBq/3.47g; 49.6 g/L fipronil, lot OP1923 FD 97, radioactivity 4.7 MBq/3.26g, a 1:100 dilution in water), were applied and spread over the surface of the epidermal samples at 10 µL/cm^2.

For human epidermis, the absorption rate for 0.5 g/L fipronil was relatively constant at 0.006-0.007 µg/cm^2/h throughout the exposure period (see Table below). At 50 g/L, absorption of radioactivity was not detected until 10 h after exposure, with an absorption rate of 0.052 µg/cm^2/h over the first 10 h and a rate of 0.032 µg/cm^2/h over 24 h. Over the 24 h experimental period, absorption rates for rat epidermis declined at 0.5 g/L, and increased marginally at 50 g/L. Absorption was not dose-proportional, with a 5-fold increase in the amount absorbed through human epidermis and a 42-fold increase in absorption through rat epidermis for a 20-fold increase in exposure concentration. Over 24 h, the rate of absorption was 12 times higher at 2.5 g/L and 94 times higher at 50 g/L through rat epidermis than through human epidermis. Less than 3% of the administered radioactivity was absorbed through human epidermis and 15 – 35% was absorbed through rat epidermis.

Table 22: Fipronil absorption through rat and human epidermis – 2.5g/L and 50 g/L

Species	Fipronil concentration	2.5 g/L				50 g/L			
	Time (h)	6	8	10	24	6	8	10	24
Human	amount absorbed (µg/cm^2)	0.041	0.050	0.059	0.134	<0.5	<0.5	0.52	0.77
	% absorbed	0.82	1.0	1.2	2.7	<0.1	<0.1	0.10	0.15
	absorption rate (µg/cm^2/h)	0.007	0.006	0.006	0.006	---	---	0.052	0.032
Rat	amount absorbed (µg/cm^2)	0.72	0.82	1.01	1.73	16.0	21.9	27.2	72.0
	% absorbed	14.5	16.3	20.2	34.5	3.2	4.4	5.5	15.0
	absorption rate (µg/cm^2/h)	0.120	0.103	0.101	0.072	2.667	2.738	2.720	3.00

Table 23: Fipronil distribution (% radioactivity recovered)

Species	Conc.	Spreader	Donor cell	Skin wash	Epidermis	Absorbed	Total
Human	**0.5 g/L**	19.1	6.28	82.8	8.68	2.68	120
	49.7 g/L	7.41	3.32	96.6	0.95	0.154	108
Rat	**0.5 g/L**	6.99	9.17	60.1	7.29	34.6	118
	49.6 g/L	8.35	6.62	36.5	7.09	14.5	73.0

Ward RJ (1998) Fipronil: *In vitro* absorption from 300 g/L EC formulation through human and rat epidermis. Study No. JV1496. Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Expt. dates: 18.11.1996-13.12.1996. Unpublished report date: 29 January 1998. (QA/GLP/Study Guidelines: none stated)

Fipronil absorption across human and rat epidermis was measured as described above (Ward 1997a). Aliquots of 10 µL/cm^2 of each radiolabelled formulation (emulsifiable concentrate formulations of 312 g/L fipronil, lot 1878 FD 96, radioactivity 4.4 MBq/3.43g; or 6 g/L fipronil, lot 1896 FD 96, radioactivity 6.8 MBq/3.45g, a 1:50 dilution in water), were applied and spread over the surface of the epidermal samples.

In human epidermis, the absorption rate for the 6 g/L formulation slightly increased over the 24 h exposure period, whereas the absorption rate at 312 g/L declined over 3-fold from 6 to 24 h (see Table below). In rat epidermis, the absorption rate for both formulations decreased from 6 to 24 h. Absorption was not dose-proportional, with 5-fold and 4-fold increases in the amount absorbed through human and rat epidermis, respectively, for a 50-fold increase in exposure concentration. Over 24 h, the rate of absorption through rat epidermis was 5-7 times higher than that through human epidermis. Less than 1.5% of the administered radioactivity was absorbed through human epidermis and 0.8 – 10.3% was absorbed through rat epidermis.

Table 24: Fipronil absorption through rat and human epidermis – 6 g/L and 312 g/L

	Fipronil concentration	6 g/L				312 g/L			
Species	Time (h)	6	8	10	24	6	8	10	24
Human	amount absorbed (µg/cm^2)	0.13	0.22	0.29	0.89	4.3	4.4	4.6	4.8
	% absorbed	0.22	0.36	0.49	1.50	0.14	0.14	0.15	0.15
	absorption rate (µg/cm^2/h)	0.022	0.028	0.029	0.037	0.717	0.550	0.460	0.200
Rat	amount absorbed (µg/cm^2)	3.8	4.1	4.3	6.2	10.6	11.7	13.4	25.6
	% absorbed	6.4	6.9	7.1	10.3	0.34	0.37	0.43	0.82
	absorption rate (µg/cm^2/h)	0.633	0.513	0.430	0.258	1.767	1.463	1.340	1.067

Table 25: Fipronil distribution (% radioactivity recovered)

Species	Fipronil concentration	Spreader	Donor cell	Skin wash	Epidermis	Absorbed	Total
Human	**6 g/L**	10.8	10.5	66.8	7.76	1.48	97.3
	312 g/L	12.4	27.3	77.0	10.7	0.15	118
Rat	**6 g/L**	0.50	6.22	31.3	50.8	10.3	99.1
	312 g/L	1.76	20.0	82.9	7.00	0.75	112

3. ACUTE STUDIES

3.1 Active Constituent

3.1.1 Median Lethal Dose Studies

Oral toxicity

Species [strain]	Sex	Group Size	Vehicle	Purity (%) /Batch	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Rats (CD)	M/F	5/sex	Corn oil	No data	50, 80, 126, 200	M: 92 (64-128) F: 103 (73-141) Combined: 97 (76-122) Deaths: (M/F): 2/2 at 80, 4/4 at 126, 5/4 at 200	Gardner (1988a) GLP
Mice (OF1)	M/F	6/sex	0.2% aqueous Tween 80	95.3 / PGS 963	25, 50, 100, 200	98 (56-169) 91 (55-151) Deaths (M/F): 1/1 at 50, 2/4 at 100, 6/5 at 200	Mondot & Dange (1995)

Abbreviations: NS=not specified; M=male, F=female. * 1,3-dioxolane-4-methanol;

The acute oral toxicity studies for fipronil are summarised in the Table above. Deaths occurred at ≥50 mg/kg bw in mice on days 2-7, and at ≥80 mg/kg bw in rats at 4 h to 3 days post dosing. Clinical signs in mice comprised hunched posture, straub tail, hypersensitivity to noise, body tremors, clonic forelimb and/or hindlimb seizures, and tonic-clonic convulsions from day 1 at 25 mg/kg bw/d and above. In rats, clinical signs occurred within 5 h of dosing, and comprised piloerection, hunched posture, abnormal gait and diarrhoea. Other signs observed in rats treated with 80 mg/kg bw or more included lethargy, decreased respiratory rate, pallor of the extremities and ptosis. Clonic convulsions and prostration preceding death occurred in 3 rats at 200 mg/kg bw. Recovery, if it occurred, was complete by day 3 (50 mg/kg bw) or by day 6 (other dose levels). With the exception of one rat that died, all rats lost bodyweight, but none showed macroscopic pathology.

Dermal toxicity

The dermal toxicity studies for fipronil are summarised in the Table below. No toxic effects were apparent in rats dermally exposed to fipronil at 2000 mg/kg bw. In contrast, deaths in rabbits given dermal doses of ≥250 mg/kg bw occurred on days 5-14 post-application. Clinical signs included sluggishness, salivation, audible breathing, spasms, tremors, vocalization, hyperactivity, prostration, a red discoloration on the perioral and perinasal fur, diarrhoea and emaciation. In addition, delayed convulsions were apparent at ≥250 mg/kg bw, initially observed on days 3-9 both in animals that died subsequently, and in survivors. The convulsions were short in duration (1-2

min) and occurred several times a day. Several affected survivors recovered within 2-14 days, and one female recovered after 28 days. Necropsy of the rabbits that died revealed liquid in the thoracic cavities, pink/dark red lungs, lungs with tan/red/brown patches or a rough surface texture, and blood in the urine. Necropsy of survivors revealed discoloured lungs or kidneys, blood in the kidneys, enlarged kidneys or spleen. No dermal reactions were evident in rats or rabbits.

Species [strain]	Sex	Group Size	Vehicle	Purity(%)/ batch	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Rats	M/F	5/sex	Water	NS	2000 (24 h; 'covered')	> 2000 No deaths	Gardner (1988b) [GLP]
Rabbits [NZW]	M/F	5/sex	Corn oil	96.7 / 78/GC/90	100, 250, 500, 1000, 2000 (24 h; occluded)	M: 445 (200-980) F: 354 (200-620) Deaths (M/F): 2/1 at 250, 2/5 at 500, 5/4 at 1000, 4/5 at 2000	Myers & Christopher (1992) [GLP]

Abbreviations: NZW=New Zealand White; M=male, F=female.

Inhalational toxicity

Species [strain]	Sex	Group Size	Vehicle/ mode	Purity (%) / Batch	Concentrations Tested (mg/m^3)	LC_{50} (mg/m^3)	Reference
Rat [SD]	M/F	5/sex	None / Nose only (dust), 4 h	96.7 / 10MTD20	330, 520, 720 (MMAD 1.66 µm, 97% < 3 µm)	M: 360 (230-550) F: 420 (340-0.510) Deaths: all at 520 and 720, 2 M at 330	Nachreiner (1995) [GLP]
Rat [SD]	M/F	5/sex	None / Nose only (dust), 4 h	95.4 / PGS 963	259, 523, 929	682 Deaths (M/F): 0/1 at 259, ½ at 523, 4/3 at 929	Cracknell (1991) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute inhalation studies for fipronil are summarised in the Table above. In the Nachreiner (1995) inhalation study, clinical signs consisted of ocular, nasal and oral wetness and encrustation, urogenital wetness, wet body fur, unkempt fur, fine whole body tremors, hypoactivity and incoordination in all groups. Hyperactivity (1 male and 1 female) and swollen penis (1 male) were observed at 720 mg/m^3, and palpebral closure (1 female) at 330 mg/m^3. Bodyweight loss or little weight gain was observed in all survivors (at 330 and 720 mg/m^3 only) in week 1, but those at 330 mg/m^3 gained bodyweight in week 2. Red/black foci, ulcerated area, or a thickened white surface, were seen in the stomach of dead rats at necropsy. Findings in the other inhalation study (Cracknell 1991) were generally similar, though deaths were fewer at comparable doses. Immediately following exposure, rats exhibited wet fur, brown staining, hypothermia, vocalisation, tremors and hunched body posture, some of which persisted for several days. Piloerection, vocalisation and hair loss were also noted during the observation period. Rats that survived the effects of exposure lost bodyweight for up to 4 days, but weight gain for the rest of the observation period was normal. There was no significant macroscopic pathology, though lung weights of rats that died were generally greater than normal. Liver and kidney weights were unaffected by treatment.

3.1.2 Skin Irritation

Myers RC & Christopher SM (1993a) MB 46030 (technical): Cutaneous irritancy study in the rabbit. Report No. 93N1217A. Lab: Bushy Run Research Center Union Carbide Chemicals and Plastics Company Inc. Export PA. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC. Expt. dates: 23.2.1993-2.3.1993. Unpublished report date: 30 April 1993. (GLP/QA: yes. Guidelines: 81-5)

Fipronil (500 mg; Batch: 78/GC/90; Purity: 96.7%) moistened with 0.5 mL of corn oil was applied to an area of preclipped skin area on the dorsal trunk of rabbits (NZW, 3/sex) for 4 h under an occlusive dressing. The animals were restrained during the treatment period. Skin reactions were scored by the Draize method at 1 h, and at 1, 2, 3 and 7 days after patch removal. Very slight to well-defined erythema was observed at the application site of all treated rabbits at 1 h (mean score 1.2), resolving in 5/6 rabbits within 1-2 days, but persisting in one rabbit for at least 72 h. Minor transient oedema was seen in 3/6 rabbits at 1 h (all scores 1), resolving within 1 day. No skin irritation was seen in any rabbit at day 7. In this study, fipronil was a slight skin irritant.

Liggett, MP (1988a) Irritant effects on the rabbit skin of M&B 46030. Report No. 881031/M&B 292/SE. Lab: Huntington Research Centre Ltd, Huntington, Cambridgeshire, UK. Sponsor: Rhone-Poulenc Ltd, Dagenham, Essex, UK. Expt. dates: 28.6.1988-1.7.1988. Unpublished report date: 8 August 1988. (QA/GLP: yes; Study Guidelines: Annex V EEC B4, OECD 404).

Fipronil (0.5 g) was applied for 4 h to a clipped area of intact skin (3 rabbits) under a gauze pad moistened with 0.5 mL distilled water, semi-occluded with an adhesive dressing. The animals were not restrained. Treated skin was examined 30 min after removal of the patches (day 1), and on days 2, 3 and 4. None of the rabbits showed any response to treatment (all scores 0). Fipronil was not a skin irritant in this study.

3.1.3 Eye Irritation

Myers RC & Christopher SM (1993b) MB 46030 (technical): Ocular irritancy study in the rabbit. Report No. 93N1217B. Lab: Bushy Run Research Center, Union Carbide Chemicals and Plastics Company Inc. Export PA. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC. Expt. dates: 1.2.1993-16.2.1993. Unpublished report date: 30 April 1993. (GLP/QA: yes. Study Guidelines: 81-4).

Fipronil (90 mg; Batch: 78/GC/90; Purity: 96.7%) was instilled into one eye of each rabbit (NZW, 3 males and 3 females), and eye reactions were scored according to the Draize scale at 1 h, and at 1, 2, 3, 7, 10 and 14 days. Minor transient corneal opacity was observed in 2/6 rabbits at 1 h only (both scores 1 for opacity, area of opacity scores 2 or 4). It is difficult to reconcile the use of the term ‘corneal opacity’ with the very early onset and transient nature of the change described here. Iritis was seen in 5 animals at 1 h (all scores 1) but subsided within 1 day. Minor to moderate conjunctival irritation was observed in all 6 rabbits within 1 h (scores 1 or 2). This resolved in one rabbit by 48 h, in another two by 72 h, but persisted in the remaining 3 for at least 10 days. All eyes had a normal ocular appearance by 14 days. Fipronil was considered a slight eye irritant in this study.

Liggett, MP (1988b) Irritant effects on the rabbit eye of M&B 46030. Study No. 881032D/M&B 293/SE. Lab: Huntington Research Centre Ltd, Cambridgeshire, UK. Sponsor: Rhone-Poulenc Ltd, Dagenham, Essex UK. Unpublished report date: 8 August 1988. (QA/GLP: yes; Study Guidelines: 79/831/EEC B5, OECD 405).

Fipronil (82 mg, purity 93%) was placed inside the lower lid of one eye of each of three rabbits (NZW). The treated eyes were examined with the aid of a hand held torch at 1 h and 1, 2, 3, 4 and 7 days after instillation. Conjunctival redness and chemosis (both grade 1) were observed in all rabbits at 1 h after treatment, with redness persisting in all rabbits at the day one observation, but only in one rabbit on day 2 (all scores 1). No damage to the cornea or iris was observed. The results of this study indicate that fipronil is a slight eye irritant.

3.1.4 Skin Sensitisation

Johnson, IR (1993) M&B 46030: Delayed contact hypersensitivity study in guinea pigs. Pharmaco-LSR Report No: 93/RHA/503/0167. Lab: Pharmaco-LSR Ltd, Eye, Suffolk, UK. Sponsor: Rhone-Poulenc 14-20 rue Pierre Baizet BP 9163 F-69263 Lyon Cedex 09 France. Expt dates: 13.1.1993-13.2.1993. Unpublished report date: 29 April 1993. (QA/GLP: yes; Study Guidelines: OECD 406)

Method This study employed the maximisation test of Magnusson & Kligman. A preliminary dose-range finding study was conducted to inform dose selection in the main study. The closely clipped dorsal skin of Dunkin-Hartley strain guinea pigs (10/sex) was subjected to intradermal injections of Freund's adjuvant, 5% w/v fipronil in propylene glycol, and 5% w/v fipronil in propylene glycol in adjuvant on day 1. Seven days later, the same area of skin was treated by topical application of 5% w/v fipronil in propylene glycol, and the test site was covered for 48 h. The same induction procedures were carried out on controls (10/sex), except that the test material was replaced by vehicle for all doses. On day 22, all guinea pigs were challenged by occluded application of propylene glycol to the left flank, and 10% w/v and 3% w/v fipronil in propylene glycol at two sites on the right flank. The occlusive dressings were removed on the following day and the condition of the test sites was assessed 24 and 48 h later.

Results and Conclusion
Intradermal injection of 5% fipronil in the adjuvant caused slight or moderate erythema, pallor and discolouration. Intradermal injection of 5% fipronil in propylene glycol caused no dermal reaction. After challenge with 10% fipronil, slight erythema (score 1) was observed in 2 test animals at 24 h, reducing to barely perceptible erythema (±) or no reaction at 48 h. Eschar formation was observed in another 2 test animals at both the 24 and 48 h observations. Exfoliation was seen in 3 of the positive responders at 48 h. Barely perceptible erythema was noted for 2 controls and one treated animal at 24 h only. Challenge with 3% fipronil elicited 3 (±) erythema responses in the treated group at 24 h, and one similar response in the controls. These had normalised at 48 h, though exfoliation was noted for 3 treated animals at this time. Challenge with propylene glycol alone resulted in slight erythema in one control animal, and barely perceptible erythema in 2 controls and 4 treated animals.

Therefore, the response rate is well below the criteria for a positive response and identification of a skin sensitisation hazard (i.e. ≥ 30% response rate). JMPR (1997) concluded that the response rate of 20% seen in this study could support the conclusion that fipronil is a mild or weak skin sensitizer.

In contrast to irritant skin reactions, skin sensitisation reactions are generally persistent, and in this study sustained erythema (i.e. of an equal or greater severity over the assessment times) was not evident in test animals at challenge. Consequently, since the observed erythema in test animals faded or was absent 48 h post challenge it is more likely that these skin reactions are irritant in nature. Additionally, two test animals challenged with 10% fipronil exhibited eschar formation at both the 24 h and 48 h readings. However, eschar formation is more often associated with severe irritation or corrosion, which was not observed in any test animal in this study. Therefore, the findings of eschar formation in two test animals does not provide reliable evidence of a skin sensitisation potential. Consequently, the results of this study provide no reliable evidence that fipronil is a skin sensitiser in guinea pigs (see Chapter 15 for additional discussion of skin sensitisation).

Smith, KD (1990) M&B 46030: Dermal sensitisation study in guinea pigs. LSR90/RHA357/0602; LSR93/RHA357/0098 – amendment 15 February 1993. Lab: Life Science Research Ltd, Suffolk, UK. Sponsor: Rhone-Poulenc 14-20 rue Pierre Baizet BP 9163 F-69263 Lyon Cedex 09 France. Expt dates: 15.3.1990-20.4.1990. Unpublished report date: 2 November 1990. (QA/GLP: yes; Study Guidelines: US EPA 81-6)

Method This study employed the Buehler method. The shaven flanks of albino guinea pigs (Dunkin-Hartley Albino, 10/sex) were subjected to a 6 h occluded topical application of 30% w/v fipronil in paraffin oil on days 1, 8 and 15. Controls were not treated in the induction phase of the study. On day 29, all tests and controls were challenged by 6 h occluded topical applications of 30% and 5% fipronil in paraffin oil on two sites of the right flank. Dermal responses to challenge were assessed 24 and 48 h post application. Dinitrochlorobenzene was applied to 10 guinea pigs as a positive control.

Results and Conclusion Induction applications of 30% fipronil in paraffin oil caused no skin reaction; and challenge applications of 30% and 5% fipronil in paraffin oil caused no significant reaction (i.e. faint erythema, or a more marked response) in test or control animals. A significant erythematous response consistent with delayed contact hypersensitivity occurred in 4/10 guinea pigs induced and challenged with dinitrochlorobenzene. Fipronil was not a skin sensitiser under the conditions of this study.

3.2 Metabolites/Degradation Products

The acute toxicity studies conducted with MB 46513 (desulfinyl fipronil), the major photodegradative product of fipronil, along with various plant and animal metabolites, are considered below. These include MB 45950 (fipronil sulfide), MB 46136 (fipronil sulfone), RPA 200766 (fipronil amide), RPA 200761 (fipronil carboxylic acid), RPA 105320 (fipronil sulfonyl amide), RPA 104615 (fipronil detrifluoromethylsulfonate) and RPA 105048 (fipronil desulfinyl amide), the latter a mammalian metabolite of MB 46513.

3.2.1 MB 46513 (desulfinyl fipronil)

Route	Species, Strain	Group Size	Vehicle/ mode	Purity (%)/Batch	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral	Rats, SD	5/sex	Corn oil	98.6 / 33 RJ 0108	3, 10, 20, 30 deaths: 0 at 3, 10, 3M/4F at 20, all at 30	M: 18 F: 15	Dange (1993a) [GLP]

Route	Species, Strain	Group Size	Vehicle/ mode	Purity (%)/Batch	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Dermal	Rats, SD	5/sex	Saline	98.6 / 33 RJ 0108	2000 2 F deaths	> 2000	Dange (1993b) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute oral and dermal studies using MB 46513 (desulfinyl fipronil) are summarised in the Table above. In the oral study, deaths occurred between days 2 and 4 post-dosing, preceded by clonic and/or tonic convulsions. Clinical signs were mainly confined to the first 5 days after dosing, and comprised hyper-reactivity to noise (all doses), reduced motor activity, dyspnea, bradypnea and nasal discharge (≥10 mg/kg bw), and hypersalivation (females, ≥20 mg/kg bw). The deaths in the dermal study occurred on days 1 and 7, the cause of death on day 1 attributed by the study author as the 'bandages were too tight'. Clinical signs were mainly noted on days 1 and 2, and were more severe in females. These included chromodacryorrhea, piloerection, polypnea and subdued behaviour in males, and lacrimation, piloerection, reduced motor activity and tremors in females. Transient weight loss was seen in the dermal study and in the oral study at ≥20 mg/kg bw. In both studies, there were gross pathology findings for the liver in the animals that died, including enlarged liver with marked lobular pattern, pale liver, or foci of necrosis with haemorrhage, early fibrosis and minimal inflammatory infiltration.

3.2.2 MB 45950 (fipronil sulfide)

Route	Species, Strain	Group Size	Vehicle/ mode	Purity (%) /Batch	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral	Rats, SD	5/sex	Corn oil	98.9% OP5502	50, 65, 90, 120 Deaths (M/F): 3/0 at 65, 4/3 at 90, 5/3 at 120	M: 69 F: 100 Combined: 83	Dange (1994a) [GLP]
Oral	Rats, SD	5/sex	Aqueous/ gavage	-	176 (M), 299 (M), 509, 865, 1471, 2500 Deaths (M/F): 2/- at 299; 4/1 at 509	M: 464 F: 732 M/F: 580	Haynes (1988a) [GLP]
Dermal	Rats, SD	5/sex	Neat/24 h	-	250, 500, 4000 Deaths (M/F): 5/4 at 4000	> 500	Haynes (1988b) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute oral and dermal studies for MB 45950 (fipronil sulfide) are summarised in the Table above. When MB 45950 (fipronil sulfide) was administered orally in corn oil, tonic or clonic convulsions and enlarged livers were noted. When water was used as the vehicle, clinical signs include piloerection, red staining of fur, hypoactivity, and convulsions, with gastric mucosal irritation and haemorrhagic enteritis sometimes reported at necropsy. Deaths were within 48 h of dosing.

Haynes G (1987b) M&B 45,950. Acute dermal irritation/corrosion study. Study no: A/S/1858. Lab: Toxicol Laboratoires Limited, Ledbury, Herefordshire, England. Sponsor: May and Baker, Dagenham, Essex UK. Expt. dates: 20.8.1987-27.8.1987. Unpublished report date: 22 October 1987. (QA/GLP: yes; Study Guidelines: OECD 404)

Doses of 0.5 g test material mixed with 1 mL distilled water were applied to a lint pad (2.5 cm x 2.5 cm) that was then secured onto the clipped skin of 6 female New Zealand White rabbits (from A. Smith, Warlingham, Surrey) by encircling the trunk of the animal with adhesive bandage. Animals were held in restraining stocks for the duration (4 h) of the treatment, after which dressings were removed and the treated areas cleansed using cotton wool soaked in warm water. Observations were made at 1, 24, 48, 72 h and 7 d after treatment. All scores for erythema and oedema were zero. Fipronil sulfide was not a skin irritant in this test.

Haynes G (1987a) Eye irritation study in the rabbit. M&B 45,950. Study no: A/E/1857. Lab: Toxicol Laboratoires Limited, Ledbury, Herefordshire, England. Sponsor: May and Baker, Dagenham, Essex UK. Expt. dates: 2.9.1987-9.9.1987. Unpublished report date: September 1987. (QA/GLP: yes; Study Guidelines: OECD 405)

Fipronil sulfide (MB 45950, 0.1 mL, ~97 mg) was instilled into the right eye of 9 New Zealand White rabbits (from A. Smith, Warlingham, Surrey). The treated eyes of 3 animals were rinsed for one minute with warm water at 2 minutes after treatment, whereas the eyes of the other 6 animals were left unrinsed. The left eye acted as the control. The treated eyes were examined with a standard light source at 1, 24, 48, 72 h and 7 days after treatment. In the rinsed group, all scores were zero. In the unrinsed group, no effects were observed on the cornea or iris. At 1 h, conjunctival redness was present in 5 animals (scores 1 or 2), persisting in 2 animals (scores 1 and 2 at 24 h; both 1 at 48 h), but resolving by 72 h. Chemosis (scores 1 or 2) was observed in 5 animals at 1 h, but had resolved by 24 h, as had the discharge seen in 1 animal at 1 h (score 1). In this test, fipronil sulfide was considered a slight eye irritant.

3.2.3 MB 46136 (fipronil sulfone)

Route	Species, strain, group size and sex	Batch / Purity	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, SD, 5/sex at 64, 100, 400, 640; 10/sex at 160, 250	WAB 212 / 98%	64, 100, 160, 250, 400, 640 in corn oil	M: 184 F: 257 Combined: 218 Deaths (M/F): 2/2 at 100, 7/4 at 160, ¾ at 250, 4/3 at 400, 5/4 at 640	Gardner (1988c) [GLP]
Dermal	Rats, SD, 5/sex	WAB 212 / 98%	2000, aqueous	> 2000 (no deaths)	Gardner (1988d) GLP

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute oral and dermal toxicity studies for MB 46136 (fipronil sulfone) are summarised in the Table above. Acute intoxication with MB 46136 (fipronil sulfone) was characterised by abnormal gait, lethargy, pallor of the extremities, diarrhoea, increased respiratory rate, ataxia, increased salivation and terminal convulsions. Deaths occurred 2-3 days post-dosing. Necropsy sometimes showed mucosal irritation of the stomach and haemorrhagic enteritis.

Liggett MP (1988c) Irritant effects on rabbit skin of M&B 46,136. Report No. 88833D/M&B 288/SE. Lab: Huntington Research Centre Ltd, Huntington, Cambridgeshire, UK. Sponsor: Rhone-Poulenc, Dagenham, Essex UK. Expt. dates: 7.6.1988-10.6.1988. Unpublished report date: 27 July 1988. (QA/GLP: yes; Study Guidelines: OECD 404)

Fipronil sulfone (MB 46136, 0.5 mg, purity ~98%, batch no. WAB 212), moistened with distilled water, was applied to the clipped skin of 3 restrained New Zealand White rabbits (2.1-2.7 kg bw, from A. Smith, Surrey) for 4 h under a semi-occlusive dressing. At the end of the exposure period, the treatment site was cleansed with water. Observations were made at approximately 30 minutes after patch removal, and on the following 3 days. All scores for erythema and oedema were zero. Fipronil sulfone was not a skin irritant under the conditions of this test.

Liggett MP (1988d) Irritant effects on the rabbit eye of M&B 46,136. Report No. 881022D/M&B 289/SE. Lab: Huntington Research Centre Ltd, Huntington, Cambridgeshire, UK. Sponsor: Rhone-Poulenc, Dagenham, Essex UK. Expt dates: 13.6.1988-27.6.1988. Unpublished report date: 11 August 1988. (QA/GLP: yes; Study Guidelines: OECD 405)

Fipronil sulfone (MB 46136, 60 mg, 0.1 mL; ~98% pure, batch WAB 212) was placed into the lower eyelid of one eye of 3 New Zealand White rabbits (2.7-3.3 kg; from Buckmasters, Henham), the contralateral eye acting as the control. Eyes were examined with the aid of a hand held torch at 1 h and 1, 2, 3, 4 and 7 days after treatment. At 1 h after treatment, 1 rabbit showed conjunctival redness and chemosis (score 1), but by 24 h, this finding was present in all animals (the chemosis score increasing to 2 in the animal for which irritation had previously been noted). These findings persisted till day 2 (all scores 1), resolving by day 3 except for conjunctival redness in one animal, which had resolved by day 4. Dulling of the normal corneal lustre was noted for one animal on days 1 and 2, but corneal opacity was not reported. Fipronil sulfone was a slight eye irritant in this test.

3.2.4 Other metabolites (oral toxicity)

Fipronil metabolite	**Species, strain**	**Group Size**	**Vehicle**	**Batch / Purity**	**Doses Tested (mg/kg bw)**	**LD_{50} (mg/kg bw)**	**Reference**
RPA 200766 (fipronil amide)	Rats (SD)	5/sex	Corn oil	57TDS62 / >98%	2000	2000 (no deaths)	Dange (1993d) [GLP]
RPA 200761 (fipronil carboxylic acid)	Rat (SD)	5/sex	Corn oil	97.5 / 57TDS11 2A	2000	>2000 (no deaths)	Katchadourian (1995) [GLP]
RPA 105320 (fipronil sulfonyl amide)	Rats (SD)	5/sex	Corn oil	48 EAR 139 / 97.8%	2000	> 2000 (no deaths)	Dange (1994e) [GLP]
RPA 104615 (fipronil detrifluoromethyl sulfonate)	Rats (SD)	5/sex	Corn oil	58 TDS 91 / 94.7%	2000	> 2000 (no deaths)	Dange (1993c) [GLP]
RPA 105048 (fipronil desulfinyl amide)	Rats (SD), M/F,	5/sex	Corn oil	57 TDS 134 / 98.6%	125, 250, 500, 1000	467 for M/F	Dange (1994f)

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute oral toxicity studies submitted for other fipronil metabolites are summarised in the Table above. In all rats treated with RPA 200766 (fipronil amide), chromodacryorrhea was observed on the day after treatment, but no other signs were reported. Bodyweights were unaffected by treatment, and necropsy revealed a moderately enlarged liver in one male only. In the study using RPA 105048 (fipronil desulfinyl amide), piloerection, reduced motor activity, hunched posture, salivation and tremors were reported. No clinical signs were reported in the other studies.

3.3 Products / Formulations

Acute toxicity studies with fipronil-based formulations/products are shown below. The formulation details are provided in Appendix IX. Median lethal dose studies are presented in tabulated form only. In all studies, unless stated otherwise, doses refer to the formulation/product.

3.3.1 Regent 200SC Insecticide (EXP60145A, 200 g/L, suspension concentrate)

Acute studies using this formulation are summarised in the Table below. Acute oral dosing with Regent 200 SC Insecticide resulted in deaths on days 1-6 post-treatment. Bodyweight loss or reduced bodyweight gain occurred, along with clinical signs of hunched posture, piloerection, lethargy, red-brown staining around the snout and pallor of the extremities. One mouse exhibited occasional body tremors. At necropsy, dark red lungs, discoloured livers and haemorrhages of the intestines were observed in rats and mice, with mice also showing pale red spleen and kidneys. Following dermal application, rats appeared unaffected, but deaths occurred in rabbits, with reduced bodyweight gain, signs of irritation at the treatment site (one with subcutaneous haemorrhage), and similar clinical signs and necropsy findings that were seen in rats and mice following oral dosing. Additional clinical signs in rabbits were hyperactivity, clonic convulsions, loss of righting reflex, decreased or increased respiration rate, increased salivation and diuresis. Deaths in the inhalation study occurred on days 1-2, again with clinical signs similar to the other acute studies. Wet fur, tiptoe gait, exophthalmos, extreme sensitivity to external stimuli, vocalisation, gasping, noisy respiration and sneezing were also reported.

Route	Species, strain, sex	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, SD, M/F	5/sex	791, 1000, 1265, 1600 (gavage, in water)	M: 977 F: 1235 Combined: 1099 Deaths (M/F): nil at 791; 3/0 at 1000; 5/3 at 1265; 5/5 at 1600	Dreher (1990a) [GLP]
Oral	Mice, BKW, M/F	5/sex	212, 300, 424, 600 (gavage, in water)	324	Dreher (1990b) [GLP]
Dermal	Rats, SD, M/F	5/sex	4192 (24 h; semi-occlusive; maximum applicable dose)	> 4192 (no deaths or clinical signs)	Dreher (1990c) [GLP]
Dermal	Rabbits, NZW, M/F	5/sex	2096, 2966, 4192 (24 h; semi-occlusive)	2493 Deaths (M/F): all at 2966; 8/10 at 4192	Dreher (1990d) [GLP]
Inhalation	Rats, SD, M/F	5/sex	520, 1030, 1920, 5000 Particle size 3.5-4.6 µm, 44-58% respirable, (<4µm)	M: 940 F: 1220 Combined: 1070 Deaths (M/F): nil at 520; 3/1 at 1030; all at 1920 and 5000	Blagden (1993a) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

Dreher DM (1993a) EXP60145A: Acute dermal irritation test in the rabbit. Project No. SLL 282/378. Lab: Safepharm Laboratories Ltd, Derby, UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt. dates: 2.2.1993-5.2.1993. Unpublished report date: 25 February 1993. (QA/GLP: yes; Study Guidelines: OECD 404; EEC B4)

Regent 200SC Insecticide (0.5 mL) was applied for 4 h to the clipped skin of three New Zealand White rabbits under a gauze pad moistened with 0.5 mL distilled water, secured with surgical adhesive tape, and with the trunk of each rabbit wrapped in an elasticated corset. Very slight erythema was noted at all treated sites at 1 and 24 h after patch removal, and at two treated skin sites at the 48-h observation. The reactions extended about 1.5 cm beyond all treatment sites. Very slight oedema was noted at all treated skin sites 1 h after patch removal and at one treated site at the 24-h observation. All scores for erythema and oedema were 1. No signs of systemic toxicity were noted. The test substance was considered a slight skin irritant in this test.

Glaza S M (1997) Primary eye irritation study of EXP 60145 in rabbits (EPA Guidelines). Document ID R011116. Lab: Corning Hazleton Inc, Madison, Wisconsin 53704. Sponsor: BASF Australia Ltd. Unpublished.

Note: The following report of this study is adapted from an OCSEH OHS assessment (14 March 2005).

The formulation EXP 60145 was instilled into the eyes of 6 rabbits. Corneal opacity occurred in one animal, there was iridal involvement in 2 animals, and moderate conjunctival irritation in all 6 animals. Positive irritation reactions, defined as any corneal opacity, an iris score of 1 or greater, or any conjunctival redness or chemosis score of 2 or greater, were observed in all 6 animals. All treated eyes were clear of positive reactions by 48 h after treatment and had returned to normal appearance by 96 h. The total mean irritation scores calculated were 9.2, 7, 3.3, 0.7 and 0 for 1 h, 24 h, 48 h, 72 h and 96 h respectively. The formulation EXP 60145 was considered a moderate eye irritant in this test.

Dreher DM (1993b) EXP60145A: Modified nine-induction Buehler delayed contact hypersensitivity study in the guinea pig. Project No. SLL282/380. Lab: Safepharm Laboratories Ltd, Derby, UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 27.1.1993-3.3.1993. Unpublished report date: 19 March 1993. (QA/GLP: yes; Study Guidelines: OECD 406, EEC B6)

Method The shaven flanks of albino Dunkin-Hartley strain guinea pigs (10/sex) were subjected to a 6 h occluded topical application of 0.5 mL undiluted Regent 200SC Insecticide on days 0, 2, 4, 7, 9, 11, 14, 16 and 18. Blank patches were applied to 10 control guinea pigs on these days. On day 28, test and control guinea pigs were challenged by 6 h occluded topical applications of the test substance (100%, or 75% in distilled water) on two separate sites of the right flank. Dermal responses to the challenge procedure were assessed 24 and 48 h post application.

Results and Conclusion Bodyweight gains were comparable in test and control guinea pigs. Induction applications of Regent 200SC Insecticide caused mild redness and desquamation at the induction sites of test animals. Challenge applications of the test substance (100% and 75% in

distilled water) caused no significant reaction in either tests or controls. It was concluded that, under the conditions of the study, Regent 200SC Insecticide was not a skin sensitiser in guinea pigs.

3.3.2 Chipco Choice Insecticide (EXP60818A, the similar test product EXP60819A, both 1 g/kg, granule)

Route	Species, strain, sex	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, SD	5/sex	2000 (gavage, in Arachis oil)	> 5000	Myers (1994a) [GLP]
Dermal	Rabbits, SD	5/sex	2000 (moistened)	> 2000	Myers (1994b) [GLP]
Inhalation	Rats, SD	5/sex	5160 (air milled dust, 4 h; MMAD 2.1 µm; GSD 1.5 µm; 81% ≤ 3 µm)	> 5160	Nachreiner (1994) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female. MMAD = Mass Median Aerodynamic Diameter; GSD = Geometric Standard Deviation

Acute studies with this formulation are summarised in the Table above. In the acute oral and dermal studies, there were no signs of systemic toxicity over the 14 day observation period. In the inhalation study, the only signs were reported on the day of exposure and included blepharospasm, perinasal wetness and brown discolouration of the fur, with reduced bodyweight gain in females during week one. Findings at necropsy were limited to multiple black foci on the lungs in 2 males, and dark brown mottling of the lungs in one female in the oral study, with brown, focal/multifocal areas in the lungs in 2 males in the inhalation study. In the dermal study, erythema and/or oedema, and brown chemical residue were evident at the treatment site in all animals on day 1, but were no longer present on day 7.

Myers RC (1994c) EXP 60819A: Ocular Irritancy Study in the Rabbit. Laboratory project ID: 93N1366D. Lab: Bushy Run Research Center, Union Carbide Corporation, PA. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC. Expt. dates: 25 Jan-1 Feb 1994. Completion date: 26 April 1994. (QA/GLP: yes; Study Guidelines: US EPA Subdivision F, 81-4).

NZW rabbits (3/sex; 2-3.5 kg; 12-18 weeks; supplied by HRP Inc, Denver, PA USA) had 100 mg (0.1 mL) of EXP60819A (Batch No. 9-MTD-7; containing 0.1% fipronil) placed into the conjunctival sac of 1 eye/rabbit. The other eye served as the control. Eye examinations were performed at 1, 24, 48 and 72 h, and 7 days after instillation. Fluorescein staining was performed at 24 h and on each subsequent examination day. Grading and scoring were based on the Draize system. All animals were killed after 14 days.

Iritis was seen in all animals at 1 h (mean score 1), and in 2 males and 1 female at 24 h (mean score 0.5), but had resolved by 48 h. Conjunctival redness (mean score 2), chemosis (mean score 0.5) and discharge (mean score 1.5) were seen in all animals at 1 h. At 24 and 48 h the respective mean scores were 1.7, 0.3 and 1, and 1.3, 0.2 and 0.5. Conjunctival redness was still evident in 3 females at 72 h (mean score 0.7), but had fully resolved by 7 days. Fluorescein staining gave a 45% response in 1 female only at 24 h (mean score 8%), which dropped to 25% at 48 h (mean score

4%), and had completely resolved by 72 h. No other corneal effects were seen. The test material was considered to be a slight eye irritant in rabbits.

Myers RC (1994d) EXP 60819A: Cutaneous Irritancy Study in the Rabbit. Laboratory project ID: 93N1366C. Lab: Bushy Run Research Center, Union Carbide Corporation, PA. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC. Expt dates: 15.2.1994-22.2.1994. Unpublished. Completion date: 26 April 1994. (QA/GLP: yes; Test Guidelines: US EPA Subdivision F, 81-5).

NZW rabbits (3/sex; 2-3.5 kg; 12-18 weeks; supplied by HRP Inc, Denver, PA) were treated with 0.5 g of ground EXP60819A (Batch No. 9-MTD-7; containing 0.1% fipronil), moistened with 0.5 mL distilled water, and placed onto a square gauze patch. The patch was placed on the clipped dorsal skin and secured with tape, then loosely covered with polyethylene sheeting which was also secured. Animals were then placed in a restraining device for 4 h, after which the coverings and as much of the test material as possible were removed. Examinations of the application site were made at 1, 24, 48 and 72 h and 7 days after exposure. Grading and scoring were based on the Draize system. All animals were killed after 7 days.

Very slight erythema was seen in all animals at 1 h, with a mean score of 1. At 24 h, very slight erythema was present in 2 males and 1 female (mean score=0.5), and at 48 h in 1 male and 1 female (mean score=0.3), resolving fully by 72 h. Very slight oedema was present in 1 male and 1 female (mean score=0.3) at 1 h, and had fully resolved by 24 h. A brown residue was seen on 4 animals, persisting from 1 through 24 h. In a previous evaluation of this study (1996) it was concluded that EXP60819A was a slight skin irritant. However, as the effects observed were very slight and transient (scores equivalent to a primary irritation index of 0.27), and no irritation was observed at 72 h, the formulation is considered to be non-irritant in this test.

Myers RC & Nachreiner DJ (1994) EXP 60819A: Dermal Sensitisation Study in the Guinea Pig using the Buehler Technique. Laboratory project ID: 94N1370. Lab: Bushy Run Research Center, Union Carbide Corporation, PA. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC. Expt. dates: 1.2.1994-3.3.1994. Unpublished. Completion date: 26 April 1994. (QA/GLP: yes; Study Guidelines: US EPA Subdivision F, 81-6, Method of Buehler EV *Arch Dermatol* 91:171-175, 1965).

Method The skin sensitisation potential of EXP60819A (Batch No. 9-MTD-7; containing 0.1% fipronil), suspended in 0.25% (w/v) aqueous methyl cellulose, was assessed in albino Hartley guinea pigs (31-41 days old; 380-494 g; supplied by HRP Inc, Denver, PA) according to the Buehler procedure. Five animals/sex were used for each group. Dosing was by means of a Hill Top Chamber attached to the clipped skin, which provided a dose area of diameter 25 mm. The chamber was covered with an adhesive patch, and a rubber dental dam was used to cover the dorsal surface. The animals were held in a restrainer. After a 6 h contact period, the animals were taken out of the restrainer and the coverings were removed. The dosed area was washed with soap and warm water and dried. One day after application the area was treated with a depilatory agent. Concentrations of the test substance used for induction and challenge doses were based on preliminary irritancy tests. The positive control was 2,4-dinitro-1-chlorobenzene (DNCB) (0.3 and 0.1% (w/v) suspensions in 0.25% (w/v) aqueous methyl cellulose). A vehicle control group was not included due reportedly to the extensive experience with 0.25% aqueous methyl cellulose.

In the induction phase, one group was dosed for 6 h with a 40% (w/v) preparation of the test substance, another group received 0.3% DNCB. All induction applications for the test material were made on the left scapular area, with DNCB applied to different locations between the left scapular and lumbosacral areas due to staining and/or irritation. This was repeated 3 times, spaced a week apart, followed by a 2 week rest period. A challenge application was performed under the same conditions, using the same concentration of test material (40%), and 0.1% DNCB as the positive control. An additional two groups of naïve controls received single 6 h exposures to the 40% test material suspension, and the DNCB suspension, respectively. Challenge doses were applied to previously undosed sites on the right scapular region. At 24 and 48 h after the induction or challenge applications, the site was assessed and scored according to a 5-point progressive system for erythema/oedema (0, 0+, 1, 2, 3). Any reaction ≥ 1 was considered positive.

Results and Conclusion During the induction phase, no skin reaction was seen in any of the test animals. After challenge, slight patchy erythema was seen in 1 test animal at 24 h only and is considered to be irritant in nature. Three animals showed areas of excoriation on the dosed area within 24 h, which is reported to probably be due to scratching. This extended outside the dose area in 2 animals. An area of eschar was also seen at 48 h in one of these animals, with a pinpoint focus of irritation seen on the dose site of one animal at 48 h. No response was seen in the naïve controls exposed to the test material, although excoriations were seen in one male at 24 and 48 h. The positive control confirmed the sensitivity of the test animal model, with all 10 animals displaying a positive response. The test material was not a skin sensitising agent under the conditions of the assay.

3.3.3 Regent 500FS Seed Dressing Insecticide (EXP80415, 500 g/L, suspension conc., equivalent to Cosmos Insecticidial Seed Treatment)

Route	Species, strain	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, SD	5/sex	125, 250, 500 (aqueous)	268 (M), 354 (F); combined: 290 Deaths (M/F): 0/1 at 125; 2/2 at 250; 5/3 at 500.	Allen (1993a) [GLP]
Dermal	Rats, SD	5/sex	2000, neat (24 h, semi-occluded)	> 2000 (no deaths)	Allen (1993b) [GLP]
Inhalation	Rats, SD	5/sex Vehicle: distilled water	100, 470, 1600 (4 h, nose-only; particle size 2.4-3.7 µm, 54-73% < 4 µm)	200 (M), 350 (F); Combined: 260 Deaths (M/F): 3/5 at 470; 5/5 at 1600	Blagden (1993b) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

Acute studies with the product Regent 500FS are summarised in the Table above. In the acute oral study, deaths occurred up to 3 days post treatment, with hunched posture and/or lethargy observed in all groups. Isolated incidents of decreased respiratory rate were noted in females treated with 125 or 250 mg/kg bw, and one female treated with 125 mg/kg bw exhibited clonic convulsions. Survivors either appeared normal throughout or recovered 3-4 days post-dosing. Necropsy of rats that died revealed haemorrhagic lungs, discoloured livers and dark red kidneys, haemorrhages in the gastric mucosa and sloughing of the non-glandular epithelium of the stomach. Haemorrhages in the small intestine occurred in one male receiving 250 mg/kg bw. No clinical signs or other toxicological effects were reported in the dermal study.

In the inhalation study, common clinical abnormalities shown at all doses included wet fur, hunched posture, piloerection and decreased respiratory rate. Lethargy, ptosis and ataxia were also noted, and occasionally rats exhibited laboured respiration, tonic convulsions, vocalisation, hyperactivity, red/brown staining on the head, high-stepping gait and diarrhoea. All low-dose rats showed signs of systemic toxicity, but regained normality 2-6 days post exposure. In the high-dose and medium-dose groups, deaths occurred 1 (8M, 8F) or 2 (2M) days post exposure. Necropsy in rats that died showed lung abnormalities (swelling and uniform or patchy dark red discolouration), patchy liver discolouration and haemorrhages in the small intestine.

Allen DJ (1993c) EXP80415: Acute eye irritation test in the rabbit. Project No. SLL282/407. Lab: Safepharm Laboratories Limited, Derby, UK. Sponsor:Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 25.5.1993-29.5.1993. Unpublished report date: 20 July 1993. (QA/GLP: yes; Study Guidelines: OECD 405).

Regent 500FS Seed Dressing Insecticide (0.1 mL) was placed in one (non-irrigated) eye of each of three rabbits, the contralateral eye acting as the control. Minimal conjunctival redness was noted in two treated eyes 1 h post-treatment. All eyes were normal 24 h post-treatment. The test substance was classified as non-irritant to the rabbit eye.

Allen DJ (1993d) EXP80415: Acute dermal irritation test in the rabbit. Project No. SLL282/406. Lab: Safepharm Laboratories Limited, Derby, UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 18.5.1993-21.5.1993. Unpublished report date: 20 July 1993. (QA/GLP: yes; Study Guidelines: OECD 404).

Regent 500FS Seed Dressing Insecticide (0.5 mL) was applied as a 4-h, semi-occluded application to the intact, clipped skin of three rabbits. Very slight erythema was noted at all treated skin sites at 1, 24 and 48 h after patch removal (mean scores of 1), and very slight oedema was noted at all treated skin sites at 1 and 24 h (mean scores of 1). No skin reactions were noted at 72 hours. There were no signs of systemic toxicity. The formulation was a slight skin irritant in this test.

Allen DJ (1993e) EXP80415: Modified nine-induction Buehler delayed contact hypersensitivity study in the guinea pig. Project No. SLL282/408. Lab: Safepharm Laboratories Limited, Derby, UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 12.5.1993-16.6.1993. Unpublished report date: 20 July 1993. (QA/GLP: yes; Study Guidelines: OECD 406).

Method The shaven flanks of 20 female albino guinea pigs were subjected to a 6 h occluded topical application of 0.5 mL undiluted Regent 500FS Seed Dressing Insecticide on days 0, 2, 4, 7, 9, 11, 14, 16 and 18. Blank patches were applied to ten female control guinea pigs on these days. On day 28, test and control animals were challenged by 6 h occluded topical applications of the test substance (100% and 75% in distilled water) on two separate sites on the right flank. Dermal responses to the challenge procedure were assessed 24 and 48 h post application.

Results conclusion Bodyweight gains were comparable in test and control guinea pigs. Induction applications of Regent 500FS Seed Dressing Insecticide caused yellow staining, mild redness, slight oedema and desquamation, and superficial scabs at the application site. Challenge applications of 100% and 75% Regent 500FS Seed Dressing Insecticide caused yellow staining, but

no skin reactions, in test or control animals at the 24- and 48-h observations. It was concluded that, under the conditions of the study, the test substance was not a skin sensitiser in guinea pigs.

3.3.4 Frontline Spray (RM1601, 2.5 g/L, Suspension conc.)

Route	Species, strain	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, SD	5/sex	5000 (fasted)	> 5000 (no deaths)	Clouzeau (1993a) [GLP]
Dermal	Rats, SD	5/sex	2000 (semi-occluded, 24 h)	> 2000 (no deaths)	Clouzeau (1993b) [GLP]
Inhalational	Rats, SD	5/sex	5060 (4 h, nose only; ~99% of particles <3.5 µm); equivalent to 12.7 µg fipronil/m^3)	> 5060 (no deaths)	Robinson (1993) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute toxicity studies for Frontline Spray are summarised in the Table above. In the acute oral study, rats that received the Frontline Spray formulation with fipronil omitted (5000 mg/kg bw) exhibited a decrease in spontaneous activity, coma and dyspnoea within the first week. One male died on day 3. The bodyweight gain of the survivors was less than expected between days 1 and 5. By day 9, behaviour had returned to normal. There was no gross pathology. Rats receiving Frontline Spray showed similar clinical signs and effects on bodyweight gain. There were no deaths, and scheduled necropsies were unremarkable. No treatment-related signs were reported in the acute dermal and inhalational studies.

Clouzeau, J (1993c) Acute dermal irritation in rabbits: RM1601C, 0.25% spray formulation. Study No. No. CIT9652 TAL. Centre International de Toxicologie, Evreux, France. Sponsor: Rhone-Merieux, Toulouse, France. Expt. dates: 9.12.92-12.12.92. Unpublished report date: 6 April 1993. (QA/GLP: Yes; Study Guideline: OECD 404)

Frontline Spray (0.5 mL) was applied as a 4 h, semi-occluded application to the intact, clipped skin of one flank of three rabbits. The Frontline Spray formulation with fipronil omitted was applied to the opposite flank under similar conditions. Frontline Spray caused very slight erythema in one animal, noted at the 1 h observation only. No skin reactions were observed in any other animal. It was concluded that Frontline Spray was non-irritant to rabbit skin in this test.

Clouzeau, J (1993d) Acute eye irritancy in rabbits: RM1601C, 0.25% spray formulation. Study No. 9653 TAL. Lab: Centre International de Toxicologie, Miserey, 27005 Evreux, France. Sponsor: Rhone-Merieux, Toulouse, France. Expt dates: 15.12.92-20.12.1992. Unpublished report date: 6 April 1993. (QA/GLP: Yes; Study Guideline: OECD 405)

Frontline Spray (0.1 mL) was placed in the conjunctival sac of the left eye of each of three rabbits. The Frontline Spray formulation with fipronil omitted (0.1 mL) was instilled into the right eye. Moderate chemosis and conjunctival redness, slight iris congestion, and corneal opacity occurred in test and control eyes. The degree of corneal opacity was maximal at 24 h (individual animal scores 1, 1, 2), persisting until 48, 72 and 96 hours post-treatment, respectively. By 5 days post-treatment, all ocular reactions had reversed. During this time, the area of opacity gradually reduced (maximum

score 4 at 24 h). On the basis of corneal opacity, reversible in 7 days, both Frontline Spray formulation and the formulation with fipronil omitted are classified as moderate eye irritants.

Clouzeau, J (1993e) Sensitisation test in guinea pigs (according to Buehler, E.V.): RM1601C, 0.25% spray formulation. Study No. CIT9654 TSG. Lab: Centre International de Toxicologie, Miserey, 27005 Evreux, France. Sponsor: Rhone-Merieux, Toulouse, France. Expt dates: 15.12.1992-14.1.1993. Unpublished report date: 5 May 1993. (QA/GLP: Yes; Study Guideline: OECD 406, 84/449/EEC Annex V B6)

Method Two groups of guinea pigs (5/sex/group) received either Frontline Spray (0.5 mL) or the Frontline Spray formulation lacking fipronil (0.5 mL) on the left flank and a dry compress on the right flank for 6 h on days 1, 8 and 15. After a 2-week rest period, challenge applications of 0.5 mL of the Frontline Spray formulation lacking fipronil (left flank) or 0.5 mL of the complete formulation (right flank) were applied to previously non-treated areas. Skin reactions were evaluated 24 and 48 h after challenge.

Results and Conclusion There were no deaths or clinical signs of toxicity, bodyweight gains were normal, and no skin reactions were observed. In this test, Frontline Spray was not a skin sensitiser in guinea pigs.

3.3.5 Frontline Spot On for Cats (100 g/L fipronil)

Route	Species, strain	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, SD	5/sex	1000, 2000, 5000 (deaths (M/F): 0/1 at 1000; 1/1 at 2000 and 4/4 at 5000)	3208 (M), 2821 (F), 3000 (combined)	De Joufrey (1995) [GLP]
Dermal	Rats, SD	5/sex	5000 (semi-occluded, 24 h)	> 5000 (no deaths)	De Joufrey (1994a) [GLP]
Inhalational	Rats, SD	5/sex	6320 (nose only; 4 h)	> 6320 (no deaths)	Kieran (1995) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute toxicity studies for Frontline Spot On are summarised in the Table above. In the acute oral study, clinical signs of sedation, reduced activity and piloerection were seen in both sexes, at all doses tested. At 5000 mg/kg bw, hypersalivation and wasting were observed in females, with dyspnea in both sexes. Signs were present from 1 h post-dosing, resolving after 8 days at 5000 mg/kg bw, but after 3 days at the lower doses. In 5 rats/sex treated with 5000 mg/kg bw of the product minus the active (RM1601E/64), there were no deaths, but sedation, piloerection and dyspnoea were observed in both sexes. In the acute dermal study, one male and 3 females treated with 5000 mg/kg bw of the product or the formulation lacking the active ingredient, appeared hyperactive four hours after treatment, but behaved normally from day 2 onwards. In the inhalation study, bodyweight gains were reduced up to day 7 for both sexes, but no clinical signs were observed.

De Jouffrey (1994b) Acute dermal irritation in rabbits. Study 12009 TAL. Lab: Centre International de Toxicology (CIT), Miserey, Evreux, France. Sponsor: Laboratoire Rhone-Merieux, Toulouse, France. Expt. dates: 3.8.1994-11.8.1994. Unpublished report date: 25 Nov 1994. (QA/GLP: yes; Test Guidelines: OECD 404, EPA Subdivision F Series 81).

Six male NZW rabbits were clipped of hair on the left and right flanks. Frontline Spot On for Cats (0.5 mL) was applied to the left flank, and the product minus fipronil (RM1601E/64) to the right flank for 4 h under a semi occlusive dressing. On day one, slight erythema was observed for all animals treated with the product, resolving between days 2 and 6. The mean scores were 0.8 for erythema (maximum 2) and 0 for oedema. The mean score for RM1601E/64 alone was 1.7 for erythema (maximum 3) and 0 for oedema. Frontline Spot On for Cats was classified as a slight skin irritant in this study.

De Jouffrey S (1994c) Acute eye irritation in rabbits. Study 12010 TAL. Lab: Centre International de Toxicology (CIT), Miserey, Evreux, France. Sponsor: Laboratoire Rhone-Merieux, Toulouse, France. Expt. dates: 1.9.1994-7.9.1994. Unpublished report date: 25 Nov 1994. (QA/GLP: yes; Test Guidelines: OECD 405, US EPA F-81).

Frontline Spot On for Cats (0.1 mL) was instilled into the left eye, and the product minus the active into the right eye of 6 male New Zealand White rabbits, and ocular reactions observed at 1, 24, 48 and 72 h. The eyes were not washed after treatment.

For the product, conjunctival redness was observed in all animals from 1 h after instillation, resolving within 6 days, with a maximum score of 2 (average 1.3). Chemosis was seen in all animals within 1 hour, resolving by day 5 (maximum score 2, average 0.8), while iritis was observed in 4 animals at 24 h, resolving by 48 h (maximum score 1, average 0.3). Corneal opacity was reported in 4 animals at 1 h, resolving by day 5 (maximum score 2, average 0.6). The product with fipronil omitted produced slight to moderate conjunctival redness (scores 1-2, average 0.9) for the first 6 days and iris congestion (score 1, average 0.1) and slight corneal opacity (score 1, average 0.1) on day 2, with all ocular lesions resolving by day 7. Based on this study, both Frontline Spot On for Cats and the product with fipronil omitted were considered moderate eye irritants in this test.

De Jouffrey S (1994d) Skin sensitization test in Guinea Pigs (Buehler test: 3 applications). Study 12011 TSG. Lab: Centre International de Toxicology (CIT), Miserey, Evreux, France. Sponsor: Laboratoire Rhone-Merieux, Toulouse, France. Expt. dates: 9.8.1994-8.9.1994. Unpublished report date: 26 Dec 1994. (QA/GLP: yes; Test Guidelines: OECD 406, US EPA F-81).

Method Male and female Dunkin Hartley Guinea Pigs were clipped to expose 16 cm^2 of skin 24 h before each application of the test preparation. Frontline Spot On for Cats or the same formulation with fipronil omitted (0.5 mL) were applied to the exposed skin on a gauze pad held in place with a semi-occlusive dressing for 6 h, once a week for 3 weeks. Fourteen days after the last application, 0.5 mL of Frontline Spot On for Cats was applied as previously, but to naïve skin on the other flank. Animals pre-treated with the product minus fipronil were challenged using the same procedure.

Results and Conclusion No skin reactions were observed during induction or at challenge. Bodyweight gains and gross pathology were normal. No abnormal clinical signs of toxicity were

observed. In this study, neither Frontline Spot On for Cats nor the product with fipronil omitted were skin sensitisers in guinea pigs.

3.3.6 Frontline Plus for Cats (100 g/L fipronil + 120 g/L (S)-methoprene)

Findlay J (1999a) Acute dermal irritation study of ML-2,095,988 508Q in rabbits. Merial Study No. PR&D 0029401. Lab: IIT Research Institute (IITRI) Life Sciences Operation, Chicago, IL 60616. Sponsor: Merial Limited, Pharmaceutical Research & Development, Development Projects, Iselain NJ, USA. Expt. dates: 20.4.1999-27.4.1999. Unpublished report date: 1 September 1999. (QA/GLP: yes; Test Guidelines: OECD 405, US EPA OPPTS 870.2500).

ML-2,095,988 508Q (Batch No. ML-2,095,988 508Q 003; 0.5 mL, undiluted) containing 10% (w/v) fipronil and 12% (w/v) (S)-methoprene was applied to a clipped area on the backs of 3 male New Zealand White rabbits (2-3 months old; 2.36-2.63 kg), and covered with a 2.5 x 2.5 cm gauze patch secured with porous tape. The mid-sections of the rabbits were bandaged to prevent removal of the test material. Dressings were removed after 4 hours' exposure, the application site cleansed with purified water, then examined for dermal irritation reactions after 30-60 minutes, and 1, 2, 3 and 7 days. An area of untreated skin on the same animal acted as a control. Dermal reactions were scored according to the Draize method.

No dermal reactions were observed at 30-60 minutes after patch removal, but at 24 h, two rabbits had very slight oedema. This resolved in one animal by 72 h, and by 7 days in the other. Erythema was present in all animals from 24 to 72 h (mean scores 1.7, 1.3 and 2.3; maximum score 3), but resolved by 7 days. At 72 h, two animals had erythema scores of 2 (well-defined erythema) and one animal had a score of 1 (very slight erythema). Therefore, Frontline Plus for Cats is considered a slight skin irritant in this test.

Findlay J (1999b) Acute eye irritation study of ML-2,095,988 508Q in rabbits. Merial Study No. PR&D 0029501. Lab: IIT Research Institute (IITRI) Life Sciences Operation, Chicago, IL 60616. Sponsor: Merial Limited, Pharmaceutical Research & Development, Development Projects, Iselain NJ, USA. Expt. dates: 20.4.1999-26.4.1999. Unpublished report date: 1 September 1999. (QA/GLP: yes; Test Guidelines: OECD 404, US EPA OPPTS 870.2400).

ML-2,095,988 508Q (Batch No. ML-2,095,988 508Q 003; 0.1 mL, undiluted) containing 10% (w/v) fipronil and 12% (w/v) (S)-methoprene was instilled into the right eye of 3 female New Zealand White rabbits (3 months old), the left eye serving as an untreated control. The treated eyes were washed with lukewarm water 24 h after introduction of the test material. Both eyes were examined for ocular reactions at 1, 24, 48 and 72 h, and for corneal lesions (using fluorescein under UV illumination) one day prior to treatment and at 24 h after treatment. Scoring was according to the Grades for Ocular Lesions (US EPA OPPTS 870.2400).

At the 1 h observation, conjunctival erythema (score 1, some blood vessels definitely hyperaemic) was noted for all 3 rabbits. This was still present in two of the rabbits at 24 h, but had resolved at 48 h. Also at 1 h post-treatment, chemosis (score 2, obvious swelling with partial eversion of lids) was present in 2 rabbits, reducing to score 1 (any swelling above normal) at 24 h, and resolving by 48 h. The remaining rabbit had a chemosis score of 1 at 1 h, which had resolved by 24 h. No effects were observed for the cornea or the iris. Frontline Plus for Cats was a slight eye irritant in this test.

Findlay J (1999c) Skin sensitization study of ML-2,095,988 508Q using the modified Buehler method in guinea pigs. Merial Study No. PR&D 0029601. Lab: IIT Research Institute (IITRI) Life Sciences Operation, Chicago, IL 60616. Sponsor: Merial Limited, Pharmaceutical Research & Development, Development Projects, Iselain NJ, USA. Expt. dates: 28.4.1999-28.5.1999. Unpublished report date: 3 September 1999. (QA/GLP: yes; Test Guidelines: OECD 406, US EPA OPPTS 870.2600).

Method ML-2,095,988 508Q (Batch No. ML-2,095,988 508Q 003; 0.3 mL, undiluted) containing 10% (w/v) fipronil and 12% (w/v) (S)-methoprene was applied in a Hill Top Chamber to the upper left quadrant of the shaved backs of 20 male Hartley albino guinea pigs (Charles River Laboratories, Portage, MI) once per week for 3 weeks. The concentrations of the test substance chosen were based on the results of preliminary experiments using 2 guinea pigs, which showed skin reactions (erythema score 1) in both the test animals when the test material was applied undiluted, but no reactions were observed with 75% or 50% dilutions of the test material. A positive control group of 10 animals was treated similarly with undiluted α-hexylcinnamaldehyde (HCA), while a negative control group of 10 animals was handled in the same way, but no substance was introduced into the chamber. The duration of each exposure was 6 h, after which the chambers were removed and the test sites cleansed with purified water. Two weeks after the third induction application, 0.3 mL of the test material (75% w/v diluted in the vehicle ML-3,967,758 000K [PR&D#0029601, batch no. ML-3,967,758 000K 001]) was applied as for the induction doses, but to the lower left quadrant of the backs of the treated and negative control guinea pigs. No information was provided to further identify this vehicle. The positive control group was challenged with 50% (w/v) HCA diluted in acetone. After the 6 h exposure, the application sites were cleansed as for the induction phase. At 24 and 48 h following the first induction exposure and the challenge exposure, the test sites were scored for erythema using the Magnusson and Kligman Grading Scale.

Results and Conclusion One negative control animal died, but as death was prior to the challenge phase, the test material was not implicated. All animals gained weight during the study period. Erythema (score 1; discrete or patchy erythema) was observed in 4/20 animals in the test group at 24 h after the first induction application, all of which had resolved by 48 h post-exposure. At challenge, a positive reaction was observed in one treated animal only (score 2, moderate and confluent erythema, at 48 h). No skin reactions were observed in the negative control group. In the positive controls, erythema (score 1 or 2) was observed in 7/10 animals at 24 and 48 h after the first induction exposure. At challenge, erythema (score 1 or 2) was observed in 9/10 positive control animals. As no information was provided regarding the potential of 50% (w/v) HCA in acetone to cause skin irritation, it is possible that some of these challenge reactions may be due to irritation. However, as 3 of the positive control animals that exhibited no skin reaction at induction after the induction application showed convincing reactions at challenge, and the skin reactions in 3 other positive control animals were increased after challenge relative to post-induction, then this is sufficient for this study to be considered robust and valid (i.e. the sensitivity of the assay has been demonstrated). Frontline Plus for Cats was not a skin sensitiser in this test.

3.3.7 Regent 25 ULV (25 g/L fipronil)

Route	Species, strain	Group Size	Doses Tested (mg/kg bw or mg/m^3)	LD_{50} (mg/kg bw) or LC_{50} (mg/m^3)	Reference
Oral	Rats, CD	5/sex	1500, 3000, 4000, 5000 (gavage, in dH_2O)	3529 (M) 3208 (combined) Deaths (M/F): 2/2 at 3000; 4/5 at 4000; 3/5 at 5000.	Warshawsky (1995a) [GLP]
Dermal	Rabbits, Hra(NZW) SPF	5/sex	4000 (24 h, semi-occluded)	>4000 (no deaths)	Warshawsky (1995b) [GLP]
Inhalation	Rats, Crl:CD BR VAF+	5/sex	5000 (4 h, nose-only; MMAD 2.6 µm; GSD 1.95 µm)	>5000 (no deaths)	Hilaski (1995) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female. dH2O = deionised water.
MMAD = Mass Median Aerodynamic Diameter. GSD = Geometric Standard Deviation.

The acute toxicity studies for Regent 25 ULV are summarised in the Table above. In the oral study, deaths occurred on days 2-3. The main clinical signs (seen on days 2-14 at >1500 mg/kg bw) were decreased activity, decreased defaecation, laboured breathing and red material around the mouth or nose, with less frequent observations of tremors, low carriage, anogenital staining, convulsions, salivation and impaired limb function. In the dermal study, clinical signs were similar, with additional findings of erythema at the test site. Signs in the inhalation study were limited to rapid respiration in 2 of the animals, normalising rapidly in one, and by day 5 in the other. Necropsy revealed red discolouration of the lungs in animals that died during the oral study, and in one male (a trace) in the inhalation study.

Warshawsky LD (1995c) Primary eye irritation study in rabbits. Study No. 347-045. Lab: International Research and Development Corporation, 500 North Main St, Mattawan, MI 49071 USA. Sponsor: Rhone-Poulenc Ag Co., PO Box 12014, 2 T.W. Alexander Drive Research Triangle Park, NC 27709. Unpublished report date: 5 July 1995. (QA/GLP: yes; Study Guidelines: US EPA Subdivision F, 81-1)

Regent 25 ULV (0.1 mL in deionised water) was placed into the conjunctival sac (right eye) of 3 male (weighing 2474-2634g, aged 4 months) and 3 female (weighing 2620-2707g, aged 4 months) NZW rabbits (Hra:NZW, SPF). The eyelids were gently held closed for 1 second. The left (control) eye was manipulated in an identical manner except no test substance was administered. The eyes remained unwashed and were observed for ocular irritation, in accordance with the Draize scale, at 1, 24, 48 and 72 h post-treatment. In addition, a sodium fluorescein examination was conducted at pretest (for animal selection purposes) and at the 48 and 72 h observation intervals (after grading the eye reaction).

Slight to moderate redness of the conjunctivae and very slight chemosis of the conjunctivae were observed at the 1 h observation point. This cleared by 48 h in 5 animals and by 72 h in the remaining animal. Based on the results of this study, Regent 25 ULV was determined to be slightly irritating to rabbit eyes.

Warshawsky LD (1995d) Primary dermal irritation test in rabbits following a 4 hour exposure period. Study No. 347-043. Lab: International Research and Development Corporation, 500 North Main St, Mattawan, MI 49071 USA. Sponsor: Rhone-Poulenc Ag Co., PO Box 12014, 2 T.W. Alexander Drive Research Triangle Park, NC 27709. Unpublished report date: 26 June 1995. (QA/GLP: yes; Study Guidelines: US EPA Subdivision F, 81-5)

Regent ULV (0.5 mL in deionised water) was applied to the intact skin of 3 male (weighing 1888-2334g, aged 3 months) and 3 female (weighing 2021-2079g, aged 3 months) NZW rabbits (Hra:NZW, SPF). The material was applied under one-inch square gauze patches secured with strips of Dermiform tape. A collar (E-Jay Saf-T Shield) was placed on each rabbit. The test substance remained in contact with the test site skin for 4 h. Following the exposure period, the bandaging materials and collars were removed and the test sites were wiped with disposable towelling moistened with water to remove any residual material. The test sites were evaluated for dermal irritation using the Draize method, at approximately 0.5-1, 24, 48 and 72 h after bandage removal.

No clinical signs were observed during the study. Dermal irritation and very slight erythema was noted on the backs of 2 animals at the 0.5-1 h observation interval. No irritation was noted at any other interval during the study. On the basis of these data, it was concluded that Regent 25 ULV is non-irritating to the skin of rabbits.

Warshawsky LD (1995e) Dermal sensitization study (Buehler) in the albino guinea pig. Study No. 347-046. Lab: International Research and Development Corporation, 500 North Main St, Mattawan, MI 49071 USA. Sponsor: Rhone-Poulenc Ag Co., PO Box 12014, 2 T.W. Alexander Drive Research Triangle Park, NC 27709. Unpublished report date: 27 July 1995. (QA/GLP: yes; Study Guidelines: US EPA Subdivision F, Guideline 81-6)

Method: Induction doses of Regent 25 ULV were administered to a group of 20 albino Hartley guinea pigs (10/sex; males weighing 352-419 g, aged 1.5 months; females weighing 321-424g, aged 1.5 months). Undiluted Regent 25 ULV was applied topically under a Webril pad to the shaved shoulder of the animals 3 times a week for 3 consecutive weeks. The patches were occluded with a medium gauge rubber dental dam. The exposure period for each induction was approximately 6 h. Another group of 10 guinea pigs (5/sex) served as the positive control group and were treated with 0.1% w/v 2,4-DNCB in acetone in a similar manner during the induction phase. The test sites were observed for a dermal response approximately 24 h post-patch removal. The negative control group of 10 guinea pigs (5/sex) remained untreated during the induction phase.

All animals were challenged 2 weeks after the induction phase by topical application to the shaven flank. Regent 25 ULV, at a concentration of 75% w/v in deionized water, was applied to the treated animals as well as the previously untreated control group. The positive control group animals were challenged with 0.05% w/v 2,4-DNCB. The exposure period was approximately 6 h. The guinea pigs were observed for a sensitisation response following the challenge phase.

Results and Conclusion: Following the fourth induction treatment with Regent 25 ULV, two to five animals manifested very slight patchy erythema at each of the remaining induction phase observation intervals. Following the last application of the positive control, moderate to severe erythema along with eschar, fissuring and oedema were observed at the test sites. One treated and one negative control animal manifested slight patchy erythema at the challenge phase. All of the positive control group animals showed a dermal response ranging from moderate to severe erythema at the challenge phase, and oedema was also noted at each test site. No apparent effect on

bodyweight gain was noted, and no signs of ill health were observed during the study. Regent 25 ULV was not a skin sensitiser in this test.

3.3.8 Goliath^{R} Cockroach Bait (0.5 g/kg fipronil)

The acute toxicity studies for Goliath Cockroach Bait are summarised in the Table below.

Route	Species, strain	Group Size	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral	Rats (OFA.SD, IOPS Caw)	5/sex	2000 (gavage, in 1% aqueous CMC)	>2000 (no deaths and no clinical signs)	Mercier (1996a) [GLP]
Dermal	Rats (OFA.SD, IOPS Caw)	5/sex	2000 (24 h, semi-occlusive)	>2000 (no deaths and no clinical signs)	Mercier (1995) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female. CMC = carboxymethylcellulose.

Mercier O (1996b) EXP 61132A – Ocular irritation and reversibility test in the rabbit (O.I.R) – 3 rabbits. Report No. 509305. Lab: Pharmakon Europe, Les Oncins BP 0118, 69593 L'arbresle Cedex France. Sponsor: Rhone-Poulenc Secteur Agro, 355 rue Dostoïevski BP 153 06903 Sophia-Antipolis Cedex, France. Unpublished report date: 5 January 1996. (QA/GLP: yes; Study Guidelines: OECD 405, EEC Annex V B5).

Three male albino rabbits (Hra:NZW) SPF, HRP, Inc. Michigan) were given a single ocular dose of 0.1 mL of EXP 61132A, then observed for up to 72 h. There were no deaths. Signs of irritation were reported for the cornea, iris and conjunctivae. At the 24 h observation point, slight chemosis and discharge in the conjunctivae and circumcorneal injection and congestion of the iris were noted, but these effects were no longer evident at 48 h. No other signs of irritation were noted. Mean scores for corneal and iridal effects were all zero. Mean scores for conjunctival redness/chemosis were 0.66/0.66 at 24h and zero thereafter. The compound EXP 61132A was classified as a slight irritant to the eye in rabbits.

Mercier O (1996c) EXP 61132A – Primary cutaneous irritation and corrosivity test in the rabbit. Report No. 509304. Lab: Pharmakon Europe, Les Oncins BP 0118, 69593 L'arbresle Cedex France. Sponsor: Rhone-Poulenc Secteur Agro, 355 rue Dostoïevski BP 153 06903 Sophia-Antipolis Cedex, France. Unpublished report date: 11 January 1996. (QA/GLP: yes; Study Guidelines: OECD 404, EEC Annex V B4)

The test material (0.5 g EXP 61132A) was applied to the skin of three male New Zealand albino rabbits (Hra:(NZW) SPF, HRP, Inc. Michigan) for 4 h. There were no deaths. Slight erythema (score 1) was seen in two of the three animals at the 1 h observation point, but these effects were not evident at the 24, 48 or 72 h post-treatment. No irritation was observed for the third animal. In a previous evaluation of this study (1997) it was concluded that EXP61132A was a slight skin irritant. However, as the effects observed were very slight and transient, and no irritation was observed even at 24 h, the formulation is considered to be non-irritant in this test.

Mercier O (1996d) EXP 61132A – Sensitizing potential in the guinea pig – modified Buehler test (9 inductions applications). Report No. 509306. Lab: Pharmakon Europe, Les Oncins BP 0118, 69593 L'arbresle Cedex France. Sponsor: Rhone-Poulenc Secteur Agro, 355 rue Dostoïevski BP 153 06903 Sophia-Antipolis Cedex, France. Unpublished report date: 11 January 1996. (QA/GLP: yes; Study Guidelines: OECD 406, EEC Annex V B6)

Method Albino Hartley guinea pigs (Charles River, France, 10/sex) were topically treated with 62% w/w EXP 61132A, prepared as a paste in water, at 0.5 mL/animal, for both induction and challenge phase. This concentration was shown to be non-irritant in a preliminary study. For the induction exposure, animals received nine applications (6 hours, occluded) on days 1, 3, 5, 8, 10, 12, 15, 17 and 19. After a 10-day rest period, the animals received a topical occlusive application of the test material for 6 hours (challenge exposure). Dermal irritation readings were scored according to the Draize scale at 24 and 48 h after the challenge dose. Five control animals/sex were treated similarly, but received only the vehicle (water) in the induction phase.

Results and Conclusion Slight erythema (score 1) was noted for days 8-19 of induction in the majority of animals in the test group. After challenge, macroscopic examination did not reveal any sensitisation reactions (all scores zero). The compound EXP 61132A was therefore non-sensitising to the skin of guinea pigs in this test.

3.3.9 Goliath^{R} Gel Cockroach Bait (0.5 g/L fipronil)

Route	Species, strain	Group Size	Doses Tested (mg/kg bw)	LD_{50} (mg/kg bw)	Reference
Oral	Rats (Crl:CD (SD)BR)	5/sex at 2000 and 5000; 5 males at 8000	2000, 5000, 8000	4400 (males) Deaths (M/F): 1/1 at 2000, 2/1 at 5000, 4 at 8000	Grunert (1996a)
Dermal	Rats (SD)	5/sex	5000 (24 h, occlusive)	>5000 (no deaths, no clinical signs)	Grunert (1996b) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

The acute toxicity studies for Goliath Gel Cockroach Bait are summarised in the Table above. In the acute oral toxicity study, clinical signs included hunched posture, apathy, ruffled fur and laboured respiration. Post-mortem examination of survivors revealed an abscess in the right area of the chest in two animals at 2000 mg/kg bw and one animal at 8000 mg/kg bw.

Grunert B (1996c) Report 96 10 42 807 C. Acute eye irritation/corrosion. CEL 261 03 I RB. Project No. 96 10 42 807. Lab: BioChem GmbH, Daimlerstr. 5b D-76185 Karlsruhe, Germany. Sponsor: CELAFLOR GmbH, Konrad-Adenauer-Str. 30 D-55218 Ingelheim, Germany. Expt. dates: 29.5.1996-3.6.1996. Unpublished report date: 11 June 1996. (QA/GLP: yes; Study Guidelines: OECD 405)

Albino rabbits (3/sex, Hra:(NZW) SPF, HRP, Inc. Michigan) were given a single ocular dose of 0.1 mL of CEL 261 03I RB, then observed for up to 72 h. There were no deaths. Sixty minutes after dosing, all of the animals were reported to have slight redness of the conjunctivae (score 1), but this effect was transient. Scores were zero for all subsequent examinations. In a previous evaluation of this study (1997) it was concluded that CEL 261 03I RB was a slight eye irritant. However, as the effects observed were very slight and transient, and no irritation was observed even at 24 h, the formulation is considered to be non-irritant to rabbit eyes in this test.

Grunert B (1996d) Report 96 10 42 807 B. Acute dermal irritation/corrosion. CEL 261 03 I RB. Project No. 96 10 42 807. Lab: BioChem GmbH, Daimlerstr. 5b D-76185 Karlsruhe, Germany. Sponsor: CELAFLOR GmbH, Konrad-Adenauer-Str. 30 D-55218 Ingelheim, Germany. Expt. dates: 7.5.1996-13.5.1996. Unpublished report date: 31 May 1996. (QA/GLP: yes; Study Guidelines: OECD 404)

Albino rabbits (3/sex) were given single dermal doses (intact skin, occlusive dressing) of 0.5 mL CEL 261 03I RB for 4 hours. Slight erythema was noted in one male one hour after removal of the bandage (Draize score 1), but all scores were zero at the other observation periods. In a previous evaluation of this study (1997) it was concluded that CEL 261 03I RB was a slight skin irritant. However, as the effects observed were very slight and transient, and no irritation was observed even at 24 h, the formulation is considered to be non-irritant in this test.

Grunert B (1996e) Report 96 10 42 814 B. Skin sensitisation study according to Magnusson & Kligman. CEL 261 03 I RB. Project No. 96 10 42 814. Lab: BioChem GmbH, Daimlerstr. 5b D-76185 Karlsruhe, Germany. Sponsor: CELAFLOR GmbH, Konrad-Adenauer-Str. 30 D-55218 Ingelheim, Germany. Expt. dates: 22.10.1996-15.11.1996. Unpublished report date: 29 November 1996. (QA/GLP: yes; Study Guidelines: OECD 406)

Method The formulation CEL 261 03 I RB was tested for sensitising potential in guinea pigs (5/sex, Pirbright White, Harlan Winkelmann GmbH, Borchen Germany) according to the Magnusson and Kligman method. For the intradermal induction, each animal received 0.1 mL injections of 50% Freund's adjuvant in water, a 5% suspension of test substance in water and a 10% suspension of test substance in a 1:1 ratio of 50% Freunds adjuvant on each side of the dorsum. For the dermal induction treatment, one week later, animals were topically exposed for 48 h to a 25% suspension of CEL 261 03 I RB in Vaseline. Two weeks later the animals were dermally challenged over a period of 24 h by administration of a 25% suspension of CEL 261 03 I RB in vaseline. Control animals (3 females and 2 males) were treated similarly, but with the test material omitted in the induction phases. Mortality, bodyweights, clinical signs and dermal responses were evaluated.

Results and Conclusion One treated guinea pig died on day 8 of the study. It was the only animal to lose weight in the week following the intradermal injections, but no explanation for this death was provided by the author. There were no adverse clinical signs of toxicity or changes in bodyweights during induction or after challenge in the other animals. None of the animals showed signs of sensitisation. A recent positive control study from the laboratory resulted in 3/10 positive reactions (score 1, scattered mild redness). The compound CEL 261 03 I RB was considered non-sensitising to the skin of guinea pigs in this test.

3.3.10 Regent 800WDG Insecticide (800 g/kg fipronil)

The acute toxicity tests for Regent 800WDG are summarized in the Table below. In the oral study in rats, deaths occurred on days 1-2 after dosing. Signs of toxicity were evident up to day 4, and included hunched posture, lethargy, ataxia, piloerection, and decreased respiratory rate. With acute dosing via the dermal route, rabbits died or were killed *in extremis* 2-10 days after dosing. Signs of toxicity were evident up to day 7 and included clonic or tonic convulsions, weight loss, hunched posture, lethargy, increased respiratory rate and salivation, red/brown stains around the mouth, body tremors and vocalisation. No signs of skin irritation were reported. In rats following acute inhalational exposure, deaths occurred within 1 day of dosing. Signs of toxicity were evident up to

day 14 and included reduced bodyweight, wet fur, hunched posture, lethargy, piloerection, decreased respiratory rate, ptosis, ataxia, red/brown staining around the mouth, tremors, clonic convulsions, tiptoe or splayed gait, laboured respiration, exophthalmos, hypersensitivity, and diuresis. Pathological examination of decedents in the three studies revealed haemorrhagic/abnormal lungs, abnormal colouring/appearance of the liver and/or kidney and spleen, plus haemorrhagic and other changes to the GIT.

Route	Species, strain	Group Size	Doses Tested (mg/kg bw)	LD50 (mg/kg bw) or LC50 (mg/m^3)	Reference
Oral	Rats (SD)	5/sex	125, 250, 500 (gavage in distilled water)	149 (M); 203 (F); combined: 177 Deaths (M/F): 1/0 at 125, 5/4 at 250, all at 500	Allen (1994a) [GLP]
Dermal	Rabbits (NZ White)	5/sex	500, 707, 1000 (24 h, semi-occluded)	530 (M); 595 (F) Combined: 569 Deaths (M/F): 2/0 at 500, 4/5 at 707, all at 1000	Allen (1994b) [GLP]
Inhalation	Rats (SD)	5/sex	200, 550, 1000, 1810 (dust, 4 h, nose-only; MMAD <2.2 µm, GSD<0.49 µm)	400 (M); 1010 (F) Combined: 630 Deaths (M/F): 1/0 at 200, 4/2 at 550, 3/2 at 1000, 5/4 at 1810	Blagden (1994) [GLP]

Abbreviations: SD=Sprague-Dawley; M=male, F=female.

Allen DJ (1994c) EXP60720: Acute eye irritation in the rabbit. SLL Proj. No. 282/415. Lab: Safepharm Lab. Ltd., Derby UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 10.11.1993-18.11.1993. Unpublished report date: 8 February 1994. (QA/GLP: yes; Study Guidelines: US EPA 81-4)

Regent 800WDG Insecticide (0.1 mL, 76 mg) was instilled into the conjunctival sac of the right eye of 6 NZW rabbits, the left eye acting as the control. Diffuse corneal opacity was observed in 5/6 rabbits at 48 h, and in 2/6 at 72 h (degree of opacity grade 1, except for a single grade 2 observation in one animal at 48 h). The area of the cornea involved was mostly less than 25%, but occasionally 25-<50%. All corneal effects were reversed by day 7. Conjunctival redness and chemosis (grade 2 from 1 h, reducing to grade 1 at 48-72 h) resolved by day 7. Discharge (grades 1-2) occurred in all animals at 1 h, but in all cases this resolved by 48 h. Effects on the iris (grade 1) were noted for variable periods from 1 h to 48 h in 4 of the rabbits. Mean Draize scores calculated for effects to the cornea, iris, and conjunctivae over the 24 to 72 h period were 4.6, 1.3 and 6.2, respectively. The formulation was a moderate eye irritant in this test.

Allen DJ (1994d) EXP60720: Acute skin irritation in the rabbit. SLL Proj. No. 282/414. Lab: Safepharm Lab. Ltd., Derby UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 2.11.1993-5.11.1993. Unpublished report date: 8 February 1994. (QA/GLP: yes; Study Guidelines: US EPA 81-5)

Regent 800WDG Insecticide (0.5 g, moistened with 0.5 mL of distilled water) was applied to the shorn skin of 6 NZW rabbits for 4 h. Erythema (grade 1) was observed at 24 h in 5 of the animals, persisting until 48 h in one animal. Oedema (grade 1) was noted at 1 h in 3 rabbits. Scores at all other observation times were zero. Mean Draize scores calculated for erythema/eschar and oedema

formation over the 24 to 72 h period were 2.3 and 0, respectively. The product was a slight skin irritant in this study.

Allen DJ (1994e) EXP60720: Magnusson and Kligman Maximisation Study in the guinea pig. SLL Proj. No. 282/416. Lab: Safepharm Lab. Ltd., Derby UK. Sponsor: Rhone-Poulenc Agrochimie, 14-20 rue Pierre Baizet 69263 Lyon, France. Expt dates: 4.10.1993-15.11.1993. Unpublished report date: 8 February 1994. (QA/GLP: yes; Study Guidelines: US EPA 81-6)

Method The skin sensitisation potential of Regent 800WDG Insecticide was determined in guinea pigs (20 test and 10 control) using the maximisation test. Intradermal injections (0.1 mL each) comprised Freund's Complete Adjuvant (FCA) in distilled water (1:1), a 5% (w/v) dilution of the test material in distilled water, and a 5% (w/v) dilution of the test material in a 1:1 dilution of FCA in distilled water. A 75% dilution of the test material in distilled water was used for topical induction. Control animals were given the same treatment, with the omission of the test material. For the challenge, 25% and 10% dilutions of the test material in water were applied dermally to separate sites 21 days after the last induction.

Results and Conclusion Two test animals were found dead (days 12 or 24), but the cause of death was not determined. No skin reactions were noted in either the test or control animals at either 24 or 48 h after challenge. In this study, Regent 800WDG was not a skin sensitizer in guinea pigs.

4. SHORT-TERM REPEAT-DOSE STUDIES

4.1. Oral Administration

4.1.1 Mice

Holmes P (1990) M&B 46030: Preliminary toxicity study by dietary administration to CD-1 mice for six weeks. Report no: 90/RHA299/0325. Lab: Life Science Research Ltd, Eye, Suffolk, England. Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Expt. dates: 13.9.1989-26.10.1989. Unpublished report date: 21 December 1990. (GLP/QA: yes. Guidelines: none stated)

Materials and Methods CD-mice (12/sex/dose) received a daily dose of fipronil (Lot No: JJW 2092/1) at 0, 15, 40, 110, 300 or 800 ppm via the diet for 6 weeks. The mice (from Charles River, UK Limited Margate, Kent, England) were 3-4 weeks old and 15-20 g on arrival, and were acclimatised for 7 days before dosing. The concentration, homogeneity and stability of the test substance in the diet were confirmed by analysis. The achieved dose levels were 2.4/2.9, 6.5/8.2, 20.3/22.0 and 36.5/42.5 mg/kg bw/d for males/females at 15, 40, 110 and 300 ppm respectively (the dose at 800 ppm was not measured due to deaths of mice during week 1).

All animals were checked twice daily for clinical signs, moribundity and death. Detailed physical examinations were performed at least once weekly. Bodyweights and food consumption were recorded at weekly intervals. All mice killed at termination, or dying during the study period, were subjected to a detailed necropsy which included gross examination, weighing of selected organs (brain, heart, kidneys, liver, lungs with mainstem bronchi, spleen, testes and uterus with cervix). Histological examination of organs/tissues (adrenals, brain, heart, kidney, liver, sciatic nerve, spinal cord, testis, thyroid with parathyroid and urinary bladder) was conducted for all mice killed or dying during the study, all males at 0, 40 and 110 ppm and all females at 0 and 110 ppm; also

thyroids, parathyroids and liver from all males at 15 ppm and all females at 15 and 40 ppm; and brain and spinal cord from all females at 40 ppm.

Results Overactivity and irritability developed in mice at 110 ppm and above from week 2, and overactivity was also seen in 1 female at 40 ppm in week 3. Convulsions were noted in 3 males and 2 females at 800 ppm, and in 1 male at 300 ppm. Other signs were thin build, hunched appearance, piloerection, respiratory abnormalities, pallor, body tremors and abnormal gait and posture in mice at 300 ppm and above. All mice at 300 and 800 ppm, and 11 males and 4 females at 110 ppm died or were killed *in extremis* during the first 2 weeks, and 2 males at 40 ppm died during week 5. Food consumption was markedly depressed (up to ~50% of control) at 300 and 800 ppm, and was also reduced (up to 15-19%) in both sexes at 110 ppm as well as in males at 15 and 40 ppm. Consequently, bodyweight loss was seen at 300 and 800 ppm, and lower bodyweight gain (60-80% of control) occurred at 110 ppm, and in males at lower doses.

Table 26: Liver weight and pathology

	Males				Females			
Dose (ppm)	**0**	**15**	**40**	**110**	**0**	**15**	**40**	**110**
Number examined	12	12	10	1	12	12	12	8
Weight, g	2.21	2.29	2.42	3.31	1.45	1.67**	1.74*	2.01**
% body	5.93	6.66**	7.36**	9.83	5.53	6.31**	6.84**	8.08**
Periacinar hepatocytic hypertrophy	1		2					1
Focal necrosis			2					
Foci of leucocytes within sinusoids			1					2
Focal telangiectasis			2					
Extramedullary haemopoiesis							2	
Hepatocytic fatty vacuolation								
- periacinar		1+++					1+	
- centriacinar	4+, 2++	1+, 1++	1++		1+	1+	1+	1++
- panacinar		1+, 7++, 1+++	1+, 3++, 4+++	1+++	5+, 2++	6+	3+, 1++, 2+++	1+, 3++, 3+++

**p<0.01 by Bartlett's test followed by Behrens-Fisher test or Dunnett's test.
Severity code: + slight, ++ moderate, +++ marked.

Absolute and/or relative liver weights were dose-relatedly increased in all treated groups. As shown in the Table below, some histopathological changes, including hepatocytic hypertrophy, focal necrosis and foci of leukocytes were revealed in the livers of males at 40 ppm and females at 110 ppm (each being the highest dose group with a meaningful number of surviving animals). The incidence and/or severity of hepatocytic fatty vacuolation were increased relative to the control in all male groups and females at ≥40 ppm. A clear dose-response was not evident, but this may have been due to deaths early in the study, particularly in males at 110 ppm. A NOEL was not established in this study due to decreased bodyweight gain in males, and increased liver weight with histopathological abnormalities in all treated groups. The LOEL was 15 ppm (equal to 2.4 mg/kg bw/d).

4.1.2 Rats

Peters DH, Stuart V, Crook D, Gibson WA, Gopinath C & Hadley J (1990) M&B 46030: Toxicity to rats by dietary administration for 4 weeks. Report no: M&B 327/891321. Lab: Huntingdon Research Centre Ltd, Huntingdon, England. Sponsor: Rhone-Poulenc Ltd, Essex England. Expt. dates: 16.3.1989-14.4.1989. (GLP/QA: yes; Study Guidelines: EU 92/69/EEC, B7 1992) *(Summary report only; original raw data were not provided).*

Materials and Methods Sprague Dawley CD rats (5/sex/dose) were given 0, 25, 50, 100, 200 or 400 ppm of M&B 46030 (fipronil; Batch: IGB 464; Purity: 93%) in the diet for 4 weeks. The intake of the chemical was determined as 0/0, 3.4/3.5, 6.9/6.7, 12.6/12.9, 24.5/24.9 and 45.3/54.9 mg/kg bw/d for males/females in ascending order of dose. Homogeneity and stability of the test chemical in the diet were confirmed by analysis. Observations were made daily for clinical signs and mortality. The rats were approximately 35 days old, and weighed 187-234 g (males), and 156-193 g (females) at the start of the study. Ophthalmoscopy was conducted on all rats at pre-test, and on the control and 400 ppm groups in week 4. Bodyweight and food consumption were recorded at least weekly. Blood and urine samples were taken in week 4 for haematology, clinical chemistry or urinalysis. At necropsy, macroscopic abnormalities of the contents of the cranium (brain, pituitary and cranial nerves), thoracic and abdominal cavities were recorded, selected organs (no details) were weighed, and a range of tissues (no details) from the 0 and 400 ppm groups, plus all macroscopic abnormalities, were examined microscopically. The liver and thyroid were examined for all groups.

Results One female at 400 ppm died in week 1, without clinical signs prior to death or microscopic findings at necropsy. On day 5, rats at 200 and 400 ppm showed dose-related bodyweight loss (up to -16/-11 g in males/females), and those at 100 ppm had a lower weight gain (44/20% of control for males/females), corresponding to reduced food consumption (up to 46-50% of control) in these groups. No treatment-related ophthalmoscopic lesions were found.

Mean platelet counts were increased at 200 and 400 ppm, with a dose-response in males at these doses, suggesting a treatment-related effect (see Table below). Total protein and globulin levels were increased to a statistically significant extent in all treated groups, but as the response across the dose groups was virtually flat, it is considered unlikely that these apparent changes were treatment-related. Though the decrease in albumin in females and the increase in calcium in males seen at 400 ppm were statistically significant, the size of these changes (~4%) was not considered to have biological significance. Cholesterol levels were increased in males at 400 ppm and in all treated female groups, the latter largely in a dose-related manner. However, as this was not reported in the 13-week rat study (Holmes 1991b), and only at the highest dose (300 ppm) in the rat chronic study (Aughton 1993), this finding, at least at the lower doses in females, is not considered toxicologically significant.

Table 27: Findings in haematology and clinical chemistry (n = 5)

	Males						Females					
Dose (ppm)	**0**	**25**	**50**	**100**	**200**	**400**	**0**	**25**	**50**	**100**	**200**	**400**
Platelets ($x10^3$/mm)	954	1027	1145	1125	1211*	1531**	1213	1277	1315	1247	1422	1439
Total protein (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**
Globulin (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**
Albumin (g/dL)	3.3	3.4	3.3	3.3	3.3	3.2	3.3	3.4	3.4	3.3	3.2	3.2*
Calcium (mEq/L)	5.5	5.6	5.6	5.6	5.6	5.7*	5.5	5.5	5.6	5.6	5.6	5.6
Cholesterol (mg/dL)	85	83	93	86	81	110*	61	84**	106**	105**	115**	139**

*p<0.05; **p<0.01.

Absolute liver weights were increased in all treated groups (see Table below). Enlargement of the liver was noted in some rats, mainly at 200 and 400 ppm, with generalised minimal hepatocyte enlargement in groups from 100 ppm. Thyroid weights were increased in all treated female groups and two male groups, but lacked a dose response. However, at the microscopic level, thyroid follicular hypertrophy (generally minimal, but moderate in some males at 200 and 400 ppm) was found in most treated animals at all doses, and not in controls, so the increase in thyroid weight was considered likely to be treatment-related. A NOEL was not established in this study due to increased liver weights, and thyroid follicular hypertrophy at all doses tested. The LOEL was 25 ppm (3.4 mg/kg bw/d).

Table 28: Findings in organ weights and pathology (n = 5)

	Males						Females					
Dose (ppm)	**0**	**25**	**50**	**100**	**200**	**400**	**0**	**25**	**50**	**100**	**200**	**400**
Bodyweight (g)	372	378	379	363	345	337	252	240	245	230	232	230
Liver (g)	18.6	20.4	21.6	21.2	21.7*	25.6**	11.6	12.3	14.3	13.6	15.6	16.6
Thyroid (mg)	20.4	20.5	24.3	24.0	20.3	21.7	16.6	19.4	19.5	18.4	19.7	18.6
Liver enlargement				1		5			1		2	3
Hepatocyte enlargement				1+	3+	5+					2+	4+
Thyroid follicular hypertrophy		5+	4+	5+	3+, 2++	2+, 3++		3+	4+	3+	5+	4+

*p<0.05; **p<0.01. + minimal; ++ moderate. Pathology findings are expressed as numbers of animals with the finding.

4.1.3 Dogs

Holmes P (1991a) M&B 46030: Preliminary toxicity study by oral (capsule) administration to Beagle dogs for four weeks. Report no: 91/RHA297/0781. Lab: Life Science Res. Ltd, Eye, England; Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France; Expt. dates: 25.7.1989-6.9.1989. (GLP/QA: yes. Guidelines: None.) *(Summary only; original raw data were not provided)*

Method Beagle dogs (2/sex/dose) were treated with 0, 1, 10 or 20 mg/kg bw/d of M&B 46030 (Fipronil; Batch: JJW2092/1; Purity: 97.2%) by gelatin capsule for 4 (at 1 and 20 mg/kg bw/d) or 6

weeks (other groups). The dogs were 23-26 weeks of age, and 9.59-10.75 kg for males and 8.85-9.69 kg for females, prior to dosing. Observations were made daily for clinical signs, with a detailed examination performed weekly. A veterinary examination was conducted prior to the start of the study and after 3 weeks of dosing. A neurological examination was performed on all dogs before dosing commenced, and on dogs in the control and 10 mg/kg bw/d groups after 3 and 5 weeks of treatment. Food consumption was measured daily, and bodyweight twice weekly. Ophthalmoscopy, haematology and clinical chemistry (including thyroid hormones) were performed before dosing and after 3 weeks of treatment. At necropsy, the dogs were given gross external and internal examinations (cranial, thoracic, abdominal and pelvic cavities and their viscera), selected organs (not specified) were weighed, and tissues (brain, spinal cord, liver, kidneys, sciatic nerve and thyroid with parathyroids) from each dog were examined histopathologically.

Results There were no deaths. At 20 mg/kg bw/d, 1 male and 1 female exhibited underactivity, hunched posture and thin appearance (the latter in the male only) during week 2, as well as other neurological signs including head nodding, facial twitching, continuous swallowing and abnormal posture or jerking of forelimbs. The female showed signs of a possible convulsive episode on day 9. Head nodding was also noted in 1 female at 10 mg/kg bw/d during weeks 4 and 5, and outstretched forelimbs during week 4. At 20 mg/kg bw/d, bodyweight losses of 0.8-1.5 kg, were seen in 1 male and 2 female dogs, particularly during the first 3 weeks, associated with markedly reduced food consumption (18-22% of control) during weeks 2 to 3.

After 3 weeks of dosing, neurological examinations revealed exaggerated flexor reflexes in 1 male and 1 female at 20 mg/kg bw/day, and occasional mild head jerks in the male also. One female at 10 mg/kg bw/d exhibited a grossly abnormal head nodding episode during the examination. Two males and 1 female at 20 mg/kg bw/d and 1 female at 10 mg/kg bw/d had increased red cell parameters (Hb and RBC). A slightly higher plasma albumin level with associated higher protein level was found at 20 mg/kg bw/d. There were no abnormal findings in organ weights, or macro- and microscopic pathology, but emaciation was seen in 1 male at 20 mg/kg bw/d. The NOEL was 1 mg/kg bw/d based on neurotoxicological signs as well as increased RBC and Hb at ≥10 mg/kg bw/d.

4.2 Dermal Application

Hermansky SJ & Wagner CL (1993) M&B 46030: Twenty-one day repeated cutaneous dose toxicity study in New Zealand White rabbits. Study no: 92N1165. Lab: Bushy Run Research Centre, Union Carbide Chemicals and Plastics Company Inc, Export, Pennsylvania, USA. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park NC. Expt. dates: 9.11.1992-10.11.1992. Unpublished report date: 23 June 1993. (GLP/QA: yes; Study Guidelines: none stated)

Materials and Methods New Zealand White rabbits (6/sex/group; from Hazeleton Research Products, Denver, PA USA) were treated cutaneously with fipronil (Lot No. 78/GC/90, purity 96.7%) suspended in a 0.5% solution of carboxymethylcellulose (CMC) in water at doses of 0, 1, 5 or 10 mg/kg bw/d. The dose volume for all groups was 1 mL/kg bw. Rabbits in the control group received the same volume of CMC in water. Seven days prior to dosing, hair was clipped from the dorsal area of the trunk from the scapular region to the rump, and the animals wrapped to simulate study conditions. Animals that did not adapt were excluded from the study. The dosing suspension was applied directly to the entire application site, and covered with a gauze pad, and the dosing site

occluded. Fifteen applications (6 h/d) were made over a 21-day period. Observations were made twice daily for mortality. Detailed clinical examinations, including dermal reactions (Draize system) were on a daily basis. Bodyweight was measured on the day of study initiation, then on days 8, 15 and 21, and food consumption was measured every 2 days. Immediately prior to sacrifice, blood was taken for haematology and clinical chemistry testing. Liver, kidneys, adrenals and testes/ovaries were weighed and tissues retained. All of these procedures were essentially as listed in Appendices III and IV.

Results No rabbits died, and there were no treatment-related changes in haematology, clinical pathology, organ weights, or macroscopic or microscopic pathology. Near the end of the study, one male and one female from the 10 mg/kg bw/d group exhibited extreme hyperactivity. In this group, the mean bodyweight of the males, and the mean bodyweight gain of both sexes were decreased throughout the study. (Bodyweight gain (g) 225/252, 269/318, 257/360, 311/346, -47**/125 for males/females in increasing order of dose; **p<0.01). Food consumption in the high dose group was decreased in the latter half of the study (male/female: 44/22%). There were no other treatment-related findings. No effects were observed at 5 mg/kg bw/d.

5. SUBCHRONIC STUDIES

5.1. Oral Administration

5.1.1 Rats

Holmes P (1991b) M&B 46030: Toxicity study by dietary administration to CD rats for 13 weeks. LSR90/RHA298/0781. Life Science Research Limited, Suffolk, UK. GLP/QA: yes.

Materials and Methods Rats (10/sex/group) received fipronil (Batch: PGS963; Purity: 95.4%) continuously in the diet at concentrations of 1, 5, 30 or 300 ppm (equal to 0.07/0.07, 0.33/0.37, 1.93/2.28 or 19.9/24.0 mg/kg bw/d for males/females respectively, by analysis) for 13 weeks.

Results There were no deaths. The appearance and behaviour of rats in the 300 ppm group were considered unaffected by treatment, though food intake and bodyweight gains were depressed in the first week. After 12 weeks at 300 ppm, neurological and ophthalmoscopic examinations revealed no treatment-related changes. At 300 ppm, relative to controls, males showed slightly lower Hb concentrations and higher urea levels, and females had a slightly lower PCV, mean cell volume, mean cell Hb, prothrombin time and higher platelet count. Plasma protein profiles were altered in both sexes (see Table below), but as the differences were small and the dose responses were flat across a wide concentration range in many cases, they were considered of dubious toxicological significance at the lower doses. This conclusion is supported by findings of increased total protein in the chronic rat study (Holmes 1991a), at the highest dose of 300 ppm (13 mg/kg bw/d), with no change in this parameter at 30 ppm (1.3 mg/kg bw/d). Decreased plasma AST and ALT were seen in females from doses of 1 and 30 ppm respectively, but this is not of clinical relevance. Increased plasma glucose levels in females at ≥5 ppm lacked a dose response, so were considered incidental to treatment. Urinalysis revealed no treatment-related abnormalities.

Table 29: Clinical chemistry findings (mean, n = 10)

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

*p<0.05; **p<0.01; ***p<0.001.

Absolute and relative liver weights were increased at ≥30 ppm (see Table below). Fatty vacuolation of the liver was seen in all groups, including controls, but with the exception of an increased incidence of panacinar vacuolation in 300 ppm males, was not considered treatment-related. Absolute and relative thyroid weights were increased in both sexes at ≥30 ppm, with an increased incidence of follicular cell hypertrophy and hyperplasia in both sexes at 300 ppm. The NOEL was 5 ppm, equal to 0.3 mg/kg bw/d, due to increased liver and thyroid weights at 30 ppm (2 mg/kg bw/d).

Table 30: Findings in organ weights and pathology (mean, n = 10)

	Males					Females				
Dose (ppm)	**0**	**1**	**5**	**30**	**300**	**0**	**1**	**5**	**30**	**300**
Liver (g)	19.1	21.0	19.4	21.8	27.2**	10.8	11.3	12.7*	13.4**	16.6**
(% body)	3.54	3.72	3.59	3.99*	5.05**	3.52	3.48	3.86	4.13**	5.57**
Thyroid (mg)	0.024	0.024	0.025	0.030	0.048**	0.019	0.019	0.021	0.023*	0.032**
(% body x 100)	0.44	0.42	0.46	0.54	0.91**	0.61	0.59	0.63	0.71	1.07**
Liver -panacinar fatty vacuolation		2		1	7**					1
-centriacinar fatty vacuolation	4	3	2	6	3	7	9	6	10	7
Thyroid -follicular epithelial hypertrophy	3	1		5	8	1				10***
-follicular cell hyperplasia	2			1	6			1	1	2

**p<0.05; **p<0.01.*

5.1.2 Dogs

Holmes P (1991c) M&B 46030: Toxicity study by oral (capsule) administration to Beagle dogs for 13 weeks. Study No: RHA/310/46030. Amendment to final report: 93/RHA310/0150. Lab: Pharmaco-LSR Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt dates: 5.2.1990-11.5.1990. Unpublished final report date: 21 November 1991; First amendment 23 February 1993. (GLP/QA: yes; Study Guideline: 82-1)

Methods Fipronil was administered in gelatin capsules to groups of Beagle dogs (4/sex/group) at doses of 0.5, 2.0 or 10 mg/kg bw/d for 13 weeks. A control group received empty capsules.

Results In dogs receiving 10 mg/kg bw/d, significant signs of toxicity included convulsions, tremors, head-nodding, hunched posture, underactivity, inappetence, emaciation, disorientation,

ataxia, apparent blindness, irregular heart rate, limb jerks and constricted pupils. In the 2 mg/kg bw/d group, inappetence was observed at times in two females, along with decreased food consumption and decreased bodyweight gain. At 10 mg/kg bw/d, 1 male and 3 females were killed during the second week of treatment, following inappetence, weight loss and/or severe clinical signs. One of the surviving males in this group also exhibited marked weight loss.

Blood chemistry tests after 6 and 12 weeks revealed increased ALP and decreased cholesterol levels at 10 mg/kg bw/d. Haematology and urinalysis gave no clear evidence of treatment-related effects. Clinical and neurological examinations revealed no findings of toxicological significance. Macroscopic examination was unremarkable. Microscopically, follicular and perifollicular atrophy of the mesenteric lymph nodes were observed in one dog at 10 mg/kg bw/d, and cortical atrophy of the thymus in another. The NOEL was 0.5 mg/kg bw/d due to clinical signs (inappetence) and decreased bodyweight gain at 2 mg/kg bw/d and above.

6. CHRONIC AND CARCINOGENICITY STUDIES

6.1 Oral administration

6.1.1 Mice

Broadmeadow A (1993) M&B 46030: Oncogenicity study by dietary administration to CD-1 mice for 78 weeks. Study no: 92/RHA313/0971. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 16.10.1990-5.5.1992. Unpublished report date: 9 March 1993. (GLP/QA: yes. Guideline: US EPA 83-5)

Materials and Methods Charles River CD-1 strain mice (72/sex/group) received fipronil (purity 95.4%) in the diet at levels of 0, 0.1, 0.5, 10 or 30 ppm, equal to (males/females) 0/0, 0.01/0.01, 0.05/0.06, 1.2/1.2, 3.4/3.6 mg/kg bw/d. Twenty/sex/group were treated for 53 weeks (toxicity phase) and 52/sex/group for 78 weeks (oncogenicity phase).

Results There were no treatment-related clinical signs. Weight gains of mice in the 30 ppm group were lower than those of controls (14% and 19% lower for males and females, respectively). Weight gains of males and females receiving 10 ppm were lower than controls for the first 13 weeks. After 76 weeks, a slightly lower proportion of neutrophils and a slightly higher proportion of lymphocytes were observed in females receiving 30 ppm. This trend was not apparent at 50 weeks; neither was it seen in males.

A sixth group of 72/sex fed 60 ppm fipronil had low weight gains and a high death rate (14 males, 7 females) by week 9, with death preceded by convulsions in 3 males. Necropsies on mice that died were unremarkable, and survivors were killed without necropsy in week 10. In all other groups including control, there were 6-9 deaths/40 mice in the toxicity phase, and 40-52 deaths/104 mice in the oncogenicity phase. Mortality was not treatment-related.

Both sexes treated at 30 ppm, and males receiving 10 ppm, generally had higher liver weights, both relative and absolute, than controls. Mice receiving 60 ppm that died also had higher liver weights relative to bodyweights. There was a statistically significant increase in the incidence of periacinar microvesicular vacuolation in the livers of males treated at 10 or 30 ppm in both the toxicity and oncogenicity phases (see Table below). This abnormality was seen in some females treated at ≥0.5 ppm, but as there was no clear relationship to dose across a broad dose range, this was not considered to represent a treatment-related effect. In the oncogenicity phase, male mice treated at ≥10 ppm showed an increased incidence of chronic degenerative changes in the liver, including

necrosis of occasional cells and apoptosis, increased ploidy, hypertrophy and degeneration of periacinar hepatocytes, chronic inflammation and bile stasis.

Male mice in the oncogenicity phase receiving 30 ppm showed a higher (but not statistically significant) incidence of malignant hepatocellular tumours. One hepatocellular carcinoma was also found in a 30 ppm mouse in the toxicity phase of the study. The incidence among controls was, however, below that observed in recent studies (range 2.9-25%), while that in treated groups was well within this range. The findings in this study were therefore not considered to be treatment-related. There was no treatment-related trend for the incidence of hepatocellular adenomas. The NOEL was 0.5 ppm equal to 0.05 mg/kg bw/d, based on increased liver weights and microscopic changes to the liver in males at 10 ppm (1.2 mg/kg bw/d).

Table 31: Histopathology findings in the liver

		Males					Females				
Dose (ppm)		**0**	**0.1**	**0.5**	**10**	**30**	**0**	**0.1**	**0.5**	**10**	**30**
Periacinar microvesicular vacuolation	Tox.	0	2	2	8**	12**	1	1	4	1	4
	Onco.	6	8	9	17*	16*	1	1	7	4	8*
Chronic degenerative changes	Onco.	14	16	12	21	25*	12	7	10	10	8
Hepatocellular carcinoma	Tox.	0	0	0	0	1	0	0	0	0	0
	Onco.	1	1	2	1	5	0	0	0	0	0
Hepatocellular adenoma	Onco.	10	3	2	6	6	0	0	0	0	1

Tox.= toxicity phase (n=20); Onco. = oncogenicity phase (n=52). Numbers represent totals for animals that survived to the end of each treatment period and premature deaths.

6.1.2 Rats

Aughton P (1993) 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. Study no: 92/RHA432/0166. Lab: Life Science Research Limited, Eye, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 2.1.1991-9.10.1992. Unpublished report date: 11 June 1993. (GLP/QA: yes; Study Guideline: 83-5)

Materials and Methods Fipronil (purity 95.4%) was administered in the diet to Charles River CD strain rats. The study was in three phases:

(i) Oncogenicity phase 50/sex/group were fed concentrations of 0, 0.5, 1.5, 30 or 300 ppm for their lifespan (89 weeks for males; 91 weeks for females). The duration was intended to be 104 weeks, but was shortened because of poor survival.
(ii) Toxicity phase 15/sex/group were treated at the same levels as above until sacrifice at 52 weeks.
(iii) Reversibility phase 15/sex/group were treated at the same levels as above for 52 weeks, then received an untreated diet for the following 13 weeks, after which they were killed. Plasma levels of thyroxin (T4), triiodothyroxine (T3) and thyroid-stimulating hormone (TSH) were measured after 1, 4, 12, 24 and 52 weeks of treatment, as well as after 2, 4, 7 and 11 weeks of recovery.

Results and Conclusion The actual achieved doses were 0, 0.02, 0.06, 1.3 and 13 mg/kg bw/d for males; and 0, 0.03, 0.08, 1.6 and 17 mg/kg bw/d for females. Several rats in groups treated at ≥1.5 ppm showed convulsive episodes, mostly during the first few weeks of treatment. During the treatment period, females at 300 ppm, and to a lesser extent at 30 and 1.5 ppm, showed signs of irritability, hyperactivity, vocalisation, salivation, aggressive behaviour and teeth grinding. These signs disappeared following cessation of treatment.

During the early part of the study, the number of rats that died or were killed was slightly increased at 300 ppm, but thereafter, mortality rate was not related to treatment. Food intakes were broadly similar in test and control groups. During the treatment period, bodyweight gain at 300 ppm was markedly lower than controls during the treatment period and at 30 ppm was slightly lower for females. There was no effect of treatment on the bodyweight gain of males and females receiving 0.5 or 1.5 ppm.

At times, rats receiving 1.5 ppm or more had decreased erythrocyte parameters, but as these changes were small, and they did not occur consistently throughout the treatment period and/or dose responses were absent, they were not considered toxicologically significant. Prothrombin time was slightly decreased in 300 ppm rats and in some 30 ppm females, but was not considered clinically significant. Blood chemistry changes of possible toxicological significance were high cholesterol values, high calcium concentrations, high total protein concentrations, low albumin and high α- and β-globulin concentrations, and low albumin:globulin ratios for the 300 ppm group. These changes were still evident in 300 ppm females at the end of the recovery period.

Circulating T4 levels were consistently lower than control values in a dose-related manner at ≥1.5 ppm, the effect being particularly marked after 1 week of treatment in 300 ppm rats, at which time T4 was undetectable in the serum. At 0.5 ppm, T4 levels were less than controls in males in weeks 4, 12 and 24 and in females in weeks 1, 4 and 12, but not at other times. As this difference did not persist till the end of the study, it was not considered toxicologically significant at this dose. Eleven weeks after cessation of treatment, T4 levels had recovered in all affected groups. Serum values of T3 in 300 ppm males were slightly lower than in controls after the first week of treatment only. TSH concentration was higher in treated rats receiving 300 ppm and for males receiving 30 ppm in weeks 1, 4 and 50, but not weeks 12 and 24. Due to the inconsistent findings for this parameter at 30 ppm, it was not considered a clear effect of treatment at this dose. After cessation of treatment, TSH levels of females (300 ppm) and males (30 ppm) recovered within one week. For 300 ppm males, progress towards recovery was evident, although not total by the end of the reversibility period.

Marginally lower urinary pH and a tendency towards higher urinary protein values were evident in 30 and 300 ppm rats. High urine volumes, associated with low specific gravities, were noted prior to termination of the study. These changes were attributed by the author to increased severity of progressive senile nephropathy, that is, enhancement of an age-related change. In support of this, pale granular and/or large kidneys were described at necropsy, with typical histopathological lesions of nephropathy. In the 1.5 ppm group, the severity of progressive senile nephropathy was greater than in controls, but this was not clearly related to treatment. At the higher doses, both the incidence and severity of this lesion were relatively increased, and may have been treatment-related. Senile nephropathy was still apparent after the recovery period.

In rats killed after 52 weeks of treatment, absolute and relative liver weights were significantly increased in the 300 ppm group. At 30 ppm, only the relative weight of the liver was increased. After 52 weeks of treatment, absolute and relative thyroid weights were increased at ≥30 ppm. With the exception of 300 ppm males, and consistent with the findings for TSH levels, this was

reversible after the 13 week recovery period. After treatment for 89-91 weeks, absolute and relative thyroid weights were increased in males at ≥1.5 ppm and females at ≥30 ppm. Absolute thyroid weight was significantly increased in 0.5 ppm males, but as there was no corresponding increase in relative weight, this change was not considered toxicologically significant at this dose.

Treatment-related neoplastic findings were restricted to the thyroids: in rats treated for 1 year and assigned to the reversibility period, six (4/15 from the 300 ppm group) had follicular cell tumours. When all rats assigned to the oncogenicity phase were considered together (irrespective of time of death), there was a significant increase in benign follicular cell adenomas for males (12/50) and females (8/50) receiving 300 ppm, and for males receiving 30 (3/50) or 1.5 (5/50) ppm. The incidence of follicular cell carcinomas was also increased in male (5/50) and female (2/50) rats receiving 300 ppm compared with controls (0/100). The apparent high incidences of thyroid follicular tumours in males receiving 30 or 1.5 ppm are well within the expected background range for recent studies at the laboratory (up to 7.2%) and reflect an abnormally low incidence in the controls. These tumours are not considered relevant to humans (see Discussion). The NOEL was 0.5 ppm, equal to 0.02 mg/kg bw/d, due to clinical signs of neurotoxicity, increased thyroid weight, decreased T4 levels, and increased severity of progressive senile nephropathy at 1.5 ppm, equal to 0.06 mg/kg bw/d.

6.1.3 Dogs

Holmes P (1992) M&B 46030: Toxicity study by oral (capsule) administration to Beagle dogs for 52 weeks. Study no: 92/RHA311/0464. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 15.1.1991-27.1.1992. Unpublished report date: 16 November 1992. (GLP/QA: yes; Study Guideline: US EPA 82-1)

Materials and Methods Fipronil (Batch: PGS963; Purity: 96.8%) was given by capsule (in lactose from day 16 to increase accuracy of dosing) to Beagle dogs (6/sex/group) at doses of 0, 0.2, 2 and 5 mg/kg bw/d for 52 weeks.

Results and Conclusion Following severe reaction to treatment, 1/6 males receiving 2 mg/kg bw/d and 2/6 males receiving 5 mg/kg bw/d were killed. Signs of neurological disturbance (convulsions, tremors, stiff limbs, gait abnormalities, lack of coordination, nervous behaviour), as well as inappetence and weight loss were observed in these dogs in weeks 11 and 31/34, respectively. Bodyweight losses averaging 1 kg occurred in the 2-6 days before euthanasia in these animals. All dogs receiving the high dose, and 5 males and 3 females receiving 2 mg/kg bw/d, showed intermittent signs of neurological disturbance (tenseness, nervous behaviour, hyperaesthesia, stiffness, abnormal gait and twitching of the facial muscles). All the dogs that were killed exhibited convulsions, as did 1 female at 2 mg/kg bw/d. One female at 0.2 mg/kg bw/d was hyperactive for weeks 13-18 of treatment, and also lost bodyweight. As this behaviour was not characteristic of responses seen at higher doses, and only one survivor at a higher dose lost weight, the effects observed in this dog were considered unlikely to be treatment related. Otherwise, with the exception of 1 female at 5 mg/kg bw/d, bodyweight gain in the survivors was similar to that of the controls. Food consumption of treated dogs that survived to the end of the study was generally similar to that of controls.

The haematology profile, blood chemistry and urinalysis of surviving dogs were considered to be unaffected by treatment. There were no ophthalmic abnormalities, and organ weights were not affected. Haematology of those killed demonstrated slight haemoconcentration, possibly signifying dehydration. Blood chemistry revealed slightly higher ALT and AST activities in the two males at 5

mg/kg bw/d, and marginally higher plasma levels of urea, total bilirubin and cholesterol for the male receiving 2 mg/kg bw/d, providing evidence of liver toxicity.

Although toxicity, including mortality, was observed at doses of 2 and 5 mg/kg bw/d, no macroscopic or microscopic pathology was noted that could be related to treatment. In particular, there were no morphological abnormalities of the nervous system to account for the neurological signs recorded. The NOEL was 0.2 mg/kg bw/d, based on clinical signs of neurotoxicity and bodyweight loss at 2 mg/kg bw/d.

Holmes P (1993) M&B 46030: Toxicity study by dietary administration to beagle dogs for 52 weeks. Report no. 93/RHA465/0243. Lab: Pharmaco-LSR Ltd. Eye, Suffolk, England. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Unpublished report date: 12 October 1993. Expt. dates: 30.12.1991-7.1.1993. (GLP/QA: yes. Study Guideline: US EPA 82-1)

Methods Groups of beagle dogs (5/sex/dose) received fipronil (Batch: PGS963, Purity: 96.8%) at 0, 0.075, 0.3, 1 or 3/2 mg/kg bw/d via the diet for 52 weeks. The high dose group received 3 mg/kg bw/d for the first 38 days, followed by a reduction in dose to 2 mg/kg bw/d in view of significant toxicity at the higher dose. The dogs were obtained from Interfauna UK Ltd., Wyton, Huntingdon, Cambridgeshire, England, and were 17-20 weeks old and 6.23-12.99 kg on arrival. The concentration (91.9-96.7% of the intended concentrations), stability and homogeneity of the test material in the diet had been demonstrated.

All animals were checked for clinical/behavioural signs, moribundity and death at intervals throughout each working day. Detailed veterinary examinations were performed before treatment commenced, and after weeks 11, 23, 35 and 47. Specific neurological examinations were performed before the initiation of treatment and after weeks 12, 24, 37 and 50. These included general reactions, cranial nerve reflexes (pupillary light, consensual light, palpebral blink, corneal reflex), segmental reflexes (flexor), and postural reactions (placing, visual, tactile). Bodyweights and food consumption were recorded at weekly intervals. Ophthalmological examinations were performed before treatment and after weeks 12, 24 and 50 in all animals. Blood samples were taken by jugular vein puncture before the start of treatment and after weeks 12, 24 and 50 for haematological and plasma chemistry analyses, as listed in Appendix III with the omission of gamma glutamyl transpeptidase, globulin and LDH. Plasma samples taken before treatment, and after weeks 1 and 13, were assayed for T3 and T4. Urine was collected before treatment and after weeks 11, 23 and 49 for analysis (as in Appendix III, plus nitrite). Plasma taken from all animals after 1, 13, 24, 38 and 50 weeks of treatment was analysed for fipronil and MB 46136 (fipronil sulfone). Survivors were killed at week 53 and subjected to necropsy. Animals found dead were also necropsied. Necropsy included gross examination, and organ weighing (as in Appendix IV, plus lungs with mainstem bronchi, pituitary, prostate with urethra sample, thymus, and uterus with cervix). A number of organs/tissues were sampled for histological examination in all groups (as in Appendix IV, plus anus, larynx and submaxillary gland). Statistical analyses were conducted on the bodyweight gain, clinical chemistry, haematology, urinalysis and organ weight data.

Results and Conclusion One female at 3 mg/kg bw/d was killed on day 32, having displayed signs of neurological disturbance from day 10, including convulsive episodes (days 11 and 30), underactivity, prostration, slow respiration and body tremors. Neurological examination showed absence of visual placing reactions, depressed menace and startle reactions, and abnormal gait. Relative to controls, high levels of Hb, Hct, RBC, ALP, total protein and cholesterol were observed in this dog, and liver weight was slightly increased, but significant micropathological findings in the liver or other organs were not observed.

From week 1 onwards, convulsions, head nodding, extensor rigidity of limbs, and/or twitching or tremors of muzzle, pinnae or facial muscles were observed in 3 males and 1 female at 3/2 mg/kg bw/d (these signs continued following dose reduction). At 1 mg/kg bw/d, 1 female had twitching of muscles of the whole body in Week 13, and another showed limb extensor rigidity in Week 20. Some dogs at 1 and 2/3 mg/kg bw/d had periods of inappetence, but bodyweight gains were not significantly affected. Neurological examinations showed exaggeration of the flexor (withdrawal) response one or more times in 1 male at 2 mg/kg bw/d, and in 2 males at 0.3 mg/kg bw/d. Isolated incidences of exaggeration of the tonic reflex were also noted in 1 male at 2 mg/kg bw/d in week 13, and in another at 0.075 mg/kg bw/d in week 51. Due to the lack of a dose response, these findings were not attributed to treatment.

There were no treatment-related effects on ophthalmoscopy, haematology, clinical chemistry (including plasma levels of T3 and T4), or urinalysis parameters. Analysis of plasma at all test points from week 1 revealed dose-related concentrations of the parent compound and MB 46136 (fipronil sulfone), and these did not change markedly over the treatment period. Fipronil and MB 46136 (fipronil sulfone) levels were essentially similar in males and females, with fipronil sulfone levels exceeding the corresponding level of fipronil.

Table 32: Plasma concentrations of fipronil and MB 46136 (fipronil sulfone) (ng/mL) (n = 4-5)

Dose (mg/kg bw/d)		Males				Females			
		0.075	**0.3**	**1.0**	**3.0/2.0**	**0.075**	**0.3**	**1.0**	**3.0/2.0**
Week 1	**Fipronil**	< 23	64	251	543	< 24	74	208	655
	MB 46136 (fipronil sulfone)	38	254	858	2255	< 33	120	461	1925
Week 50	**Fipronil**	31	105	328	525	33	101	303	538
	MB 46136 (fipronil sulfone)	43	424	1006	1903	< 69	221	822	1686

Results expressed as means.

Absolute and relative spleen weights were increased (24-26%) in males at 3/2 mg/kg bw/d, with a higher incidence of swollen or large spleens, and hyperplasia of the splenic red pulp (3/5 vs 1/5 in control and other groups). The NOEL was 0.3 mg/kg bw/d based on clinical signs of neurotoxicity at 1 mg/kg bw/d.

7. REPRODUCTION STUDY

7.1 Rats

King VC (1992) M&B 46030 Reproductive performance study in rats treated continuously through two successive generations. Study No: 92/RHA425/0309. Lab: Life Science Research Limited, Eye, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 22.10.1990-11.7.1991. Unpublished report date: 26 June 1992. (GLP/QA: yes; Study Guideline: US EPA 83-4)

Materials and Methods Fipronil (95.4% purity) was administered to two generations of Charles River CD strain rats at dietary levels of 0, 3, 30 or 300 ppm, respectively equal to (males/females) 0/0, 0.25/0.27, 2.5/2.7 and 26/28 mg/kg bw/d, which represent the average intake of test material in the 10-week pre-pairing periods for both parental generations. The F_0 generation comprised 30/sex/group. A further 30/sex/group were selected from F_1 litters to form the F_1A generation. To

provide additional data, F_0 rats receiving 0, 3 and 30 ppm were mated again to produce an F_1B generation.

Results Seven F_0 300 ppm rats (2 males, 5 females) died or were killed for humane reasons. Most had exhibited convulsions. A further two females had convulsions but survived. One 30 ppm male was killed after showing decreased muscular control. Food intake and bodyweight gain were reduced in F_0 rats receiving 300 ppm, as was mean litter size at day 1 *post partum* and pup viability up to day 4. Convulsions were observed in 13 offspring in 9 litters. The bodyweight of 300 ppm F_1 offspring at day 1, and subsequent weight gain to weaning, were reduced, and tooth eruption was delayed. Litter size and viability of offspring in the 3 and 30 ppm groups was similar to that of controls, as was survival. Necropsy of F_0 adults and F_1 offspring revealed no gross changes attributable to treatment. Liver and thyroid weights were increased in F_0 males and females receiving ≥30 ppm, and ovarian weight was lower at 300 ppm. Livers of 300 ppm females showed increased centriacinar fatty vacuolation. Thyroids of 30 ppm males, and of both males and females receiving 300 ppm, showed follicular epithelial hypertrophy.

F_1 generation

Four females died or were killed for humane reasons (300 ppm, 3 females; 30 ppm, 1 female). At 300 ppm, bodyweight gain was reduced; mating performance was slightly reduced, with a consequent reduction in fertility index; F_2 offspring had a reduced post-implantation survival index (81% *vs* controls 90%) and viability index at day 4 *post partum* (73% *vs* controls 98%); four offspring in three litters showed convulsions; mean bodyweight at day 1 *post partum* and pup weight gain to weaning were lower; and there was a slight delay in unfolding of the pinna. The post-implantation survival index was also slightly reduced at 3 (84%) and 30 (85%) ppm compared with controls (90%), but postnatal viability was unaffected. Given the slightness of the reduction in post-implantation survival at 3 and 30 ppm relative to the control, and the flat dose response, this was not considered a toxicologically significant finding. Necropsy of F_1 adults and F_2 offspring revealed no gross changes attributable to treatment. Liver and thyroid weights were increased at both 30 and 300 ppm in F_1 males and females, and pituitary gland weight was reduced in females at 30 and 300 ppm. Livers of 300 ppm females showed increased centriacinar fatty vacuolation. Thyroids showed increased follicular epithelial hypertrophy in males from all treated groups (2/30 at 3 ppm, equal to 0.25 mg/kg bw/d), and in 30 and 300 ppm females. This change was not present at 3 ppm in the F_0 adults, and was observed in the 13 week rat dietary study at 20 mg/kg bw/d, but not at 2 or 0.3 mg/kg bw/d. Also, at 52 weeks in the chronic rat study, thyroid follicular cell hypertrophy was not reported, but thyroid follicular cell tumours occurred in several rats at 13 mg/kg bw/d, but not at 1.3 mg/kg bw/d. As the thyroid follicular cell hypertrophy seen at 3 ppm in the F_1 appears to be an isolated finding at that dose, it is unlikely that it was related to treatment. Therefore, the parental NOEL was 3 ppm (equal to 0.25 mg/kg bw/d), due to increased thyroid and liver weights, decreased pituitary weight, and increased incidence of follicular epithelial hypertrophy of the thyroid at 30 ppm (equal to 2.5 mg/kg bw/d). The NOEL for effects on the offspring was 30 ppm, equal to 2.5 mg/kg bw/d, due to clinical signs of neurotoxicity, reduced pup viability and bodyweight gain, and developmental delay at 27 mg/kg bw/d. The reproductive NOEL was 30 ppm (equal to 2.5 mg/kg bw/d) due to reduced litter size and pup viability in the F_0 and a reduction in mating performance and fertility index in the F_1 at 27 mg/kg bw/d.

8. DEVELOPMENTAL STUDIES

8.1 Rats

Brooker AJ and John DM (1991) the effect of M&B 46,030 on pregnancy of the rat. Study no: M&B 335+326/90582. Lab: Huntington Research Centre, Cambridgeshire, UK. Sponsor: Rhone-Poulenc, Dagenham, Essex UK. Expt. dates: 4.9.1989-19.9.1989. Unpublished report date: 13 August 1991. (GLP/QA: yes, Study Guidelines: not stated)

Specific pathogen free, female Charles River rats (CD^R(SD)BR VAF/Plus strain) (5/group) were administered 0, 1, 4 or 20 mg/kg bw/d fipronil (93% purity) in 0.5% methylcellulose by gastric intubation on days 6-15 of pregnancy. On day 20 the rats were killed, litter values determined, and foetuses preserved for subsequent examination.

Dams in the 20 mg/kg bw/d group showed slightly increased water consumption during days 8-20, reduced food consumption for days 6-11, and reduced bodyweight gain for days 6-10. Maternal toxicity was not observed at 1 or 4 mg/kg bw/d. There were no treatment-related effects on litter values or on the incidence of abnormal offspring. Fipronil at 20 mg/kg bw/d caused maternal toxicity, but did not cause embryo- or foetotoxicity, or affect embryofoetal development. The maternal NOEL was 4 mg/kg bw/d due to reduced bodyweight gain at 20 mg/kg bw/d. The developmental NOEL was 20 mg/kg bw/d, the highest dose tested.

8.2 Rabbits

King VC (1990) M&B 46030: Teratology study in the rabbit. Report No: 90/RHA321/0722. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Expt. dates: 8.3.1990-10.7.1990. Unpublished report date: 29 November 1990. (GLP/QA: yes. Study Guideline: US EPA 83-3)

Fipronil (purity 95.4%) was administered by oral gavage to groups of 22 pregnant NZW rabbits on gestation days 6-19 at doses of 0, 0.1, 0.2, 0.5 or 1 mg/kg bw/d, in 0.5% w/v aqueous methylcellulose mucilage and 0.5% w/v Tween 80. Dams were killed on day 29 and their uterine contents examined.

Maternal bodyweight gain was reduced in all treated groups at the beginning of the dosing period (gestation days 6-10: 110±50, 50±40***, 70±60*, 60±50***, 60±50**g in increasing order of dose; *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$), and was associated with decreased food consumption. However, neither of these parameters showed a dose response for this period. When the entire dosing period was considered, only rabbits at ≥0.5 mg/kg bw/d showed weight gain that was less than the control group to a statistically significant extent (gestation days 6-20: 300±100, 220±120, 220±230, 150±150**, 90±170***), and as a dose response was present for the full treatment period, this was considered to represent a toxicological effect. The two lower dose groups gained weight at a similar rate to controls after the initial few days of the dosing period.

One control female and one female in each of the 0.1 and 1 mg/kg bw/d groups exhibited total litter resorption on gestation day 29. One 0.1 mg/kg bw/d female aborted during the study. Embryo and foetal survival, growth, and morphological development *in utero* were unaffected by treatment with fipronil at doses up to and including 1 mg/kg bw/d. The maternal NOEL was 0.2 mg/kg bw/d due to reduced bodyweight gain at 0.5 mg/kg bw/d. The developmental NOEL was 1 mg/kg bw/d, the highest dose tested.

9 GENOTOXICITY STUDIES

Summaries of findings in submitted genotoxicity studies are provided in the Tables below. Full evaluations are provided for studies that resulted in positive or equivocal findings.

9.1 Active constituent (Fipronil)

Table 33: Summary of *in vitro* genotoxicity studies with fipronil

Assay	Bacterial strain or cell type	Conc. or Dose	Batch / Purity	Metab. Act.	Result	Reference
Gene Mutation						
Reverse mutation in bacteria (Ames test)	S. typhimurium TA 98 TA 100 TA1535 TA 1537	Test 1 (±S9): 0, 0.8, 4, 20, 100, 500 µg/mL; Test 2 (±S9): 0, 25, 50, 100, 200, 400 µg/mL	IGB 438 /95-97%	+, -	-,-	Clare (1988a) [GLP]
Gene mutation at HGPRT locus	CHO V/79 cells	+/-S9: 0.8, 4, 20, 100, 500 µg/mL	JJW2092 /1 / 97.2%	+, -	-,-	Lloyd (1990) [GLP]
Chromosomal Effect Assays						
Chromosomal Aberration	Human lymphocytes	±S9: 0, 75, 150, 300 µg/mL	IGB 438 /95-97%	+, -	-,-	Marshall (1988a) [GLP]
	Chinese hamster lung	-S9/6h: 0, 30, 45, 60 µg/mL; -S9/24 or 48h: 0, 7.5, 15, 22.5, 30 µg/mL; +S9/6h: 0, 15, 30; 60 µg/mL	TAK174 7/98.3%	+, -	+,+	Wright (1995) [GLP]

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Wright NP (1995) Chromosome aberration test in CHL cells in vitro. Report No: 282/456. Safepharm Laboratories Limited, Derby, UK. Expt. dates: 13 Feb 1995–15 March 1995. (GLP: yes. Guidelines: JMAFF 4200 (1985) *(Only a summary provided)*

Note The materials and methods for this study are summarised in the Table above.
Results and Conclusion In the absence of S9 mix, the percent aberrant cells in cultures treated with fipronil for 6 h at 0, 30, 45 or 60 µg/mL were respectively 0.5, 0.5, 3.5* and 14.5*** (excluding gaps) or 1.0, 0.5, 3.5, 15.0*** (including gaps). Under the same incubation conditions, but in the presence of metabolic activation, the percent aberrant cells at 60 and/or 30 µg/mL also increased, but to a lesser (and generally not statistically significant) extent (i.e. 2.0, 0.5, 1.0, 5.5 %, excluding gaps; and 2.5, 1.0, 3.0, 6.5* %, including gaps, for concentrations of 0, 15, 30, 60 µg/mL respectively). No effects of treatment were observed following incubation times of 24 or 48 h. Positive controls gave appropriate responses. Only a summary of this study was provided, so it was not possible to make an independent assessment of the cytotoxicity of the fipronil concentrations that produced clastogenic effects in this test. As clastogenicity was not observed after 24 or 48 h exposures, it appears unlikely that the increase in aberrations seen after the 6 h exposures represent a toxicologically significant effect.

Table 34: Summary of *in vivo* genotoxicity studies with fipronil

Assay	Species (Strain)	Dose	Batch / Purity	Result	Reference
Chromosomal Effect Assays					
Micronucleus (marrow cells)	Mouse (CD-1)	0, 6.95, 16.1, 48 mg/kg bw	DA832/96.2%	-	Edwards (1995) [GLP]
Micronucleus (marrow cells)	Mouse	0, 1, 5, 25 mg/kg, po	JJW2092/97.2%	-	Edwards (1991) [GLP]

Results are expressed as +, positive; -, negative; +/-, equivocal.

Note: The Edwards (1991) study did not produce any observable effects at the chosen doses, so a follow-up study was performed (Edwards 1995). In a pre-test conducted at relatively high doses (30-120 mg/kg bw), 2/4 mice died at 70 and 120 mg/kg bw, with weight loss and clinical signs observed in survivors. In the main test, mice at 48 mg/kg bw lost weight, and hunched posture, piloerection, convulsions, underactivity and slow respiration were also observed, but not at lower doses.

9.2 Metabolites/degradates of fipronil

9.2.1 MB 46513 (fipronil desulfinyl)

Table 35: Summary of *in vivo* genotoxicity studies with fipronil desulfinyl

Assay	Species (Strain)	Dose	Batch / Purity	Result	Reference
Chromosomal Effect Assays					
Micronucleus (marrow cells)	Mouse (SPF Swiss CD-1)	2-16 mg/kg bw, po	CHO89 / 99.5	-	Proudlock (1996) [GLP]

Results are expressed as +, positive; -, negative; +/-, equivocal.

Table 36: Summary of *in vitro* genotoxicity studies with fipronil desulfinyl

Assay	Bacterial strain or Cell type	Conc. or Dose	Batch / Purity	Metab. Act.	Result	Reference
Gene Mutation						
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537 TA 1538	1st test & 2nd test: 10-250 µg/plate (±S9), in DMSO	33RJO 108 / 98.6%	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Percy (1993b) [GLP]
Mutation in mammalian cells	CHO-K1-BH4 cells with HPRT locus	5-125 µg/mL (-S9) 15-625 µg/mL (+S9), in DMSO	CHO89 / 99.5%	+, -	-, -	Adams (1996a) [GLP]
Chromosomal Effect Assays						
Chromosomal Aberration	Human lymphocytes	Test 1(±S9): 30-625 µg/mL; Test 2(±S9): 30-100 µg/mL	CHO89 / 99.5%	+, -	-, -	Adams (1996b) [GLP]

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation.

9.2.2 MB 45950 (fipronil sulfide)

Table 37: Summary of *in vitro* genotoxicity studies with fipronil sulfide

Assay	Bacterial strain or Cell type	Batch / Purity	Conc. or Dose	Metab. Act.	Result	Reference
Reverse mutation in bacteria (Ames test)	S. typhimurium TA 98 TA 100 TA1535 TA 1537 TA 1538	DXH 1379/3 /not stated	1.6-1000 µg/plate (±S9)	+, -	-, -	Asquith (1987) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537	OP5502 / 98.9%	10-250 µg/plate (±S9)	+, - +, - +, - +, -	-, - -, - -, - -, -	Percy (1994a) [GLP]
Chromosomal Aberration	Human lymphocytes	37JJW1898/>99%	6.25-400 µg/plate (±S9)	+, -	-, -	Marshall (1988b) [GLP]

9.2.3 MB 46136 (fipronil sulfone)

Table 38: Summary of *in vitro* genotoxicity studies with fipronil sulfone

Assay	Bacterial strain or Cell type	Batch / Purity	Conc. or dose	Metab. Act.	Result	Reference
Reverse mutation in bacteria (Ames test)	S. typhimurium TA 98 TA 100 TA 1535 TA 1537	WAB 202/1A / 98.7%	-S9: 0.32-200 µg/plate; +S9: 0.8-500 µg/plate	+, -	-,-	Clare (1988b) [GLP]
Chromosomal Aberration	Human lymphocytes	WAB 202/1A / 98.7%	±S9: 4.69-300 µg/mL	+, -	-,-	Marshall (1989) [GLP]

9.2.4 Other metabolites

Table 39: Summary of *in vitro* studies using other metabolites as listed

Assay	Bacterial strain or Cell type	Chemical (Batch / Purity)	Conc. Or Dose	Metab. Act.	Result	Reference
GENE MUTATION						
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537 TA 1538	**RPA 200766** (fipronil carboxylic acid) (57TDS62/ 98%)	1st test: 50-2500 (+S9) or 50-1000 µg/plate (-S9); 2nd test: 50-1000 µg/plate (±S9)	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Percy (1993a) [GLP]

Table 39: Summary of *in vitro* studies using other metabolites as listed, cont...

Assay	Bacterial strain or Cell type	Chemical (Batch / Purity)	Conc. Or Dose	Metab. Act.	Result	Reference
GENE MUTATION, cont...						
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537 TA 1538	**RPA 104615** (fipronil detrifluoromethyl sulfonate) (58 TDS 91 / 94.7%)	250-5000 µg/plate (±S9)	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Percy (1993c) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537	**RPA 105328** (48EAR139 / 96.4%)	50-1000 µg/plate	+, - +, - +, - +, -	-, - -, - -, - -, -	Percy (1994b) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537	**RPA 105040** (fipronil desulfinyl amide) (57TDS134/98.6%)	250-5000 µg/plate	+, - +, - +, - +, -	-, - -, - -, - -, -	Percy (1994c) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537 TA 102	**RPA 097920** (fipronil detrifluoromethyl sulfinyl) (943026 / 99.7%)	25-2500 µg/plate (- S9) 100-2500 µg/plate (+ S9)	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Percy (1996) [GLP]
Reverse mutation in bacteria	*S. typhimurium* TA 98 TA 100 TA1535 TA 1537 TA 102	**RPA 200761** (fipronil carboxylic acid) (57TDS112A/ 97.5%)	100-5000 µg/plate	+, - +, - +, - +, - +, -	-, - -, - -, - -, - -, -	Percy (1995) [GLP]
CHROMOSOMAL EFFECT ASSAYS						
Chromosomal Aberration	Human lymphocytes	**RPA 097920** (fipronil detrifluoro-methylsulfinyl) (94 3026 DA 942 / 99.7%)	Test 1: 12.5-150 µg/mL (-S9) 50-800 µg/mL (+S9) Test 2: 25-150 µg/mL (-S9) 100-600 µg/mL (+S9) (20 and 44 h sampling times)	+, -	- , +	Johnson (1995)
Chromosomal Aberration	Human lymphocytes	**RPA104615** (fipronil detrifluoromethyl sulfonate) (58TDS91 / 94%)	Test 1: 39.06-5000 µg/mL (±S9) Test 2: 312.5-5000 µg/mL (-S9) 625-5000 µg/mL (+S9)	+, -	-, -	Allais (2002a) [GLP]
Chromosomal Aberration	Human lymphocytes	**RPA200766** (fipronil amide) (73PAC1 / 99.8%)	Test 1: 9.77-1250 µg/mL (±S9) Test 2: 3.91-500 µg/mL (-S9) 78.13-800 µg/mL (+S9)	+, -	-, -	Allais (2002b) [GLP]

Results (+, positive; -, negative or +/-, equivocal) are expressed relative to the presence (+) or absence (-) of metabolic activation.

Johnson AL (1995) Pyrazole / MB 45897 / RPA097920: An in vitro test for induction of chromosome damage: Cytogenetic study in cultured human peripheral lymphocytes. Report No. 94/RHA534/1034. Lab: Pharmaco-LSR Ltd, Eye, Suffolk, England. Sponsor: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. Expt. dates: 1.8.1994-3.10.1994. Unpublished report date: 21 March 1995. (QA/GLP: yes; Study Guidelines: OECD 473) *(Only summary provided)*

Note The materials and methods for this study are summarised in the table above.
Results and Conclusion In the absence of S9 mix, cultures treated with RPA 097920 (fipronil detrifluoromethylsulfinyl) at 150 μg/mL (the highest concentration used) for both tests at the 20 h sampling time showed a statistically significant increase in aberrant cell frequency over concurrent solvent control values, both including and excluding gaps. Also in the absence of S9 mix, an increase was apparent in the second test at 125 μg/mL (but not at 150 μg/mL) at the 44 h sampling time, both including and excluding gaps.

Table 40: Results of *in vitro* tests with RPA 097920 (–S9)

Solvent or test material (μg/mL)	Incub. time (h)	No. of cells scored	Mean mitotic index	Cells with aberrations (+ gaps)	Cells with aberrations (- gaps)
Test 1					
DMSO	20	200	12.9	5	4
RPA097920 (150)	20	200	3.3	16 (8%)*	12 (6%)*
Test 2					
DMSO	20	200	11.4	6	3
RPA097920 (150)	20	200	2.7	27 (13.5%)***	23 (11.5%)***
DMSO	44	200	9.7	7	6
RPA097920 (125)	44	200	1.8	26 (13%)***	15 (7.5%)*

*p<0.05; ***p<0.001

It is concluded that under the test conditions, evidence of clastogenic activity was only apparent at concentrations causing marked cytotoxicity, with no dose response. In the presence of S9 mix, RPA 097920 (fipronil detrifluoromethylsulfinyl) showed no evidence of clastogenic activity, with or without inclusion of gaps.

Table 41: Summary of *in vivo* genotoxicity studies for other metabolites

Assay	Species (Strain)	Chemical (Batch / Purity)	Dose	Result	Reference
Chromosomal Effect Assays					
Micronucleus (marrow cells)	Rat (SD CD)	**RPA 200766 (fipronil carboxylic acid)** (73PAC1/99.8%)	500, 1000, 2000 mg/kg (in corn oil), po	-	Mehmood (2002) [GLP]

Results are expressed as +, positive; -, negative; +/-, equivocal.

10 NEUROTOXICITY

Gill, MW, Wagner CL, Driscoll CD (1993) M&B 46030: Single exposure peroral (gavage) neurotoxicity study in Sprague-Dawley rats. Report No. 91N0099. Lab: Bushy Run Research Centre, Union Carbide Chemicals and Plastics Co. Ltd Pennsylvania, USA. Sponsor: Rhone-Poulenc Ag Co. 2 T. W. Alexander Dr, Research Triangle Park, NC USA 27709. Unpublished study completion date: 27 April, 1993. (GLP/QA: yes. Study Guideline: US EPA 81-8)

Sprague-Dawley rats (15/sex/group) were dosed once by oral gavage with fipronil in corn oil at 0, 0.5, 5 or 50 mg/kg bw, and killed 16-19 days later. Clinical signs of toxicity, functional observations (see Appendix VI), motor activity, gross pathology and histological neuropathology were assessed. Six deaths (5 males, 1 female) occurred at 50 mg/kg bw, and survivors showed decreased bodyweight gain for days 7-14, and a variety of abnormalities in nervous system function. This included convulsions, tremors, head bobbing, myoclonic movements and/or decreased hind leg splay, as well as decreases in open field activity, various reflexes, muscle tone and/or body temperature, and motor activity at 7-8 h post-treatment. Also at this test point, decreased hind leg splay was observed at 5 mg/kg bw. On day 7, at 50 mg/kg bw, open field activity was increased in a few animals (changes in arousal, rearing, exaggerated reflex response to sound and tactile stimulation), but there were no concomitant changes in motor activity. The NOEL was 0.5 mg/kg bw due to decreased hind leg splay at 5 mg/kg bw.

Hughes EW (1997a) Fipronil: Neurotoxicity to rats by acute oral administration (including a time to peak effect study). Report No. RNP 536/973345. Lab: Huntingdon Life Sciences Ltd, Cambridgeshire, England. Sponsor: Rhone-Poulenc Inc, 355 rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France. R010419. Study duration: July-August, 1997. Unpublished report date: 6 November 1997. (GLP/QA: yes. Study Guidelines: EPA FIFRA: 81-8)

Materials and Methods: Rats (11/sex/dose) received a single oral gavage dose of fipronil (Batch: TAK 1747, purity 97.9%) at 0, 2.5, 7.5 or 25 mg/kg bw as a suspension in corn oil. Rats (Crl: CD BR, from CR Breeding Laboratories, Kent, UK) were 42 days old for males and 35 days old for females, and within a 15 g weight range for each sex.

The concentration, homogeneity and stability of the suspensions were confirmed by analytical means. After dosing, the animals were observed for 14 days. Individual animals were observed daily for clinical signs. Additional checks were made in the morning and afternoon for dead or moribund animals. Bodyweights and food consumption were recorded weekly. All animals were subjected to a FOB (see Appendix VI) prior to treatment and at the time of peak effect (determined as 7 h post-dosing), and on day 7 and 14 post-treatment. The motor activity of each animal was also quantitatively assessed at the same intervals. At termination on day 15, all rats were subjected to whole body perfusion. Gross examination was confined to designated tissues of the nervous system which were subsequently examined microscopically.

Results and Conclusion: Some rats at 25 mg/kg bw showed staining of the fur of the head/muzzle/nasal regions, and soiled/stained anogenital region on day 2. Bodyweight gains were significantly lower for males at 25 mg/kg bw (53% of control) and females at 7.5 and 25 mg/kg bw (44% and 25% of control respectively) in week 1, corresponding to reduced food intake (up to 26% lower) in these groups.

Seven hours after treatment at 25 mg/kg bw, 1 male and 1 female showed unusual behaviour/posture (chewing in the arena, a posture in the arena, resting or rubbing of the chin on the floor, or moderate ear twitches, awkward to handle, and limpness). Stationary position following positioning for tail pinch was observed, and forelimb grip strength was increased at the same dose. Temperature was reduced relative to the controls (37.6°C *vs* 37.9-38.2°C) in both sexes at 25 mg/kg bw. There was a statistically significant decrease in locomotor activity at 25 mg/kg bw during the first 10 minutes of assessment. Landing footsplay was decreased in males at 7.5 and 25 mg/kg bw, and in females at 25 mg/kg bw. The incidence of vocalisation was increased in males at 7.5 mg/kg bw (14 days only) and 25 mg/kg bw (7 h, days 7 and 14), and the incidence of grooming was reduced in females at 7.5 and 25 mg/kg bw on days 7 and 14. There were no treatment related

effects on brain weight and brain measurement, and no neuropathological findings. The NOEL following a single oral exposure to fipronil was 2.5 mg/kg bw, based on reduced bodyweight gain and decreased footsplay at 7.5 mg/kg bw.

Driscoll CD & Hurley JM (1993) MB46030: Ninety-day dietary neurotoxicity study in Sprague-Dawley rats. Report No. 92N1074. Lab: Bushy Run Research Centre, Union Carbide Chemicals and Plastics Co. Ltd Pennsylvania, USA. Sponsor: Rhone-Poulenc Ag Co. 2 T. W. Alexander Dr, Research Triangle Park, NC USA 27709. Unpublished study completion date: 27 April, 1993. (GLP/QA: yes. Study Guideline: US EPA 82-5 b).

Sprague-Dawley rats (15/sex/group) were administered fipronil in the diet at concentrations of 0, 0.5, 5 or 150 ppm for a minimum of 13 weeks, equal to 0.02/0.03, 0.3/0.3, and 7.2/8.6 mg/kg bw/d for males/females. Rats were killed during the fourteenth week. Clinical signs of toxicity, bodyweight, food consumption, functional observations (see Appendix VI), motor activity, perfused brain weights, gross pathology and histological neuropathology were assessed. At 150 ppm, food consumption and bodyweight gain were reduced during the initial 1-2 weeks of the study. There were no treatment-related gross findings at any dose, or microscopic findings for rats in the high dose group. In week 4, there was an increased incidence of urine in the observation area (statistically significant at 150 ppm), but as this was seen against a high background incidence in the controls, and was present in many rats pre-treatment, it was unclear if this was due to treatment. Also at this time, an exaggerated startle and tail pinch response was seen in 4/10 males at 150 ppm, but not in any other group, so this may have been related to treatment. At week 13, forelimb strength was increased in 150 ppm females. The NOEL was 5 ppm, (equal to 0.3 mg/kg bw/d) due to neurobehavioural changes (exaggerated startle and tail pinch, increased forelimb strength) at 150 ppm.

Holmes P (1991d) 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. Report No. 90/RHA371/0790. Lab: Life Science Research Limited, Suffolk, UK. Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Unpublished. Completion date 28 March 1991. (GLP/QA: yes, Study Guidelines: none applicable)

Fipronil (20 mg/kg bw/d) was administered in gelatin capsules to 4 female Beagle dogs until signs of neurotoxicity were apparent. Treatment was for 5 days for 2 dogs, and 7 or 13 days for the other 2 dogs. All dogs were then held untreated for at least 28 days. Signs of neurotoxicity included abnormal gait, tremors, stiffening of limbs or body, convulsions, head nodding and facial twitches. One dog displayed an apparent lack of vision. Such signs continued for 2-10 days post-treatment. Other signs shown by treated animals included underactivity (which continued for up to 12 days in the reversibility period), inappetence (recovery complete by 7 days into the reversibility period), bodyweight loss (recovery within 17 days), abnormal gait, behavioural changes, hunched posture and peripheral vasodilatation. Neurological examination revealed a range of abnormalities, and there was evidence of slow recovery during the reversibility period. Signs included depression of the blink, tactile and visual placing, pupillary, consensual light, extensor postural thrust, tonic neck, hindlimb hopping and flexor responses. Macroscopic and microscopic pathology were unremarkable.

Mandella RC (1995) A developmental neurotoxicity study of fipronil in the rat via dietary administration. Report No. 93-4508. Lab: Pharmaco-LSR/Huntingdon Life Sciences East Millstone, New Jersey. Sponsor: Rhone-Poulenc Inc, Research Triangle Park, NC USA. Expt. dates: 14.3.1994-3.5.1994. Unpublished report date: 28 December 1995. (GLP/QA: yes. Study Guideline: US EPA 83-6)

Materials and Methods Mated female SD rats (30/dose) received 0, 0.5, 10 or 200 ppm of fipronil in the diet (Lot no: 6ADM93, Purity: 96.1%) from gestation day 6 through lactation day 10. The rats (from Charles River Breeding Laboratories, Portage, MI USA) were 9-10 weeks old, weighing 208–321 g on gestation day 0. The concentrations, homogeneity and stability of the test chemical in the diet were confirmed by analysis. Achieved dose levels of the test substance were 0.05, 0.9-0.92 and 8.7-18.5 mg/kg bw/d at 0.5, 10 and 200 ppm respectively. The wide range at 200 ppm reflected fluctuations in food consumption during gestation. Physical observations, bodyweight and food consumption measurements were performed on all dams at selected intervals throughout the gestation and lactation periods. Maternal females were sacrificed following weaning of the last litter.

Litters were culled to 8 pups on lactation day 4. Pinna detachment, incisor eruption, eye opening, vaginal patency and preputial separation were evaluated for all surviving pups. Motor activity, auditory startle response and swimming, learning and memory evaluations were performed on 1 male and 1 female pup per litter. Pups (1/sex/litter) were sacrificed on days 11 or 60 *post partem* for gross examination, determination of brain weight, and/or neuropathological examination (the latter in the control and 200 ppm groups only).

Results and Conclusion One 200 ppm dam died on each of lactation days 6 and 9, with discolouration of lungs observed at necropsy. Reduced food consumption (51% lower) and bodyweight loss (10%) were noted in dams at 200 ppm during gestation days 6-10. At later stages, both food consumption and bodyweight gain were comparable with, or higher than the controls, with bodyweight remaining low during the entire treatment period, but recovering by lactation day 21.

No clinical signs were observed in any group, and pregnancy status, gestation length, litter size and pup sex distribution were not affected. At 200 ppm, 32 pups were found dead at birth (*vs* 4 in the control), 4 litters lost all pups by lactation day 4 (*vs* 0 in the control), and a total of 170 pups (*vs* 35 in the control) died during lactation days 0-21, resulting in a reduced pup live birth index, pup viability index and pup weaning index. No treatment related findings were revealed at necropsy of the dead pups. Reduced pup weight was observed at 10 and 200 ppm throughout the lactation period (see Table below). The reduction in mean pup bodyweight at 10 ppm relative to the control was approximately 3-9%. Given that these changes were small, there is uncertainty as to their biological significance. The historical control ranges supplied for F_1 pup bodyweights in multigenerational studies were 8.5-10.6 g on postnatal day 4 and 37.7-56.1 g on postnatal day 21, suggesting that the pup weights at 10 ppm may be in the normal range. Leaving aside the different type of study from which these figures were obtained, the studies were conducted in 1978-1991, and so are not contemporaneous with the present study. On these grounds, the use of this data is not considered appropriate. Therefore, it is not possible to dismiss the finding of decreased pup weight at 10 ppm. Pup bodyweight gain in the post-weaning period was similar in all groups.

Table 42: Litter data

Dose (ppm)	Total or male/female			
	0	**0.5**	**10**	**200**
Pups dead at birth	0.0	0.2	0.2	1.1**
Pup live birth index (%)	99.1	98.9	98.4	93.0
Pups alive on day 4 (pre-cull)	14.6	14.7	15.0	12.9*
Pup viability index (%)	98.9	95.7	96.3	75.5**
Pup weaning index (%)	86.9	86.8	87.1	81.9
Mean pup weight; Lact. Day 0	6.5 / 6.2	6.5 / 6.1	6.3 / 5.9*	5.9** / 5.7**
Lact. Day 4	10.7 / 10.3	10.4 / 9.7	10.0* / 9.4**	7.7** / 7.5**
Lact. Day 11	27.4/26.3	26.1/24.9	25.6/24.3*	18.0**/17.4**
Lact. Day 17	41.7/40.3	41.2/39.2	38.9*/36.7*	31.3**/29.5**
Lact. Day 21	53.9 / 51.6	52.1 / 49.4	50.4 / 47.8*	41.3** / 38.5**

*p<0.05; **p<0.01.

Delays in lower incisor eruption occurred at 200 ppm, and in sexual development at 10 (males only) and 200 ppm. At 200 ppm, delays of approximately 0.5, 1.5 and 4.5 days occurred for lower incisor eruption, vaginal patency and preputial separation respectively, with a delay of ~1.4 days for preputial separation at 10 ppm (times to preputial opening 44.0 ± 2.5, 44.7 ± 2.5, 45.4 ± 2.9, 48.8 ± 3.3 days in increasing order of dose). Historical control data for the only other developmental neurotoxicity study conducted in this laboratory gave a range of 49.7-41.8 days (mean 45 ± 1 days), suggesting that the delay at 10 ppm was unlikely to have biological significance.

Auditory startle response was evaluated on postnatal days 22 and 60±2. A significant decrease in the maximum voltage response was noted at 200 ppm on day 22. Evaluations of swimming development on postnatal days 6, 8, 10, 12 and 14 revealed a slight delay at 200 ppm as determined by direction and angle evaluations. Results of water "Y" maze time trials on postnatal days 24, 25, 30, 60, 61 and 65 were consistent with treatment not having affected learning and memory. At necropsy of pups on postnatal days 11 and 60, the lower absolute brain weight at 200 ppm was associated with lower bodyweight, and the brain/bodyweight ratio was comparable with the control. Sporadic gross and microscopic findings in all pup groups were not attributed to treatment.

The maternal NOEL was 10 ppm, equal to 0.9 mg/kg bw/d due to bodyweight loss at 8.7 mg/kg bw/d. The pup NOEL was 0.5 ppm, equal to 0.05 mg/kg bw/d, due to reduced pup weight during lactation at 0.9 mg/kg bw/d.

Anadon A, Pita R, Garcia-Uzcategui Y, Diaz MJ, Martinez-Larranaga MR (2004) Decrease of 5-HT levels after fipronil treatment. The Toxicologist. 78 (1-S): 228.

Wistar rats (6/group) were dosed orally with fipronil at 5 or 10 mg/kg bw/d for 6 days. Controls received corn oil. Rats were sacrificed 24 h after the last dose, their brains were rapidly removed, and the hypothalamus, hippocampus and striatum were dissected and analysed by HPLC for 5-hydroxytryptamine (5-HT = serotonin) and its metabolite 5-hydroxy-3-indole acetic acid (5-HIAA). Relative to the controls, fipronil-treated animals had lower levels of serotonin in each of the brain structures examined. The authors stated that this effect was dose-related, but only results for the 10 mg/kg bw/d dosing regime were provided. Relative to the negative control, the percentage reductions in serotonin and its metabolite were respectively: hypothalamus (29%, 26%), hippocampus (40%, 34%), and striatum (45%, 45%).

11 TOXICITY STUDIES ON FIPRONIL METABOLITES

11.1 MB 46513 (desulfinyl fipronil)

MB 46513 (desulfinyl fipronil) [(5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethyl pyrazole] is a plant photolytic metabolite of fipronil. An extensive data package has been submitted for the assessment of the toxicology of this compound.

11.1.1 Metabolism and Toxicokinetics

Totis M (1996) MB 46513: Absorption, Distribution, Metabolism and Excretion in the Rat (Report Part A). Study No: SA 95304. Lab: Rhone-Poulenc Agrochemie, Centre de recherché, 355 rue Dostoievski, BP 153 F-06903 Sophia Antipolis and Rhone-Poulenc Agriculture Ltd. Fyfield Rd, Ongar, Essex, England. Sponsor: Rhone-Poulenc Ag Company, Research Triangle Park, NC. Study period: 7.9.1995-10.5.1996. Unpublished report date: 15 May 1996. (QA/GLP: yes. Guidelines: US EPA 85-1, 1984)

Materials and Methods A series of experiments was designed to obtain information on the absorption, distribution, metabolism and elimination (ADME) of MB 46513 (fipronil desulfinyl) in 5 animals/sex Iffa Credo CD (Sprague Dawley origin) strain rats (from Iffa Credo, France). Unlabelled MB 46513 (fipronil desulfinyl) (Batch no. 10DGM22; purity 99.7%) and [^{14}C]-phenyl-MB 46513 (desulfinyl fipronil) (Batch no. GHS847; radiopurity estimated as approximately 100%) were used in the experiments. For the high dose experiments, a mixture of unlabelled and [^{14}C]-MB 46513 (desulfinyl fipronil), suspended in aqueous methyl cellulose (0.5% w/w) and Tween 80 (0.01% w/v), was used. The low dose experiments used the labelled material alone. Each dosing suspension was prepared on the day of treatment, and assayed for amount of test material by HPLC and LSC before, mid-way through, and after the dosing procedure. The following experiments were performed:

(i) *Single oral high dose - ADME*

Food was withheld from the rats for 18 h prior to dosing, and replaced 1 h after dosing. The animals received a single oral dose of 10 mg/kg bw by gavage, then remained in metabolism units for 168 h for the collection of urine and faeces. Carbon dioxide was not collected as a preliminary study at 15 mg/kg bw showed that < 0.1% was eliminated by this route within 48 h. Urine and faeces were collected at 24 h intervals. The liver, kidneys, heart, lungs, thyroid, brain, spleen, muscle, abdominal fat, ovaries, testes, intestinal tract with contents, stomach with contents, bone and marrow, adrenals, uterus, pancreas, and skin and fur were collected from each animal. The residual carcass was also retained. Plasma was obtained from cardiac blood samples, and the metabolism cages were washed with distilled water every 24 hours, and the rinsate collected.

(ii) *Single oral low dose - ADME*

Procedures were as for the high dose, except the rats received a single oral dose of 1 mg/kg bw by gavage.

(iii) *Repeat oral low dose - ADME*

Animals received 14 consecutive daily gavage doses of unlabelled test material at 1 mg/kg bw. Five animals/sex were then randomly selected on a weight basis and transferred to a metabolism cage 24 h before an oral gavage dose of ^{14}C-labelled material at 1 mg/kg bw. Subsequent procedures were as for the single high dose study.

(iv) *Single oral dose - pharmacokinetics*

Five to seven animals/sex were selected randomly on a weight basis 24 h prior to the administration of a single oral gavage dose of 1 or 10 mg/kg bw [^{14}C]-labelled test material. The procedure was as for the single high dose study. Blood samples were taken prior to dosing, at approximately 0.5, 1, 2, 3, 4, 6, 8 and 24 h after dosing, and at 24 h intervals thereafter up to 15 days (low dose) or 17 days (high dose), and finally at intervals of 2-3 days up to day 27 post-dosing.

(v) *Supplementary single oral dose - tissue metabolism*

Three males were selected randomly on a weight basis 24 h prior to the administration of a single oral gavage dose of 1 mg/kg bw. The liver and abdominal fat were removed from each animal after exsanguination 7 days post dosing. Other organs were removed, as indicated for ADME studies, and the residual carcass retained.

Radioactivity levels in the samples were determined by LSC. Liquid samples were analysed directly, while solid samples were extracted and combusted, or combusted directly, prior to scintillation counting. Urine samples were subjected to enzyme hydrolysis with β-glucuronidase and/or sulfatase. Urine and faecal samples also underwent acid hydrolysis prior to analysis. Quantification of metabolites present in various samples was performed using HPLC. Metabolite characterisation and identification was via HPLC, LC/MS, and GC/MS.

Results

(i) Following a single oral dose of 10 mg/kg bw, urinary elimination of radioactivity for 7 days post-treatment (mean ± SD) was 8.8 ± 2.5% in males, and 10.7 ± 7.1% in females. Elimination of the bulk of the label in the urine occurred during the first 96 hours. In the faeces, the mean elimination figures were 69.6 ± 7.1% in males, and 56.0 ± 8.1% in females, with the bulk of the label in the faeces eliminated in the first 120 hours (although 2 males and 1 female eliminated high levels in the first 24 hours). In males, mean tissue concentrations (expressed as µg [^{14}C]-MB 46513 (desulfinyl fipronil) equivalents/g ranged from 1.25 in stomach and contents and 1.44 in muscle, up to 6.4, 7.0 and 18.3 in adrenals, liver and fat respectively. In females, mean tissue concentrations ranged from 1.52 in blood to 6.7, 7.4, 9.7, 10.4 and 50.8 in liver, adrenals, gonads, uterus and fat respectively. In both sexes, no tissues had radiolabel below the limit of detection at 7 days post dosing. The mean total tissue levels of radiolabel (% dose) were 19.9% (males) and 30.0% (females). The residual carcass contained 8.2% (males) and 13.0% (females) of the dose. In males, the mean tissue:plasma ratios were <1, except for liver, kidneys, lung, fat, intestine, adrenals, skin/fur and pancreas, ranging from 0.4 (stomach) to 8.2 (fat). In contrast, the majority of mean tissue:plasma ratios in females were >1, except for brain, spleen, muscle, bone and marrow, blood and stomach, ranging from 0.6 (blood) to 23.2 (fat). The mean total recovery of radiolabel in both sexes was 100.2%, range 98-102.9%.

(ii) Following a single oral dose of 1 mg/kg bw, urinary elimination of radioactivity over 7 days was (mean ± SD) 6.1 ± 0.4% in males, and 4.4 ± 1.2% in females. The bulk of the label in the urine was eliminated in the first 4 days. In the faeces, the mean elimination figures were 60.1 ± 2.8% in males, and 46.4 ± 4.2% in females, with the bulk of the label in faeces eliminated in the first 120 hours. In males, mean tissue concentrations (expressed as µg ^{14}C-MB 46513 (desulfinyl fipronil) equivalents/g) ranged from 0.04 in stomach and contents and 0.05 in bone/marrow and spleen, up to

0.28, 0.3, 0.34 and 1.54 in liver, adrenals, skin/fur and fat respectively. In females, mean tissue concentrations ranged from 0.06 in blood and 0.07 in stomach and contents, up to 0.6 and 2.7 in skin/fur and fat respectively. In both sexes, no tissues had radiolabel below the limit of detection at 168 hours post dosing. The mean total tissue levels of radiolabel (% dose) were 26.6% (males) and 41.1% (females). The residual carcass contained 11.0% (males) and 17.4% (females) of the dose. In males, the mean tissue/plasma ratios ranged from 0.3 (stomach) to 12.8 (fat). In females, the majority of mean tissue/plasma ratios were >1, except for stomach, spleen, blood and bone and marrow. The range of the mean ratios in females was 0.6 (stomach) to 25.2 (fat). The mean total recovery of radiolabel in both sexes was 93.2%, range 88.8-96.3%.

(iii) After repeated oral doses of 1 mg/kg bw/d, urinary elimination of radioactivity (mean ± SD) was 10.3 ± 3.5% in males, and 10.8 ± 1.9% in females over 7 days. In the faeces, the mean elimination figures were 61.1 ± 2.7% in males, and 53.3 ± 4.4% in females. The bulk of the label in both urine and faeces was eliminated in the period from 2 to 7 days. In males, mean tissue concentrations (expressed as µg ^{14}C-MB 46513 (desulfinyl fipronil) equivalents/g) ranged from 0.07 in stomach and contents, up to 1.97 in fat, while in females, the range was from 0.18 in blood and bone/marrow, up to 3.15 in fat. In both sexes, no tissues had radiolabel below the limit of detection at 7 days post dosing. The mean total tissue levels of radiolabel (% dose) were 22.4% (males) and 31.6% (females). The residual carcass contained 8.3% (males) and 13.9% (females) of the dose. In males, the mean tissue:plasma ratios ranged from 0.3 (stomach) to 6.2 (fat), and in females from 0.6 (blood and stomach) to 16.4 (fat). The mean total recovery of radiolabel in both sexes was 97.1%, range 93.3-103.7%.

(iv)The radioactivity in whole blood samples was converted to µg [^{14}C]-MB 46513 (desulfinyl fipronil) equivalents/g whole blood. Mean values for each sex were then used to calculate pharmacokinetic parameters for the high and low dose groups given in the Table below. The increases in C_{max}values were approximately proportional to dose. The elimination $t_{1/2}$ for ^{14}C-MB 46513 (desulfinyl fipronil) and/or its metabolites was estimated as approximately 195 h at the high dose, and 183 h at the low dose, with values for the males slightly lower than females.

Table 43: Pharmacokinetics parameters

MB 46513	**10 mg/kg bw**				**1 mg/kg bw**			
	Males		**Females**		**Males**		**Females**	
Parameter	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
C_{max} (µg equiv./g)	2.0	0.5	2.3	0.9	0.14	0.02	0.15	0.03
T_{max} (h)	72.5	9.1	70.5	8.3	45.9	13.6	60.6	17.1
t ½ (h)	170.1	21.2	220.6	55.7	156.3	17.9	209.9	13.7
AUC (0-648 h)	503.4	55.5	539.9	79.3	33.2	5.1	49.4	7.3

(v) Only one radioactive fraction was obtained by LC in tissue extracts from liver, fat, skin/fur and residual carcass extracts. This was identified as unchanged MB 46513 (fipronil desulfinyl), accounting for approximately 22% of the dose.

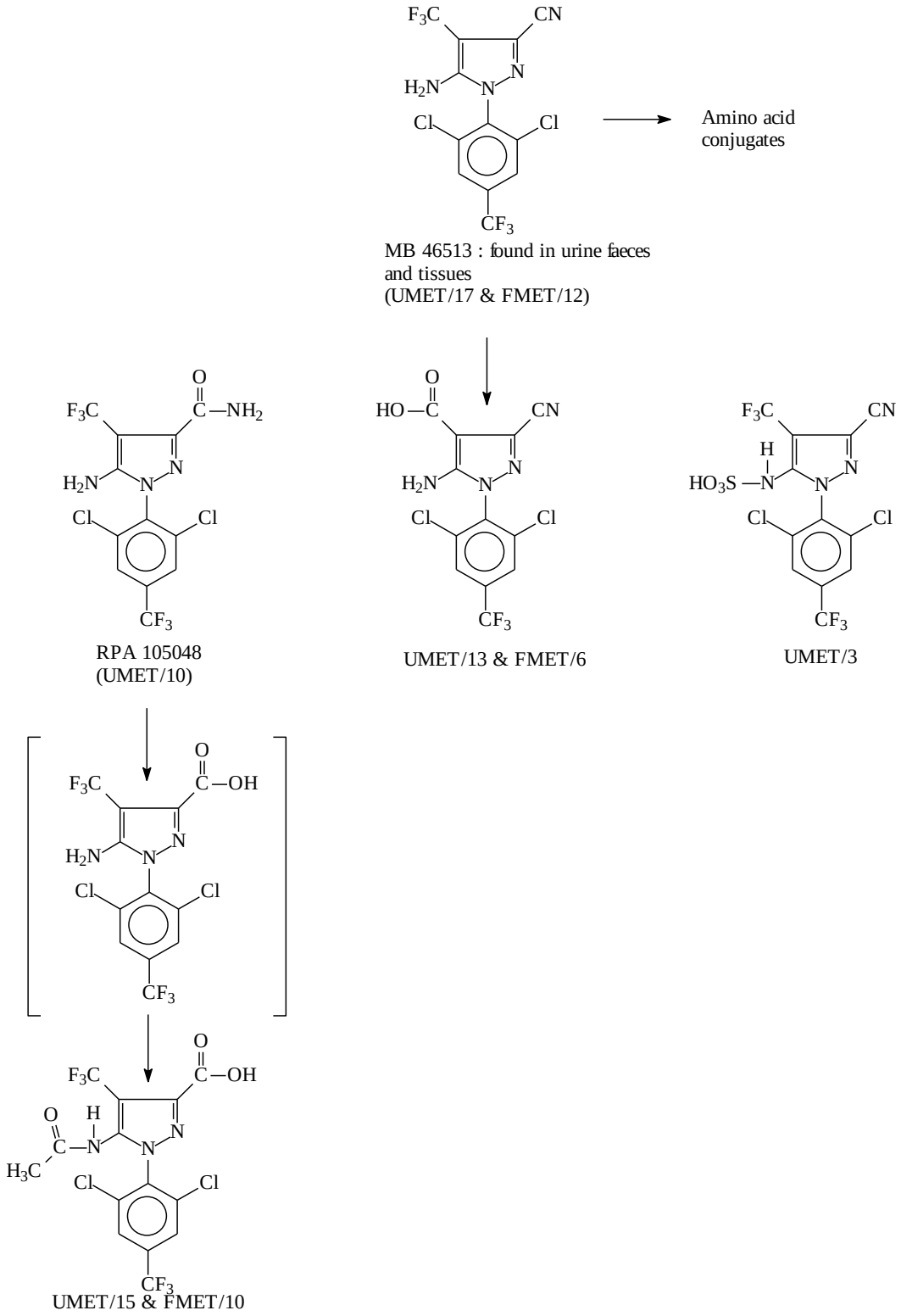
Metabolism

Up to 17 or 13 radioactive components were present in the urine and faeces, respectively, in the single high, single low and repeat low dose studies. The proposed metabolic schema is provided in Figure 2.

In the urine, unchanged MB 46513 (desulfinyl fipronil) (urinary metabolite, UMET/17) was detected only at trace levels (0.01-0.09% of the dose). A pyrazole-4-carboxylic acid derivative of MB 46513 (desulfinyl fipronil) (UMET/13) accounted for 5.5/4.2%, 2.2/1.3% and 4.6/3.0% of the dose in males/females in the single high, single low and repeat low dose studies, respectively. The majority of other fractions typically accounted for <1% (total radioactivity excreted via urine was only approximately 4-11% of the dose). A sulfate conjugate of MB 46513 (desulfinyl fipronil) (UMET/3) accounted for 0.6/0.9%, 0.2/0.1% and 2.3/2.3% of the dose in the single high, single low and repeat low dose studies respectively in males/females. The author suggested that two other polar metabolites (UMET/4 and 6) were amino acid conjugates of MB 46513 (desulfinyl fipronil), while 2 others were thought to be conjugates of the pyrazole-4-carboxylic acid derivative. Other identified urinary metabolites included an amide derivative (UMET/10), and a pyrazole-3-carboxylic acid, 4-N acetyl derivative (UMET/15). An unidentified metabolite (UMET/14) contained a carboxylic group. The compounds of urinary origin given in the proposed metabolic pathway below account for up to 7% of the administered dose (89% of the radioactivity in the urine).

In the faeces, the main radiolabelled compound was unchanged MB 46513 (desulfinyl fipronil) (UMET/17 = faecal metabolite, FMET/12), accounting for (males/females) 43.9/39.5%, 44.1/38.5%, and 28.5/35.4% of the dose in the single high, single low and repeat low dose studies respectively. The high levels of unchanged MB 46513 (desulfinyl fipronil) suggested that a large proportion of the administered dose may be excreted directly in the faeces, or excreted as a conjugate in the bile which undergoes subsequent deconjugation, or direct elimination into the intestine. The second most abundant faecal metabolite was the pyrazole-3-carboxylic acid, 4-NPDSC acetyl derivative (UMET/15 = FMET/10), accounting for (males/females) 14.2/7.1%, 7.1/3.2%, and 12.2/7.5% in the single high, single low and repeat low dose studies respectively. The pyrazole-4-carboxylic acid derivative of MB 46513 (desulfinyl fipronil) (UMET/13 = FMET/6), accounted for (M/F) 5.1/2.8%, 3.4/1.7%, and 5.2/2.5% in the single high, single low and repeat low dose studies respectively. Other metabolites included FMET/9 (= UMET/14), and FMET/1, 2 and 4, which were possible conjugates. The compounds of faecal origin given in the proposed metabolic pathway below account for up to 55% of the administered dose (92% of the radioactivity in the faeces).

Figure 2: Proposed General Metabolic Pathway for MB 46513 (fipronil desulfinyl) in the Rat



Note: UMET = urinary metabolite; FMET = faecal metabolite; MB 46513 = desulfinyl fipronil; RPA 105048 = desulfinyl fipronil amide

11.1.2 Percutaneous absorption

Cheng T (1996) Dermal absorption of ^{14}C-MB 46513 in male rats (preliminary and definitive phases). Report no. CHW 6224-230. Lab: Corning Hazleton Inc, Madison, Wisconsin. Sponsor: Rhone-Poulenc Inc, Research Triangle Park, NC USA. R010401. Study duration: 3.4.1996–30.5.1996. Unpublished report date: 27 September 1996. (GLP/QA: yes; Study Guidelines: US EPA 40 CFR Part 58, 85-3)

Materials and Methods The extent of absorption of MB 46513 (desulfinyl fipronil) was studied following application of [^{14}C]-MB 46513 (Lot No: GHS-847.2; Radiopurity: 95.9%; Specific activity: 22.6 mCi/mM) mixed with unlabelled MB 46513 (Lot No: 805 DAP DA 999; Purity: 99.2%) in 1% CMC to the skin of male rats. Male Charles River Crl:CD BR rats (from the Portage, Michigan); ~7 weeks old; 162 to 190 g bw at the beginning of the study), were assigned to 6 groups (2 groups in the preliminary phase and 4 groups in the definitive phase) and dosed at levels shown in the table below. Approximately 100 µL of the test suspension was applied onto pre-shaved and washed intact skin (area ~12.5 cm^2) on the back and shoulders of each rat under a non-occlusive cover.

The accumulated faeces and urine were collected from each rat at 0.5 h post-application for groups 1 & 2, at 24 h for group 3, and at 0.5, 1, 2, 4, 10 or 24 h for each of groups 4-6 (4 rats/group/timepoint). Animals were sacrificed at the conclusion of each collection period. Residual urine was collected from the urinary bladder at sacrifice and added to the corresponding excreted urine sample. Before sacrifice, the rats were anaesthetised with ketamine, and after removing the non-occlusive cover, the application site was washed with gauze pads and cotton-tipped applicators, which were collected and saved for radioanalysis. Rats were exsanguinated by cardiac puncture and the skin from the application site excised. All samples or solvent extractions from skin wash, faeces, urine, blood, skin at the application site, residual carcass, spreader and plastic enclosure for the test material, dressings, cage wash and wipes, were analysed by LSC, with or without combustion as required.

Results The mean recovery of radioactivity ranged from 94.3 to 100.6%. The majority of radioactivity (≥91.7%) was detected in the skin wash. For all treatment groups, total radioactivity detected in the blood, urine, excreta (including cage wash and wipe), and carcass amounted to less than 3% of the applied radioactivity. Mostly, radioactivity in the blood and carcass was below the limit of detection.

A comparison of the amount of MB 46513 (desulfinyl fipronil) absorbed at different doses is shown in the Table below. The term 'direct absorption' represents the total amount of radioactivity detected in the blood, eliminated in excreta, and retained in the carcass. 'Indirect absorption' includes both direct absorption and the amounts left on/in the skin of the test site after the skin wash. The maximum amount of direct absorption (0.01 mg) was noted in Group 6 at 0.5 h. Due to the low amount absorbed systemically, no correlation between systemic absorption and the length of exposure could be determined for this group. The maximum amount of indirect absorption (0.047 mg; 0.7% of the dose) was measured in Group 6 at 4 h (not shown in Table), suggesting that the amount of radioactivity at the application site had become saturated at that point. For the lower dose groups (4 and 5), there was some indication of an increasing amount of indirect absorption with increasing exposure time. For the high dose group (6), indirect absorption was low but somewhat variable, and likely to have reached saturation by 4 hours of exposure. Over the concentration range tested

(0.08-8%), the level of dermal absorption for MB 46513 (desulfinyl fipronil), including the amount retained in the skin, was up to 9.3% of the applied dose.

Table 44: Dermal absorption of ^{14}C-MB 46513 (fipronil desulfinyl

Group number	Dose concentration (mg/mL) [a]	Dose (mg/rat)	Dose (µg/cm^2)	Direct absorption [b]		Indirect absorption [b]	
				% dose	mg equiv	% dose	mg equiv
Preliminary							
1	0.696	0.0931	7.45	NC		0.69	
2	79.3	11.2	899	NC		0.17	
Definitive							
3	Vehicle						
4	0.80	0.0808	6.46	2.64	0.002	6.61	0.005
5	8.10	0.881	70.5	0.35	0.003	1.40	0.012
6	80.3	7.17	574	NC (0.14)	NA (0.010)	0.39 (0.27)	0.028 (0.019)

a. dilution of the test formulation with 1.0% carboxymethylcellulose (vehicle).
b. the absorption data are mean distribution of radioactive dose at 24 h post-dosing for the definitive groups and at 0.5 h for the preliminary groups. (Data in parentheses for Group 6 represent absorption at 0.5 h).
NC: not calculated. NA: not applicable.

11.1.3 Short-term repeat-dose studies

Mice

Dange M (1994b) MB 46513: Preliminary 28-day toxicity study in the mouse by dietary administration. Report No. SA 93228. Lab: Rhone-Poulenc – Secteur Agro, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc-Secteur Agro, Direction Internationale de la Toxicologies, Lyon, France. Expt dates: 3.8.1993-1.2.1994. Unpublished report date: 9 May 1994. (GLP/QA: no; Guidelines: none stated)

Materials and Methods OF-1 mice (10/sex/dose) received MB 46513 (desulfinyl fipronil) (Batch: 10 DGM 22, Purity: 97.5%) in the diet at 0, 0.5, 3, 30 or 60 ppm for 28 days. Intakes of the test substance were determined by sample analyses, and were 0.08/0.10, 0.49/0.61 and 5.02/5.65 mg/kg bw/d for males/females at 0.5, 3 and 30 ppm respectively, and for the 60 ppm group, were 7.05 mg/kg bw/d for males (week 1 only, due to reduced food consumption and deaths) and 12.10 mg/kg bw/d for females. The mice (from Iffa-Credo, 69210 L'Arbresle, France) were 6-7 weeks old, bodyweights 29.8-35.8 g for males and 22.0-29.5 g for females at the start of dosing.

Mice were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Bodyweight and food consumption were recorded weekly. At termination, blood samples were taken from fasted mice for the determination of a limited number of clinical chemistry parameters (albumin, bilirubin, cholesterol, glucose, urea nitrogen, total protein, AST, ALT and ALP). Selected organs (brain, heart, kidneys, liver, spleen, thymus) were weighed and a range of tissues as listed in Appendix IV fixed and examined microscopically.

Results Mortalities occurred at 30 (7 males; 2 females, days not stated) and 60 ppm (all males, 6 females, days 6-16). Among the mice found dead at 60 ppm, 1 male and 1 female

exhibited a posture suggesting that they may have died during or following convulsions. Increased motor activity, excessive jumps, irritability to touch and compulsive biting were seen at 30 and 60 ppm.

Table 45: Incidence of main clinical signs (n = 10)

	Males					Females				
Dose (ppm)	**0**	**0.5**	**3**	**30**	**60**	**0**	**0.5**	**3**	**30**	**60**
Increased motor activity				10	8			2	4	8
Prostration				1	1					
Excessive vocalisation								2	2	1
Irritability to touch				3	6					1
Tremors					1					1
Excessive jumps				4	8				1	2
Hunched posture					1					
Suspected convulsions*					1 (d10)					1 (d10)
Clonic convulsions*										1 (d16)
Compulsive biting*				8 (d7)	8 (d5)					6 (d7)
Death				7	10				2	6

*incidence (the day when the sign first seen).

Dose-related bodyweight loss occurred in males at 30 and 60 ppm in week 1 (respectively 10% and 24% lower than the corresponding day 1 weight), with lower bodyweights persisting in survivors at 30 ppm (16% lower than control). Females at 60 ppm also showed lower bodyweights (up to 10% less than control). Week 1 food consumption was decreased by 50% in 60 ppm males, and was also lower (up to 28%) in males at 30 ppm during the first two weeks. Females at 30 and 60 ppm occasionally had lower food consumption (up to 12% and 22% respectively).

One surviving female at 60 ppm had elevated urea (46 *vs* 9.3 mmol/L in control) at termination. As shown in the Table below, liver weight relative to bodyweight was increased in 30 ppm males and 60 ppm females. At necropsy, no treatment-related gross changes were noted, but centrilobular hypertrophy of the liver was revealed by microscopic examination in some mice at 30 and 60 ppm.

Table 46: Liver weight and pathology (n = 10)

	Males					Females				
Dose (ppm)	**0**	**0.5**	**3**	**30**	**60**	**0**	**0.5**	**3**	**30**	**60**
Weight as g	1.39	1.43	1.35	1.37	-	1.07	0.98	1.02	1.14	1.18
as % bodyweight	4.11	4.35	4.21	4.91**		4.38	4.25	4.34	4.77	5.34**
Centrilobular hypertrophy				5	2				1	1

**p<0.01 by Bartlett's test and Dunnett's test.

The dose at which no effects were seen was 3 ppm (equal to 0.5 mg/kg bw/d), based on treatment-related clinical signs and deaths, decreased bodyweight gain, and increased liver weight with microscopic changes to the liver (centrilobular hypertrophy) at 30 ppm (equal to 5 mg/kg bw/d).

Rats

Dange M (1994c) MB 46513: Exploratory 14-day toxicity study in the rat by gavage. Report no: SA 93063. Lab: Rhone-Poulenc-Secteur Agro, Centre de Recherche, 355, rue Dostoievski, BP 153, 06903 Sophia Antipolis Cedex, France. Sponsor: Rhone-Poulenc-Secteur Agro Agrochimie Lyons, France. Unpublished report date: 9 May 1994. (GLP/QA: no. Guidelines: none applicable)

Materials and Methods Sprague Dawley rats (5/sex/dose) received MB 46513 (desulfinyl fipronil) (Batch: 33RJO108, Purity: 98.6%) at 0, 0.3, 1, 3 or 10 mg/kg bw/d by gavage for 14 days. The rats (from Iffa-Credo, 69210 L'Arbresle, France) were 7 weeks old, weighing 245-294 g (males) and 191-222 g (females) at the start of treatment.

Rats were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Bodyweights were recorded on days 1, 5, 8, 12, 14 and 15, and food consumption weekly. At termination, blood samples were taken from fasted rats for haematology (as in Appendix III, but omitting clotting parameters) and limited clinical chemistry (albumin, bilirubin, cholesterol, glucose, urea nitrogen, total protein, AST, ALT and ALP). Selected organs (kidney, liver, ovary, spleen and thyroid/parathyroid) were weighed, a range of tissues (adrenals, brain, kidney, liver, thyroid, thymus and uterus) were given gross examinations, and the liver and thyroid gland examined microscopically.

Results One 3 mg/kg bw/d female died, and all 10 mg/kg bw/d rats died or were killed moribund between days 5 and 8. Prior to death, 3 males and 1 female at 10 mg/kg bw/d had convulsions on day 5 or 6 at approximately 2 to 5 h after dosing. As tabulated below, clinical signs observed at 3 and 10 mg/kg bw/d, were mostly piloerection, chromodachryorrhea, prostration, excessive reaction to noise, curled up at handling, hunched posture, nasal discharge and few faeces.

Table 47: Incidence of main clinical signs (n = 5)

	Males					Females				
Dose (ppm)	**0**	**0.3**	**1**	**3**	**10**	**0**	**0.3**	**1**	**3**	**10**
Piloerection				1	4				4	4
Few faeces					4				3	5
Nasal discharge					4					2
Increased salivation										1
Few faeces					4				3	5
Tonic convulsions*					2 (d5)					1 (d4)
Clonic convulsions*					1 (d5)					1 (d5)
Suspected convulsions*					1 (d7)					
Death					5				1	5

*incidence (the day when the sign first seen).

Food consumption was reduced at 3 and 10 mg/kg bw/d (up to 69% and 32% lower respectively). This was associated with significant bodyweight loss in rats at 10 mg/kg bw/d (males 20%, females 23%, week 1), and lower bodyweight gain at 3 mg/kg bw/d, particularly in females, which led to slightly lower bodyweights (3-10% lower than control) by the end of study.

White blood cell counts lower than controls, largely accounted for by a fall in lymphocyte numbers, were noted in some females at 1 and 3 mg/kg bw/d (see Table below). Relative to controls, neutrophils (%) were increased in 3 mg/kg bw/d females, but absolute neutrophil numbers were similar. Given the lack of statistical significance for the reductions in total WBC and lymphocyte numbers due to high variability, and that the WBC values are within the mean control range for SD rats[8], these changes are not considered to be biologically significant. Lower total plasma bilirubin was observed in females at ≥1 mg/kg bw/d, and was possibly related to treatment, though high variation in control values, and small numbers/group suggest that this was unlikely to be treatment-related at 1 mg/kg bw/d. In addition, increased total protein was observed in females at 3 mg/kg bw/d, though this was slight and considered unlikely to have biological significance.

Table 48: Findings in haematology and clinical chemistry

	Males					Females				
Dose (ppm)	**0**	**0.3**	**1**	**3**	**10**	**0**	**0.3**	**1**	**3**	**10**
WBC (10^9/L)	13.3	14.3	12.9	13.5	-	8.65	10.3	6.33	4.87	-
Neutrophil (10^9/L)	1.56	1.26	0.95	1.47	-	0.76	0.98	0.77	1.00	-
(%)	11.9	8.00	7.40	11.0	-	9.00	9.40	13.5	22.0**	-
Lymphocyte (10^9/L)	11.4	12.7	11.6	11.6	-	7.55	9.00	5.41	3.77	-
(%)	86.4	88.8	89.0	85.8	-	87.4	87.2	83.8	75.9*	-
Total bilirubin (μmol/L)	1.70	1.84	1.50	1.26	-	2.38	2.86	1.62	1.20*	-
Total protein (g/L)	60.4	61.2	62.2	61.2	-	60.5	61.2	61.2	64.0*	-

*p<0.05; **p<0.01 by Bartlett's test and Dunnett's test.

Necropsy revealed pale livers in females at 1 mg/kg bw/d and above, including decedents at 10 mg/kg bw/d, but there were no supportive findings at microscopic examination. Pinpoint dark spots were noted on the glandular stomach of the decedent female at 3 mg/kg bw/d. Atrophic (inactive) follicles, characterised by a very flattened follicular epithelium, were noted in the thyroid gland in all groups including the control, though the most severe atrophy occurred in 1 male at 3 mg/kg bw/d and 2 females at each of 3 and 10 mg/kg bw/d (tabulated below). The no-effect level was 1 mg/kg bw/day based on death, clinical signs and reduced bodyweight gain at ≥3 mg/kg bw/d.

Table 49: Histopathology findings (n = 5)

Dose	**Males**					**Females**				
(mg/kg bw/day)	**0**	**0.3**	**1**	**3**	**10**	**0**	**0.3**	**1**	**3**	**10**
Thyroid, Atrophic follicles	1##	2#, 2##	1#, 3##	3##, 1###		4#	4#, 1##	3#, 1##	2#, 1##, 2###	1##, 2###

#: slight; ##: mild; ###: marked.

Dange M (1995a) MB 46513: Preliminary 28-day toxicity in the rat by dietary administration. Report No. SA 93138. Lab: Rhone-Poulenc-Secteur Agro, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agrochimie Toxicology. Research Triangle Park, NC USA. Expt. dates: May 1993–February 1994. Unpublished report date: 6 November 1995. (GLP/QA: no Guidelines: none stated)

Materials and Methods Sprague-Dawley rats (10/sex/dose) received MB 46513 (desulfinyl fipronil) (Batch: 10 DGM 22, Purity: 97.5%) in the diet at 0, 0.5, 3, 30 or 100 ppm (0,

0.04/0.04, 0.23/0.24, 2.20/2.32 and 3.74/3.80 mg/kg bw/d respectively for males/females, determined by sample analyses) for 28 days. The rats (from Charles River France, St Aubin-les-Elbeuf, France) were 5-6 weeks old, bodyweights 223-260 g (males) and 168-206 g (females) at the start of exposure period.

Rats were observed daily for mortality and clinical signs. Physical examinations were performed weekly. Bodyweight and food consumption were recorded weekly. A blood sample was collected from each rat on days -1, 7 and 23/24 for T3, T4 and TSH measurements. Blood samples were taken from fasted rats on days 23/24, and overnight urine samples were collected on days 29/30/31, for urinalysis and haematology (as in Appendix III, except for clotting parameters), and clinical chemistry (most parameters in Appendix III). Selected organs (as in Appendix IV, plus epididymis, pituitary gland, prostate, thymus, uterus) were weighed and a range of tissues (as in Appendix IV for the 0 and 30 ppm groups, and thyroid/parathyroid, mammary glands, uterus and vagina for all groups) were fixed and examined microscopically.

Results Mortalities occurred in 1 male at 30 ppm on day 6 and all rats at 100 ppm over days 5 to 15. One female at 100 ppm had tonic and clonic convulsions on day 7, five days before death. Other clinical signs in survivors at 30 and 100 ppm consisted of piloerection, thin appearance, emaciation, excessive vocalisation and curling up upon handling. Bodyweight loss occurred in rats at 100 ppm (18% and 13% weight loss for males and females respectively) in week 1, and lower bodyweights were seen at 30 ppm (up to 17% and 13% lower than control for males and females respectively). These effects were associated with reduced food consumption (30-34% and 68-73% lower than control) at 30 and 100 ppm in the first 2 weeks.

On Day 7, relative to controls, plasma T3 (females) and T4 levels (both sexes) were reduced in surviving rats at 100 ppm, as well as T4 levels in males at 30 ppm (see Table below). Decreased T3 (males) and T4 levels (both sexes) were also observed at 30 ppm on Day 23. The statistically significant low levels of T4 in 3 and 30 ppm females at pre-test relative to the controls, suggest that this parameter was highly variable, or there were technical problems with the assay. However, as 7/10 values in the 30 ppm female group were lower than concurrent controls on day 23, the reduction in T4 in females was considered an effect of treatment at ≥30 ppm. There were no effects on TSH.

Table 50: Percent change in T3, T4 and TSH plasma levels relative to control (n=10)

		Males					Females				
Dose (ppm)		**0**	**0.5**	**3**	**30**	**100@**	**0**	**0.5**	**3**	**30**	**100**
T3	pretest	0	- 7	- 13	- 8	- 13	0	2	6	- 6	- 5
	day 7	0	0	0	0	- 17	0	0	0	0-18	-46 ***
	day 23	0	-23	-25	-40 **	ND	0	0	0	-23	ND
T4	pretest	0	-5	-14	-2	7	0	-18	-37***	-41***	-19
	day 7	0	0	0	-33***	-63*	0	0	0	-21	-50**
	day 23	0	0	0	-49**	ND	0	0	0	-61***	ND

@: n=3 due to deaths. ND: no data due to deaths.
*p<0.05; **p<0.01; ***p<0.001 by Dunnett's t test or Wilcoxon U test.

At 30 ppm, mean total bilirubin was lower than controls by 28% and 33% (p<0.01) in males and females respectively. No other significant treatment-related changes were noted in haematology, clinical chemistry or urinalysis. At termination, female thymus weights were

lower by 28% ($p<0.01$), and male kidney weights were lower by 13% ($p<0.01$), both at 30 ppm, without corresponding microscopic changes. Microscopic examination revealed minimal changes in the uterus (endometrial hyperplasia, luminal dilatation) and mammary gland (hyperplasia, increased tissue) in some females in each group, without a relationship to treatment. The dose at which no effects were seen was 3 ppm (equal to 0.23 mg/kg bw/d) based on clinical signs and deaths, reduced bodyweight gain, and decreased plasma T3, T4 and total bilirubin at 30 ppm (equal to 2.2 mg/kg bw/d).

Dogs

Dange M (1995b) MB 46513: Preliminary 28-day toxicity in the dog by dietary administration. Report No. SA 94143. Lab: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc-Secteur Agro, Direction Internationale de la Toxicologies, 14-20 rue Pierre Baizet, BP 9163, 69263 Lyon Cedex 09, France. Expt. dates: 11.5.1994-1.9.1994. Unpublished reort date: 5 September 1995. (GLP/QA: no; Study Guidelines: none)

Materials and Methods Beagle dogs (2/sex/dose) received MB 46513 (desulfinyl fipronil; Batch: 10 DGM 22, Purity: 97.5%) via the diet at 0, 27, 80 or 270 ppm for up to 28 days. In ascending order, the achieved doses were 0, 1/1, 1.9/1.7 and 2.3/2.3 mg/kg bw/d respectively for males/females in the first week, but decreased to 0.7/0.4 and 0.1/0.1 mg/kg bw/d for the mid and high dose groups in the second week due to inappetence. The dogs (from CEDS, 89130 Toucy, France) were 19-21 weeks old, and weighed 5.7-7.8 kg (males) and 5.7-6.6 kg (females) at the start of dosing. Dogs were observed daily for mortality and clinical signs. Bodyweights were recorded weekly, and food consumption daily. Physical examinations were performed weekly. Additionally, a specific neurological examination was performed which included cranial nerve reflexes (pupillary light, consensual light, palpebral blink, corneal reflex), segmental reflexes (flexor), and postural reactions (placing, visual, tactile). Ophthalmological examinations were performed at pre-test and at the end of treatment. Blood samples were collected on days -12, -5 and 28 for haematology and clinical chemistry, and urine samples were collected overnight on days -5 and 28 for urinalysis. Testing was essentially as in Appendix III, with the omission of clotting parameters, and a few clinical chemistry parameters. Selected organs (epididymis, pituitary gland, prostate, thymus, uterus, and others as in Appendix IV) from all groups were weighed and a range of tissues (as in Appendix IV) from all groups were fixed and examined microscopically.

Results Treatment of dogs at 270 ppm was terminated on day 10 due to a lack of food consumption since day 5, and the dogs were sacrificed without necropsy. At 80 ppm, 1 male and 1 female were sacrificed moribund on day 10, and the remaining dogs in this group were sacrificed moribund on day 15. All dogs at 27 ppm survived the study period. 'Fear' and severe clonic convulsions were observed in 1 male at 27 ppm shortly before the scheduled sacrifice. Reduced motor activity, staggering step, irritability, increased salivation, absence of or few faeces, and emaciation were observed at 80 ppm. Few or absence of faeces and emaciation were also observed at 270 ppm.

All dogs lost weight at 80 (0.3-1.2 kg) and 270 ppm (0.3-1.8 kg). Dogs at 80 ppm had decreased food consumption starting on day 4 or 6 (up to 4-13% of that on day 1), and dogs at 270 ppm had a marked decrease in food consumption from day 2, and practically stopped eating on day 6 until the treatment was terminated on day 10. In surviving dogs, no treatment-

related changes were observed in ophthalmology, haematology, clinical chemistry or urinalysis.

At gross necropsy, one female at 27 ppm had a pale liver. For the 4 dogs sacrificed moribund at 80 ppm, pale, multifocal whitish areas or mottled appearance were observed in the livers of 2 males and 1 female, and diffuse sinusoidal leukocytosis and centrilobular hepatocytic enlargement were revealed microscopically in the livers of all dogs at this dose, with mild multifocal hydropic degeneration of the hepatocytes and chronic hepatitis along with periportal fibrosis in 1 male and 1 female. Observations of small thymus (3/4 dogs) correlated with marked thymic atrophy seen in all dogs at 80 ppm. Also at this dose, multifocal red areas were seen on the lungs of 1 female, and pinpoint black spots on the gastric mucosa of the other female in this group. A no-effect level was not established in this study due to clonic convulsions at the lowest dose (equal to 1 mg/kg bw/d).

11.1.4 Subchronic studies

Mice

Bigot D (1996) MB 46513: 90-day toxicity study in the mouse by dietary administration. Study No. SA 95055. Lab: Rhone-Poulenc Agrochimie, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Expt. dates: 23.3.1995-29.8.1995. Unpublished report date: 12 January 1996. (QA/GLP: yes; Guidelines: EPA/FIFRA 82-1, 1984)

Materials and Methods Groups of OF1 mice (10 /sex/dose from Iffa-Credo, L'Arbresle, France; 6-7 weeks old; 22.2-32.6 g) were fed certified rodent diet AO4CP1 *ad libitum* mixed with MB 46513 (desulfinyl fipronil) (Batch: 805-DAP; Purity: 96%) at 0, 0.5, 2 or 10 ppm for at least 90 days. The measured dietary intake of the test material (mg/kg bw/d) was 0, 0.08/0.11, 0.32/0.43 and 1.74/2.15 in males/females in increasing order of dose. The stability of the test material in the diet had been demonstrated in a previous study, where samples of 0.5 and 100 ppm diets were stable at -15°C for 52 days. Homogeneity and stability were checked in the current study at 0.1 and 10 ppm, and were acceptable at ambient temperature and below -15°C over 94 days. Diet formulations were prepared twice during the course of the study. All animals were checked for clinical signs, moribundity and deaths twice daily. Detailed physical examinations were performed at least once weekly. Bodyweights and food consumption were recorded at weekly intervals. Blood samples were taken on days 91-93 for plasma chemistry analysis (bilirubin, urea, total protein, albumin, cholesterol, AST, ALT and ALP) prior to necropsy. Animals found dead were also necropsied. Necropsy included gross examination, and weighing of brain, heart, kidneys, liver, spleen and testes. Organs/tissues were sampled for histological examination (only minor variations to those listed in Appendix IV). Histological examinations were performed on all tissues from the control, mid and high dose groups. Liver, lung and kidney were examined in all groups.

Results Ten mice at 10 ppm were found dead (1 female on day 5; nine males on days 20-62). The last surviving male at 10 ppm was killed moribund on day 84. Excessive jumps, aggression and/or irritability to touch occurred sporadically in treated animals, but at a low incidence (1-2 animals in each group) and without an apparent relationship to dose. However, as 5 of the 10 ppm males died relatively early in the study (5 on day 28 or before), and the remainder by day 62, this may have decreased the likelihood of observing these signs at the

top dose. The single 10 ppm male that survived until close to the end of the study, was irritable to touch on 5 occasions between days 70 and 78 inclusive, and showed aggression on days 77 and 78. In males at 2 ppm, one mouse was irritable to touch and showed aggression on 3 occasions (days 70-72), while another was irritable to touch, with increased motor activity on day 29 and showed aggression on day 33. At 0.5 ppm, aggression was observed in one male on day 78, and in one female on 3 occasions between days 42 and 78. Given that these signs were associated with MB 46513 (desulfinyl fipronil) treatment in rat studies (Dange 1994d, Bigot 1998), the signs in the 2 mice at 2 ppm cannot be dismissed, and are therefore considered treatment-related.

No significant differences were noted in mean bodyweights or bodyweight gains, although 10 ppm males had slightly lower mean bodyweight after day 45 approximately. A statistically significant increase in ALP activity relative to controls (74 ± 25, 95 ± 32, 102 ± 39, 119* ± 57 IU/L in ascending order of dose, $p \leq 0.05$) was seen in the 10 ppm females (no data available for the 10 ppm males due to deaths). However, the wide ranges of values reported for all treatment groups, the absence of changes in other liver enzymes, and the lack of supporting pathology in 10 ppm females, suggest that the change in ALP was not treatment-related.

No treatment related organ weight changes were seen at up to 2 ppm in males and 10 ppm in females. Gross examination revealed liver enlargement in 3/10 decedent males, and small thymus in four. In the unscheduled deaths, histological examination showed mild centrilobular hypertrophy of the liver in 6/10 males. Hepatocellular mitotic figures were also seen in one decedent male in which hypertrophy was slightly more marked. Autolysis prevented examination of the decedent female. No treatment related histological changes were seen in other groups after scheduled sacrifice. The NOEL was 0.5 ppm (equal to 0.08 mg/kg bw/d), based on clinical signs of aggression and irritable to touch at 2 ppm (0.3 mg/kg bw/d).

Rats

Dange M (1994d) MB 46513: 90-day toxicity study in the rat by dietary administration. Study No. SA 93226. Lab: Rhone-Poulenc - Secteur Agro, Sophia Antipolis, France. Sponsor: Rhone-Poulenc - Secteur Agro, Lyon, France. Expt. dates: 24.8.1993-24.2.1994. Unpublished report date: 17 June 1994. (QA/GLP: yes; Guidelines: EPA/FIFRA 82-1, 1984)

Materials and Methods Sprague Dawley rats (from Charles River, France; 10 /sex/dose; 6-7 weeks old; 176-296 g weight range) were fed certified rodent diet AO4CP1 *ad libitum* mixed with MB 46513 (desulfinyl fipronil) (Batch 10DGM22; 97.5-99.7% purity) at 0, 0.5, 3, 10 or 30 ppm for at least 90 days. The measured dietary intake of the test material (mg/kg bw/d) was 0.03, 0.18, 0.59 and 1.77 in males, and 0.035, 0.21, 0.71 and 2.1 in females in increasing order of dose. The stability of the test material in the diet had been demonstrated in a preliminary 28 day study, where samples of 0.5 and 100 ppm diets were stable at -15°C for 52 days. Stability was checked in the current study at 0.5 and 30 ppm after storage (frozen) for 6 weeks. In addition, samples were taken from food pots (0.5 and 30 ppm) at weeks 4, 8 and 12. The stability in the diet was satisfactory. Homogeneity and dietary concentrations were also found to be satisfactory. Diet formulations were prepared approximately every 3 weeks during the course of the study, and stored at -18^0C.

All animals were checked for clinical signs, moribundity and deaths twice daily. Detailed physical examinations were performed at least once weekly. Bodyweights and food consumption were recorded at weekly intervals. Ophthalmological examination was performed before treatment, and at week 12 in control and 30 ppm animals. Blood samples were taken on days 85, 86 or 87 prior to necropsy for haematological (as in Appendix III, but not platelets) and plasma chemistry analyses (as in Appendix III, omitting GGT, LDH, CPK and globulin). Additionally, blood samples were taken during weeks 2 and 10 for analysis of T3, T4 and TSH. Urine was collected for analysis on days 91-94 prior to necropsy (as in Appendix III). Animals found dead were also necropsied. Necropsy included gross examination, and weighing of adrenals, brain, epididymis, heart, kidneys, liver, ovary, pituitary gland, prostate, spleen, testes, thyroid and uterus. A number of organs/tissues were sampled for histological examination in all groups (listed in Appendix IV).

Results At 30 ppm, one male was killed moribund on day 45 and 3 females died (days 11, 13 and 64). Clinical signs included aggression, irritability to touch and excessive vocalisation, seen mainly in the 10 and 30 ppm groups, and occasionally in one male at 3 ppm. At ≥10 ppm, these signs were mainly apparent during weeks 3-5 but occurred from time to time throughout the study. In the 3 ppm rat, aggression was observed once on day 41, irritability to touch on 3 occasions between days 50 and 84, with excessive vocalisation noted for the same animal on day 70. As there were similar findings at the higher doses (though earlier in the treatment period), the clinical signs at 3 ppm cannot be dismissed.

Mean bodyweights were lower in 10 and 30 ppm males throughout the study, attaining significance ($p<0.01$) from week 2 in 30 ppm males, and from week 7 in 10 ppm males. In females, mean bodyweight was significantly lower ($p<0.01/0.05$) at 30 ppm for weeks 2-5, and thereafter showed some recovery. Bodyweights were also lower in 10 ppm females but the effect did not reach significance. Food consumption was significantly lower ($p<0.01$) in 30 ppm animals in weeks 1-2, but thereafter was comparable to controls.

No ophthalmological abnormalities were reported. Haematological and urine examinations revealed no treatment related effects. At 30 ppm in females, significantly lower total bilirubin (-43%; $p<0.05$), total cholesterol (-24%; $p<0.05$), and triglycerides (-25%; $p<0.05$) were seen (relative to controls). The triglyceride results varied considerably across the dose range, and as they displayed no relationship to dose, they were not considered to be due to treatment. However, the findings for bilirubin (1.82, 1.67, 1.58, 1.38 and 1.04* µmoles/L) and cholesterol (1.02, 0.98, 0.97, 1.08, 0.78* mmol/L), listed in ascending order of dose, cannot be dismissed on these grounds. The study author indicated that the individual values for these parameters at 30 ppm were within their respective historical control ranges, and therefore were not of toxicological significance. However, it is still possible that these changes represent an effect of treatment. Thyroid hormone analysis revealed decreases relative to controls in the following: T4 in 30 ppm males at week 2 (-48%; $p<0.01$) and week 10 (-25%; not statistically significant); T4 in 30 ppm females at week 10 (-29%; not statistically significant); T3 in 30 ppm males at week 10 (-29%; $p<0.05$). In all these cases, the T3 or T4 values were largely within a quite broad concurrent control range, albeit it the low end, or not far below the lowest control value, possibly representing a threshold effect.

Significant increases in brain to bodyweight ratios at 10 and 30 ppm in males were considered to be related to decreased terminal bodyweights in these animals. Other significant increases in heart (3 ppm males) and liver (10 ppm females) weights relative to bodyweight showed no dose relationship, and were not considered to have toxicological significance. In the

unscheduled deaths, gross examination revealed enlarged adrenals in all animals, necrotic areas of the liver in two females, and focal gastric ulcerations/erosions in the male and one female. No gross findings were reported in the animals sacrificed on schedule. Histological examination revealed no treatment related changes. The NOEL was 0.5 ppm (equal to 0.03 mg/kg bw/d), based on treatment related clinical signs at 0.2 mg/kg bw/d.

Dogs

Dange M (1996) MB 46513: 90-day toxicity study in the dog by dietary administration. Study No. SA 95100. Lab: Rhone-Poulenc Agrochimie, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Study period: 3.5.1995-24.10.1995. Unpublished report date: 14.5.1996. (QA/GLP: yes. Guidelines: EPA/FIFRA 82-1, 1984)

Materials and Methods Groups of purebred beagle dogs (5/sex/dose; from HARLAN FRANCE, France; approx. 35 weeks old) were fed certified canine meal JAPE21 (300 g/d) mixed with MB 46513 (desulfinyl fipronil) (Batch 805DAP; 96% purity) at 0, 3.5, 9.5 or 35 ppm for 90 days. The measured dietary intake of the test material (mg/kg bw/d) was 0.1, 0.27 and 0.95 in males, and 0.1, 0.29 and 1.05 in females in increasing order of dose. The stability (frozen and room temp. for 35 days) and homogeneity of the test material in the diet had been demonstrated in a preliminary study at 4 and 40 ppm. Diet formulations were prepared 7 times during the course of the study, and stored at room temperature. Dietary concentrations were verified on preparations for weeks 1, 2, 3, 4, 8 and 12, and were within target ranges. Prior to treatment, all animals were subjected to a detailed clinical examination (including haematology, clinical chemistry and urinalysis - see below for details).

All animals were checked for clinical/behavioural signs, moribundity and deaths twice daily. Detailed physical examinations were performed at least once weekly. Additionally, a specific neurological examination was performed which included cranial nerve reflexes (pupillary light, consensual light, palpebral blink, corneal reflex), segmental reflexes (flexor), and postural reactions (placing, visual, tactile). Bodyweights and food consumption were recorded at weekly intervals. Ophthalmological examination was performed before treatment, and at weeks 6 and 13 in all animals. Blood samples were taken by jugular vein puncture at weeks 6 and 13 for haematological (as in Appendix III, omitting reticulocyte count) and plasma chemistry analyses (as in Appendix III, omitting GGT, LDH, CPK and globulin). Urine was collected on days -8, 42 and 85 for analysis (as in Appendix III). Survivors were killed on days 91-94 and subjected to necropsy. Animals found dead were also necropsied. Necropsy included gross examination, and organ weighing (adrenals, brain, epididymis, heart, kidneys, liver, ovary, pituitary gland, prostate, spleen, testes, thymus, thyroid and uterus). Organs/tissues were sampled for histological examination in all groups (essentially as in Appendix IV).

Results One 35 ppm female was killed on day 28, after exhibiting increased salivation, prostration, writhing, tremors, absence of rotular reflex, noisy breathing and dyspnea. Necropsy revealed marked coronary arteritis and myocardial necrosis, so the necessity for this animal to be put down may not have been due to treatment. However, as another 35 ppm female exhibited excessive barking and aggressiveness on day 28, and salivation, irritability and tremors on day 86, the clinical signs in the prematurely sacrificed dog may have been treatment-related. Signs in other animals occurred with comparable frequency between

groups. Mean bodyweights and food consumption were not affected by treatment, and no ophthalmological or haematological abnormalities were reported.

In week 13, a 9.5 ppm male had high ALT (178 *vs* 34 in control) and ALP activities (374 *vs* 52), but in the absence of effects in other animals at the same dose or at higher doses, this was considered incidental to treatment. A slight but statistically significant ($p<0.01$) increase in urinary pH was seen in 35 ppm males at week 13, but in the absence of related findings, this is not considered toxicologically significant. At terminal sacrifice, no significant differences were seen in organ weights, or at gross or microscopic examination (including the female killed on day 28). The NOEL was 9.5 ppm (equal to 0.27 mg/kg bw/d), due to clinical signs in females at 35 ppm (equal to 1 mg/kg bw/d).

11.1.5 Chronic studies

Rats

Bigot D (1998) Chronic toxicity and carcinogenicity study of MB 046513 in the Sprague Dawley rat by dietary administration. Report No. SA 95156. Lab: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agro, Lyon, France. Expt. dates: 8.6.1995-12.12.1997. Unpublished report date: 23 June 1998. (GLP/ QA: yes. Guidelines: EPA/FIFRA, 83-5; OECD 453, 1981; EEC B33, 1992)

Materials and Methods MB 46513 (desulfinyl fipronil) (Batch: 805DAP; Purity: 96.0-99.2%) was administered in the diet at 0, 0.5, 2 or 10 ppm to 10 rats/sex/dose designated for interim sacrifice in week 54, and to 60 rats/sex/dose designated for final sacrifice in week 105 or 106. The dose for female rats in the 10 ppm (0.853 mg/kg bw/d) group was reduced to 6 ppm after 26 weeks of treatment due to an increase in mortality rate. The mean achieved test material intake was 0.025/0.032, 0.098/0.127 and 0.497/0.546 mg/kg bw/d for males/females at 0.5, 2 and 10/6 ppm respectively. The rats (Sprague Dawley, Ico:OFA SD, IOPS Caw, from Iffa-Credo, St Germain-sur-L'Arbresle, France) were 6 to 7 weeks old at the start of treatment. The homogeneity, concentration and stability of the test chemical in the diet were analysed and were generally within acceptable ranges.

All animals were checked for clinical signs, moribundity and deaths daily. Detailed physical examinations were performed weekly or fortnightly. Bodyweights and food consumption were recorded weekly during the first 13 weeks of treatment and once every 4 weeks thereafter. Ophthalmological examination was performed before treatment, and after 1 and 2 years. Blood samples were taken from fasted and anaesthetised animals (10/sex/group) in weeks 26, 52, 78 and 104 for haematology (see Appendix III) and clinical chemistry analyses (as in Appendix III, omitting GGT, LDH, CPK and globulin). Urine samples were collected overnight at weeks 25, 51, 77 and 103 for urinalysis (Appendix III). All animals, either found dead or moribund, or killed by design, were necropsied and selected organs/tissues (adrenals, brain, epididymis, heart, kidneys, liver, ovary, pituitary gland, prostate, spleen, testes, thymus, thyroid with parathyroid and uterus) were weighed. Histopathological evaluations were performed on selected tissues (essentially as in Appendix IV) from all animals found dead or moribund, all terminal sacrifice animals, and all interim sacrifice animals in the control and high dose groups, and on the liver, lung, kidney and spleen from all animals in the low and intermediate dose groups, and on macroscopic findings.

Results Prior to dose reduction, 7 females at 10 ppm died, compared to 1 death in the control group. By study termination, the mortality rate in all treated male groups was higher than the corresponding control. However, given the lack of a dose-response relationship across the treated male groups, the increased mortality in males was not considered to be an effect of treatment (see Table below). For females, there was a statistically significant positive trend for increasing mortality, but pairwise comparison with the control was statistically significant only at the high dose. Kaplan-Meier estimations of survival (95% confidence intervals) showed that the adjusted survival rates in females were outside the historical control range at ≥2 ppm, and therefore it is possible that the increased mortality at these doses was related to treatment.

Table 51: Mortality

	Males				**Females**			
Dose (ppm)	**0**	**0.5**	**2**	**10**	**0**	**0.5**	**2**	**10 / 6**
Week 26	1	0	1	3	1	0	0	7
Week 53	2	1	4	6	5	1	3	8
Week 104 (n) (%)	28 (40%)	40 (57%)*	37 (53%)	40 (57%)*	28 (40%)	32 (46%)	37 (53%)	41 (59%)*
Adjusted survival# (%)	53.6%	33.4%	38.7%	35.9%	54.0%	46.8%	40.6%	35.4%
Historical control of adjusted survival	38.3-58.8%				43.6-61.8%			

*p<0.05 by Cox's test.
Kaplan-Meier estimated survival rates at the end of the study after adjusting for censored animals.

At ≥2 ppm in males and in 10/6 ppm females, the number of animals displaying aggression and/or irritability to touch was increased (M/F: 3/0, 3/4, 7/1, 12/28, in increasing order of dose), and this was considered treatment-related. Though these signs occurred in more females than males at the top dose, they mostly occurred only once in females, but were generally reported on multiple occasions in males (up to 14 times). Tonic or clonic convulsions were observed in all groups, including the control. For the rats in which convulsions were observed, this finding was usually reported on more than one occasion, but the number of convulsive episodes reported per animal was not dose-related. The maximum number of reported convulsions in a single animal was 14, this being achieved in one female in each of the control and 0.5 ppm groups. One male at 10 ppm and 1 female at 2 ppm were found dead after convulsions. Though there was an apparent dose-related trend of an earlier onset of convulsions in treated females, there was wide intra-group variability, with the average first day of convulsions not significantly different in any treated group when compared to the control group, using the Mann-Whitney-Wilcoxon test. The incidence of females showing convulsions at 2 and 10/6 ppm was statistically significantly higher than the concurrent control, as well as above the range of the historical controls provided by the sponsor (see Table below), and is considered treatment-related.

Table 52: Incidence of convulsions (n = 70)

Dose (ppm)	Males				Females			
	0	**0.5**	**2**	**10**	**0**	**0.5**	**2**	**10 / 6**
Tonic convulsions, n	2	1	6	3	2	5	9	11
Clonic convulsions, n	7	2	9	9	5	8	13	16
Total Incidence (tonic/ clonic convulsion), n (%)	7 (10)	2 (2.8)	9 (12.8)	10 (14.3)	5 (7.1)	8 (11.4)	13 (18.6)*	20 (28.6)**
Historical control (tonic and/or clonic), %	1.2 – 13.3%				2.5 – 16.7%			
First day of onset (mean ± SD)					394 ± 61	356 ± 61	344 ± 171	301 ± 169
(median)					412	370	308	255

*p<0.05, **p<0.01 by Fisher's exact test (one-tail).

Because of the highly unusual background incidence of convulsions in the control animals, the task of determining the toxicological significance of the increased incidence in the treated groups, particularly the 0.5 ppm females, is very difficult. Also, given that the frequency of observing the animals was a maximum of twice per day, for the observers to be present when a convulsion was occurring was a matter of chance. The actual observations can only reflect a sample of the number of convulsions that may have occurred, though all things being equal, this should have a similar effect on all groups. The experimental dates for the studies that comprise the historical control data were not provided, but their study numbers indicate that the earliest studies in which convulsions were observed in controls commenced in 1994. Therefore, this information would not have been available at the time the present study commenced. Up to the conclusion of the present study, the animal supplier had not observed convulsions in stock rats up to 24 months old, but suggested that this may have been due to the very limited handling that rats are subjected to in this environment, relative to the experimental situation. However, Iffa-Credo indicated that they had been aware of spontaneous convulsions in OFA-SD rats used in studies in both France and abroad, and had 'closely monitored the situation which appears to be stable'. Whether this information was conveyed to the sponsor prior to study initiation is not known. Though less than ideal, the study and historical control data will therefore be accepted on their merits, with 0.5 ppm considered the no-effect level for convulsions.

There was no treatment-related effect on food consumption and bodyweight, and no treatment related ophthalmoscopic findings. Males at 10 ppm had increased neutrophils (%) and decreased lymphocytes (%) in week 104, but as there were no changes in absolute WBC numbers, this was not considered to represent a toxicologically significant finding. Temporary or sporadic changes in haematology, clinical chemistry or urinalysis, including significant decreases in total bilirubin (20%, $p<0.05$), triglyceride concentrations (44%, $p<0.01$), and an increase in glucose concentration (20%, $p<0.01$) in females at 6 ppm in week 26, and elevated plasma phosphate concentration in females at 6 ppm (18%, $p<0.05$) at week 104 (a few individual values outside the range of concurrent control values), were considered incidental to treatment.

As shown in the Table below, at interim sacrifice, absolute uterine weight was increased in 10/6 ppm females (44%, $p<0.05$), accompanied by a non-statistically significant increased relative weight. However, as similar differences were not apparent at terminal sacrifice, this was not considered toxicologically significant. At terminal sacrifice, prostate gland weights were higher than controls in males at 10 ppm, the difference significant only for absolute

weight (33%, p<0.05). Also, adrenal gland weight relative to bodyweight was increased to a statistically significant extent (26%, p<0.05) in the high dose female group. Given the absence of pathological findings in these organs, and the non-dose-related variability in adrenal weight across the dose range, these changes were not considered to have toxicological significance.

Table 53: Selected organ weights (n = 7-10)

Dose (ppm)	Males				Females			
	0	0.5	2	10	0	0.5	2	10/6
Interim, Uterus, g					0.73	0.84	0.76	1.05*
% body					0.18	0.21	0.18	0.24
Final, prostate, g	0.89	0.92	1.02	1.18*				
% body	0.183	0.179	0.185	0.164				
Adrenal, g	0.084	0.100	0.098	0.099	0.094	0.077	0.092	0.105
% body	0.014	0.015	0.014	0.016	0.019	0.015	0.018	0.024*

*p<0.05 by Dunnett's test or Mann-Whitney's test.

Overall, macroscopic examination did not reveal any treatment-related findings. The incidence of spongiosis hepatis was increased in males at 2 and 10 ppm relative to the control group (see following Table). When compared with the findings from a concurrent study in the same laboratory, which showed an incidence of 5/30 for this lesion in terminal sacrifice control males, and in control decedent and terminal sacrifice males together of 7/70, then the incidences in the present study are only slightly greater. It therefore seems likely that the changes in the present study are within the normal limits for this strain of rat. Diffuse hepatocyte hypertrophy (slight or mild) was also a finding of relatively high incidence, but given the absence of a dose-relationship, this is not considered a consequence of treatment. The incidence of ovarian atrophy was increased in all treated groups, particularly in the decedents, but given the flat dose response, this is also unlikely to be linked to exposure to the test material.

Table 54: Microscopic findings

Dose (ppm)	0		0.5		2		10	
	D	T	D	T	D	T	D	T
Males, number examined	27	33	39	21	36	23	40	22
Spongiosis hepatis, n (%)	0 (0)	2 (6)	1 (3)	1 (5)	0 (0)	5 (22)	1 (3)	7 (32)
Females, number examined	28	32	32	28/27	37	25/24	41/40	22
Diffuse hepatocyte hypertrophy, n (%)	4 (14)	1 (3)	4 (13)	6 (21)	0 (0)	6 (24)	4 (10)	4 (18)
Ovary atrophy, n (%)	4	3	8	4	13	5	13	5

D=decedent; T=terminal.

There was an overall low incidence of neoplastic changes in all organs, including the thyroid. A slight, but statistically significant higher incidence (in age-adjusted analysis) of pituitary gland adenomas (pars distalis) was observed in males at 10 ppm (see Table below). Statistical analysis by the logistic prevalence method did not reveal any treatment-induced, dose-related or statistically significant increase in the incidence of tumours in the pancreas (adenoma, adenocarcinoma, islet cell), adrenal gland (adenoma, cortex), mammary gland (adenocarcinoma), pituitary gland (adenocarcinoma, pars distalis), thyroid gland (adenoma, carcinoma, C-cell) or uterus (polyp, endometrial stromal). The NOEL was 0.5 ppm (equal to 0.03 mg/kg bw/d), based on increased mortality and convulsions in females at 2 ppm, and an

increased incidence of aggression and irritability to touch in males at the same dose (equal to 0.1 mg/kg bw/d).

Table 55: Neoplastic findings (n = 47-70)

	Males				Females			
Dose (ppm)	**0**	**0.5**	**2**	**10**	**0**	**0.5**	**2**	**6**
Pituitary gland, adenoma, pars distalis, n (%)	24 (35.3)	27 (45.8)	20 (34.5)	31 (44.3)*	30 (43.5)	31 (49.2)	34 (53.1)	29 (42.0)

**p<0.05 by the pairwise comparison one-sided tests*

11.1.6 Developmental studies

Rats

Foulon O (1997) MB 046513: Developmental toxicology study in the rat by gavage. Report No. SA 96227. Lab: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 8.7.1996-12.9.1996. Unpublished report date: 10 April 1997. (GLP/QA: yes, Study Guidelines: OECD 414 (1981), EEC 92/69-Annex V-method B31 (1992), EPA/FIFRA 83-3 (1984)

Materials and Methods Sperm-positive female CD rats (25/dose) were dosed with 0, 0.2, 1.0 or 2.5 mg/kg bw/d of MB 46513 (desulfinyl fipronil) (Batch 805 DAP/DA999, Purity 99.2%) in aqueous solution of 0.5% methylcellulose 400 by gavage during gestation days 6 to 15. Maternal bodyweights and food consumption were recorded daily during dosing and on days 0 and 20. Clinical observations were recorded daily. At necropsy on day 20, the gravid uterine weight was recorded and the dams were evaluated for number of corpora lutea, and number and status of implantations (resorptions, dead and live foetuses). Live foetuses were removed, counted, weighed, sexed and examined externally. Placental weights of live foetuses were also recorded. Approximately half of the live foetuses from each litter was fixed and dissected for internal examination. The remaining half was eviscerated, fixed and stained for skeletal examination.

Results Reduced food consumption (17%) and reduced bodyweight gain (27.5 *vs* 47.3 g in controls, $p<0.01$), were observed at 2.5 mg/kg bw/d during the dosing period. An increased incidence of hair loss (severe in 2 dams) for days 11-20 was also seen at this dose. At 1 mg/kg bw/d, bodyweight gain was less than controls during days 9-12 ($p<0.05$), but this difference was small (8%), so was not considered toxicologically significant. At necropsy, one female at 1 mg/kg bw/d and 3 females at 2.5 mg/kg bw/d had fused placentae. The study author considered that this finding was probably related to the high number of implantations (11-14) in the same uterine horns for these litters. As no deleterious effects appeared to be associated with the fused placentae, this was not considered a toxicologically significant finding.

Foetal bodyweights at 2.5 mg/kg bw/d were 2-4% less than controls, and this was statistically significant ($p=0.05$ for males, $p=0.01$ for females). As the dose response was flat across the dose range, this was considered unlikely to have resulted from treatment. There were no biologically relevant or treatment-related findings for other litter parameters.

External and internal examinations indicated no treatment-related abnormalities. As shown in the Table below, skeletal examination revealed a slightly delayed ossification of bones at 2.5 mg/kg bw/d, including sternebrae, pubic bones and caudal vertebrae, frequently associated with foetuses of reduced bodyweights. At 1 mg/kg bw/d, the slightly increased incidence of incomplete ossification of various bones was mainly accounted for by 2 runt foetuses from the same dam, and therefore was not considered a biologically relevant finding. The NOEL for maternal toxicity was 1 mg/kg bw/d, based on reduced bodyweight gain and severe hair loss at 2.5 mg/kg bw/d. The NOEL for embryo-foetal developmental toxicity was 1 mg/kg bw/d, based on retarded skeletal ossification at 2.5 mg/kg bw/d.

Table 56: Foetal skeletal observations

	Number of foetuses affected / Number of litters affected			
Dose (mg/kg bw/d)	**0**	**0.2**	**1.0**	**2.5**
No of foetuses / litters examined	187 / 24	182 / 24	192 / 25	186 / 24
Nasal/frontal/parietal bones incompletely ossified	1 / 1		3 / 2	3 / 3
5^{th} /6^{th} sternebrae not ossified	54 / 21	66 / 18	73 / 24	82** / 21
1^{st} thoracic vertebral body not ossified	1 / 1	1 / 1	3 / 2	4 / 4
Pubic bone(s) incompletely ossified	3 / 3	3 / 3	2 / 2	6 / 5
Less than 5 caudal vertebrae	2 / 2		3 / 2	8 / 6
1^{st} distal phalanges of forepaws not ossified	1 / 1		2 / 1	3 / 3

***p<0.01 by chi-square test.*

11.1.7 Neurotoxicity

Hughes EW (1996) MB 46513 (fipronil desulfinyl): Neurotoxicity to rats by acute oral administration (including a time to peak effect study). Report No. RNP 471/951489. Lab: Huntingdon Life Sciences Ltd, Cambridgeshire, England. Sponsor: Rhone-Poulenc Inc, 355 rue Dostoievski, BP 153, F-06903 Sophia Antipolis Cedex, France. Unpublished report date: 11 January 1996. (GLP/QA: yes. Study Guidelines: EPA FIFRA 81-8).

Materials and Methods Groups of Crl: CD BR rats [12/sex/dose; from CR Breeding Laboratories, Kent, UK; 6-7 weeks old and weighing 281-293 g (males) or 180-183 g (females) at study initiation] received a single gavage dose of MB 46513 (desulfinyl fipronil) (Batch No. CH089; white powder; purity 99.5%) as a suspension in corn oil at 0 (corn oil alone), 0.5, 2 or 12 mg/kg bw/d. The doses were based on a dose range finding study (RNP/450) performed in the same laboratory (see below). The dosing suspension was prepared on the day prior to use. Analysis of the suspension indicated that the mean concentration was typically within 9% of nominal concentrations, and that the suspension was homogeneous and stable. After dosing, the animals were observed for 14 days. Individual animals were observed for signs of ill health and palpated at least once daily. Additional checks were made in the morning and afternoon for dead or moribund animals. Bodyweights and food consumption were recorded weekly. A functional observation battery (FOB) (see Appendix VI) and motor activity assessment were performed on all animals before treatment, at 6 hours post dosing, and on days 7 and 14. Not all animals were tested on one day, but time of testing was balanced across groups. The FOB comprised 4 sets of observations (home cage, in the hand, arena, manipulations). Motor activity was assessed over a 1 hour period using a Coulbourn infra-red activity monitoring system. For testing, the animals were placed singly

into observation cages, and the following recorded: no movement, locomotor and non-locomotor activities.

The study was terminated on day 15. The animals were anaesthetised with pentobarbital and perfused *in situ* with heparinised 0.7% sodium nitrite followed by a 1.5% glutaraldehyde: 4% paraformaldehyde solution. The skull was removed, followed by the brain, which was weighed and measured (length from the rostral part of the cerebral hemispheres to the most caudal part of the cerebellum, and width across the widest part of the cerebral hemispheres). The sciatic, tibial and sural nerves were exposed. The spinal cord and column were fixed *in toto* integral with the carcass. After overnight storage, CNS tissue samples were taken and stored in buffered formalin for at least 48 hours prior to H&E preparation. Peripheral nerve samples were processed for epon/toluidine blue sections. All sections were examined microscopically. Tissue samples were taken from all animals, but initial histological examination was restricted to 5 animals/sex from the control and high dose groups. Statistical analyses were conducted on the bodyweight and food consumption data, and certain FOB parameters.

Dose range finding study results (Study dates: 13 Feb - 8 March 1995)

Behavioural changes and their time of peak effect was assessed in 4 groups of 4 animals/sex Crl: CD (SD) BR rats given single oral doses of 0.5, 2, 10 or 13.5 mg/kg bw MB 46513 (desulfinyl fipronil) (Batch no. CH089; purity 99.5%) by gavage. A FOB was performed prior to treatment and at 2, 4, 6 and 24 hours post-dosing. Animals were maintained for 7 days, then killed and subjected to a routine macroscopic examination. Other details were similar to those described for the main study.

Signs seen on the day of treatment included piloerection (one male at 13.5 mg/kg bw, one 10 mg/kg bw female), quiet when held (one 13.5 mg/kg bw male), salivation (one 13.5 mg/kg bw female), hunched posture (two 13.5 mg/kg bw females), and cold to touch (two 13.5 mg/kg bw females). Slight weight losses were seen in both sexes at 13.5 mg/kg bw, and in females at 10 mg/kg bw for days 1-2. Thereafter they gained weight. Signs determined during the FOB at 13.5 mg/kg bw included: clonus of jaws, mild clonic convulsion (one female), decreased arousal, decreased rearing and activity counts, palpebral closure, decreased rectal temperature (females only), changes in posture, gait and motor patterns. At the lower doses, observations were typically limited to decreased arousal, decreased rearing and activity counts and changes in motor patterns. Due to the low animal numbers and the absence of controls, it could not be determined if the changes at 0.5 and 2 mg/kg bw were associated with treatment. The majority of signs occurred within 4-6 hours of dosing. Terminal necropsy revealed no effects considered to be treatment related.

Main study results

No unscheduled deaths occurred. The only clinical signs were soft faeces and consequent changes in fur appearance. These were considered attributable to the use of corn oil as the vehicle. A statistically significant ($p<0.01$) lower bodyweight gain was reported for both sexes at 12 mg/kg bw over the first week. In week 2, weight gains were generally comparable in all groups, with the 12 mg/kg bw males gaining slightly but significantly more weight than controls ($p<0.05$). No effect on bodyweight was seen at lower doses, and final mean bodyweights were comparable between groups. Effects seen were probably related to lower

food intakes in both sexes at 12 mg/kg bw over the first week ($p<0.01$), which had returned to control levels in week 2.

Decreased rectal temperature and decreased group mean footsplay values seen in both sexes at 12 mg/kg bw at 6 h post-dosing (both $p<0.05$ and $p<0.01$ in males and females respectively) were considered treatment related. A decreased number of 12 mg/kg bw males displayed an immediate righting reflex on days 7 and 14, statistically significant ($p=0.004$) on day 14. This occurred against a background of similar responses in all groups including the control, but as the righting responses of two of the 12 mg/kg bw males were also unusual (right hindlimb tucked under/hindlimbs outstretched), this was possibly a treatment-related effect. Locomotor activity was decreased in 12 mg/kg bw males and females at 6 h (statistically significant in females; $p<0.01$), and when the data were analysed for the first 30 minutes of the 1 hour observation period, the difference was significant for both sexes ($p<0.01$). This was also considered possibly related to treatment. Due to high intra-group variability, apparent increased mean activity counts in females at ≥2 mg/kg bw on day 7 and at 12 mg/kg bw on days 7 and 14, and an increase in mean rearing counts in 12 mg/kg bw females on day 14, were not considered to have toxicological significance. Also, due to the inconsistency in the direction of change, the increased forelimb grip strength in 12 mg/kg bw females at 6 h, but decreased forelimb grip strength in 12 mg/kg bw males on day 7, were considered unlikely to have toxicological significance.

There were no significant differences in brain weights or dimensions. Initial microscopic examination revealed trace or minimal axonal degeneration in lumbar dorsal root fibres and/or sciatic nerve (sciatic notch and/or mid-thigh) in 4/5 of the 12 mg/kg bw males cf. none in controls. As a result, lumbar dorsal root fibres and sciatic nerve samples were processed and examined for the remaining control and 12 mg/kg bw males, and read blind. In the control males, 4/12 showed trace axonal degeneration in one or two of the tissues (lumbar dorsal root fibres and/or sciatic nerve [sciatic notch and/or mid-thigh]). At 12 mg/kg bw, 6/12 males showed trace axonal degeneration in one, two or all three of the tissues. The incidences of axonal degeneration were not significantly increased in the treated group, and are provided in the Table below. Taking into account the minimal severity of the findings, and that the lesions were also found in the control group, with only a slightly increased incidence at 12 mg/kg bw, these findings were not considered toxicologically significant. The same conclusion was reached for females, where 1/5 control and 2/5 of the 12 mg/kg bw animals showed trace axonal degeneration in the sciatic nerve. The no-effect level in this study was 2 mg/kg bw due to reduced bodyweight gain in the first week after treatment, and findings in the FOB tests (decreased footsplay, decreased rectal temperature, slow righting reflex, decreased locomotor activity) at 12 mg/kg bw.

Table 57: Axonal degeneration in males (n=12)

Region	Finding	Control	12 mg/kg bw
Lumbar dorsal root fibre	trace	1	3
	minimal	0	1
Sciatic nerve (notch)	trace	1	3
	focal thickening of myelin sheath - trace	2	2
Sciatic nerve (mid thigh)	trace	3	5
	focal thickening of myelin sheath - trace	0	2
Spinal cord (L1-4)	trace	0	2

11.2 MB 45950 (fipronil sulfide)

Broadmeadow A (1991c) M&B 45950: preliminary toxicity study by oral (capsule) administration to Beagle dogs for four weeks. Report no. 90/RHA322/0222. Lab: Life Science Research Ltd., Eye, England; Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Expt. dates: 22.11.1989–21.12.1989. (GLP/QA: yes, Study Guidelines: None) *(Only study summary provided)*

Materials and Methods Beagle dogs (2/sex/dose) were given 0, 1, 5 or 15 mg/kg bw/d M&B 45950 (fipronil sulfide; Batch: JJW2126; Purity: 97.6%) by gelatin capsule for 28 days. The dogs were 28-51 weeks of age, and 9.6-14.1 kg at study initiation. Observations were made daily for clinical signs and a detailed examination was performed weekly. Ophthalmoscopic, veterinary and neurological examinations, and haematology and clinical chemistry tests were performed before and after the treatment period. Neurological examinations comprised evaluations of cranial nerve reflexes (pupillary, palpebral and gag), segmental reflexes (flexor, patellar and extensor tone), postural reactions (placing, righting, tonic neck, hopping reflex and extensor postural thrust) and various observations (behavioural changes, abnormalities of gait and stance and presence of tremor or other dyskinesias). At necropsy, the dogs were given gross external and internal examinations (cranial, thoracic, abdominal and pelvic cavities and their viscera), selected organs (not specified) were weighed, and a wide range of tissues (not specified) from each dog were examined histopathologically.

Results and Conclusion There were no deaths or treatment-related clinical signs. At 15 mg/kg bw/d, the 2 female dogs showed very little bodyweight gain (0.1 or 0.2 kg *vs* 0.4 or 0.9 kg in control), one of them showing reduced food consumption, but the males of this group were not affected. At the end of treatment, these 2 females also showed slightly higher Hct, Hb, and erythrocyte counts. Slightly, but statistically significant higher plasma ALP activity (90 *vs* 64 IU/L in control, $p<0.05$) was found in males at 15 mg/kg bw/d. There were no other abnormalities detected. No effects were observed at 5 mg/kg bw/d.

Broadmeadow A (1991d) M&B 45950: toxicity study by dietary administration to CD rats for 13 weeks. Report no. 90/RHA323/0415. Lab: Life Science Research Ltd., Eye, England. Sponsor: Rhone-Poulenc Agrochimie, Lyon, France. Expt. dates: 6.12.1989-13.3.1990. Unpublished. (GLP/QA: yes. Study Guidelines: USEPA 82-1, 1984) *(Only study summary provided)*

Materials and Methods Sprague-Dawley CD rats (10/sex/dose) received a daily dose of MB 45950 (fipronil sulfide; Batch: JJW2126; Purity: 97.6%) at 0, 10, 25, 50 or 300 ppm in the diet for 13 weeks. The animals were 4-5 weeks old, weighing 120-153 g (males) and 110-142 g (females) at the start of dosing. The concentrations, homogeneity and stability of the chemical in the diet were verified by analysis. The mean achieved test material intakes were 0, 0.7/0.8, 1.8/2.2, 3.5/4.1, 21.5/24.6 mg/kg bw/d for males/females in ascending order of dose. Clinical signs and mortality were recorded daily, and a detailed physical examination was performed weekly. Bodyweight and food consumption were measured weekly. Ophthalmological examinations were performed prior to commencement of dosing and after 12 weeks of treatment. A neurological examination after 12 weeks' dosing comprised evaluations of cranial nerve reflexes (pupillary, palpebral and startle), segmental reflexes

(flexor withdrawal), postural reactions (placing, righting and grasping) and general observations (behavioural changes, abnormalities of gait and stance and presence of tremor or other dyskinesias). After 12 weeks, blood samples were collected from each rat for haematology and clinical chemistry, and after 11 weeks urine samples were collected for urinalysis, details of which were not provided. At necropsy, the rats were given gross external and internal examinations (cranial, thoracic, abdominal and pelvic cavities and their viscera), selected organs (not specified) were weighed, and a wide range of tissues (not specified) from the control and 300 ppm rats were examined histopathologically, plus thyroids/parathyroids from the lower dose levels.

Results and Conclusion There were no deaths. Males at 300 ppm had damaged vibrissae in week 2, and nasal (discharge) staining from weeks 3/4 to 11. Relative to controls, food consumption was reduced by ~15% in males and females at 300 ppm during the first week, with bodyweight gain for the entire treatment period reduced in males only (89% of control, $p<0.05$).

There were no abnormal findings for neurological examinations, ophthalmology, haematology or urinalysis. As shown in the Table below, total plasma protein and α-1 globulin were increased to a statistically significant extent at 50 and 300 ppm in females, and in all treated male groups. The statistical significance in males at 10 and 25 ppm was attributed by the study author to the unusually low control value. However, given the flat dose response for these parameters in males at 10 to 50 ppm, the findings for these groups are unlikely to be treatment-related. Females at 300 ppm also had cholesterol and phosphorus levels higher than controls. Making an assumption regarding the meaning of the units (see note below Table), total protein levels for all groups and cholesterol levels for all but 300 ppm females (which is just outside the range), lie within the historical mean control range for SD rats, and the phosphorus levels for all groups are relatively low[8]. Overall, given the nature and extent of the changes in clinical chemistry, it appears unlikely that any are of toxicological significance.

Table 58: Selected clinical chemistry parameters (n = 10)

	Males					Females				
Dose (ppm)	**0**	**10**	**25**	**50**	**300**	**0**	**10**	**25**	**50**	**300**
Total protein (g%)	6.9	7.3**	7.3**	7.4***	7.7***	7.5	7.7	7.6	8.0**	8.2**
α-1 globulin (g%)	1.3	1.6***	1.5**	1.6***	1.7***	1.2	1.2	1.3	1.4**	1.5***
Cholesterol (mg%)	45	48	51	48	51	60	60	54	63	104***
Phosphorus	2.3	2.2	2.2	2.1	2.3	1.7	1.8	1.6	1.7	2.0**

*p<0.05; **p<0.01; ***p<0.001.
The SI equivalent of g% and mg% was not provided, but the results suggest that they are equivalent to g/dL and mg/dL respectively. No unit was provided for phosphorus.

Absolute and relative liver and thyroid weights were increased at 50 and 300 ppm, with liver weights also increased in females at 25 ppm, all considered an effect of treatment. Thyroid follicular cell hypertrophy as well as hyperplasia was seen in almost all males and half the females at 300 ppm, with a few cases of hypertrophy at 25 and 50 ppm which, given that this lesion was seen at higher doses, could not be dismissed. The NOEL was 10 ppm, (equal to 0.7

[8] Derelanko MJ (2000) Toxicologist's Pocket Handbook. CRC Press. Table 84. Mean control ranges of typical serum clinical chemistry measurements in CD rats.

mg/kg bw/d) based on increased liver weight in females and thyroid follicular cell hypertrophy in one male at 25 ppm (equal to 1.8 mg/kg bw/d).

Table 59: Findings in organ weights and histopathology (n = 10)

	Males					Females				
Dose (ppm)	**0**	**10**	**25**	**50**	**300**	**0**	**10**	**25**	**50**	**300**
Liver (g)	21.5	23.0	22.6	23.4	26.1**	11.2	12.1	12.6	13.0	15.5**
Rel. to bw (%)	3.58	3.87	3.74	4.15**	4.79**	3.62	3.66	4.17**	4.07*	5.00**
Thyroid (g)	0.020	0.022	0.024	0.030**	0.040**	0.023	0.025	0.024	0.031*	0.033**
Rel. to bw (%) x 100	0.34	0.37	0.39	0.53**	0.73**	0.75	0.76	0.79	0.96	1.09**
Thyroid, -follicular cell hypertrophy			1	2	10***				1	5*
-follicular cell hyperplasia					9***					4

*p<0.05; **p<0.01; ***p<0.001.

11.3 RPA 200766 (fipronil amide)

Berthe P (1996) RPA 200766: 28-day toxicity study in the rat by dietary administration. Report No. SA 95273. Lab: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 11.7.1995–20.12.1995. Unpublished report date: 23 April 1996. Amendment No. 1, 10 May 1996. (GLP/QA: yes. Guidelines: OECD 407 (1981); EEC 92/69 B7, 1992)

Materials and Methods Sprague-Dawley rats (10/sex/dose) received a daily dose of RPA 200766 (fipronil amide; Batch: NM1878; Purity: 96.2%) at 0, 50, 500, 5000 or 15000 ppm in the diet for 28 days. The animals were 213-237 g (males) and 147-177 g (females) at the beginning of dosing. The concentrations, homogeneity and stability of the chemical in the diet were verified by analysis. The mean achieved doses were 0, 3.8/4.4, 38/44, 385/387 and 1087/1063 mg/kg bw/d for males/females in ascending order of dose. Clinical signs were recorded daily. Bodyweight and food consumption were measured weekly. Ophthalmological examinations were performed on all groups prior to dosing and on the control and the high dose groups during week 4. The week before necropsy, blood samples were collected for haematology and clinical chemistry testing (as in Appendix III, but not CPK), and frozen samples were kept for possible future measurements of TSH, T3 and T4. Samples were collected for urinalysis overnight prior to sacrifice (as in Appendix III). At necropsy, selected organs were weighed (as in Appendix IV, plus epididymis, pituitary gland, prostate, thymus, uterus). Microscopic examinations were performed on a range of tissues (as in Appendix IV, plus articular surface of coxo-femoral, anus, larynx, and tongue) from the control and 5000 ppm groups, as well as from all decedents, and sections from the liver, lung, kidney, adrenal, thyroid and tissues with significant gross findings in 50 and 500 ppm groups. Based on reduced bodyweight gain, the dose level of 15000 ppm was considered to be excessively high, so tissues from this group were not processed for microscopic examination.

Results and Conclusion One female at 15000 ppm was found dead on day 24 with no clinical signs prior to death and no macroscopic changes at necropsy. The study author considered this death was attributable to trouble with blood collection rather than an effect of treatment. Overall, there were no treatment related clinical signs or ophthalmological

findings. At 5000 and 15000 ppm, food consumption was reduced (by up to 33%), and bodyweights were lower than control from day 8 (10/16% and 23/28% lower at 5000 and 15000 ppm respectively for males/females at the end of the study).

Relative to the corresponding control groups, Hb concentrations were slightly reduced at ≥500 ppm in males, accompanied by similar decreases in Hct and MCH (see following Table). A similar trend occurred in females at ≥5000 ppm, but results were highly variable. As these changes were slight and results within normal limits, they were not considered biologically significant. Also, there were increases in urea in females at ≥5000 ppm, creatinine in males at ≥500 ppm, which in concert with the high urine volume mentioned below may indicate effects of treatment on the kidney. Cholesterol and triglycerides were increased at ≥500 ppm and ≥5000 ppm respectively in both sexes, and total protein was increased in 15000 ppm males, indicative of toxic effects on the liver. The 15000 ppm males also showed decreased AST but increased ALT, the opposite directions of change suggesting that neither was toxicologically significant. Phosphorus was decreased in males at ≥5000 ppm, and potassium was increased in females at 15000 ppm, but as these were not accompanied by changes in other serum ions, they were considered of unlikely toxicological significance. Except for a higher urine volume (up to 160% of control) for males at 5000 ppm and both sexes at 15000 ppm, no significant changes were apparent in the urinalysis results.

Table 60: Changes in haematology and clinical chemistry (n = 10)

	Males					Females				
Dose (ppm)	**0**	**50**	**500**	**5000**	**15000**	**0**	**50**	**500**	**5000**	**15000**
Hb (g/100 mL)	16.5	16.0	15.8*	15.2*	15.1**	15.5	15.3	15.0*	14.4**	14.6
Hct	0.50	0.48	0.48	0.46*	0.46**	0.46	0.45	0.45	0.43**	0.44
MCH (pg)	19.4	19.5	19.0	18.7	18.6*	19.7	19.7	19.8	18.9*	18.7*
Urea (mmol/L)	5.03	5.07	5.49	4.81	4.91	4.62	4.87	5.08	6.18**	6.25**
Creatinine (μmol/L)	60.5	61.3	64.7*	68.9*	72.3*	62.5	61.7	65.4	66.2	66.2
Cholesterol (mmol/L)	1.18	1.24	1.54**	3.26**	4.53**	1.26	1.35	1.88**	4.18**	4.88**
Triglycerides (mmol/L)	0.53	0.65	0.58	1.60**	3.26**	0.58	0.64	0.63	1.97**	1.82**
Total protein (g/L)	58.8	59.0	61.4*	61.2	64.9**	59.1	59.3	63.4**	62.4*	61.2
AST (IU/L)	55.8	52.1	50.8	48.5	46.9*	48.1	45.4	42.5	41.6*	42.2
ALT (IU/L)	20.8	19.0	19.1	25.9	28.4**	16.8	16.3	17.2	19.3	18.2
Phosphorus (mmol/L)	2.46	2.40	2.23*	2.16*	2.16**	2.19	2.18	1.98	1.97	2.18
K (mmol/L)	3.58	3.62	3.56	3.74	3.64	3.44	3.49	3.34	3.71	3.82**

*p<0.05; **p<0.01; ***p<0.001.

At necropsy, liver weight (absolute and relative to bodyweight) was increased at ≥500 ppm, and adrenal weights were increased in males at all doses (see Table below). Adrenal weights lacked a dose response in females, and were actually decreased in the 15000 ppm group. Thyroid weights (absolute and relative to bodyweight) were increased in all treated male groups, and prostate weights were decreased at ≥5000 ppm, with no microscopic changes observed in either of these organs. At 50 ppm, only 3/10 male rats had thyroid weights outside the concurrent control range, so this was considered unlikely to represent a toxicologically significant change. The absence of a dose response at 500 ppm, suggests that treatment-related increases in thyroid weights were limited to ≥5000 ppm, though no hypertrophy was reported in the microscopic findings at 5000 ppm. Macroscopic findings included enlarged liver and adrenals at 5000 and 15000 ppm, and hepatic dark abnormal colour was reported at 500 ppm in one male, and at higher doses in both sexes. Microscopic examination revealed centrilobular or diffuse hepatocellular hypertrophy and adrenal extra-medullary haemopoiesis

at 5000 ppm. Fine/coarse vacuolation of the adrenal zona fasciculata was observed in males (slight changes at 50 and 500 ppm, increasing in severity at 5000 ppm), and in females at 5000 ppm. Given the dose response for vacuolation of the adrenals, both with respect to incidence and severity, the changes to the adrenals in 50 ppm males cannot be dismissed. Because of this, a NOEL was not established in this study. The LOEL was 4 mg/kg bw/d.

Table 61: Organ findings (n=10, except n=9 for females at 15000 ppm)

	Males					Females				
Dose (ppm)	**0**	**50**	**500**	**5000**	**15000**	**0**	**50**	**500**	**5000**	**15000**
Liver										
Weight, g	9.4	9.8	10.9*	16.0**	17.8**	6.8	7.4	8.8**	12.6**	12.9**
Rel to bw, %	2.73	2.87	3.30**	5.21**	6.80**	2.87	3.06	3.82**	6.33**	7.63**
Enlargement				8	10				10	9
Dark colour			1	8	10				9	9
Centrilobular hypertrophy				6	n.e.				6	n.e.
Diffuse hypertrophy				4	n.e.				4	n.e.
Adrenals										
Weight, g	0.058	0.071	0.081**	0.080*	0.072	0.083	0.080	0.098	0.080	0.046**
Rel to bw, %	0.017	0.020*	0.024**	0.026**	0.027**	0.035	0.033	0.042*	0.040	0.027*
Enlargement				5	3				3	2
Extra-medullary haemopoiesis				5	n.e.				4	n.e.
Fine/coarse vacuolation		2	5	10	n.e.				7	n.e.
Thyroid										
Weight, g	0.016	0.021*	0.021**	0.026**	0.028**	0.019	0.019	0.016	0.014	0.017
Rel to bw, %	0.0049	0.0064 *	0.0066 **	0.0084 **	0.0105 **	0.0081	0.0079	0.0069	0.0070	0.0102
Prostate										
Weight, g	0.52	0.61	0.46	0.30**	0.20**					
Rel to bw, %	0.152	0.178	0.138	0.097 **	0.074 **					

n.e. = not evaluated

11.4 RPA 104615 (fipronil detrifluoromethyl sulfonate)

Dange M (1998) RPA 104615: 28-day toxicity in the rat by dietary administration. Report No. SA 96368. Lab: Rhone-Poulenc Agrochimie, Centre de Recherche, Sophia Antipolis, France. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 4.9.1996-22.5.1997. Unpublished report date: 19 December 1997, amended on 27 January 1998. (GLP/QA: yes, Study Guidelines: OECD 407, 1981; EEC Directive 92/69–Annex V–Method B7, 1992)

Materials and Methods Sprague-Dawley rats (10/sex/dose) received a daily dose of RPA 104615 (fipronil detrifluoromethyl sulfonate; Batch: CBL24; Purity: 930 g/kg) at 0, 50, 500, 5000 or 10000 ppm in the diet for 28 days. The animals (Iffa-Credo, St Germain-sur-L'Arbresle, France) were 6 weeks old, with bodyweights of 127-185 g (males) and 129-160 g (females). The concentrations, homogeneity and stability of the chemical in the diet were

verified by analysis. The mean achieved test material intake was 0, 4.5/4.7, 45.7/50.4, 458/487 and 916/950 mg/kg bw/d for males/females in increasing order of dose.

Clinical signs were recorded daily. Bodyweight and food consumption were measured weekly. Ophthalmological examinations were performed on all groups before dosing commenced, and on the control and the high dose groups during week 4. The week before necropsy, blood samples were collected for haematology and plasma chemistry analyses (see Appendix III). The levels of TSH, T3 and T4 were apparently measured in each plasma sample by radioimmunoassay, but these results were not provided. Overnight urine was collected prior to sacrifice for analysis (as in Appendix III). Survivors were killed on days 29-31 and subjected to necropsy. Animals found dead were also necropsied. This included gross examination, with organs weighed (as listed in Appendix IV, plus epididymis, pituitary gland, prostate, thymus, uterus). A number of organs/tissues were sampled for histological examination in all groups (as in Appendix IV, plus articular surface (coxo-femoral), larynx and tongue).

Results and Conclusion There were no mortalities, and no treatment-related clinical signs or ophthalmoscopic findings. Bodyweight and food consumption were unaffected by treatment. Mean prothrombin times were greater in males by 8% and 16% at 5000 and 10000 ppm respectively (see Table below). A significant increase in plasma ALP activity was noted in males and females at 5000 ppm (respectively 134% and 294% of control) and 10000 ppm (332% and 433% of control). In females, total cholesterol was increased at 10000 ppm, and triglyceride levels were increased at 5000 and 10000 ppm relative to controls. All of these findings were indicative of toxic effects on the liver. The increased ALP levels and prothrombin times suggest effects of treatment on liver function, consistent with the increases seen in liver weight, though this was not supported by microscopic findings (see below). A tendency towards slightly higher values was observed in urinary pH in treated male groups, but in the absence of related findings, the toxicological significance of this is not clear.

Table 62: Selected haematology, clinical chemistry and urinalysis findings (n = 10)

	Males					**Females**				
Dose (ppm)	**0**	**50**	**500**	**5000**	**10000**	**0**	**50**	**500**	**5000**	**10000**
Prothrombin time (s)	13.2	13.2	13.5	14.3*	15.4**	14.2	14.3	14.3	13.7	13.3
ALP (IU/L)	163	170	167	220*	545**	126	127	122	371**	674**
Cholesterol (mmol/L)	1.34	1.41	1.47	1.50	1.54	1.31	1.49	1.39	1.52	1.78**
Triglyceride(mmol/L)	0.78	0.98	0.93	0.81	1.15	0.50	0.76	0.63	1.13**	1.08**
Urine pH (unit)	6.4	6.8	7.0*	7.3**	7.2**	6.1	6.6	6.6	6.1	6.4

*p<0.05; **p<0.01 by Dunnett's test or Mann-Whitney's test.

Absolute and/or relative liver weights were increased (10-15%) in both sexes at 5000 and 10000 ppm to a statistically significant extent (tabulated below), and though the dose response was modest, this was considered treatment-related, at least in females at these doses and males at the top dose. Statistically significant increases in thymus weight, and decreases in testis or ovary weights were reported at 5000 ppm. Given the absence of dose-relatedness and corresponding histopathological findings, these were not considered to be of toxicological significance.

Table 63: Selected organ weight findings (n = 10)

	Males					Females				
Dose (ppm)	**0**	**50**	**500**	**5000**	**10000**	**0**	**50**	**500**	**5000**	**10000**
Liver, g	8.76	9.49	9.77	9.71	10.1*	6.21	6.56	6.12	6.87	6.95
% bw	2.84	2.97	3.05	3.10*	3.19**	2.92	3.06	2.93	3.27**	3.27**
Thymus, g	0.56	0.66	0.66	0.72*	0.65	0.41	0.44	0.44	0.52	0.47
% bw	0.18	0.21	0.20	0.23**	0.21	0.20	0.21	0.21	0.24*	0.22
Testis/ovary, g	3.60	3.51	3.41	3.33*	3.45	0.13	0.13	0.13	0.11*	0.12
% bw	1.17	1.10	1.07*	1.07*	1.09	0.063	0.059	0.063	0.053*	0.055

*p<0.05; **p<0.01 by Dunnett's test or Mann-Whitney's test.

In the liver, there was a slight increase in the incidence and severity of sinusoidal lymphoid aggregations at 10000 ppm, associated with occasional degenerate hepatocytes in both sexes, and fine vacuolation of hepatocytes, mainly periportal and typically diffuse throughout the liver of females (see Table below). These foci were graded as minimal or slight, and given the lack of a dose response, are not considered to have toxicological significance. In the thyroid, there was an increased incidence of hypertrophy of the follicular epithelium in males at 10000 ppm, which was possibly treatment-related. The NOEL was 500 ppm (equal to 45 mg/kg bw/d) based on increased liver weights as well as increased plasma ALP and triglyceride levels, and prothrombin times at 5000 ppm (equal to 460 mg/kg bw/d).

Table 64: Histopathological findings (n = 10)

	Males					Females				
Dose (ppm)	**0**	**50**	**500**	**5000**	**10000**	**0**	**50**	**500**	**5000**	**10000**
Liver										
Sinusoidal lymphoid aggregation with occasional degenerate hepatocytes	5	4	4	4	7	6	2	6	2	8
Fine vacuolation of hepatocytes, mainly periportal	1				1	5	6	7	5	9
Thyroid										
Hypertrophy of follicular epithelium	2		1	1	5					1

11.5 MB 45897/RPA 097920 (fipronil detrifluoromethylsulfinyl)

Johnson IR (1995) Pyrazole/MB 45897/RPA 097920: Intermediate of fipronil (MB 46030): Four week oral toxicity study in the rat. Report No. 95/RHA535/0684. Lab: Pharmaco-LSR, Eye, Suffolk, England. Sponsor: Rhone-Poulenc. Expt. dates: 30.8.1994-27.9.1994. Unpublished report date: 28.6.1995. (QA/GLP: yes; Study Guidelines: EEC B7)

Materials and Methods RPA 097920 (fipronil detrifluoromethylsulfinyl; Batch No. 94 3026 DA 942; Purity 99.7%; Source: Rhone-Poulenc), was administered by gavage to CD rats (5/sex/dose) at 0, 50, 200 or 1000 mg/kg bw/d in maize oil for 4 weeks. The rats (from Charles River UK Limited Margate, Kent, England) were 28-35 days old and 15-20 g on arrival, and were acclimatised for at least 5 days before dosing. The concentration, homogeneity and stability of the test substance were confirmed by analysis.

All animals were checked daily for clinical signs, moribundity and deaths. Detailed physical examinations were performed at least once weekly. Food consumption was recorded weekly

and bodyweight twice weekly. On Day 29, blood samples were collected for haematology (RBC, Hb, Hct, MCV, MCH, MCHC, total and differential WBC count, platelets) and clinical chemistry (ALT, AST, urea, creatinine, glucose, total bilirubin, total protein, electrophoretic protein fractions, Na, K, Cl). All rats were killed on day 29, and given a detailed necropsy. Selected organs were weighed (adrenals, kidneys, liver and testes), and histological examination of organs/tissues (adrenals, heart, kidney, liver, spleen, testes and all macroscopic abnormalities) was carried out.

Results and Conclusion There were no deaths. Salivation was observed from day 2 at 1000 mg/kg bw/d, from day 3 at 200 mg/kg bw/d, and from day 8 at 50 mg/kg bw/d. Hunched posture and underactivity were seen after dosing at 200 and 1000 mg/kg bw/d. Rats at 1000 mg/kg bw/d also showed areas of fur loss from Day 3. Bodyweight gain was lower (84% of control) in males at 1000 mg/kg bw/d, and in all treated female groups (83-88% of control) without a dose-relationship.

Erythrocyte count, Hb and Hct were slightly decreased at 1000 mg/kg bw/d in females relative to the control (see Table below), and may have been related to treatment, particularly as these results were below or at the low end of the historical control range for CD rats. Total protein was increased in both sexes at 1000 mg/kg bw/d, along with albumin, α2-globulin and β-globulin, and was considered a treatment-related effect, though the absence of a dose response for albumin in females suggests that this may have not been an effect of treatment. The percentage increase in total protein in males at 200 mg/kg bw/d was not considered toxicologically significant, as it resulted from slight but not biologically significant increases in both albumin and β-globulins. The possibility that the decreased levels of K and Cl, and increased urea in 1000 mg/kg bw/d males were related to treatment, could not be discounted.

Table 65: Selected haematology and clinical chemistry findings (n = 5)

	Males				Females			
Dose (mg/kg bw/day)	**0**	**50**	**200**	**1000**	**0**	**50**	**200**	**1000**
RBC (10^6/mL)	7.99	7.60	7.74	7.63	7.47	7.70	7.69	6.99*
Hb (g%)	15.7	14.9	15.2	14.6*	14.6	14.9	14.8	13.1***
Hct (%)	47	45	46	45	43	43	43	40*
Urea (mg%)	20	24	25	32**	29	29	31	28
Glucose (mg%)	90	93	90	75	102	98	86**	83**
Total protein (g%)	5.8	5.8	6.3**	6.9***	6.3	6.2	6.5	6.9***
Albumin (g%)	2.9	3.1	3.2	3.4**	3.2	3.7**	3.6**	3.6**
α2-globulins (g%)	0.5	0.5	0.4	0.7***	0.4	0.3	0.4	0.5*
β-globulins (g%)	1.2	1.2	1.4	1.5*	1.4	1.2	1.5	1.5
K (mmol/L)	3.7	3.6	3.6	3.3***	3.3	3.6	3.5	3.1
Cl (mmol/L)	102	102	101	99**	105	106	103	103

*p <0.05; **p<0.01; ***p<0.001 by *Student's t-test*. The SI equivalent of g% and mg% was not provided, but the results suggest that they are equivalent to g/dL and mg/dL respectively.

Absolute and relative liver weights were increased at 1000 mg/kg bw/d (see Table below), and this was associated with periacinar hepatic hypertrophy in the livers of some rats. In this group, relative testes weight was slightly increased, but this was considered secondary to lower bodyweight. No other macroscopic or microscopic changes were observed. The NOEL was 200 mg/kg bw/d based on decreased bodyweight gain, changes in haematology (decreased RBC, Hb and Hct), clinical chemistry (total protein, albumin and α2 & β-globulins), and increased liver weight with associated pathological changes at 1000 mg/kg bw/d.

Table 66: Selected organ weights (n = 5)

Dose (mg/kg bw/day)	Males				Females			
	0	50	200	1000	0	50	200	1000
Terminal bodyweight (g)	313	313	297	274**	222	198**	204*	206*
Liver (g)	12.1	13.3	12.8	16.0**	8.8	8.4	8.7	13.9**
(relative to bw)	3.83	4.24	4.31	5.86**	3.97	4.24	4.28	6.76**
Testes (g)	3.06	3.03	3.15	3.17				
(relative to bw)	0.98	0.97	1.06	1.16*				

*p<0.05 and p<0.01 by Dunnett's test.

12 HUMAN STUDIES

12.1 Case Studies

Chodorowski Z, Anand JS (2004) Accidental dermal and inhalation exposure with fipronil – a case report. Journal of Toxicology. Clinical Toxicology. 42: 189-2004.

This paper describes the clinical observations for a 50-year-old male admitted to a clinic following his reported dermal and inhalation exposure to spray prepared from the fipronil product Regent 200 SC. The subject reported headache, nausea, vertigo and weakness that occurred approximately 2 h after applying the spray over a period of 5 h, then having a shower and changing his clothes. He wore no PPE during the spraying operation, but claimed that his clothes did not become moist from the spray, nor did he eat, drink or smoke while working. Physical examinations and biochemical results were normal. All symptoms resolved spontaneously after 5 h, and he remained asymptomatic over the next 3 weeks. Interpretation of this report is confounded by the patient's history of cardiac disease.

Mohamed F, Senarathna L, Percy A, Abeyewardene M, Eaglesham G, Cheng R, Azher S, Hittarage A, Dissanayake W, Sheriff MHR, Davies W, Buckley N, Eddleston M (2004) Acute human self-poisoning with the N-phenylpyrazole insecticide fipronil – a $GABA_A$-gated chloride channel blocker. Journal of Toxicology. Clinical Toxicology. 42: 943-951.

Seven prospectively recorded cases of self-poisoning with fipronil, and one retrospectively identified death are reported. All of the exposures were to the fipronil product Regent 50 SC (50 g/L fipronil). In three of the cases, it appears that this product was ingested alone, whereas the other patients had also ingested other pesticides and/or alcohol. Estimated amounts of the product ingested were 50 or 100 mL in 4 cases (approximately 2.5-5 g fipronil), otherwise the amount was unknown. In 6 of the patients, exposure to fipronil was proven by detection of fipronil and/or its metabolites in the blood. In one of the blood analysis methods used, only fipronil sulfone and desulfinyl fipronil could be distinguished from the parent, though desulfinyl fipronil was not found in any of the samples. The other test method used on samples from 2 of the patients could not distinguish any metabolites from the parent. The highest level measured was 3.74 mg/L fipronil equivalents. Patients were asymptomatic at blood levels of ~1 mg/L fipronil + metabolites.

The patient that died had ingested 100 mL of the fipronil product (approximately 5 g fipronil), and was admitted unconscious to intensive care, where he had several episodes of tonic-clonic fits. He developed pneumonia and died without regaining consciousness 17 days after

exposure. The weight of the patient was not provided, but this lethal dose is approximately equal to 50-100 mg/kg bw for an adult in the weight range 100-50 kg, consistent with acute toxicity studies in animals). Another patient who ingested a similar amount of the product was drowsy, sweating profusely and vomiting on admission, had one generalised tonic-clonic seizure, and subsequently became more alert, but also agitated. After treatment with activated charcoal and diazepam, he was asymptomatic within 12 h. A patient who had ingested alcohol in addition to the pesticide, had similar symptoms and outcome. All other patients recovered quickly, all but one having been given forced emesis.

In the experience of the authors, fipronil poisoning is characterised by vomiting, agitation and seizures, and normally has a favourable outcome, with only resuscitation and supportive care required to achieve this. Based on the limited data available in this study, the authors concluded that in humans, fipronil is absorbed rapidly, with clinical toxicity peaking in the first few hours, and correlating with peak blood levels of fipronil and its metabolites. After 15-20 h, during which blood fipronil levels fell, but levels of fipronil sulfone increased, total fipronil concentrations reached a plateau. The apparent elimination half-life in the two patients for which this could be followed, was 36 and 47 h.

12.2 Exposure studies - Dog and cat stroking studies

Note: The results of studies in this section were reported as percentages of the applied dose. To provide an estimate of the amount of fipronil and its metabolites that this represents, some values (usually maximum values, or the range) have been converted to µg fipronil equivalents. A more comprehensive summary of the results expressed as µg fipronil equivalents is tabulated in Chapter 14.

Hughes DL (1997b) Dislodgeable residues of fipronil following application of Frontline® Top Spot™ to cats. Covance 6848-103, Merial Metxt 168. Lab: Covance Laboratories Inc. 3301 Kinsman Boulevard, Madison, Wisconsin 53704. Sponsor: Merial. Laboratoire de Toulouse, 4 chemin du Claquet, Toulouse Cedex, France. Expt dates: 1 July 1997-8 August 1997). Unpublished report date: 30 October 1997. (QA/GLP: yes; Test Guidelines: No specific guidelines applicable, but followed the general provisions of US EPA guideline 'Occupational and Residential Exposure Test Guidelines, Series 875').

Materials and Methods Frontline Top Spot (Batch: M02881AY-D) containing 9.7% w/w fipronil as the active constituent was applied using an applicator constructed to deliver approximately 0.5 mL of the product to 5 mixed breed cats (from Liberty Research, New York, ~8-11 months old, bw 2.9-3.3 kg) according to label instructions. Following HPLC analysis, the amount of fipronil in the application tube was calculated to be 49.47 mg. The cats were maintained in individual cages indoors, with a 12 h light/dark cycle. Dislodgeable residues were sampled before dosing on day 1, and at approximately 4, 8, and 12 h, and 2, 3, 5, 8, 15, 22 and 29 days post-dosing, and were analysed for fipronil, fipronil sulfone, fipronil sulfide and desulfinyl fipronil. The first time point of 4 h was chosen to enable the animals' coats to dry before sampling commenced. Sampling was performed by a person stroking the animal with the dominant hand (clothed in a pure cotton dye-free glove that also covered the wrist area), using uniform medium pressure and motions that ran with the lay of the haircoat, for a total of 4 strokes to cover the whole surface of the animal, commencing at the head and ending at the base of the tail (one stroke on the back line, one on each of the flanks, and one over the ventral zone). Any dislodged hair was removed from the glove prior to placing it in a

storage container, which was then frozen until extraction and analysis were carried out (details not supplied).

Results All values for desulfinyl fipronil were below the LOQ, and were considered to be zero, as the study author considered that this photodegradate could not form during a study of this design. The dislodgeable residues were therefore considered as the sum of fipronil, fipronil sulfide and fipronil sulfone, and were detectable throughout the study period (see Table below). One cat was reportedly damp at 4 h, and the relatively high residues for this animal at this time point (1.6% of fipronil applied) possibly reflected this. In all, fipronil residues were maximal in 3/5 cats at 4 h, and in the other 2 cats on day 2. The highest dislodgeable residue level of 1.7% (occurring on day 2) was equivalent to 843 µg fipronil. Fipronil sulfone was detected at very low levels for up to 2 days (maximum ~20 µg), and small amounts of fipronil sulfide were detected in 2 animals, but at 4 h only (maximum ~20 µg).

Table 67: Dislodgeable residue on gloves (% of amount applied) - Hughes (1997b)

Time	% Mean dislodgeable residue ± SD (n=5)	Range (%)
4 h	**0.750 ± 0.550**	**0.276 – 1.61**
8 h	0.311 ± 0.168	0.133 – 0.555
12 h	0.215 ± 0.134	0.101 – 0.405
Day 2	**0.635 ± 0.625**	**0.155 – 1.70**
Day 3	0.134 ± 0.053	0.062 – 0.186
Day 5	0.047 ± 0.018	0.025 – 0.067
Day 8	0.022 ± 0.006	0.016 – 0.029
Day 15	0.011 ± 0.002	0.009 – 0.013
Day 22	0.009	NA
Day 29	0.009	NA

NA=not applicable

De Fontenay G, Magloire B, Suberville S, Birckel Ph, Weil A (1997a) Dislodgeable residues of fipronil following a topical application of Frontline® Spot-On treatment to cats. Study No. MET417. Lab: Merial, Toulouse France and Chrysalis, L'Arbresle, France. Sponsor: Merial 4, chemin du Calquet, 31057 Toulouse Cedex. Expt dates: commenced 22 March 1997. Unpublished report date: 2 December 1997. (GLP/QA: yes; Guidelines: none)

Materials and Methods Dislodgeable residues of fipronil, its metabolites RM1502, RM1602, and desulfinyl fipronil were measured after Frontline spot-on (Batch M02711AYD, 9.77% w/v fipronil, in 0.5 mL applicator; ~50 mg fipronil/cat) was applied to cats (3/sex, ~2.5 kg bw) according to label instructions. The identification of RM1502 and RM1602 was not provided, but each was found in very low amounts relative to the parent. Cats were crossbreeds (European type household x Abyssinian), 5 months old, 2.0-2.7 kg bw, from Iffa Credo, France. Samples were collected as for the above study (Hughes 1997a), with slight modification to the sampling time points (see results Table below). After extraction with acetonitrile, the residues were quantified by HPLC (LOQ=3 µg/glove for each of the residue components).

Results

Table 68: Dislodgeable residue on gloves (% of amount applied) – Fontenay *et al.* (1997a)

Time	Mean ± SD	Range
0	BLQ	-
1 h	6.70 ± 5.59	2.20 – 14.1
4 h	1.66 ± 0.85	0.662 – 2.88
8 h	1.02 ± 0.38	0.470 – 1.48
Day 1	0.630 ± 0.236	0.341 – 0.912
Day 2	0.232 ± 0.097	0.119 – 0.377
Day 4	0.044 ± 0.010	0.029 – 0.056
Day 7	0.015 ± 0.006	0.009 – 0.024
Day 14	0.005 ± 0.004	0.000 – 0.010
Day 21	0.001 ± 0.003	0.000 – 0.007
Day 28	BLQ	-

BLQ: below the limit of quantification; - (not applicable)

The highest levels of fipronil residues were detected at 1 h post-application (see Table above). The residues transferred to the glove at this point ranged from 1039 to 6658 µg, illustrating the high variability between animals. Desulfinyl fipronil was not detected at all, while the other metabolites (maximum levels 192 µg for RM 1602 and 62 µg for RM 1502) were not detected after day 2.

Hughes DL (1997c) Dislodgeable residues of fipronil following application of Frontline® Spray treatment to cats. Covance 6848-102, Merial Metxt 167. Lab: Covance Laboratories Inc. 3301 Kinsman Boulevard, Madison, Wisconsin 53704. Sponsor: Merial. Laboratoire de Toulouse, 4 chemin du Claquet, Toulouse Cedex, France. Expt dates: 1 July 1997-13 August 1997). Unpublished report date: 30 October 1997. (QA/GLP: yes; Test Guidelines: No specific guidelines applicable, but followed the general provisions of US EPA guideline 'Occupational and Residential Exposure Test Guidelines, Series 875').

Materials and Methods This experiment was performed essentially as described above for Frontline Top Spot (Hughes 1997b), except Frontline Spray (0.29% w/w fipronil; Batch No. L697111A from Merial) was applied to groups of 5 cats (mixed breed from Liberty Research New York, 8-11 months old, 2.8-3.5 kg bw). The spray is supplied in 100 mL containers with spray nozzles constructed to deliver approximately 0.5 mL of the product per full depression of the trigger pump. Each cat was sprayed according to the maximum label rate of 6 mL/kg. The actual amount delivered was determined by weighing the container before and after treatment. An earlier sampling point at 2 h replaced the 8 h time point in the Top Spot study, as this appears to have been considered sufficient to allow this product to dry on the animal.

Results The maximum residue level found on a glove was equivalent to 0.592% of the applied active, equal to 325 µg fipronil, at 12 h post-treatment (see Table below). However, maximum levels for the other 4 cats were achieved at 4 h, with one of the cats having very similar levels at the first 3 test points. Metabolite levels were low (maximum levels 4 µg and 9.5 µg for fipronil sulfide and fipronil sulfone, respectively), or for desulfinyl fipronil, below the LOQ.

Table 69: Mean dislodgeable residue on gloves (% of amount applied) - Hughes (1997c)

Time	% Mean dislodgeable residue ± SD (n=5)	Range
2 h	0.337 ± 0.096	0.210 -0.473
4 h	**0.443 ± 0.139**	0.212 – 0.559
12 h	0.384 ± 0.158	**0.204 – 0.592**
Day 2	0.370 ± 0.156	0.176 – 0.567
Day 3	0.156 ± 0.088	0.077 – 0.290
Day 5	0.055 ± 0.043	0.019 – 0.115
Day 8	0.019 ± 0.010	0.009 – 0.037
Day 15	0.009 ± 0.001	0.008 – 0.010
Day 22	0.009 ± 0.001	0.008 – 0.010
Day 29	0.009 ± 0.001	0.008 – 0.010

De Fontenay G, Magloire B, Suberville S, Birckel Ph, Weil A (1997b) Dislodgeable residues of fipronil following a topical application of Frontline® Spray treatment to cats. Study No. MET415. Lab: Merial, Toulouse France and Chrysalis, L'Arbresle, France. Sponsor: Merial 4, chemin du Calquet, 31057 Toulouse Cedex. Expt dates: commenced 25 March 1997. Unpublished report date: 2 December 1997. (GLP/QA: yes; Guidelines: none)

Materials and Methods Frontline spray (2.50 mg/mL fipronil; Batch No. L697111A, from Merial) was applied to 6 cats (3/sex, 1.8-2.5 kg bw) at the maximum dose of 6 mL/kg bw, according to label instructions. Samples were collected as for the above study (Hughes 1997b), with slight modification to the sampling time points (see results Table below). The dislodgeable residues of fipronil, desulfinyl fipronil, and metabolites RM1502 and RM1602 (unidentified metabolites) were measured. The two undefined metabolites were found in very low amounts relative to the parent.

Results Dislodgeable residue levels, though variable between animals, were essentially similar for the samples taken on the day of application, reducing rapidly over the next couple of days, with no detectable residues on day 14 (tabulated below). Levels of the metabolites tested for were very low (maximum levels of 7 and 3 µg of fipronil sulfide and sulfone respectively), and desulfinyl fipronil was not detected at all. The maximum amount of fipronil + metabolites found on any glove was 0.939% of the amount applied, equal to 341 µg fipronil (achieved at 8 h post-application).

Table 70: Dislodgeable residue on gloves (% of amount applied) – De Fontenay *et al.* (1997b)

Time	Mean ± SD	Range
0	BLQ	-
1 h	0.553 ± 0.064	0.487 – 0.669
4 h	0.638 ± 0.183	0.405 – 0.819
8 h	0.532 ± 0.210	0.387 – 0.939
Day 1	0.394 ± 0.171	0.184 – 0.638
Day 2	0.206 ± .0165	0.058 – 0.477
Day 4	0.056 ± 0.036	0.018 – 0.120
Day 7	0.014 ± 0.008	0.000 – 0.023
Day 14, 21, 28	-	-

BLQ: below the limit of quantification; - (not applicable)

Hughes DL (1997d) Dislodgeable residues of fipronil following application of Frontline® Top Spot™ to dogs. Covance 6848-101, Merial Metxt 166. Lab: Covance Laboratories Inc. 3301 Kinsman Boulevard, Madison, Wisconsin 53704. Sponsor: Merial. Laboratoire de Toulouse, 4 chemin du Claquet, Toulouse Cedex, France. Expt dates: 2 July 1997-26 August 1997). Unpublished report date: 3 November 1997. (QA/GLP: yes; Test Guidelines: No specific guidelines applicable, but followed the general provisions of US EPA guideline 'Occupational and Residential Exposure Test Guidelines, Series 875').

Materials and Methods Frontline Top Spot (Batch No. M02853BY-D from Merial) containing 9.7% w/w fipronil as the active constituent, was applied using an applicator constructed to deliver approximately 1.34 mL of the product to 5 short-haired purebred Beagle dogs (from Covance Research Products, Inc) and 5 long-haired mixed breed dogs (from LBL Kennels) according to label instructions. The dogs were ~8 months to 2 years old, and weighed 10.2-19.6 kg. Following HPLC analysis, the amount of fipronil in the application tube was calculated to be 134.5 mg. The dogs were maintained in individual cages indoors, with a 12 h light/dark cycle. Dislodgeable residues were sampled before dosing on day 1, and at approximately 4, 8, and 12 h, and on days 2, 3, 5, 8, 15, 22 and 29 post-treatment, and were analysed for fipronil, fipronil sulfone, fipronil sulfide and desulfinyl fipronil. The first time point of 4 h was chosen to enable the animals' coats to dry before sampling commenced. Sampling and analysis were performed as in Hughes (1997b), except that a total of 5 strokes were used to cover the whole surface of the animal, commencing at the head and ending at the base of the tail (one stroke on the back line, one to each of the flanks, and one to each side of the ventral zone).

Results All values for fipronil desulfinyl were below the LOQ, and were equated to zero, as it was not expected that this photodegradate could form in a study of this design. Therefore, the dislodgeable residues were the sum of fipronil, fipronil sulfide and fipronil sulfone. In all, fipronil residues were somewhat variable (see Table below), with maximal levels detected on short-haired dogs at 8 h post-application (2 dogs), day 3 (2 dogs) and day 5 (1 dog) and in long-haired dogs at 4 h (3 dogs), 8 h (1 dog) and day 3 (1 dog). Maximum levels of residues on the glove were 838 µg and 1816 µg for short-haired and long-haired dogs, respectively, at 4 h. At later time points when it would be expected that the treated area had dried, 1655 µg of residues were detected on day 3 for the short-haired dogs and 977 µg on day 2 for the long-haired dogs. Fipronil sulfone was detected at very low levels throughout the sampling period, and small amounts of fipronil sulfide were detected up to day 22.

Table 71: Mean dislodgeable residue on gloves (% of amount applied) – Hughes (1997d)

	Short-haired dogs		Long-haired dogs	
Time	**Mean ± SD (n=5)**	**Range**	**Mean ± SD (n=5)**	**Range**
4 h	0.416 ± 0.150	0.227 – 0.623	**0.618 ± 0.417**	**0.357 – 1.35**
8 h	**0.596 ± 0.354**	**0.320 – 1.03**	0.437 ± 0.121	0.320 – 0.614
12 h	0.486 ± 0.155	0.266 – 0.703	0.370 ± 0.183	0.199 – 0.683
Day 2	0.320 ± 0.063	0.222 – 0.372	0.321 ± 0.231	0.152 – 0.726
Day 3	**0.614 ± 0.357**	**0.354 – 1.23**	0.473 ± 0.183	0.180 – 0.653
Day 5	0.455 ± 0.299	0.175 – 0.875	0.424 ± 0.094	0.314 – 0.510
Day 8	0.265 ± 0.220	0.061 – 0.631	0.276 ± 0.089	0.147 – 0.346
Day 15	0.149 ± 0.175	0.029 – 0.456	0.127 ± 0.042	0.079 – 0.171
Day 22	0.070 ± 0.061	0.026 – 0.174	0.067 ± 0.030	0.038 – 0.105
Day 29	0.038 ± 0.030	0.014 – 0.071	0.049 ± 0.032	0.019 – 0.097

De Fontenay G, Campagna S, Suberville S, Birckel Ph, Weil A (1997d) Dislodgeable residues of fipronil following a topical application of Frontline® Spot-On treatment to dogs. Study No. MET416. Lab: Merial, Toulouse France and Chrysalis, L'Arbresle, France. Sponsor: Merial 4, chemin du Calquet, 31057 Toulouse Cedex. Expt dates: commenced 25 March 1997. Unpublished report date: 1 December 1997. (GLP/QA: yes; Guidelines: none)

Materials and Methods This experiment was conducted essentially as described above in Hughes (1997c), using Frontline spot-on (batch M02463AY, 9.83% w/v fipronil from a 1.34 mL applicator), though 6 beagle dogs were used (3/sex), and there were slight changes to the sampling times as shown in the Table below. As in de Fontenay *et al*. (1997 a,b) fipronil, desulfinyl fipronil and metabolites RM1502 and RM1602 were measured, but the two fipronil metabolites were not identified by chemical name.

Results Fipronil and its metabolites were recovered from gloves used for stroking the dogs up to and including day 28. Desulfinyl fipronil was not detected, while the maximum levels of fipronil sulfide and fipronil sulfone were 68 and 11 µg, respectively. Dislodgeable residues were maximal at 1-4 h post-application. These levels were ~50% lower at 8 h, then remained fairly constant up to day 2, after which they declined steadily. The maximum dislodgeable residue levels for individual dogs ranged from 841 to 4297 µg.

Table 72: Dislodgeable residue on gloves (% of amount applied) – De Fontenay *et al.* (1997d)

Time	Mean ± SD	Range
0	BLQ	-
1 h	0.834 ± 1.231	0.055 – 3.26
4 h	**1.078 ± 0.639**	0.407 – 2.16
8 h	0.474 ± 0.157	0.230 – 0.724
Day 1	0.505 ± 0.121	0.294 – 0.630
Day 2	0.469 ± 0.199	0.257 – 0.734
Day 4	0.232 ± 0.072	0.166 – 0.320
Day 7	0.175 ± 0.053	0.113 – 0.261
Day 14	0.042 ± 0.016	0.024 – 0.064
Day 21	0.020 ± 0.008	0.013 – 0.030
Day 28	0.005 ± 0.004	0.000 – 0.010

BLQ: below the limit of quantification; - (not applicable)

Hughes DL (1997e) Dislodgeable residues of fipronil following application of Frontline® Spray treatment to dogs. Covance 6848-100, Merial Metxt 165. Lab: Covance Laboratories Inc. 3301 Kinsman Boulevard, Madison, Wisconsin 53704. Sponsor: Merial. Laboratoire de Toulouse, 4 chemin du Claquet, Toulouse Cedex, France. Expt dates: 2 July 1997-28 August 1997). Unpublished report date: 3 November 1997. (QA/GLP: yes; Test Guidelines: No specific guidelines applicable, but followed the general provisions of US EPA guideline 'Occupational and Residential Exposure Test Guidelines, Series 875').

Materials and Methods This experiment was performed essentially as described above for Frontline Top Spot (Hughes 1997c), except Frontline Spray (0.29% w/w fipronil; Batch No.

L663151B from Merial), supplied in 250 mL containers with spray nozzles constructed to deliver approximately 1.5 mL of the product per full depression of the trigger pump, was applied to groups of 5 short-haired purebred Beagle dogs (from Covance Research Products Inc and Ridglan Farms, Inc) or 5 long-haired mixed breed dogs (LBL Kennels). The animals weighed 9.5 to 19.2 kg and were at least 8 months old. Each dog was sprayed according to the maximum label rate of 6 mL/kg. The actual amount delivered was determined by weighing the container before and after treatment. An earlier sampling point of 2 h replaced the 8 h time point in the Top Spot study. Results on day 3 for two of the long-haired dogs were from a second stroking on that day, as it was possible that a fresh glove was not used for each animal in the first instance. This may have reduced the score for the group on that day.

Results In the short-haired group, maximum dislodgeable residues were found at 12 h post-treatment in all but one dog, for which dislodgeable residues peaked at 4 h (see Table below). The maximum residue level of 1.45% equated to 2068 µg fipronil. For the long-haired dogs, peak residue levels were attained at 4 h, except for one dog which had similar maximum levels at both 2 and 12 h. The maximum level in the long-haired dogs of 0.883% was equivalent to 2231 µg fipronil. Metabolite levels were low (maximum 50 µg and 68 µg for fipronil sulfide and sulfone, respectively; or below the LOQ for desulfinyl fipronil.

Table 73: Mean dislodgeable residue on gloves (% of amount applied) – Hughes (1997e)

	Short-haired dogs		Long-haired dogs	
Time	**Mean ± SD (n=5)**	**Range**	**Mean ± SD (n=5)**	**Range**
2 h	0.629 ± 0.206	0.405 – 0.878	0.575 ± 0.090	0.454 – 0.702
4 h	0.772 ± 0.277	0.486 – 1.10	**0.688 ± 0.190**	**0.375 – 0.883**
12 h	**0.886 ± 0.422**	**0.469 – 1.45**	0.592 ± 0.139	0.451 – 0.757
Day 2	0.687 ± 0.364	0.245 – 1.07	0.509 ± 0.229	0.266 – 0.793
Day 3	0.486 ± 0.246	0.161 – 0.743	0.351 ± 0.208	0.138 – 0.685
Day 5	0.299 ± 0.152	0.070 – 0.420	0.275 ± 0.153	0.182 – 0.546
Day 8	0.208 ± 0.110	0.449 – 0.310	0.145 ± 0.084	0.085 – 0.291
Day 15	0.118 ± 0.048	0.035 – 0.149	0.069 ± 0.033	0.037 – 0.122
Day 22	0.059 ± 0.017	0.032 – 0.075	0.030 ± 0.014	0.019 – 0.054
Day 29	0.030 ± 0.009	0.022 – 0.045	0.016 ± 0.006	0.012 – 0.025

De Fontenay G, Campagna S, Suberville S, Birckel Ph, Weil A (1997c) Dislodgeable residues of fipronil following a topical application of Frontline® Spray treatment to dogs. Study No. MET 414. Lab: Merial, Toulouse France and Chrysalis, L'Arbresle, France. Sponsor: Merial 4, chemin du Calquet, 31057 Toulouse Cedex. Expt dates: commenced 25 March 1997. Unpublished report date: 2 December 1997. (GLP/QA: yes; Guidelines: none)

Materials and Methods This study was performed essentially as described above in Hughes *et al*. (1997 d), except that 6 beagle dogs were used (3/sex). The spray contained 2.42 mg/mL fipronil (Batch No. L713151A from Merial). As in the other dog stroking studies by de Fontenay *et al*., fipronil, desulfinyl fipronil, and metabolites RM1502 and RM1602 were measured.

Results Desulfinyl fipronil was not detected. Maximum levels of dislodgeable residues were detected at 4 h post-application, with low levels still detectable at 28 days (tabulated below). The maximum amounts of fipronil residues recovered on the gloves ranged from 2778 to

1095 µg. The maximum amounts of metabolites were 85 and 19 µg for fipronil sulfide and sulfone, respectively. Desulfinyl fipronil was not detected.

Table 74: Dislodgeable residue on gloves (% of amount applied) – De Fontenay *et al.* (1997c)

Time	Mean ± SD	Range
0	BLQ	-
1 h	0.893 ± 0.193	0.539 – 1.10
4 h	**1.43 ± 0.497**	**0.871 – 2.14**
8 h	0.684 ± 0.181	0.379 – 0.932
Day 1	0.568 ± 0.121	0.343 – 0.701
Day 2	0.464 ± 0.093	0.310 – 0.560
Day 4	0.239 ± 0.079	0.142 – 0.339
Day 7	0.124 p 0.029	0.088 – 0.155
Day 14	0.039 ± 0.011	0.027 – 0.052
Day 21	0.017 ± 0.006	0.010 – 0.026
Day 28	0.009 ± 0.002	0.000 – 0.011

BLQ: below the limit of quantification; - (not applicable)

Jennings KA, Canerdy TD, Keller RJ, Atieh BH, Doss RB, Gupta RC (2002) Human exposure to fipronil from dogs treated with frontline. Veterinary and Human Toxicology 44(5): 301-303

The aim of this study was to determine the amount of fipronil residues that could be transferred to personnel handling dogs subsequent to treatment with 1.34 mL of Frontline Top Spot formulation (9.8% fipronil). The product was applied to 4 household dogs between the shoulder blades at the nape of the neck according to the directions for use. The dogs had not been treated with fipronil for at least 3 months prior to the study and were not bathed during the 3 days before and after treatment. Another group of 3 dogs acted as controls. At 1, 8, 15, 22, 29 and 36 days after application, sampling of transferable residues was performed by vigorously petting the coat of each dog both forward and back along its back and both sides for a period of 5 minutes. Cotton gloves were worn during sampling, after which they were stored at -20°C for <2 days until analysed for fipronil by GC/MS. It was not stated if the stroking was performed with one hand or two.

The maximum level of fipronil transferred to the gloves (expressed as mean ± SEM) was detected at 1 day post-application (589 ± 206 ppm), falling to 448 ± 118, 250 ± 146, 304 ± 43, 30 ± 39 ppm on days 8, 15, 22 and 29 respectively, with undetectable levels on day 36. Taking into account that the extract volume was 4 mL, and the density of the combined solvents was approximately 1 g/cm^3, the maximum amount of fipronil transferred to the gloves in this study was approximately 2.4 mg.

Astruc B, Suberville S, Bosc F, Weil A (1998) Evaluation of the presence of M&B46513 on the hair after topical application (spot-on or spray) of fipronil to the dog. Study No. MET419. Lab: Merial, Toulouse, France. Sponsor: Merial, 4, chemin du Calquet, 31057 Toulouse Cedex, France. Unpublished report date: 13 January 1998. (GLP/QA: yes)

Materials and Methods Frontline Spot On (10% w/v fipronil, batch no. L0406; dose 0.1 mL/kg) or Frontline Spray (0.25% w/v fipronil, batch no. L713151A; dose 6 mL/kg) were applied to Beagle dogs (3/group). The dogs were kept in individual cages inside an animal house, but were allowed to go outside under natural light for a period of around 6 hours/day, weather permitting. This was to assess whether the photodegradative product of fipronil, desulfinyl fipronil, formed on the coat of a treated animal when it was exposed to sunlight. The achieved hours per day outside were 5.9 ± 0.86 during the week, and 3.3 ± 1.8 h at weekends and on a public holiday. However, though days on which there was rain ('dogs slightly wet') were noted, the actual hours of sunshine to which the dogs were exposed was not provided. Hair samples were collected before treatment, and on days 3, 7, 14, 21 and 28 post-treatment. The samples were taken from the lumbar zone up to the shoulder blades from an area ~3cm x 4 cm, using one sampling site per time point. The hairs were cut with scissors so that the upper part of the hairs most in contact with the light were collected. The pre-treatment sample was taken from the jugular region. The concentrations of fipronil residues (fipronil, desulfinyl fipronil, RM1502 and RM1602) on the dog hair were quantified using HPLC.

Results As shown in the Table below, desulfinyl fipronil was detected on dog hair from the first sampling point (day 3) until days 14 or 21 post-treatment for the spot-on and spray formulations respectively. Along with fipronil and its metabolites, absolute levels of this photometabolite appeared to decrease over time, though it is not clear how comparable the sampled areas of hair were, as the description of the sampling technique was limited in detail. As a proportion of the fipronil + RM1602 components of the residues, desulfinyl fipronil was reported to increase from days 3 to 14 following spot-on application (~3.7-6.2%), and to fall overall following spray application (~4.5-2.4%), having peaked on day 3. In this study, fipronil residues were much greater for the spray than the spot-on, which contrasts with the dislodgeable residue studies above, where levels were similar for the two types of formulations. This suggests that much of the material measured on the hair of dogs in the present study may not be dislodgeable when stroking the treated animal.

Table 75: Mean hair concentrations of fipronil residues (µg/g)

	Spot-on				Spray			
Time (days)	**Fipronil**	**Desulfinyl fipronil**	**RM 1502**	**RM 1602**	**Fipronil**	**Desulfinyl fipronil**	**RM 1502**	**RM 1602**
0	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ	BLQ
3	23.6	1.56	0.636	18.5	581	27.0	6.89	86.3
7	8.23	0.788	BLQ	10.1	140	5.13	2.22	44.2
14	1.98	0.424	BLQ	4.91	15.7	0.797	0.291	20.1
21	0.659	BLQ	BLQ	2.53	4.26	0.250	BLQ	5.88
28	0.415	BLQ	BLQ	1.63	1.65	BLQ	BLQ	1.72

BLQ=below the limit of quantification

12.3 Occupational exposure and indoor air levels following termiticide treatment

Honeycutt RC (2001) Determination of inhalation exposure to house occupants and pest control operators from fipronil during and after application of Termidor 80 WG as a termiticide treatment to homes. Aventis CropScience Study No. RP99V16852. H.E.R.A.C., Inc., 220-1 Swing Road, Greensboro, North Carolina 27409, USA. Unpublished report date: 9 January 2001.

Materials and Methods Houses, distributed throughout the USA, were treated with 0.07-0.08% Termidor 80WG termiticide (Termidor) in water at 4.968 L/m on outer and interior walls and 2.484 L/m injected into foundation walls, using a variety of spray probes and injectors. Sixteen male pest control operators in good health were monitored for inhalation exposure to fipronil in 16 crawl-space houses, using personal air-monitoring pumps attached to air sorbent tubes and air-filter cassettes. Five crawl-space, 6 cellar, and 5 slab construction houses were monitored for indoor air residues of fipronil. Indoor air was sampled for 24 h/d before and on the day of application (day 0), and 1, 2 and 7 days after application. Air sorbent tubes and sampling pumps were installed in the kitchen, family room, one bedroom, and cellar (if applicable) of each monitored house. Samples were analysed by gas liquid chromatography (GLC) against known standards.

Results Pest control operator exposures varied between individuals from 0.03 to 5.5 µg/kg bw/h (more than 180-fold), with a mean of 0.95 ±1.60 µg/kg bw/h (see Table below). No fipronil residues were detected in household air prior to treatment. Household air residues of fipronil were detected in 12 of the 16 monitored houses, 4 with cellars, 3 with crawl-spaces and 5 with slabs. Airborne fipronil residues were generally detected on the day of application (day 0) although residues were detectable in some houses up to 7 days after application. Fipronil residues were generally higher in houses with cellars (0.005 - 0.042 ng/L) than in crawl-space houses (0.004 - 0.011 ng/L), with the highest residues in slab-construction houses (0.006 - 0.081 ng/L).

Table 76: Pest control operator exposure

Pest control operator	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
ng fipronil/L[@]	3.57	6.02	1.28	11.26	1.29	10.00	7.40	21.20
total µg fipronil/h[#]	6.21	10.48	2.23	19.59	2.25	17.40	12.88	36.89
Exposure µg/kg/h[&]	0.09	0.15	0.03	0.28	0.03	0.25	0.18	0.53
Pest control operator	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**
ng fipronil/L[@]	1.42	11.20	9.52	16.10	157.80	221.40	95.60	36.70
total µg fipronil/h[#]	2.47	19.49	16.57	28.01	274.57	385.24	166.34	63.86
Exposure µg/kg/h[&]	0.04	0.28	0.24	0.40	3.92	5.50	2.38	0.91

[@] ng fipronil per L of air collected [#] µg fipronil/L of air collected x 29 L per minute x 60 min/hr
[&] µg/kg/hr = total ng fipronil/hour ÷ 70 kg bodyweight x 1000/µg

Table 77: Concentrations of fipronil in household air (ng fipronil/L air) after treatment with Termidor[#]

Type of house	Site No.	Day	Bedroom	Den	Kitchen	Cellar
Cellar construction	1	0				0.017
		1		0.008		
		2				0.006
	6	0			0.005 0.006	0.005
		7			0.005	
	25	0	0.030 0.031	0.030 0.035	0.028 0.026	0.042 0.039
	26	0		0.005 0.005		0.007 0.008
Crawl-space construction	2	0		0.009 0.011	0.007 0.005	
	3	0	0.004 0.005	0.008 0.008	0.009 0.010	
	8	0			0.006	
Slab construction	13	0	0.012 0.014	0.008	0.009 0.011	
	14	0	0.048 0.047	0.024 0.026	0.023 0.022	
	15	0	0.035 0.038	0.027 0.032	0.022 0.027	
		2	0.011			
		7		0.010		
	16	0	0.081 0.076	0.029 0.027	0.029 0.030	
	17	0		0.011	0.010 0.011	
		7	0.006			

[#] the absence of a value indicates there were no detectable fipronil residues.

13 OTHER STUDIES

13.1 Mechanism of action

Bushey DF (1993) Mode of action of fipronil. RTP Biochemistry Group Report. Rhône-Poulenc Ag Company, North Carolina, USA. Unpublished report date: 8 April 1993.

Several experiments were undertaken to determine the mode of action of fipronil.
(i) The nervous system of the housefly maggot was dissected out and connected to electrodes to enable the measurement of the electrical impulse transferred down the CNS. Fipronil at 10 nM effectively reversed the effect of 10 mM gamma-aminobutyric acid (GABA).

(ii) A biochemical assay that measures GABA-induced influx of chloride ions across the cell membrane was used in rat brain microsacs to measure the effect of fipronil on GABA receptor function. Fipronil (IC_{50} = 6-8 µM) inhibited the GABA-activated transport of radiolabelled chloride ions across the cell membrane.

(iii) Radioligand binding assays were used to determine fipronil's binding site on the GABA receptor in vertebrate tissue (rat brain) and insect tissue (housefly head). Results indicated that the fipronil binding site was within the GABA-gated chloride channel. Fipronil displayed greater avidity for invertebrate tissue relative to vertebrate tissue.

(iv) In oocytes expressing the *Drosophila* GABA receptor, fipronil at concentrations of 3 µM to 10 nM inhibited the GABA-induced electrical response in a dose-related manner.

Fitzgerald M (1993) The significance of fipronil metabolite residues. RPA Report (September 1993). Rhône-Poulenc Agrochimie, Lyon.

In this paper, selected data were reported on the GABA-receptor binding of several phenylpyrazole compounds. Fipronil was demonstrated to bind *in vitro* to GABA-gated chloride channels in rat and mouse brain tissue, with IC_{50} values for the binding of the GABA-gated chloride channel ligands TBPS (t-butylbicyclophosphorothionate) and EBOB (ethynylbicycloothobenzoate) shown in the Table below. The author stated that both MB 45950 (fipronil sulfide) and MB 46136 (fipronil sulfone) exhibit good binding activity, which correlates with their insecticidal and mammalian acute toxicity, but data were not provided for these metabolites. Two other fipronil metabolites, RPA 200766 (fipronil amide; found in plants and animals) and RPA 105048 (desulfinyl fipronil amide; found in plants) were inactive in two rat GABA-binding assays.

Table 78: Mammalian receptor binding in vitro - IC_{50} (nM)

Phenylpyrazole	Rat brain, TBPS ligand[9]	Rat brain, EBOB ligand[9]	Mouse brain EBOB ligand[10]
Fipronil	43	772	4300
RPA 200766 (fipronil amide)	>10,000	>10,000	ND
RPA 105048 (desulfinyl fipronil amide)	>10,000	>10,000	ND

TBPS: t-butylbicyclophosphorothionate; EBOB: ethynylbicycloorthobenzoate. These ligands are probes for the GABA-gated chloride channel. ND, not determined

In a GABA-induced $^{36}Cl^-$ influx assay in rat brain membranes, fipronil had an IC_{50} of 13 µM, correlating well with the published value of 20 µM in mouse brain membranes[10]. In contrast, neither RPA 200766 (fipronil amide) nor RPA 105048 (desulfinyl fipronil amide) showed any activity in the rat brain assay at 100 µM, demonstrating that these metabolites do not interfere with GABA receptor function.

13.2 Blood pressure, heart rate and electrocardiogram

Richard S and Champeroux P (1990) M&B 46030: Effects of M&B 46030 on blood pressure, heart rate and ECG in conscious rabbits. CERB No. 900145E. Lab: Centre De Recherches Biologiques, Baugy, France. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Unpublished report date: 23 July 1990. (GLP/QA: no.

Fipronil (4 mg/kg bw) was administered orally to a group of eight female New Zealand White rabbits. A second group was treated with the vehicle (0.5% methylcellulose and 0.01% Tween 80). Arterial blood pressure, heart rate and ECG were recorded before dosing and 1, 24, 48,

[9] Cole LM, Nicholson RA, Casida JE. (1993) Action of phenylpyrazole insecticides at the GABA-gated chloride channel. Pesticide Biochemistry and Physiology **46**(1): 47-54.

[10] Gant DB, Bloomquist JR, Ayad HM, Chalmers AE (1990) 7th International Congress of Pesticide Chemistry (IUPAC), Hamburg, Germany, August 5-10, 1990. Pesticide Science **30** (3): 355-357.

72, 96 and 120 h after dosing. Under the conditions of the study, fipronil did not influence any of these three parameters. Neither were there signs of toxicity.

13.3 Electroencephalogram studies

Algate DR, Beard DJ, Templeton D (1991a) M&B 46030: Assessment of the effects on the electroencephalogram of conscious rabbits following single-dose oral administration. Report no. RNP 360/90865. Lab: Huntington Research Centre Limited, Cambridgeshire, England. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 11.5.1990-19.5.1990. Unpublished report date: 30 January 1991. (QA: yes; GLP: not stated)

Two groups of New Zealand White rabbits (2.7-3.3 kg, 6-9 months old, from Ranch Rabbits, Susses, England) received a single oral dose of vehicle (0.5% methylcellulose containing 0.01% Tween 80) or fipronil (4 mg/kg bw; Batch No. PGS 963, supplied by the sponsor) by gavage. The cortical EEG was recorded prior to dosing and at 24 h intervals post-dosing for 1 week, over a 30 min time period. Treatment with the vehicle failed to induce behavioural or clinical abnormalities; and there were no treatment-related effects on bodyweights during the 2-week dosing period. Fipronil induced a significant 'right shift' (towards higher frequencies) in the mean and median frequency of the cortical EEG, significantly higher than the control in 3/5 rabbits at 72 h. An increase in percentage change in total electrical activity (from pre-dose values) was also noted at 72, 144 and 168 h. The EEG waveforms were not considered 'pathological', and were possibly attributable to slight CNS activation, though this was not accompanied by behavioural changes. It is possible that 4 mg/kg bw was a threshold dose, enough to induce slight changes in the EEG, but insufficient to cause behavioural effects.

Algate DR *et al* (1991b) M&B 46030: Assessment of the effects on the encephalogram of conscious rabbits following daily oral administration at 4 and 8 mg/kg/day. Report No. RNP 369/901076. Lab: Huntington Research Centre Limited, Cambridgeshire, UK. Sponsor: Rhone-Poulenc Agrochimie SA, Lyon, France. Expt. dates: 23.6.1990-28.6.1990. Unpublished report date: 31 January 1991. (QA: yes, GLP: not stated)
Two groups of five female New Zealand White rabbits (3.09-3.47 kg, 6-9 months old, from Ranch Rabbits, Sussex, England) received daily oral doses of 4 or 8 mg/kg bw fipronil (Batch No. PGS 963, from the sponsor) suspended in 0.5% methylcellulose containing 0.01% Tween 80. The cortical EEG was recorded prior to the first treatment and at 1 h post-dosing for the next 4 days.

The study was terminated on day 5 due to toxic effects and loss of bodyweight, following the deaths of three rabbits (two in the 4 mg/kg bw/d group). Clinical signs, which commenced on days 2-3, included agitated behaviour, hyperventilation or gasping, abnormally lowered body carriage, increased skin temperature and cutaneous blood flow to the ears, tremors, ataxia, and convulsions. Apart from a small amount of blood in the thoracic cavity of one animal, no pathological changes to major organs were noted at gross macroscopy for the two decedent animals examined (the third was not examined due to autolysis). Both groups of animals showed bodyweight loss, and by day 5 losses were 130-320 g at 4 mg/kg bw/d and 290-590 g at 8 mg/kgbw/d.

Detailed EEG analysis indicated a general dose-related increase in total activity after the first dose, followed by a dose-related reduction in total activity over the next few days. Analysis of individual spectra suggested that significant changes had taken place in both groups at various frequencies, which could be consistent with slight CNS activation. These changes peaked at 72 h, and were more notable in the 4 mg/kg bw/d group. Visual examination of the EEGs revealed no pathological wave forms.

13.4 Enzyme induction

Shavila Y, Barnett CR, Wilson J, Ioannides C (1990) Induction of hepatic cytochrome P-450 proteins and phase II conjugation enzymes by M&B 46030. Research report. Lab: Department of Biochemistry, University of Surrey, Guildford, Surrey, UK. Sponsor: Rhone-Poulenc Agrochemie, Lyon, France. Unpublished report date: September 1990. (QA: yes, GLP: no)

In a comparative study, the ability of fipronil to induce hepatic microsomal cytochrome P-450 proteins and phase II conjugation enzymes in the mouse, rat and rabbit was investigated following 4-day or 14-day repeated gavage administration. Various doses were employed: 1.2 and 5.0 mg/kg bw/d in the rat and mouse, and 0.3 and 1.2 mg/kg bw/d in the rabbit. Animals were sacrificed 24 h after the final dose, at which time livers were removed and microsomal and cytosolic fractions prepared. Rats (5/sex/group, 129-150 g) were CD strain from Charles River, UK; NZW rabbits (3/sex/dose, 2.9-3.5 kg) were from Rhone-Poulenc Agro, UK and mice (5/sex/dose, CD-1, 22-29 g) were from Charles River UK. A range of enzymes was assayed. Positive controls were performed for 1 animal/sex that had been dosed with phenobarbitone (150 or 75 mg/kg bw) by the same route, and negative controls (1/sex) were administered the vehicle only.

Bodyweight was not affected in rats and rabbits, but one male rat died after administration of the 5th dose of 5 mg/kg bw/d in the 14-day study. A number of mice died under each of the treatment regimes, females being more affected than males, and this was preceded by substantial weight loss. However, the other mice in these groups appeared normal throughout. Liver weight relative to bodyweight was increased in 5 mg/kg bw/d female rats for both the 4- and 14-day treatments, but there was no consistent pattern for the other species. Total cytochrome P450 was increased in male and female rats in a dose-dependent fashion after the 4-day treatment, but after the 14-day treatment was much less marked in male rats, and was no longer present in females. No inductive response was apparent in the rabbit, but 14-day administration of fipronil in mice resulted in a rise in microsomal protein content, and thereby an increase in some mixed function oxidase activities on a per g liver basis. In all groups, other changes in the microsomal mixed function oxidase system were occasional, with no consistent pattern. The significance of these changes was difficult to interpret due to variability and low numbers of animals per group. Phase II enzymes were generally unaffected, but otherwise decreased in rats (cytosolic glutathione S-transferase, 14 days), and male rabbits and mice of both sexes (4-methylumbelliferone glucuronyl transferase, after 4 days in rabbits, and after both 4 and 14 days in mice). Microsomal glucuronidation of 1-naphthol was decreased in mice after treatment for 14 days. The positive control gave the expected results. Though the findings in this study were not clear cut, it provides limited evidence that induction of cytochrome P450 occurs in fipronil-treated rats and mice, but not in rabbits.

13.5 Thyroid function

Peters DH, Stuart V, Hall M, Chasseaud LF, Chanter DO (1991a) M&B 46,030: An investigation into the potential effects on thyroid function in male rats by using the 'perchlorate discharge test'. Report no. M&B 353/90920. Lab: Huntington Research Centre Limited, Cambridgeshire, UK. Sponsor: Rhone-Poulenc Ltd, Dagenham, Essex. Expt. dates: 24.11.1989-8.12.1989. Unpublished report date: 12 April 1991. (GLP/QA: yes)

The effect of fipronil on thyroid function was investigated in Charles River CD strain male rats. Fipronil was compared with propylthiouracil (PTU), a known inhibitor of thyroid iodide organification in many species, and noxyflex, another thiourea compound that lowers serum thyroxine levels and reduces iodide organification in cultured porcine thyrocytes *in vitro*.

Four groups (27 males/group) were treated for 14 days with either 10 mg/kg bw/d fipronil, 200 mg/kg bw/d PTU, 50 mg/kg bw/d noxyflex, or 0.5% methylcellulose (controls). At termination, each rat received $Na^{125}I$, followed 6 h later by 0.9% saline and potassium perchlorate (10 or 25 mg/kg bw). (Note: Perchlorate releases free iodide present in the thyroid.)

A large reduction in the ^{125}I content of the thyroid glands, and relatively less ^{125}I radioactivity in the thyroid than in the blood, was observed in PTU-treated animals given perchlorate. There was no evidence of inhibition of iodide organification by either noxyflex or fipronil. Administration of potassium perchlorate to fipronil-treated rats resulted in no change in blood or thyroid ^{125}I levels. Fipronil and noxyflex enhanced the accumulation of radioactive iodide in the thyroid and triggered a stimulation of thyroid functional activity by a mechanism not involving direct inhibition of iodide organification.

Peters DH, Stuart V, Crook D, Chanter DO, Colman KA, Gopinath C (1991) M&B 46030: 4-week dietary study to investigate thyroid hormone levels in the rat. Report No. M&B 360/901275. Lab: Huntington Research Centre Limited, Cambridgeshire, UK. Sponsor: Rhone-Poulenc, Secteur Agrochemie, Lyon, France. Expt dates: 26.4.1990–25.5.1990. Unpublished report date: 20 May 1991. (GLP/QA: yes)

Charles River CD strain rats (10/sex/group) were treated with fipronil at 0, 0.1, 1, 5 or 30 ppm (equal to 0, 0.01, 0.1, 0.5 and 2.9 mg/kg bw/d, respectively) in the diet for 4 weeks. There were no signs of toxicity, and food intake and weight gain were not affected. At 30 ppm, T3 levels fluctuated, T4 levels were reduced and TSH levels were increased. Marginal increases in liver and thyroid weights were recorded for both sexes. There was minimal periacinar hepatocyte enlargement in males, trace/minimal centriacinar fat in the livers of females, and a minimal increase in the height of follicular epithelium in the thyroid glands of both sexes. At 5 ppm, a decrease in T3 and T4 and/or increased TSH levels and a marginal increase in thyroid weights were recorded for males only. Histopathology revealed trace/minimal centriacinar fat in the livers of females and a minimal increase in the height of thyroid follicular epithelium in males. The thyroid findings were attributed to an increase in T4 clearance from the blood, resulting in a reduction of the feedback inhibitory control of thyroxine on thyroid function. At 1 or 0.1 ppm, an effect on T3, T4 or TSH was not

established, and there were no compound-related organ weight differences or microscopic findings. The no-effect level was 1 ppm (equal to 0.1 mg/kg bw/d).

Taylor T (1993) The effect of single and repeated oral doses of MB46030 (1 mg/kg/day and 10 mg/kg/day) on the biliary excretion of intravenously administered ^{125}I-thyroxine (T4) from bile-duct-cannulated rats. Report no. HRC/ITT 2/930645. Lab: Huntington Research Centre Limited, Cambridgeshire, UK. Sponsor: Investitox Ltd, Great Barford, Bedfordshire, England. Expt. dates: 15.6.1992-17.10.1992. Unpublished report date: 7 July 1993. (GLP/QA: yes)

Materials and Methods Groups of Sprague-Dawley CD VAF$^+$ strain rats (3/group) were given fipronil as a single dose, or repeated daily doses for 14 days, orally in water with 0.5% methylcellulose at 1 or 10 mg/kg bw/d. Others were treated with the corresponding number of doses of phenobarbital intraperitoneally at 80 mg/kg bw/d (positive controls), or 0.5% methylcellulose orally (negative controls). Immediately after the last (or only) administration, rats were anaesthetised, common bile ducts cannulated and sodium iodide (1 mg) administered. Four hours later, 10 µCi ^{125}I-thyroxine (T4) was administered intravenously. Bile and whole blood samples were collected at intervals during the next 5 h.

Both fipronil and phenobarbital treatment for 2 weeks enhanced the biliary clearance of T4. Excretion of T4-conjugated products during the interval 0-5 h increased about 3-fold for 1 mg/kg bw fipronil; 4-fold for 10 mg/kg bw fipronil; and 5-fold for phenobarbital. Single doses of fipronil at both levels, or phenobarbital, also raised biliary excretion of conjugated ^{125}I during 0-5 h (1 mg/kg bw, 48%; 10 mg/kg bw, 56%; and phenobarbital, 74%, greater than the control). The output of bile was increased following fipronil treatment at 10 mg/kg bw or phenobarbital treatment for 14 days.

Peters DH, Stuart V, Hall M, Chasseaud LF, Chanter DO (1991b) M&B 46030: An investigation into the potential effects on thyroid function in male rats by studying thyroxine clearance. Report no: M&B 352/90958. Lab: Huntington Research Centre Limited, Cambridgeshire, UK. Sponsor: Rhone-Poulenc Ltd, Dagenham, Essex. Expt. dates: 22.11.1989-7.12.1989. Unpublished report date 12 April 1991. (GLP/QA: yes)

Charles River male rats (6/group) were administered fipronil (10 mg/kg bw/d p.o.) or phenobarbital (80 mg/kg bw/d ip) as a single dose, or daily for 14 days. Four hours after the single or final dose, each rat was given ^{125}I-T4 i.v. (10 µCi/kg bw). Levels of ^{125}I in whole blood were monitored for up to 30 h following thyroxine administration and were used to estimate the pharmacokinetic parameters of T4 terminal half-life, clearance and volume of distribution. Fipronil increased T4 clearance from whole blood when administered orally for 14 days at a level of 10 mg/kg bw/d. This was paralleled by a statistically significant decrease in terminal half-life of T4 in blood. After only 1 day of dosing with fipronil (10 mg/kg bw), similar, but smaller, effects were noted. Phenobarbital (80 mg/kg bw/d) produced changes similar to those affected by fipronil after 1 or 14 days' dosing.

14 NON-DIETARY PUBLIC EXPOSURE TO FIPRONIL

Products available for home veterinary/home garden (HV/HG) use that are considered in this review include the spray and spot-on products for use in cats and dogs, and insect baits. The risk assessment for the exposure to the insect baits is straightforward, due to the low concentrations of fipronil in these products. Therefore, this is addressed briefly in Chapter 15 along with a review of the safety directions for these products in Chapter 16.

(i) HV products – spray and spot-on formulations

Dermal and inhalation exposure during application

The method of application of the Frontline spot-on range of products involves breaking the snap-off top from the pipette, then while holding the pipette tip against the skin of the animal, squeezing the pipette several times until it is emptied of its contents. This procedure is unlikely to lead to the user being dermally exposed to the product, but given the unpredictable behaviour of pets, accidental exposure cannot be ruled out. However, as the duration of the procedure is brief and performed at most on a fortnightly basis, the opportunity for exposure during application is limited. If the safety direction 'wash hands after use' is followed, any exposure should be transitory and not of toxicological concern. As fipronil is not volatile, exposure by inhalation is not expected.

Label instructions for the Frontline Spray ready-to-use product require the user to spray the entire animal against the lay of the hair, using a pump nozzle applicator held 10-20 cm from the animal's coat. The coat is to be ruffled as the spray is applied, so the product penetrates down to the skin, and the coat is thoroughly wet. The most likely route of user exposure is dermal, when carrying out the actions just described, restraining or carrying the wet animal, other incidental contact, or being sprayed with the product if a wet animal shakes itself nearby. The safety directions recommend that rubber gloves be worn, and this is considered appropriate. As well as protection from exposure to the active constituent, gloves are necessary to protect the user from the skin irritancy potential of this product. Taking into account that fipronil is present in the product at a low concentration (0.25%); the shortest re-treatment interval is 3 weeks; and the vapour pressure of fipronil is low, the level of user exposure is not expected to be of toxicological concern. This conclusion is supported by the findings from an exposure study of pet groomers using Frontline Spray to treat 8 animals consecutively[11], and thereby providing a conservative estimate of exposure to a householder treating domestic pets. In that study, inhalation exposure was minimal and large MOEs resulted for total exposure via the dermal and inhalation routes, using toxicological endpoints from repeat dose studies (see OHS Review of Fipronil for this report).

Exposure from handling a treated pet

It should be noted that the following discussion relates to the possibility of systemic effects in humans following repeated dermal exposure to dogs or cats that have been treated with

[11] Meo NJ, Gonzalez CM and Mester TC (1997) Dermal and inhalation exposure of commercial pet groomers during application of Frontline Spray Treatment. Study No. SAFXT046, Merial Limited (formerly Rhone Merieux, Inc.), Georgia USA.

fipronil products. It does not include a discussion on the potential for the development of an allergic response. This is considered separately in Chapter 15.

Eight pet stroking studies were submitted (Hughes 1997b,c,d,e and de Fontenay 1997a,b,c,d), and a related published study was also taken into account (Jennings *et al*. 2002). Full details of the studies summarised here can be found in Chapter 12 of this review. In brief, cats or dogs treated with the spot-on or spray formulations were stroked with cotton gloves and dislodgeable residues (fipronil and its main metabolites) were measured at various times following treatment. The amounts of the spot-on product applied to the animals in these studies corresponded to the label directions for the treatment of cats (0.5 mL) and medium dogs weighing 10-20 kg (1.34 mL; both short and long-haired dogs in the Hughes studies). The spray was applied at the maximum label rate (6 mL/kg) to cats and medium dogs. As it is not expected that a person handling a treated dog would be exposed at the maximum level at each encounter with the animal, it was considered appropriate to use the mean values when performing an assessment of likely exposure, and these are provided in the Table below. Higher levels of dislodgeable residues are likely if more than one treated pet is handled, or if large or extra large dogs are involved, for which the treatment level is respectively 2 and 3 times the dose for medium dogs. The results of the published study (Jennings *et al*. 2002) in which 1.34 mL of the spot-on formulation was applied to dogs, and which employed a much more vigorous and prolonged stroking procedure (5 min), are not included in the Table below. In this study, the maximum levels of fipronil dislodged were approximately equivalent to 2.4 mg at the first measurement point, on the day after application. Taking into account the differences in the stroking protocol between this and the unpublished studies, the results from the published study are consistent with the other findings. However, the extensive and prolonged hand contact with the dogs in the published study is likely to overestimate exposure in the real life situation.

Both the Hughes and de Fontenay studies obtained 3 samples on the day of application. The Hughes reports indicated that the timing of the first sampling point was chosen to allow for the applied material to dry. This was not addressed in the de Fontenay study reports, in which the first sampling point was always 1 h post-treatment. Sampling at this early stage did not appear to affect the results for the spray product, as all studies reported fairly similar results for the 3 samples taken in the first 12 h. However, a possible explanation for the high level of dislodgeable fipronil at 1 h in the de Fontenay spot-on study in cats is that the application site was still wet at this point. In this study, dislodgeable residues were similar to the corresponding Hughes study on day 2 and thereafter.

Table 79: Stroking study results – mean dislodgeable residue levels (mg fipronil equivalents*)

	Top spot					Spray				
Approx. dose (mg fipronil)	50		134			51	34.7	154	247	137
Species	**Cats**		**Dogs**			**Cats**		**Dogs**		
			SH	**LH**	**SH**			**SH**	**LH**	**SH**
Reference	Hughes 1997a	DF 1997a	Hughes 1997c		DF 1997c	Hughes 1997b	DF 1997b	Hughes 1997d		DF 1997d
Time										
1 h	-	3.42	-	-	1.12	-	0.19	-	-	1.22
2 h	-	-	-	-	-	0.17	-	0.97	1.43	-
4 h	0.38	0.85	0.56	0.83	1.44	0.23	0.22	1.19	1.71	1.97
8 h	0.16	0.52	0.80	0.59	0.64	-	0.18	-	-	0.94
12 h	0.11	-	0.65	0.50	-	0.20	-	1.36	1.47	-
Day 2	0.32	0.32	0.42	0.43	0.68	0.19	0.14	1.06	1.27	0.78
Day 3	0.07	0.12	0.82	0.63	0.63	0.08	0.07	0.74	0.87	0.64
Day 5	0.02	0.02	0.61	0.57	0.31	0.03	0.02	0.46	0.69	0.33
Day 8	0.01	0.008	0.35	0.37	0.23	0.01	0.005	0.24	0.36	0.17
Day 15	0.005	0.003	0.20	0.17	0.06	0.005	-	0.18	0.17	0.05
Day 22	0.005	0.001	0.09	0.09	0.03	0.005	-	0.09	0.07	0.02
Day 29	0.005	BLQ	0.05	0.07	0.07	0.005	-	0.04	0.04	0.01

* sum of fipronil, fipronil sulfenyl and fipronil sulfonyl (Hughes studies); or sum of fipronil, 'RM1502' and 'RM1602' (de Fontenay studies)
L = long-haired; S = short-haired; DF = de Fontenay *et al*.
A dash indicates that samples were not taken at that time point.

With the exception of the de Fontenay *et al* spot-on study in cats, discussed above, the dislodgeable residues in cats were low relative to dogs, with very low levels of exposure likely from day 3. For dogs, results from short-haired and long-haired animals were similar, but on days 1 and 2, dislodgeable residues were higher for the spray groups relative to the spot-on groups, though very similar thereafter. This may reflect the distribution of the spray fairly evenly over the animal's coat, compared to the more localised application for the spot-on product. It is even possible that the stroking episodes in the first 2 days aided the distribution of fipronil over the coats of the dogs in the spot-on studies, in which the levels did not fall appreciably until day 8, whereas the levels in the spray groups were falling steadily from day 2 in most instances.

While these stroking studies appear to have been well-conducted and provide some information on the likely exposure levels, there is still some difficulty in applying this to real-life situations. The number of times an individual handles an animal in a day, and the extent of this contact is going to be highly variable, though for a procedure such as thoroughly checking a dog for ticks on a daily basis, it is possible that an adult may experience levels of exposure approaching those seen in the stroking studies. Also, the amount of dislodgeable fipronil decreases over time, making comparison with endpoints from repeat dose animal studies that use constant dose levels, less straightforward. In most cases the products are to be applied on at least a monthly basis, so exposure will be ongoing. Frontline Top Spot for dogs may be applied at two-weekly intervals for control of paralysis ticks, so increased exposure is expected with this treatment regime.

Choosing an appropriate animal study with which to compare predicted human exposure is therefore problematic. For example, the 3-week dermal study in rabbits (NOEL = 5 mg/kg bw/d) provides the appropriate route of exposure. This is a very conservative choice on the basis that it represents exposure for 6 h/d, 5 days/week, occluded, compared with the relatively transitory nature of contact between humans and pets. Given that the dislodgeable residues reduce over time between treatments, with little exposure expected after one week post-treatment for dogs, and after 2 days for cats, the use of a short-term study rather than one of longer duration is considered adequate. Taking the worst case scenario, in which all of the residues on the animal (maximum mean residue level of 3.4 mg at 1 h in the de Fontenay cat spot-on study) are transferred a 60 kg person, the dose is 0.06 mg/kg bw/d, giving a MOE of 83. Taking into account the inbuilt conservatism in this risk assessment, this is considered adequate. On this basis, exposure of an adult to more than one treated pet, or to a very large dog, is also not of toxicological concern.

For the following risk assessments for children, the early sampling points in the de Fontenay spot-on studies will be discussed separately. As the product is likely to have not dried on the animal at that stage, and is expected to have been located mainly at the application site, this is considered to represent a different scenario to when the data for the later time points were obtained. It is expected that adults do not willingly touch treated animals while they are still wet.

The highest mean level of transferable residues later than 1 h post-treatment is ~2 mg at 4 h. When small children interact with treated pets it is expected that residues would be dislodged onto the child's clothing as well as exposed areas of the body. In the unlikely event that all of the dislodgeable residues are transferred to a 15 kg child, and 50% is transferred to the child's skin (hands, arms, legs comprise 40-50% of total body surface area for 1 to 4-year-old children[12]), this is equivalent to a dermal dose of 0.07 mg/kg bw, giving a MOE to the NOEL from the rabbit dermal study (5 mg/kg bw/d) of ~70. Considering the conservative nature of the chosen NOEL as argued above, this is acceptable.

It is well accepted that toddlers may be exposed to toxicants through hand-to-mouth transfer. Considering the relatively short interval of a child's life during which mouthing activities are a significant behaviour, a NOEL from a short-term study would be adequate for risk assessment purposes. On this basis, the NOEL of 1 mg/kg bw/d from a 4-6 week study in dogs will be used here (Holmes 1991a). Only a proportion of the dislodgeable residues will be transferred to the child's hands, and therefore be available for transfer to the mouth. If 20% of the dislodgeable residues are transferred to the child's hands (assuming more on the hands than other exposed body parts), and 50% of the residues on the hands are transferred to the child's mouth and are ingested, this results in an oral dose of 0.01 mg/kg bw (2 mg÷15 kg x 10%), giving a MOE of 100 (1 ÷ 0.01).

The worst case scenario for a child exposed via contact with a treated animal is expected to be through touching the treated site shortly after the spot-on product has been applied, and is therefore, in effect, exposure to the product itself. Because transfer of fipronil in liquid to the child's skin is likely to be more efficient than any subsequent exposure once the product is dry on the animal, this will be considered to represent an acute exposure. The NOEL from the acute neurotoxicity study in rats (Hughes 1997) of 2.5 mg/kg bw/d will therefore be used

[12] US EPA (August 1997) Exposure Factors Handbook. National Centre for Environmental Assessment. Office of Research and Development.

here. The de Fontenay study (1997a) provided a dislodgeable residue level of 3.4 mg fipronil at 1 h post-application to cats. If 3.4 mg were deposited on the skin of a 15 kg child, the dermal dose would be 0.2 mg/kg bw. It is expected that less than 1% of fipronil coming into contact with human skin will be absorbed (see Discussion section of the Review). Therefore, using a dermal absorption factor of 1%, this equates to a MOE of 1250 (2.5 ÷ 0.2 ÷ 1%), so dermal exposure in this situation is not of toxicological concern. If half of the dermal dose was ingested, the oral dose is 0.1 mg/kg bw, giving a MOE of 25 (2.5 ÷ 0.1). Taking into account the chance nature of the child touching the wet spot rather than other areas of the dog's coat, this margin is considered acceptable.

The addition of (S)-methoprene to the Frontline Top Spot formulation does not alter the product hazard profile, so no additional consideration is required for exposure to the Frontline Plus product range (see Chapter 16). These products are packaged similarly and have the same method of application as for Frontline Top Spot (see Chapter 14).

15 FIPRONIL: SKIN REACTIONS IN HUMANS

The decision to review and reconsider the active fipronil and products containing fipronil included concerns over human health. The APVMA has received a number of adverse experience reports involving products containing fipronil, including skin reactions in humans, therefore the toxicity of fipronil primarily in relation to dermal irritation and the induction of skin sensitisation is of particular interest. This chapter adressess this issue, focusing on sensitisation. For completeness, a summary of the available animal data is also provided below.

Animal skin irritation studies

Fipronil was tested for skin irritation potential in two rabbit studies, one in which the fipronil was applied semi-occluded under a gauze pad moistened with water, and the other in corn oil under an occlusive dressing (Liggett 1988b, Myers & Christopher 1993). The latter study indicated that fipronil was a slight skin irritant, whereas the former reported no reactions. The effect of the different protocols on the outcomes of these studies is not known, but given that skin irritation was observed in 6/6 rabbits in the Myers & Christopher (1993) study, compared to the 3/3 showing no irritation in the other study, it is concluded that fipronil is a slight skin irritant. Of skin irritation studies conducted on formulations containing fipronil, 5/10 were slight skin irritants and 5/10 were not skin irritants.

Animal sensitisation studies

The allergenic potential of fipronil and its products were investigated by means of animal skin sensitisation adjuvant and non-adjuvant tests in guinea pigs. These were the maximisation test (GPMT) of Magnusson & Kligman using an adjuvant, or the occluded patch non-adjuvant test developed by Buehler.

This review has considered 12 skin sensitisation studies in guinea pigs; two for the active constituent, and the remainder for products containing fipronil at concentrations ranging from 0.1% to 80%. Nine of these studies used the Buehler or modified Buehler test method, six of which reported no dermal skin reactions at challenge. In three of the Buehler studies, erythema was reported in a single test animal at challenge (Myers & Nachreiner 1994; Warshawsky 1995e; Findlay, 1999c). In two studies (Myers & Nachreiner 1994; Warshawsky 1995e) the observed erythema was minimal (i.e. slight patchy erythema) and had resolved 48 hours after challenge or the same severity or skin reactions was seen in a control animal. Consequently, the limited erythema seen in test animals in both these studies is considered irritant in nature. Erythema was observed in only a single study that was considered not likely to be irritant in nature (Findlay, 1999c). In this study, moderate erythema was reported in one animal at 48 h only. No skin reactions were observed in negative control animals. Thus, this study provides limited evidence of a weak delayed skin sensitisation potential.

The remaining three studies, using technical fipronil (Johnson 1993), or products containing 80% (Grunert 1996e) or 0.05 % fipronil (Allen 1994e), employed the GPMT method of Magnusson and Kligman. In the two studies using fipronil products, no skin reactions were seen at challenge. In the study using technical fipronil, slight erythema was observed in two test animals at 24 h post challenge to 10%, which had diminished to barely perceptible erythema in both animals at 48 h. Thus, as the severity of the erythema was not sustained over

the post challenge observation times, it is considered likely to be irritant in nature. Additionally in this study, the eschar formation observed in 2 test animals in the absence of severe skin irritation or corrosion is also not considered to provide reliable evidence of a skin sensitisation potential. Furthermore in this study, no skin reactions were observed in test animals challenged with 3% fipronil.

Thus, the weight of evidence from laboratory animal studies of fipronil and its formulated products indicates that fipronil is not a skin sensitiser in guinea pigs. On the basis of these animal studies, fipronil is concluded to have no sensitising potential in humans.

Human adverse experience information is also available which is reported to have occurred after the use of fipronil products. Some of these adverse experiences have been cutaneous in nature and a discussion of these reports follows.

Allergenicity of the excipients in Frontline products

Information available on the allergenicity of each of the excipients in Frontline Spray, and those common to the Frontline Spot-on range of products, is provided in Appendix XI. No animal skin sensitisation data were located for the alcohols present in these products. Limited human testing was generally of poor quality, but there was some evidence to suggest that human sensitisation to these alcohols may occur in some individuals. However, in the context of their long history of widespread use, such reactions are very rare, and on this basis they are not expected to have contributed to the adverse reactions under consideration in this report.

Human adverse experience reports for Frontline products in Australia

Up to February 2006, the OCSEH was aware of 73 adverse experiences reports associated with Frontline products, which dated back to 1998. Of these, 30 were due to accidental exposure or related to off-label use, and are not discussed further in this report. Another 12 reports are not pursued here due to confounding factors such as underlying chronic illnesses and associated medication, symptoms inconsistent with the toxicology profile of fipronil (taking route of exposure into account), or insufficient information. A Table summarising the remaining human adverse reactions to the Frontline products to date (31 in total) is provided in Appendix X.

Of the tabulated cases, the majority (23, or 74%) were cutaneous in nature, with pruritus, rashes, hives, blisters and/or angioedema reported. Other reported symptoms included burning sensation of the eyes/face (3), sore/watery eyes (3), puffy or swollen face, lips, tongue or limbs (8), headache (2), shortness of breath (1) chemical taste (1), persistent shortness of breath (1), and facial numbness (1). There was a single case that reported various symptoms including diarrhoea, lethargy, nausea, cramps, fever and incoordination. The severity of symptoms varied from slight (resolved overnight with no intervention other than washing the affected area) to severe reactions involving hospitalisation (two cases). With a few exceptions, adverse reactions were of sufficient concern to the affected party that a medical practitioner was consulted.

The time and onset of symptoms was generally less than 48 hours after contact with the product or a treated animal, and these symptoms were frequently treated with cortisone and/or antihistamines. In many cases the outcome of this treatment was not provided in the report, though a positive response to treatment was documented for ten cases. Seven individuals

reported a recurrence of symptoms that could potentially be associated with exposure to the product or a treated pet. While the recurrence of symptoms was only seen in 7 individuals, this may be due to other individuals associating their symptoms with the Frontline product and, thus, discontinuing using it.

In several cases, the product had previously been used without incident for an extended period of time, but in other instances (6/31 cases) reactions were reported after using the product for the first time. The two events that involved hospitalisation occurred in 1999 and 2002. It should be noted, that another report from the USA involving hospitalisation that resulted in death was also provided to the OCSEH. This incident involved a 64-year-old woman who presented at a hospital emergency department with swelling of the neck and throat after reportedly using Frontline Spray 'sometime that day'. The patient received treatment and she was later discharged (no details provided). The woman died from asphyxiation 'shortly after discharge'. Her death was attributed to a hypersensitivity reaction, but apparently a link was not made to any particular trigger. Autopsy revealed evidence of swelling of the vocal chords, and 'there was not a toxic level of the product (fipronil) in her system'.

Of the 31 individuals reporting cutaneous effects, 13 (42%) had a history of intolerances or allergies to other substances such as food, plants, medicines or other chemicals. That is, 13 individuals had pre-existing conditions influencing their immune response (i.e. atopic). Consequently, these individuals are considered susceptible to allergic contact dermatitis, and care should be taken in using such data to determine the skin sensitisation potential of Frontline products. For the remaining 18 individuals, it cannot be definitively determined from the limited details in the adverse experience reports whether the absence of statements on intolerances or allergies means these individuals are not susceptible to allergic contact dermatitis (i.e. non-atopic).

Assessment of the adverse experience report program (AERP) descriptions by an expert clinical dermatologist was undertaken. The dermatologist was of the opinion that:

> "...there is an impression of an urticarial morphology of some of these eruptions, as suggested by the use of terms "hives" and "welts", but that it is difficult to make accurate clinical diagnoses on the information provided. There are many different causes of urticaria...."

Opinion was that irritant contact dermatitis is another possible cause of these dermal reactions, particularly because fipronil is present in a vehicle which is alcohol-based. Considering the possibility of sensitisation, it was stated that "allergic contact dermatis [ACD] typically presents with symptoms such as itching, redness, swelling and even blisters followed by scaling and dryness. Some of the reports of adverse reactions might be consistent with ACD, but it is difficult to be sure....ACD is generally tested for by dermatologists with patch testing." The advisor was of the opinion that the AERP provided a much needed surveillance for adverse reactions, however, the information is not of sufficient detail to identify a sensitiser, but is useful in identifying areas of concern which require follow-up and appropriate testing.

It is the nature of adverse experience reports that they lack the detail that could potentially provide a more definitive link between the symptoms experienced and exposure to Frontline products. Consequently, the nature and time of onset of many of the reported symptoms and their recurrence upon repeated exposure in a small number of individuals, provides only limited evidence to suggest that Frontline products may be the causative agent.

For observed cutaneous effects, the limited information in adverse experience reports (i.e. a lack of diagnostic patch testing) also means that it cannot be conclusively determined whether the observed skin effects were irritant or allergic in nature. However, it is noted that in skin irritation tests on rabbits, fipronil provided divergent results (i.e. slight skin irritation in one study and no skin irritation in another), while Frontline spot-on was a slight irritant, and Frontline Spray was not a skin irritant. Therefore, the data suggests that direct contact with the spot on product during application, or while still wet on the animal, may produce skin reactions that are irritant in nature. However whether the amount of fipronil on a treated animal's coat would be sufficient to provoke skin irritation in persons coming into contact with the animal is not known.

It is should be noted that the reported incidents may represent only a proportion of similar responses in the community, as adverse reactions to agricultural and veterinary chemicals are typically under-reported. On the other hand, it is noteworthy that the number of Australian adverse experience reports for Frontline Top Spot, Frontline Plus, and Frontline Spray for cats and dogs **(**omitting those due to accidental exposure or off-label use; when confounding factors were present; if they were inconsistent with the toxicological profile of fipronil, or for which there was insufficient information), peaked in 1999 (8/10 reports clustered in January-March), and has since decreased (summarised in the Table below). The reason for this is not known.

Table 80: Annual incidence of Australian adverse reactions to Frontline Top Spot, Frontline Plus and Frontline Spray for cats and dogs

1998	1999	2000	2001	2002	2003	2004	2005
1	10	4	5	4	3	2	2

Comparison of human adverse experience reports for Frontline products world wide and in Australia

To assist in the determination of the skin sensitisation potential of Frontline products, Merial submitted their international database of human adverse experiences with these products for the years 2000-2003 inclusive. Of the 1772 suspected human adverse experiences reported for this period world wide, 76% can be divided into two main categories; cutaneous reactions (815/1772; 46%) and ocular exposure incidents (536/1772; 30%). This distribution was similar for the intervals 2000-2001 and 2002-2003. The Merial Australian database for 2000-2003 showed that cutaneous incidents were high (23/33; 70%) relative to the world wide incidence shown above, though this difference is unlikely to be significant given the relatively few Australian reports. Compared to the spot-on formulations, there were much fewer adverse experiences associated with Frontline Spray. However, the number of human adverse reactions reported for each formulation type was approximately in proportion to the number of doses sold, both world wide and for the Australian subset of results, and this is likely to have influenced the distribution of adverse outcomes.

Merial has stated that the global incidence of reactions to Frontline products that could possibly be ascribed to allergenicity is insignificant in comparison to the number of doses sold. Though sales figures do not provide an accurate representation of the number of households that use these products and, thus, the number of people potentially exposed, data

from industry suggest that around 1.92 million households have used a fipronil containing ectoparasiticide. In Australia, it is this demographic (i.e. the household) that nearly all human adverse reports for Frontline products arise. Using the maximum number of AERP's of 10 reported in one year (mean of 4), the annual incidence is low at one per 500,000 households. The proportion of doses applied by veterinarians, who are more likely to be wearing protective clothing when using the product and handling a treated animal, is not known, however is probably only a very small proportion of the doses applied. The EMEA report (2006) refers to human incident data for veterinary products in general, but states that it is unlikely to be established beyond a reasonable doubt that incidences were caused by the products. The report also refers to skin reactions from products containing fipronil and gives a possible explanation for this as increased awareness of the reporting system.

Of the 1772 adverse reports in the Merial Worldwide Database 2000-2003, Merial has extracted 920 as 'Reports with signs even potentially consistent with hypersensitivity occurring after Frontline exposure 2000-2003'. The vast majority of these were cutaneous reactions. Examination of this subset of reports reveals that the presence of a hypersensitivity reaction was unlikely in about half of these cases (e.g. stinging when product entered a laceration, localised tingling and numbness, chemical taste, and various confounders), but in the remainder, representing approximately 25% of reported incidents, it is agreed that the reported reactions, mostly comprising hives, rashes, and pruritus, are possible immune reactions. Approximately one third of these cases represented reactions in individuals who did not apply the product, but came into contact with a treated pet. For the latter, at times it was reported that contact was made prior to the product drying on the animal, but in many instances contact was recorded as having occurred after the product was dry, or at a sufficient length of time after application for this to be assumed. Approximately 10% of reports consistent with allergic reactions stated that the affected person had other allergies, but it is not known if this information was routinely requested.

The French afssa/afsse report (2005) examined human adverse experiences reported worldwide. The data included cases of exposure to both veterinary and agricultural products. Around 300 cases of adverse experiences following exposure to veterinary products during treatment of an animal or handling a treated pet were provided to the French CAP-TV (a Poison and Toxicant Monitoring Center). Of these, most were the result of accidental ocular exposure, and only 10 reported cutaneous symptoms. Of those exhibiting dermal lesions, 3 persons were known to have other allergies and one had concurrent illness considered responsible for the cutaneous lesions. One other person failed to develop lesions following re-exposure to the product. The remaining 5 people exhibited erythema, non-characterised rash persisting for four days, skin irritation, burning lips, blisters at point of contact, and/or hives and oedema of the eyelids. Aside from one case, which reported the absence of cutaneous lesions following subsequent exposure, there is no information regarding follow up or a definitive diagnosis by an expert clinician.

Overall, the types of scenarios and symptoms described in the overseas adverse reports, and the level of detail provided in the reports, were similar with those seen in Australian reports.

Human adverse experiences with other fipronil products

The APVMA's Adverse Experience Reporting Program for Agricultural Chemicals did not commence until the end of 2003, so information from this sector is much less complete than for veterinary products. In 1999, the OCSEH reviewed an incident that occurred the previous

year, in which two pilots involved in the aerial sowing of rice treated with fipronil reported stinging lips, irregular taste in mouth, inflamed feeling in throat, watering eyes, sore throat, burning eyes and throat, and stinging on the forehead, cheeks, ears and neck. At the time, these symptoms were considered inconsistent with the toxicity profile of fipronil or the fipronil product used for treatment of rice. The OCSEH is not aware of any similar subsequent events.

In 2005 an incident involving pest control operators applying a fipronil product in a sub-floor space was reported. One worker (apparently wearing the prescribed personal protection equipment) experienced swelling of the face and neck that commenced one hour into a treatment process of approximately 1.5 hours duration, with symptoms subsiding one hour after completion of the job. Another worker reported burning of the skin around the eyes, mouth and cheeks. In these instances, the symptoms were consistent with non-active constituents in the product, so the possible role of fipronil in their aetiology is not clear.

The French AFSSA/AFSSE report (2005) examined human adverse experiences reported worldwide, including exposure to agricultural products. In the French CAP-TV (Association des Centres Antipoison et de Toxicovigilance) data consisting of 417 acute cases, only 5 professionals are reported to have developed dermal symptoms (pruritus), with limited additional information provided. Agricultural products were also represented in the domestic situation with 4 cases reported of dermal symptoms evident in persons occupying treated premises. The symptoms reported include 'skin eruptions', hives and a burning sensation. However, in all 9 cases, the CAP-TV rated fipronil as being 'doubtful' for being responsible for the reported effects. Likewise, other international databases assessed in this French report, including data from the US and UK, indicate exposure to fipronil containing products is largely associated with 'benign lesions', such as irritation to the eye and skin following topical exposure.

The AFSSA/AFSSE report included a summary from a study assessing neurobehavioural effects in 76 workers in production plants in the US and Indonesia. This showed blood serum levels of 6 – 308 ng/mL for fipronil and its primary metabolite. Although this is indicative that workers are in fact being exposed to fipronil, despite (the assumed) use of PPE, the actual route of exposure and presence or absence of dermal lesions is unknown from the summary provided. Documentation was provided to the OCSEH from occupational physicians in a fipronil manufacturing plant in France (124 employees) and formulating plant in Brazil (53 employees). This documentation states there have been no cases of skin irritation or sensitisation since operations began in 1997 in France and 2005 in Brazil, as noted in routine medical surveillance.

Overall, despite the widespread use of fipronil in agricultural products used in large volumes and varied use patterns, there are only limited reports of cutaneous adverse effects. From the current Australian and International monitoring databases, there is no robust evidence that fipronil causes skin sensitisation.

Fipronil metabolites

The possibility that the reported sensitisation reactions could be attributable to metabolites of fipronil present on the coats of treated animals should also be considered. Studies that quantified the levels of fipronil that could be dislodged by stroking treated animals showed that fipronil metabolite levels (fipronil sulfone and fipronil sulfide) in these samples were very low, and frequently below the limit of detection. Also, photodegradates of fipronil are formed on exposure to sunlight. The primary photodegradate, desulfinyl fipronil, was found to be associated with the coats of treated dogs that had been allowed access to sunlight for around 6 hours per day for two weeks, with levels of the photodegradate reaching up to 6% of fipronil present (Astruc 1998). No information is available regarding the sensitisation potential of fipronil metabolites.

Summary and Conclusions

- The results of 12 skin irritation studies in rabbits, two of which were on the active, indicate fipronil is a slight skin irritant.
- The results of 12 skin sensitisation studies in guinea-pigs, two of which were on the active (Buehler and Magnusson & Kligman method), indicate that fipronil and its products were not skin sensitisers in guinea-pigs.
- Though sales figures do not provide an accurate representation of the number of households that use these products and, thus, the number of people potentially exposed, the number of human adverse reports to Frontline products is relatively low compared to sales figures.
- The Australian adverse experience reports provide limited information with little or no follow-up information. Taken individually, each individual adverse report does not provide a definitive causative link to the product. When considered together, the evidence is only suggestive that the observed adverse effects may be due to exposure to Frontline products. The International data is similarly limited.
- For the observed cutaneous effects in the adverse experience reports, it cannot be reliably determined from the limited information whether they were irritant or allergic in nature. No human patch tests for allergenicity to fipronil appear to have been performed. Similarly, although some reports indicated that cutaneous effects were seen in some individuals using the product for the first time (i.e. would be irritant in nature), given the potential widespread use of Frontline products prior exposure through contact with treated pets or their environs cannot be ruled out (i.e. reported skin reactions may be allergic in nature).
- Although it is not known if an individual's history to allergies was routinely requested, approximately 10% and 40% of world wide and Australian adverse reports respectively were seen in individuals who were susceptible to allergic contact dermatitis. Care should be taken in using such data to determine the skin sensitisation potential of Frontline products, as skin reactions seen in such individuals may not be reflective of what would be observed in the general population.
- An irritation and sensitisation study on the primary photodegradate, desulfinyl fipronil, would provide additional data on the irritant and skin sensitisation potential of this photodegradate, which may be of some assistance in interpreting the cutaneous reactions described in adverse experience reports.

Thus, the animal data provides no robust evidence of a skin sensitisation potential for fipronil or Frontline products. In humans, although cutaneous effects have been seen in adverse experience reports their incidence is relatively low compared to sales figures, the information contained in such reports do not provide a causative link to Frontline products, and the nature of the skin reactions (irritant or allergic) cannot be reliably determined. Furthermore, although it cannot be established if an individual's history to allergies was routinely determined, skin reactions were often seen in individuals with pre-existing conditions influencing their immune response (40% of individuals reporting an adverse cutaneous effect in Australia). Thus, the available practical experience in humans does not reliably demonstrate Frontline products to be capable of inducing a skin sensitisation reaction in a substantial number of people.

Therefore, both the available animal and human data provide no reliable evidence that fipronil or Frontline products are skin sensitisers, however fipronil is associated with slight skin irritation.

16 ASSESSMENT OF SAFETY DIRECTIONS FOR SOME FIPRONIL PRODUCTS

This section considers First Aid Instructions and Safety Directions for the fipronil products that are included in this Review (listed in Appendix II). Toxicology studies have been provided for many of the products, or for formulations that are sufficiently similar to Australian registered products that they may be used in establishing their acute toxicity profiles of those products. Where no product toxicity data were available, the acute toxicity profiles were estimated from the characteristics of the individual constituents and their respective concentrations in each product (see Appendix XI).

Consideration of Safety Directions

Note: Throughout this section, statements that have been added to the existing safety directions are shown in italics.

Regent 800WG Insecticide (800 g/kg fipronil)

This product is for the control of various insect pests in bananas, brassicas, cotton, mushrooms, pasture, potatoes, sorghum and sugarcane. It is applied in most situations as a dilute spray by air or ground rig, sometimes with the inclusion of a wetting agent. Repeated applications in some situations are recommended. For mushroom cultivation, it is mixed with a small volume of water prior to incorporation into peatmoss.

Acute toxicity studies using the formulation EXP60720 ('Regent 800WDG') indicated that it had moderate oral and inhalation toxicity in rats, and moderate dermal toxicity in rabbits. It was a moderate eye irritant and slight skin irritant in rabbits, and did not cause skin sensitisation in guinea pigs. The current formulation differs slightly from that tested (see Appendix IX), in that one of the non-active constituents is now present at a lower concentration. As this ingredient is of low toxicity, and is not considered to contribute significantly to the overall toxicity of the product (see Appendix XI), the use of these studies as a basis for the hazard statements for Regent 800WG is considered appropriate.

First aid instruction '**a**' is considered appropriate for this product, and is included on the current label. The safety directions on the label are consistent with the entry in the FAISD Handbook for 'WG 800 g/kg or less', and this entry remains appropriate.

WG 800 g/kg or less	
Poisonous if absorbed by skin contact, inhaled or swallowed.	130 131 132 133
Will irritate the eyes and skin.	161 162 164
Avoid contact with eyes and skin.	210 211
Do not inhale dust.	220 221
If product in eyes, wash it out immediately with water.	340 343
After use and before eating, drinking or smoking, wash hands, arms and face thoroughly with soap and water.	350

[Note: The fipronil OH&S review will provide advice on any amendments to personal protective clothing and equipment before this entry is included in the FAISD Handbook.]

Cosmos Insecticidal Seed Treatment (SC, 500 g/L fipronil)

Cosmos Insecticidal Seed Treatment is for the control of various pests in canola, rice, sorghum and sunflowers, and to protect seedlings from attack from black field earwig in sorghum and sunflowers. The maximum application rate is 400 mL product, added to 600 mL of water/100 kg seed. Directions on the label indicate the use of commercial seed coating equipment for application to sorghum or sunflowers and 'apply as a spray to seed prior to sowing' for rice. No method is specified for canola seed treatment.

Studies provided for a formulation that has essentially equivalent toxicity to Cosmos Insecticidal Seed Treatment, indicated that this product has moderate acute oral and inhalational toxicity, and low acute dermal toxicity in rats. It is expected to be a slight skin irritant in rabbits, but not an eye irritant. It did not cause skin sensitisation in guinea pigs.

First Aid Instruction '**a**' is appropriate for this product, and is present on the current label. The safety directions on the label are consistent with the entry in the FAISD Handbook for fipronil 'SC 500 g/L or less, more than 200 g/L'. However, it is appropriate for the statement 'will irritate the skin' to be included also (see Allen 1993d). It is therefore recommended that the entry 'SC 500 g/L or less, more than 200 g/L' be amended to the following.

SC 500 g/L or less, more than 200 g/L	
Poisonous if inhaled or swallowed	130 132 133
Will irritate the skin	*161 164*
Avoid contact with skin	210 164
Wash hands after use	351

[Note: The fipronil OH&S review will provide advice on any amendments to personal protective clothing and equipment before this entry is included in the FAISD Handbook.]

Regent 200 SC and PRESTO Insecticide (200 g/L fipronil)

Regent 200 SC is for the control of a range of insect pests in a variety of crops (bananas, brassicas, cotton, wine grapevines, mushrooms, potatoes, sorghum, sugarcane) and pasture.

According to the label instructions, this product is applied by aircraft, or using various other spray techniques (mechanical or hand held equipment) after mixing with water to a concentration up to the equivalent of 300 mL of product per 100 L of water, sometimes with the inclusion of a wetting agent, with re-treatment in some situations as recommended.

PRESTO Insecticide is for the control of mushroom flies in mushroom houses. It is to be mixed with a 'small quantity of water' and applied to peat moss at a rate of 16 mL product per 300 L bale of peatmoss.

First aid instruction '**a**' is appropriate for these products, and is included on the current labels. The hazard statements on the labels are consistent with the entry in the FAISD Handbook for fipronil 'SC 200 g/L or less, more than 100 g/L'. Results of acute toxicity studies available for Regent 200 SC (EXP 60145) indicate that it had low acute oral toxicity in rats, low dermal toxicity in rats and rabbits, and moderate inhalational toxicity in rats. It was a slight skin irritant and a moderate eye irritant in rabbits, and was not a skin sensitiser in guinea pigs according to the Buehler method. As this product is a moderate eye irritant, this review recommends the amendment of the entry 'SC 200 g/L or less, more than 100 g/L', to include safety directions '340 343' as shown below.

SC 200 g/L or less, more than 100 g/L	
Harmful if inhaled or swallowed	129 132 133
Will irritate the eyes and skin	161 162 164
Avoid contact with eyes and skin	210 211
If product in eyes, wash it out immediately with water	*340 343*
Wash hands after use	351

[Note: The fipronil OH&S review will provide advice on any amendments to personal protective clothing and equipment before this entry is included in the FAISD Handbook.]

Termidor Residual Termiticide (SC, 100 g/L fipronil)

This product is for the protection of structures from subterranean termite damage and for the control of subterranean termites around domestic and commercial structures. It is applied by licensed pest control operators. The product is diluted at a rate of 600 mL in 100 L water for the creation of chemical soil barriers by a combination of conventional spraying and trenching, or soil injection equipment, though an increased concentration (up to 600 mL/60 L water) may be required to minimise run-off in soils that are difficult to wet.

Termidor Residual Termiticide is applied to the soil by conventional spraying and trenching, or using soil injection equipment (rodding) if this is not possible. It may be injected through concrete slabs via pre-drilled holes, ensuring a strong seal with the top of the drill hole to minimise leakage and that the drill holes are plugged after treatment. Precautions on the label indicate that residents and pets should not be allowed in a room being treated, that any spills should be cleaned up before leaving the room, and that the all ducts, pipes, drains, and conduits are identified and not punctured or contaminated. Application around edible plants is to be avoided. A re-entry period 'until spray has dried' applies. A study has been reviewed in which household air was monitored following treatment with fipronil (Honeycutt, 2001). In that study, spray was applied to the interior and exterior walls, and injected into foundation

walls. In most cases, fipronil was detectable in household air on the day of treatment (maximum 0.08 ng/L in a house of slab construction), with only occasional detections thereafter (maximum 0.011 ng/L). Fipronil has low volatility and the spraying of interior walls as in the Honeycutt (2001) study would be expected to result in higher indoor air levels of fipronil than for the methods of application for Termidor Residual Termiticide described above. Therefore, if the risk mitigation measures on the label are followed, exposure of householders via the inhalation route is not expected to be of toxicological concern. As Termidor Residual Insecticide is applied mainly in uninhabitable areas of buildings and through drill holes, exposure of building occupants is not expected by any other route.

Hazard statements on the label are consistent with those recommended in the earlier OCSEH and NOHSC evaluations of this product (i.e. low acute oral, dermal and inhalation toxicity; not a skin irritant, but a slight eye irritant). Though the safety directions on the label reflect the recommendations of these reports, the entry in the FAISD Handbook includes hazard statements for skin irritation, and does not include a hazard statement for the oral toxicity of the product. As no product-specific acute toxicity data are available, the acute toxicity of the product has been derived from the toxicity of its individual constituents and their respective concentrations. Therefore, while the hazard statements on the current label are correct, the entry in the FAISD Handbook is not, and should be amended to the following:

SC 100 g/L or less	
Harmful if swallowed	*129 133*
May irritate the eyes	*160 162*
Avoid contact with eyes	*210 162*
Wash hands after use	351

[Note: The fipronil OH&S review will provide advice on any amendments to personal protective clothing and equipment before this entry is included in the FAISD Handbook.]

Presto 100 Insecticide (SC, 100 g/L fipronil)
(NB. This product is no longer registered.)

Presto 100 Insecticide is for the control of mushroom flies in mushroom houses. It is to be mixed with a 'small quantity of water' and applied at a rate of 32 mL product per 300 L bale of peatmoss. This process is performed mechanically. The diluted product contains the same concentration of fipronil as the in-use concentration for Presto Insecticide, and the application rates of the two products are the same.
First aid instruction '**a**' is appropriate for this product, and is included on the current label. The hazard statements on the label are consistent with the entry in the FAISD Handbook for fipronil 'SC 200 g/L or less, more than 100 g/L'. However, Presto 100 Insecticide is expected to have low acute oral, dermal and inhalational toxicity. It is likely to be a slight eye irritant, but not a skin irritant (see Appendix XI for further discussion). Therefore, the entry for fipronil 'SC 100 g/L or less' recommended above is also appropriate for this product.

Semevin Super Seed Dressing Insecticide (SC, 400 g/L thiodicarb, 80 g/L fipronil)

This product is used for the control of false wireworm and thrips in cotton. It is applied at a rate of 625 mL/100kg seed using commercial seed coating equipment.

A second active ingredient, thiodicarb, is included in this formulation. As this is a cholinesterase-inhibiting compound, First Aid Instruction '**m**' is appropriate. [If swallowed, splashed on skin or in eyes, or inhaled, contact a Poisons Information Centre (Phone Australia 131126) or a doctor at once. Remove any contaminated clothing and wash skin thoroughly. If swallowed, activated charcoal may be advised. Give atropine if instructed.] This First Aid Instruction should replace the instructions '**a,h**' [If poisoning occurs contact a doctor or Poisons Information Centre (telephone 131126). If swallowed, give one atropine tablet every 5 minutes until dryness of the mouth occurs - if poisoned by skin absorption or through lungs, remove any contaminated clothing, wash skin thoroughly and give atropine tablets as above. Get to a hospital or doctor quickly.] that are currently on the label.

The acute toxicity of Semevin Super Seed Dressing Insecticide has been estimated from the toxicity of its individual constituents and their concentration in the product (see Appendix XI). The product is expected to have high oral and inhalation toxicity and low dermal toxicity. It is expected to be a moderate eye irritant, but not a skin irritant. The amended safety directions for **thiodicarb** are tabulated below. Statements '340 343' should be added to the existing SDs due to the moderate eye irritancy of this product.

SC 400 g/L or less, with fipronil 80 g/L or less	
Poisonous if inhaled or swallowed	130 132 133
Will irritate the eyes	161 162
Avoid contact with eyes	*210 162*
If product in eyes, wash it out immediately with water	*340 343*
Do not inhale vapour	220 222
Repeated minor exposure may have a cumulative poisoning effect	190
After use, and before eating, drinking or smoking, wash hand, arms and face thoroughly with soap and water	350

[Note: The fipronil OH&S review will provide advice on any amendments to personal protective clothing and equipment before this entry is included in the FAISD Handbook.]

Adonis 8.5UL (8.5 g/L fipronil) and Adonis 3UL Insecticide (3 g/L fipronil)

These products are intended for the control of locusts and grasshoppers in pasture and sorghum. The respective labels state that Adonis 8.5UL is only available for use by the Australian Plague Locust Commission (APLC) or APLC approved applicators, and Adonis 3UL Insecticide is supplied for use by the APLC or the appropriate government authority undertaking locust control. These products are to be applied by aircraft either undiluted, or in the case of Adonis 8.5UL, diluted 4-20 fold with a compatible spraying oil.

First aid instruction '**a**' is appropriate for these products, and is included on the current labels. No data have been submitted that are specific to these products. However, acute studies are available for a product with a very similar formulation, but containing 25 g/L fipronil (see Appendix IX). These studies indicate that the two products under consideration here are likely to have low oral, dermal and inhalational toxicity. They are expected to be slight eye irritants, but not skin irritants. The current entry for UL 25 g/L or less remains appropriate.

UL 25 g/L or less	
Will irritate the eyes	161 162
Avoid contact with eyes	210 162
Wash hands after use.	351

[Note: The fipronil OH&S review will provide advice on any amendments to personal protective clothing and equipment before this entry is included in the FAISD Handbook.]

Chipco Choice Insecticide and Impede Insecticide (GR, 1 g/kg fipronil)
(NB. Chipco Choice Insecticide is no longer registered).

These granular products are for the control of Argentine stem weevil, funnel ants and mole crickets in recreational, domestic and commercial turf, including bowling greens, golf courses, parks and playing fields. The rates of application are 30-75 kg/ha. The granules are distributed evenly on the surface, then incorporated into the turf with at least 6 mm of rainfall or overhead irrigation immediately after application. Chipco Choice Insecticide is available in sizes from 1 kg to 22.68 kg, while Impede Insecticide is available in a range of pack sizes less than 1 kg. The label stipulates that application to domestic turf is to be performed by authorised lawn-care specialists only. As the product is watered-in immediately after application, public exposure is not expected.

First aid instruction '**a**' is appropriate for these products, and is included on the current labels. However, the Chipco Choice Insecticide label includes the First Aid Instruction 'If swallowed, and if more then 15 minutes from a hospital, induce vomiting, preferably using Ipecac Syrup APF.' This statement is inappropriate and should be deleted. It is justifiable to base the safety directions for these products on the results of the acute toxicity studies submitted for the formulation EXP60819A (see Appendices VII and VIII). The products are therefore considered to have low acute oral, dermal and inhalation toxicity, and to be slight eye irritants, but not the skin irritants. The current entry for GR 1g/kg remains appropriate.

GR 1 g/kg or less	
Will irritate the eyes	161 162
Avoid contact with eyes	210 162
Wash hands after use	351

Goliath Cockroach Bait (BA, 0.5 g/kg fipronil)

This is a home garden product for the control of cockroaches in buildings. The baits are enclosed in containers that are to be placed in areas where cockroaches are likely to be, and replaced at monthly intervals.

Two formulations are provided (see Appendix IX). Formulation 1 was used for the acute toxicity studies for this product. The differences in formulation relate to constituents of low toxicity that are not considered to affect the toxicity profile of the product. Studies showed Goliath Cockroach Bait had low acute oral and dermal toxicity in rats and rabbits respectively. In rabbits, it was a slight eye irritant, but not a skin irritant. It was not a skin sensitiser in guinea pigs.

The presentation of the product (welded plastic bait station) is such that the user is not expected to come into contact with the bait itself. The amount of bait per station is not provided. If a 15 kg child consumed 25 mg of fipronil (50 g of the bait), equal to 1.7 mg/kg

bw, this would provide a MOE of 15 to the dose at which neurotoxic signs, but no deaths were seen in an acute neurotoxicity study in rats (25 mg/kg bw; Hughes 1997a), which is acceptable. Therefore, as the product label for a pack of 6 bait stations indicates a net weight of 20 g, this product is not of concern regarding accidental ingestion by a child. The acute toxicity profile of the product does not indicate any other concerns in the unlikely event that a child gains access to the bait (e.g. child inserts a finger into the bait station).

First aid instruction '**a**' is appropriate for these products, and is included on the current labels. No changes are recommended to the 'nil' safety directions for HG BA 0.5 g/kg or less in plastic labyrinth.

HG BA 0.5 g/kg or less in plastic labyrinth	
Nil	

Goliath Cockroach Gel (BA, 0.5 g/kg fipronil)

This is a crevice, crack, or spot treatment for the control of cockroaches, available in a pack size of 35 g. It is to be applied at up to 3 spots per m^2 (recommended spot size 30-60 mg). To apply the gel, the cap is removed from the nozzle of the cartridge, and after touching the tip of the nozzle to the surface to be treated, the plunger is depressed slightly. The dispenser is to be re-capped after use. The product is to be re-applied when the bait is no longer visible. The label states that the product is for use by professional pest control operators. This is at variance with the original OCSEH evaluation of this product, where it was considered for home garden use.

Acute toxicity studies using Goliath Cockroach Gel showed it to have low acute oral and dermal toxicity in rats. It was not a skin sensitiser in guinea pigs. First aid instruction '**a**' is appropriate for these products, and is included on the current labels. The safety directions on the current label are consistent with the entry in the FAISD Handbook (HG BA gel 0.5 g/kg or less). The previous OCSEH evaluation of this product concluded that it was a slight skin and eye irritant in rabbits. On re-examination of the relevant studies, these conclusions are considered to be overly conservative. This review concludes that this product is neither a skin irritant nor an eye irritant. Although the product has a low concentration of fipronil, there is some potential for occasional minor contamination of the fingers when the cap is removed or replaced. It is recommended that the current entry (HG BA gel 0.5 g/kg or less) be replaced with the following entry:

BA gel 0.5 g/kg or less	
Wash hands after use	*351*

Goliath Gold Gel Insecticide and Maxforce Gold Gel Insecticide (BA, 0.3 g/kg fipronil)

These products are for the treatment of cockroach infestations in domestic, commercial and public service buildings including farm buildings, food processing establishments, factories, hospitals, homes, kitchens, offices, restaurants, retail outlets, storerooms vehicles and other situations that fall within these definitions. The gel is to be applied at up to 5 x ~0.1g spots per m^2. The gel spots are to be replenished when consumed while cockroaches remain a problem. The products are applied after removing the cap from the nozzle of the dispenser, touching the tip of the nozzle to the surface to be treated, then slowly depressing the plunger. The nozzle is

to be re-capped after use. Both products are presented in tubes of 5, 7.5, 10, 12.5, 15, 20, 30 and 35 g net. Unlike Goliath Cockroach Gel, it is not stipulated on the labels of Goliath Gold Gel Insecticide or Maxforce Gold Gel Insecticide that they are for use by professional pest control operators. It is therefore likely that members of the public will use these products, so they will be considered here for home garden use.

Taking into account the toxicity of the individual consitituents in these products, and their concentrations, the toxicity profile of Goliath Gold Gel Insecticide/Maxforce Gold Gel Insecticide is expected to be equivalent to Goliath Cockroach Gel (see Appendix IX). If a 15 kg child accidentally ingested the largest tube in the range (35 g), this is equivalent to a dose of ~0.7 mg/kg bw fipronil (0.03% of 35 g ÷ 15), which provides a margin of safety of 35 (25÷0.7) to the fipronil dose at which clinical signs but no deaths occurred in a rat acute oral neurotoxicity study (25 mg/kg bw; Hughes 1997a), which is acceptable. This, along with the predicted low dermal toxicity of the products, and that they are unlikely to cause skin or eye irritation, indicates that this product is suitable for HG use.

First aid instruction '**a**' is appropriate for these products, but is not included on the current labels. This should be added. The safety directions on the label are consistent with the current entry in the handbook. However, as these products are very similar to Goliath Cockroach Gel, the same safety directions apply. It is recommended that SDs for all three products be included under the entry 'BA gel 0.5 g/L or less' given above.

Combat Ant-Rid Relief from Tough Ant Problems 4 Ant Baits (BA, 0.1 g/kg fipronil)

This product is for use by the householder to control ants indoors. The baits, housed in welded plastic stations, are presented in packs of 4, to be placed next to ant trails. They have openings to allow ants to access the bait, but these are expected to be sufficiently small to deny access to other organisms. The welds of the plastic bait station are tested at manufacture to an internal standard as part of the quality control process, but not to an Australian Standard. Provision has been made for adhering the bait stations to walls and under benches. The label recommends that all 4 stations be used at the one time.

Given the nature of the excipients and their expected toxicity (see Appendix XI), and the very low concentration of fipronil in this product, it is considered to have low oral and dermal toxicity. Skin and eye irritation is expected to be minimal. However, as the users of this product are not expected to come into contact with the bait, this is not an issue in practice. This type of product does not present an inhalational hazard. A pack of 4 baits (6 g net) contains approximately 0.6 mg fipronil in total, delivering a dose of 0.04 mg/kg bw if a 15 kg child ingested all 4 baits. This yields a MOE of 62 to the acute oral NOEL for fipronil in rats, which is acceptable. Therefore, safety directions for this product are not considered necessary. There are no safety directions on the label, and this is consistent with the corresponding entry in the FAISD Handbook 'HG BA 0.5 g/kg or less in plastic labyrinth', which is considered to accommodate this product. First aid instruction '**a**' is appropriate for this product, and is included on the current label.

HG BA 0.5 g/kg or less in plastic labyrinth	
Nil	

Frontline Spray (LD, 2.5 g/L fipronil)

Frontline Spray is for the treatment and prevention of flea infestations, control of flea allergy dermatitis and control of ticks and biting lice on dogs and cats. It is available in 100, 250 and 500 mL pump packs. The maximum rate of application of 6 mL/kg (0.5 mL per pump action).

Acute toxicology studies performed using this product showed that it had low oral, dermal and inhalational toxicity in rats, it was a non-irritant to rabbit skin, and a moderate eye irritant in rabbits, but not a skin sensitiser in guinea pigs.

First aid instruction '**a**' is appropriate for this product, and is included on the current label. The use of gloves when using the product is remains appropriate, due to the considerable dermal exposure as compared to the spot-on product. The entry in the FAISD Handbook for fipronil 'HV LD 2.5 g/L or less' is amended as follows:

HV LD 2.5 g/L or less	
Will irritate the eyes	161 162
Avoid contact with eyes	210 162
When using the product wear rubber gloves	279 283 290 312
If product in eyes, wash it out immediately with water	340 343
Wash hands after use	351
After each day's use, wash gloves	360 361

The Frontline Top Spot, Frontline Plus and Startgard ranges of spot-on products (SA, 100 g/L fipronil)

Frontline Top Spot for small dogs up to 10 kg, medium dogs 10-20 kg, large dogs 20-40 kg and extra large dogs 40-60 kg, is supplied in unit sizes of 0.67 mL, 1.34 mL, 2.68 mL and 4.02 mL respectively, in packs of 3 or 6 foil-backed trays of single-use pipettes. A separate peeling action is required to access each individual pipette, and the end of the pipette must be snapped off prior to squeezing the contents onto the skin of the animal. The packaging is such that it is difficult for a small child to access the product. Frontline Top Spot is applied monthly for flea treatment and at fortnightly intervals for paralysis tick control. The unit size for Frontline Top Spot for cats is a 0.5 mL pipette, also available in packs of 3 or 6. Frontline Plus is identical to the Frontline Top Spot range of products, except that it contains the additional active constituent (S)-methoprene, at 90 g/L or 120 g/L for dogs and cats respectively. This is intended to provide the added benefit of preventing the development of flea eggs, larvae and pupae produced by adult fleas. The Startgard range comprises kits of one tablet from the Heartgard range (active constituents ivermectin, or ivermectin and pyrantel) and one pipette from the Frontline Top Spot or Frontline Plus range. As these kits are intended for the treatment of puppies and kittens, the pipettes are the smallest sizes in the Frontline ranges (0.5 mL for kittens and 0.67 mL for puppies).

Data specific to the Frontline Top Spot formulation indicated that it had low oral, dermal and inhalational toxicity in rats, it was a slight skin irritant and a moderate eye irritant in rabbits, and was not a sensitiser in guinea pigs. The presence of (S)-methoprene in the Frontline Plus formulation is not expected to affect the acute oral, dermal and inhalation toxicity of these products relative to Frontline Top Spot. In rats, (S)-methoprene exhibited very low acute oral toxicity (LD_{50} >49,000 mg/kg bw), low acute dermal toxicity (LD_{50} = 3038-10250 mg/kg bw) and low acute inhalation toxicity (LC_{50} >210,000 mg/m^3). The Frontline Plus for Cats

formulation was a slight skin irritant in rabbits, and was not a skin sensitiser in guinea pigs. It was less irritating to the eyes of rabbits (i.e. a slight irritant) relative to the moderate eye irritancy of Frontline Top Spot, and therefore the safety directions '340 343' (if product in eyes, wash it out immediately with water) do not apply. However, as it is outside the scope of this review to reduce safety directions, the same safety directions should apply to all the spot-on products (see below).

The statement on the label 'Do not use FRONTLINE Plus if you or your pet have a known hypersensitivity to insecticides or alcohol' is considered misleading and should be deleted. First aid instruction '**a**' is appropriate for this product, and is included on the current label. Gloves are not considered necessary for the spot-on products due to the limited dermal exposure afforded by the packaging and application method. The current FAISD Handbook entry for fipronil 'HV SA 100 g/L or less' is no longer appropriate, and will be amended as follows:

HV SA 100 g/L or less	
Will irritate the eyes and skin	161 162 164
Avoid contact with eyes and skin	210 211
If product in eyes, wash it out immediately with water	340 343
Wash hands after use	351

Products Assessed For Necessity Of '180' Safety Direction Only

Termidor Dust Termiticide (DU 5g/kg fipronil)

This product is for the control of termites in various situation. The dust is applied by hand puffers or powered equivalents into holes drilled into the termite nest in trees, wall cavities etc., or directly onto termites. The hole is then sealed and treatment may need to be repeated in 2-4 weeks. It is available in 5 g and 15 g packs.

Based on the most recent assessment of this product by the OCSEH, the acute toxicology profile of this product was extrapolated from the acute toxicity of the individual product constituents. Based on this, the product is likely to have low oral, dermal and inhalational toxicity. It is not expected to be a skin irritant, but will be irritating to the nose and throat and is likely to be a slight eye irritant. At the time of the last assessement, it was concluded that the product is likely to be a skin sensitiser, based only on the fipronil content. However, following this Review, fipronil is not considered a skin sensitiser.

The current label safety directions are inconsistent with those listed in the FAISD Handbook, with the label omitting the 180 statement. However, following a reassessment of fipronil as non-sensitiser in this Review, the 180 statement is no longer considered necessary. Therefore, the following safety directions are recommended:

DU 5 g/kg or less	
160 162 163	May irritate the eyes, nose and throat.
210 162	Avoid contact with eyes.
220 221	Do not inhale dust.
351	Wash hands after use.

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Canovas M, Vilageliu J, Lopez S, German M, Moya D (1994) Percutaneous absorption and hair, skin and fat distribution after spot on application of 14C RM1601 in cats. Report No. CD-93/3748F. 25.10.1994 [M; Sub: CR108-1, Vol. 5 of 9] *(Dermal absorption in target species)*

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Gasmi A, Chataigner D, Garnier R, Lagier G (2001) Toxicity of fipronil-containing insecticides. Report of 81 cases from Paris Poisons Center. Veterinary and Human Toxicology 43(4): 247. *(No significant additional information)*

APPENDIX I: FIPRONIL TOXICOLOGY DATA SUBMISSION DETAILS

Sponsor/Provider	Submission Number	Data Details	ABBREV.
BASF Australia Ltd	CR 108-1	58 volumes (2004)	BASF
Merial Australia Pty Limited	CR108-1	7 volumes (2004)	ME
Clorox Australia Pty Ltd	CR108-1	1 volume (2004)	CL
Bayer CropScience Pty Ltd	CR108-1	1 volume (2004)	BAY
Public submissions	CR108-1	1 volume (2004)	
Aventis CropScience Pty Ltd	12092	4 volumes (2002)	ACS
Rhone-Poulenc Rural Australia Pty Ltd	11530	6 volumes (1997)	RPA
Rhone-Poulenc Rural Australia Pty Ltd	11443	4 volumes (1997)	RPA
Rhone Poulenc	11346, 11369, 11409	13 volumes (1996)	RP
Rhone Merieux	11288	5 volumes (1996)	RM
Rhone Poulenc	10810	4 volumes (1995)	RP
Rhone Poulenc	10528	4 volumes (1995)	RP

N.B. Submission 11580 contained data for a formulation which is not currently registered, so these studies were not evaluated in the review.

APPENDIX II: AUSTRALIAN REGISTERED PRODUCTS CONTAINING FIPRONIL

(Adapted from APVMA PUBCRIS. Only products that are included in the Review are listed.)

Veterinary products:

APVMA Product Code	Product Description	Product Name
46828	HV LD, 2.5 g/L fipronil	Frontline Spray
48523	HV SA, 100 g/L fipronil	Frontline Top Spot Cat
48606	HV SA, 100 g/L fipronil	Frontline Top Spot Small Dog
49825	HV SA, 100 g/L fipronil	Frontline Top Spot Medium Dog
49826	HV SA, 100 g/L fipronil	Frontline Top Spot Large Dog
51304	HV SA, 100 g/L fipronil	Startgard For Puppies *
51530	HV SA, 100 g/L fipronil	Startgard For Kittens *
52043	HV SA, 100 g/L fipronil	Frontline Top Spot Extra Large Dog
52327	HV SA, 100 g/L fipronil	Frontline Top Spot For Dogs
54523	HV SA, 100 g/L fipronil, 90 g/L (S)-methoprene	Frontline Plus (Fipronil Plus (S)-Methoprene) For Dogs
54524	HV SA, 100 g/L fipronil, 120 g/L (S)-methoprene	Frontline Plus (Fipronil Plus (S)-Methoprene) For Cats
56123	HV SA, 100 g/L fipronil, 90 g/L (S)-methoprene	Startgard Plus for Puppies
56124	HV SA, 100 g/L fipronil, 120 g/L (S)-methoprene	Startgard Plus for Kittens

*These are combination products containing Frontline Top Spot and Heartgard in the one package. The Frontline Top Spot pipettes in these kits are the size equivalent of the pipettes for the 'small dog' and 'small cat' products.

Agricultural products:

APVMA Product Code	Product Description	Product Name
46793	SC, 200 g/L fipronil	Regent 200SC Insecticide
47407	WG, 800 g/L fipronil	Regent 800WG Insecticide
48921*	GR, 1 g/kg fipronil	Chipco Choice Insecticide
49434	SC, 500 g/L fipronil	Cosmos Insecticidal Seed Treatment
49646	HG BA, 0.5 g/kg fipronil	Goliath Cockroach Bait
49647	BA gel, 0.5 g/kg fipronil	Goliath Cockroach Gel
50285	UL, 8.5 g/L fipronil	Adonis 8.5ul Insecticide
51371	SC, 400 g/L thiodicarb, 80 g/L fipronil	Semevin Super Seed Dressing Insecticide
51720	HG BA, 0.1 g/kg fipronil	Combat Ant-Rid Relief From Tough Ant Problems 4 Ant Baits
53156	UL, 3.0 g/L fipronil	Adonis 3ul Insecticide
53264*	SC, 200 g/L fipronil	Presto Insecticide
53737*	SC, 100 g/L	Presto 100 Insecticide
54587	HG BA gel, 0.3 g/kg	Goliath Gold Gel Insecticide
54624	SC, 100 g/L fipronil	Termidor Residual Termiticide
55553	HG BA gel, 0.3 g/kg	Maxforce Gold Gel Insecticide
57764	GR, 1 g/kg fipronil	Impede Insecticide

*= Registered at initiation of review, no longer registered.

Briefly included in revised safety directions, but not fully reviewed as registered after data call-in:		
60654	DU, 5 g/kg fipronil or less	Termidor Dust Termiticide

APPENDIX III: LIST OF CLINICAL CHEMISTRY, HAEMATOLOGY & URINALYSIS PARAMETERS

Clinical Chemistry	Haematology	Urinalyses
Albumin alkaline phosphatase bilirubin (total) calcium chloride cholesterol (total) creatinine (blood) gamma-glutamyl transpeptidase globulin glucose (blood) LDH (serum lactate dehydrogenase) phosphorus potassium protein (total) SGPT (serum alanine aminotransferase) SGOT (serum aspartate aminotransferase) sodium triglycerides urea nitrogen (blood) CPK (creatinine phosphokinase)	clotting parameters (clotting time, prothrombin time) erythrocyte count haematocrit (packed cell volume) haemoglobin leucocyte differential count leucocyte total count platelet count reticulocyte count MCH MCHC MCV blood smear	appearance specific gravity glucose ketones sediment (microscopic) occult blood pH protein volume bilirubin urobilinogen

APPENDIX IV: LIST OF ORGANS WEIGHED AND TISSUES EXAMINED

Organs Weighed	Tissues Examined		
Adrenals	Adrenals	heart	salivary gland
Brain	aorta	ileum	seminal vesicle
Gonads	blood smear	jejunum	skin
Heart	bone	kidneys	spinal cord (cervical
Kidneys	bone marrow	lacrimal gland	thoracic, lumbar)
Liver	brain (3 levels)	liver	spleen
Spleen	caecum	lungs	sternum
Thyroid	colon	lymph nodes	stomach
(w/parathyroid)	duodenum	mammary gland	testes
	epididymes	muscle (smooth)	thymus
	eyes	muscle (skeletal)	thyroid
	eyes (optic nerve)	nerve (peripheral)	(w/parathyroid)
	gall bladder	oesophagus	trachea
	Harderian glands	ovaries	urinary bladder
	head - 3 sections	pancreas	uterus
	(nasal cavity, para-	pituitary	vagina
	nasal sinus, tongue,	prostate	Zymbal's gland
	oral cavity, naso-	rectum	gross lesions
	pharynx, inner-ear)		

APPENDIX V: REPRODUCTIVE AND DEVELOPMENTAL INDICES

$$\textbf{Male/female mating index } (\%) = \frac{\text{number of males/females with confirmed mating*}}{\text{number of males/females placed with females/males}} \times 100$$

* defined by females with vaginal sperm or that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Male fertility index (\%)} = \frac{\text{number of males proving their fertility*}}{\text{number of males placed with females/males}} \times 100$$

* defined by a female giving birth to a litter or with pups/foetuses in utero

$$\textbf{Female fertility index (\%)} = \frac{\text{number of females pregnant*}}{\text{number of females mated**}} \times 100$$

* defined as the number of females that gave birth to a litter or with pup/foetuses in utero
** defined as the number of females with vaginal sperm or that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Gestation index (\%)} = \frac{\text{number of females with live pups on the day of birth}}{\text{number of females pregnant*}} \times 100$$

* defined as the number of females that gave birth to a litter or with pups/foetuses in utero

$$\textbf{Live birth index (\%)} = \frac{\text{number of liveborn pups at birth}}{\text{total number of pups born}} \times 100$$

$$\textbf{Viability index (\%)} = \frac{\text{number of live pups on day 4* after birth}}{\text{number of liveborn pups on the day of birth}} \times 100$$

* before standardisation of litters (i.e. before culling)

$$\textbf{Lactation index (\%)} = \frac{\text{number of live pups on day 21 after birth}}{\text{number of live pups on day 4* after birth}}.$$

* after standardisation of litters (i.e. after culling)

$$\textbf{Sex ratio} = \frac{\text{number of live male or female pups on day 0/21}}{\text{number of live male and female pups on day 0/21}} \times 100$$

$$\textbf{Conception rate (\%)} = \frac{\text{number of pregnant animals}}{\text{number of fertilised animals}} \times 100$$

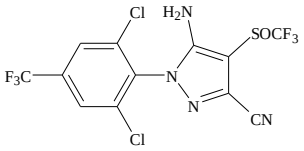
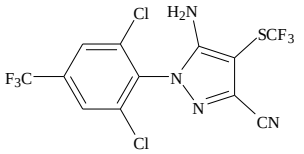
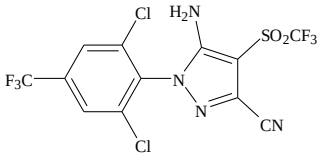
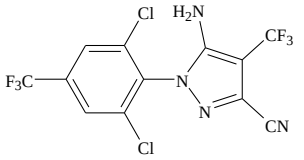
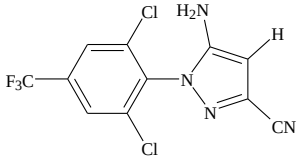
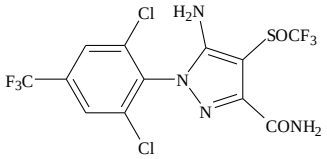
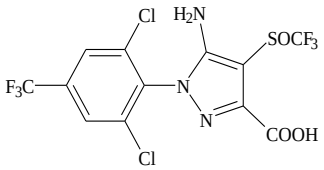
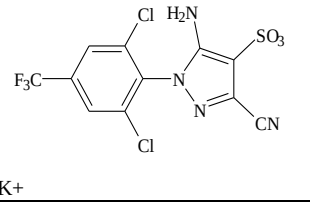
$$\textbf{Preimplantation loss (\%)} = \frac{\text{number of corpora lutea – number of implantations}}{\text{number of corpora lutea}} \times 100$$

$$\textbf{Postimplantation loss (\%)} = \frac{\text{number of implantations – number of live foetuses}}{\text{number of implantations}} \times 100$$

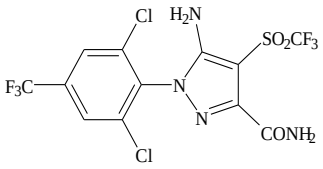
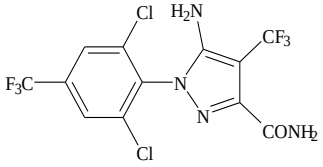
APPENDIX VI: STANDARD FUNCTIONAL OBSERVATION BATTERY (FOB) PARAMETERS

Observations	**Parameters**
Home cage observations	Posture, piloerection, gait abnormalities, involuntary motor movements, vocalisations and any other abnormalities
Handling observations	Ease of removal from cage, reaction to being handled, muscle tone, palpebral closure, pupil size, pupil response, lacrimation, salivation, stains and any other abnormalities
Open field observations	Piloerection, respiratory abnormalities, posture, involuntary motor movements, stereotypy, bizarre behaviour, gait abnormalities, vocalisations, arousal, rearing, defecation, urination and any other abnormalities
Sensory observations	Approach response, startle response, pupil response, forelimb extension, air righting reflex, touch response, tail pinch, eye blink response, hindlimb extension, olfactory orientation
Neuromuscular observations	Hindlimb extensor strength, hindlimb foot splay, grip strength hind- and forelimb and rotarod performance
Physiological observations	Catalepsy, body temperature, bodyweight

APPENDIX VII: CHEMICAL STRUCTURES OF RELEVANT COMPOUNDS AND METABOLITES USED IN STUDIES

Chemical Structure	Code and common name	Chemical Name
	MB 46030 fipronil	(±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile
	MB 45950 fipronil sulfide	1H-Pyrazole-3-carbonitrile, 5-amino-1-(2,6-dichloro-4-(trifluoromethylphenyl)-4-((trifluoromethyl)thio)
	MB 46136 fipronil sulfone	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole-3-carbonitrile
	MB 46513 desulfinyl fipronil	5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl phenyl)-4-trifluoromethylpyrazole
	MB 45897 detrifluoro methylsulfinyl fipronil	5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-1H-pyrazole-3-carbonitrile
	RPA 200766 fipronil amide	5-amino-3-carbamoyl-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinyl-pyrazole
	RPA 200761 fipronil carboxylic acid	5-amino-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-trifluoromethylsulfinylpyrazole-3-carboxylic acid
	RPA 104615 fipronil detrifluoromethyl sulfonate	5-Amino-3-cyano-1-(2,6-dichloro-4-trifluoromethyl phenyl) pyrazole-4-sulfonic acid, potassium salt

Chemical structures of relevant compounds and metabolites used in studies, cont...

	RPA 105320 fipronil sulfone amide	5-Amino-3-carbamyl-1-(2,6-dichloro-4-trifluoro methylphenyl)-4-trifluoromethylsulfonylpyrazole
	RPA 105048 desulfinyl fipronil amide	1-(2,6-dichloro-4-trifluoromethylphenyl)-3-amido-5-amino-4-trifluoromethylpyrazole

APPENDIX VIII: DECLARATION OF COMPOSITION FOR THE ACTIVE

[DELETED AS CONTAINS CIC INFORMATION]

APPENDIX IX: COMPOSITION OF AUSTRALIAN REGISTERED PRODUCTS (AND SOME FORMULATIONS USED IN TOXICITY STUDIES)

[DELETED AS CONTAINS CIC INFORMATION]

APPENDIX X: Summary of Australian adverse reactions to Frontline Top Spot, Frontline Plus, and Frontline Spray for cats and dogs.*

(* Reports were not included that were due to accidental exposure or off-label use; when confounding factors were present; if they were inconsistent with the toxicological profile of fipronil, or for which there was insufficient information)

Frontline Spray for Dogs and Cats (2.5 g/L Fipronil)			
NRA Report No.	**Date of reported incident or OCSEH response date**	**Time of onset of symptoms after using product or exposure to treated animal**	**Symptoms**
H-00-004-V	01/10/99	< 12 h	Veterinary nurse developed pruritis on arms, chest, torso. Had been using product for a number of years. Wears gloves.
H-05-10162-V	/9/2005	2 days	Shortness of breath persisting for 3 weeks. 12 dogs treated. Had been using product every 4-6 weeks for 3 years.
Frontline Top Spot for Dogs and Cats (100 g/L Fipronil)			
*M-98-170V100	17/11/98	< 24 h	Sore eyes, papular red rash over arms, legs and face. Person has history of allergies.
*H-99-001-V	5/01/99	< 24 h	Diarrhoea and lethargy, progressing to nausea, leg cramps, fever, headache, incoordination, burning sensation in eyes and face, atrial fibrillation and a breast abscess.
*H-99-003-V	27/01/99	< 24 h	Hives on legs & stomach. Treated with antihistamine tablets.
H-99-005-V	04/03/99	Contact with dog 3 d post-treatment	Swollen face, and red papular rash on face progressing to arms and chest. Reaction commenced 2 days after contact with treated dog. Treated with antihistamines.
H-99-006-V	22/03/99	Contact 3 h post-t	Cheek and chin numbness on day of contact (brushed face against dog). Washed face a number of times. No symptoms on the next day.
H-99-007-V	11/02/99	< 48 h	Rash on forearm. Using product for 12 months. No previous side effects. Treated with cortisone cream and tablets. Rash resolved with treatment.
*H-99-008-V	13/03/99	< ½ h	Tingling in arm progressing to papular rash on wrist. Wore gloves during application. Previously administered total of 4 doses of product to pets – feels 'shaky' after each application. Treated with cortisone cream.
H-99-009-V	23/03/99	< 48 h	Localised skin inflammation on arm progressing to hard, painful and itchy inflammation. Subsequently developed welts, scarring and systemic disease (low white blood cell count, increased liver enzymes, joint pain). Hospitalised 7 days.
*H-99-013-V	01/06/99	1 week post-treatment	Hives, redness, pruritis on hands, arms and back. Responded to antihistamines. Previous similar but more severe reaction to an imidacloprid spot-on, that did not respond to antihistamines.

H-99-014-V	11/03/99 29/5/99	< 12 h < 2 h	Swollen upper lip, progressing to involve nose, cheek, and area below eye. Eye pruritic within 12 h. Has experienced total of 4 reactions within minutes of touching own or neighbour's treated dog, reaction increasing in severity each time.
H-99-017-V	22/10/99	< 48 h	Welts, erythema, pruritis on legs, arms & stomach, also swollen lips. Product used monthly without previous side effects. Treated with antihistamines, recovered in 24 h.
H-00-003-V	07/02/00	< 8 h	First time use of product. Angioedema (upper lip), cat slept close to face overnight. Resolved in 2 days after treatment with antihistamines.
*AU00RDA2080 (from Merial summary) #	13/11/00	4 h	Product used intermittently for several years. Husband applied product. Wife experienced burning eyes and could detect smell on cats. Three days later, wife had not been touching cats but got watery and stinging eyes when cat came close. A child who visited 2 days post-application developed watery eyes and blotches after nursing one of the cats.
H-01-004V	05/03/01 (Merial report date)	< 8 h	Pet owner suspects that the red itchy spots, local wheals (arms, legs, shoulders, ears) occur after coming into contact with cat. Spots 6-7 mm diameter, cover areas up to 15 cm. Allergy tests did not reveal a cause. Recently applied some Frontline to herself and red wheals occurred within 10 minutes. Uncertain if reaction worse after recent animal application. Has been using product for 2.5 years.
*H-01-005-V	18/03/01	24 – 48 h	Watery eyes, puffy face, headache, when treated dog comes inside house (but did not touch dog). Symptoms persist for 2 hours, but no problems for 48 h after treating dog, for which period it is usually kept outside. Takes many medicines.
*H-01-006-V	30/04/01	< 1 h	Itchy rash on wrist progressing to welts in armpit and groin regions the next day, but resolved by pm. Took antihistamine tablets. Has been using product for years. Used gloves when applying the product, but played with dog 1 h afterwards this time (usually sends outside). Has had similar reaction to insect bites, medication.
H-00-009-V	10/03/00	< 48 h	Red rash on hand, no reaction at next treatment, but subsequent use associated with red rash over entire body. Resolved in 6 weeks with antihistamine and cortisone.
H-01-010-V	12/07/01	< 1 h	Patted cat shortly after it was treated (did not realise product had been applied). Itchy red rash, hands, arms, upper body. Treated with antihistamines from within half-hour of incident for 10 days.
*H-01-014-V	16/10/01	1 h	Burning eyes and nose after dog sat on lap 1 h post-application. Thirsty, sweaty, disoriented during night. Product used monthly for 3 years without previous side effects. Recovered the next day. Has 'extreme chemical sensitivities'.
H-02-005-V	21/3/2002	3 days	Feet swollen, itchy, skin rashes, welts, after first use. These symptoms continued. Product was used in the next 2 months without symptoms worsening, but subsequently progressed to severe symptoms including swollen tongue, requiring hospitalisation, intravenous administration of adrenalin and steroid tablets. Discharged without further incident.

*H-02-006-V	16/10/2002	1 week post-treatment	Child developed vesicles/blisters on fingers, spreading to most of body, later 'scabbing'. Treated with cortisone ointment, but continued to spread. Unclear when contact with treated pet occurred. Same scenario had occurred previously, subsequent to handling another dog one week after it had been treated with Frontline. Child suffers from 'acute food chemical intolerances'.
H-02-3816-V	8/05/2002	< 24 h	First use of product. Son (10 years) developed itchy red papular rash on arms, legs, chest, and back the morning after having handled a cat that had just been treated. Responded to antihistamines.
*H-02-5255-V	11/9/2002	Immediate	Swollen tongue, dizziness, disorientation and burning sensations – immediate reactions on opening box (symptoms last overnight) each time product applied, but not when handling treated animal. Sensitive to various household chemicals.
*H-03-6641-V	8/04/2003	A few minutes	Red, pruritic, papular, erythematous skin rash after embracing treated dog. Prescribed cortisone treatment. Recovered over following 3 weeks. Has had previous skin reactions to plants and other chemicals.
Frontline Plus (fipronil plus S-methoprene) for dogs and cats			
H-03-6639-V	8/04/2003	24 h	Skin irritation – lumpy spots and hives. Generalised reaction, with significant swelling around ankles. First use of product. Diagnosed as allergic reaction.
*H-03-8238-V	9/1/2004	?	Three separate applications. Wrinkling sensation in bottom lip and chemical taste in mouth upon touching treated animal for 2-3 weeks after treatment (unclear if after ALL treatments). Suffers allergies to multiple chemicals.
H-04-8610-V	23/7/2004	<24 h	Treated dog with Frontline Plus for first time. Next morning, small lumps appeared around ankles that spread over much of the rest body over the next 'couple of days'. Treated with antihistamines at 5-6 days post-exposure, at which point condition did not worsen.
H-04-8501-V	23/4/2004	Morning after sleeping with treated dogs	Treated dogs, left them with a friend over the weekend, then allowed them to sleep on her bed on her return. In the a.m., had redness, pruritis and swelling of skin between thighs, with redness and swelling later on hands/inside of arms. Symptoms resolved when treated with oral prednisolone. Continued to feel 'irritated' when touching dogs. Had been using Frontline Top Spot for 3 years without any adverse reaction.
H-05-20436-V	/2/2006	?	Red and itchy skin after treating dog with the product for the first time. Recurrence on a subsequent application. Reported skin felt sensitive immediately upon contact with treated dog.

V: veterinary product

* = The person affected has history of allergic responses.

A corresponding report could not be identified in the OCSEH files.

APPENDIX XI: ESTIMATED TOXICOLOGICAL CHARACTERISTICS OF AUSTRALIAN REGISTERED PRODUCTS

[DELETED AS CONTAINS CIC INFORMATION]

APPENDIX XII: DETAILS OF NON-TOXICOLOGICAL DATA SUBMISSIONS

A XI.1 Public submissions

CONFIDENTIAL

Submission No. CR108-1. Vol 66 of 68

National Toxics Network (undated) NTN submission of data for reconsideration of approvals and registration related to fipronil. NTN, 47 Eugenia St, Rivett ACT 2611.

This submission voiced concern about the continued use of fipronil, and the domestic uses of fipronil in products such as pet care, home pest control and granular turf products. The point of view was also expressed that lack of further evidence of human health damage is not justification for assuming that fipronil is safe, and that reproductive toxicity and neurotoxicity in mammals is a clear indication that human babies may be susceptible to permanent developmental damage. The NTN expressed the belief that continuous low level exposure may lead to adverse outcomes for people, particularly for vulnerable subpopulations such as developing humans, the chemically ill, and the elderly. Information in the submission, quoting several sources, is summarised below. Copies of the referenced publications were not provided.

Fipronil is carcinogenic to rats at doses of 300 ppm, causing thyroid cancer related to disruption in the thyroid-pitutary status, and is classified as a Group C (possible human) carcinogen based on rat carcinogenicity studies. (*Evaluation on: fipronil use as a public hygiene insecticide, Issue No. 187, The Health and Safety Executive, UK, 1999*)

Fipronil is neurotoxic in both rats and dogs. Severe skin reactions to Frontline Top Spot for cats and dogs have occurred. Organs affected by chronic exposure may include liver, thyroid and kidney. Reproductive effects including reduced fertility, decreased litter size, decreased bodyweights in litters, and foetus mortality occurred at the highest dose tested. (*Pesticide Action Network – UK (PAN) 2000. Active ingredient fact sheet: fipronil. June. Pesticide News 48:20-22. London, England*)

Concerns were raised about exposure of pet groomers to Frontline Spray treatment in 1996, leading to the denial of registration. (*Fipronil for use on rice (Regent, Icon) and Pets (Frontline), HED Risk Assessment, Chemical 129121, Barcodes D242090, D245656, D245627, & D241676, Cases 288765, 031271, 060305, & 061662, Submissions S535772, S541670, S541551, S534929, USEPA Washington DC 20460, US, Office of Prevention, Pesticides and Toxic Substances, 1998, 90 pp + 3 attachments*)

No actual toxicological data were provided in this submission. Except for the reactions in cats and dogs which are an animal safety matter, the toxicological endpoints referred to have been addressed in this review. Frontline Spray is currently registered in the USA.

APPENDIX XIII: EXTRACT MINUTES OF THE ADVISORY GROUP ON CHEMICAL SAFETY (AGCS)

1. AGCS 1st Meeting. March 2006.

AGENDA ITEM 5: CHEMICALS UNDER REVIEW

5.1: Fipronil – Skin sensitisation

Members:

- Considered **whether there was sufficient evidence that fipronil was a skin sensitiser in humans;**
- Noted **that reported incidents of human adverse reactions were relatively high compared to other actives but were relatively low compared to sale figures;**
- Expressed **concerns about the poor quality of human adverse reaction reports;**
- Suggested **that OCSEH should encourage sponsors to provide more information on human adverse reactions observed overseas.**
- Suggested **it would be beneficial if sponsors provided sensitisation data on fipronil and its main photo-degradate, preferably through testing in the localized lymph node assay (LLNA);**
- Anticipated **further discussions at future meetings as more data became available.**

2. AGCS 8th Meeting. December 2008.

AGENDA ITEM 3: MATTERS ARISING FROM PREVIOUS MEETINGS AND UPDATE ON OUT OF SESSION ACTIVITES

3.1: Fipronil – Skin sensitisation

Meeting notes not ratified at present. Draft version:
Members **noted** that:

- The sponsor provided a number of international reports, including medical surveillance data, occupational physician reports and a report collaborated on by the French Food Health Safety Agency (AFSSA) and the French Environmental Health and Safety Agency (AFSSE) to support the argument that fipronil is not a skin sensitiser in humans. Only a translated summary of the French report was available for this AGCS meeting; the full copy of the French report is currently being translated.

- The quality of adverse experience reports obtained through the APVMA AERP are, in general low in detail, often subjective and are therefore of limited value in distinguishing whether reported skin reactions were irritant or allergic in nature.

- The specialist community is not reporting evidence that would suggest sensitisation caused by fipronil.

- The use of fipronil is not limited to home-use veterinary preparations but also includes large scale agricultural use; suggesting that if sensitisation was significant, this may be associated with incident data.

Members **suggested** that:

- The solvent base used in fipronil products, may be an irritant to people with a pre-existing skin condition which undermines the integrity of the skin barrier (e.g. eczema).

- Given the wide-spread use of fipronil products, if fipronil was causing significant skin sensitisation then more adverse experience reports or other incident data would have been received.

Members **agreed** that:

- The use pattern and current reporting from occupational use suggest that there is insufficient evidence to class fipronil as a sensitiser. Cases of adverse reactions should continue to be monitored but ideally assessed by an expert clinician as to the true nature of these reactions.