Safety of Fipronil in Dogs and Cats : a review of literature

Conducted on behalf of the
Australian Pesticides and Veterinary Medicines Authority
(APVMA)

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1 Introduction

Fipronil[5-amino-3-cyano-1-(2,6-dichloro-4-trifluoromethylphenyl)-4-fluoromethylsulfinyl pyrazole] is a second-generation phenylpyrazole insecticide used extensively as a agricultural and veterinary pesticide (US Environmental Protection Agency, 1996; Hovda and Hooser, 2002; Tingle et al., 2003). In Australia, fipronil was first registered as a veterinary pesticide in 1995 as Frontline Spray, with the spot-on form of fipronil (Top Spot) registered from 1996 onwards for dogs, cats, puppies and kittens (Australian Pesticides and Veterinary Medicines Authority (APVMA) website). At the commencement of the review there were four active constituent approvals and 29 registered products containing the active constituent fipronil for agricultural and veterinary use (Australian Pesticides and Veterinary Medicines Authority, 2003). Fipronil was first registered for animal health in the USA in 1996, consisting of Frontline Spray, containing 0.29% w/w fipronil (Technical Information Sheet, 1997b), and Frontline Top Spot, containing 9.7% fipronil w/w (Technical Information Sheet, 1997a).

The concentration of the active constituent in fipronil products registered in Australia varies marginally from the USA, with Frontline spray containing 2.5 g/L (0.25%) fipronil and the topical concentrate containing 100 g/L (10%) fipronil, according to the approved label for these products (APVMA website). An insect growth regulator, (S)-methoprene [90 g/L (9%) for dogs or 120 g/L (12%) for cats], has also been included with several of the products containing fipronil (e.g. Frontline Plus for dogs or cats and Startgard Plus for puppies or kittens).

Fipronil-containing products have proven an effective therapy to control fleas (Hutchinson et al., 1998; Ritzhaupt et al., 2000b, a; Cadiergues et al., 2001; Jacobs et al., 2001; Mehlhorn et al., 2001; Moyses and Gfeller, 2001; Medleau et al., 2002; Medleau et al., 2003), ticks (Estrada-Pena and Ascher, 1999; Young et al., 2003), biting lice (Pollmeier et al., 2002; Pollmeier et al., 2004), trombiculid mites (Nuttall et al., 1998) and other mites (Curtis, 1996; Bordeau and Hubert, 2000; Curtis, 2004) in dogs and cats. Veterinary pesticides containing fipronil have the following label claims: Spray: up to 12 wks for fleas on dogs and 8 wks for fleas on cats; Flea Allergy Dermatitis (FAD) apply monthly; paralysis ticks apply every 3 wks, brown dog tick up to 4 wks. Dog spot ons: monthly for fleas, FAD and brown dog tick; 2 wks for paralysis tick. Cat spot-ons: monthly for fleas and FAD; note NO claim for paralysis tick.)

There have been a number of reports of Adverse Drug Experiences (ADEs) following the use of fipronil-containing products. These ADEs have been reported in target and non-target animal species and in humans either applying the product or handling the target animal after application. The purpose of this review is to summarize the published and unpublished information concerning the safety of fipronil in the target species (dogs and cats), as well as off-label use in non-target species, and to present an objective assessment of the potential risks.

2 Methodology of assessment

The information used to form this review was obtained from published and unpublished sources of information, including international databases, scientific publications, web-based peer review (e.g. Veterinary Information Network (VIN) website), the APVMA Adverse Experience Reporting

Program (AERP) and, also, information provided by the applicant regarding global suspected ADE reports. Two reviews that were particularly helpful in summarizing the available information on toxicological studies concerning fipronil and/or its metabolites were: (i) 'Pesticide Residues in Food – 1997', resulting from a joint meeting of FAO experts on pesticide residues in food and the environment and the WHO Core assessment group, with the support of the International Programme on Chemical Safety (IPCS); (ii) 'Pesticide Residues in Food 2000: Fipronil', prepared by Virginia Dobozy for the Environmental Protection Agency (EPA), Washington DC, USA. These studies included *in vivo* acute and chronic toxicology studies conducted in laboratory animals (rats, mice, rabbits, dogs) and *in vitro* studies.

Many of the toxicological studies, particularly chronic dosing studies, were performed in laboratory animals. It could be expected that there may be some overlap between information obtained from the search of published and unpublished literature. A majority of the toxicological studies originated from unpublished reports supplied by Rhone-Poulenc Inc. to international regulatory agencies, including the World Health Organisation (WHO) and the US Environmental Protection Agency (EPA).

3 Mechanism of action

Fipronil exhibits high insecticidal activity against many insects and other arthropod pests (Tingle et al., 2003). The principle mechanism of action is against the γ-aminobutyric acid (GABA) receptor-chloride complex. Ligand-gated chloride channels, such as GABA, act to inhibit excitable membranes. Blockage of the GABA-gated chloride channels by fipronil reduces neuronal inhibition and leads to hyper-excitation of the central nervous system, convulsions and death (Bloomquist, 1996, 2003; Zhao et al., 2003; Zhao et al., 2004).

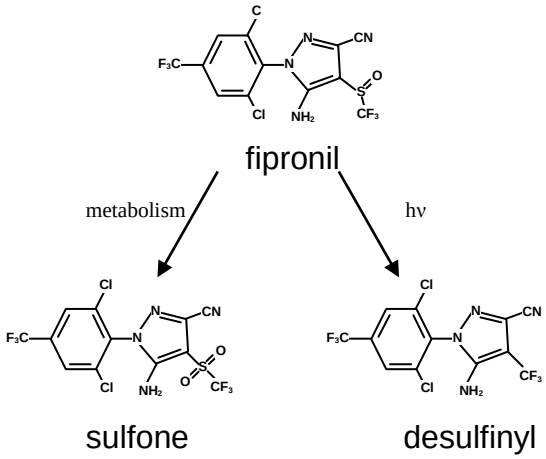
Ligand-gated GABA chloride channels are also essential to vertebrate nervous function, but fipronil appears to be selective for insecticidal forms of this membrane-bound protein complex (Bloomquist, 2003; Zhao et al., 2003). Several ligands may act as "gates" for the GABA-associated chloride channels, including glycine and glutamate (Bloomquist, 1996, 2003). Glutamate-gated GABA chloride channels appear to be a critical target for fipronil (Zhao et al., 2004) and, since these channels are only found in invertebrates (Raymond and Sattelle, 2002), possibly explains the high selectivity of fipronil for invertebrate pests (Zhao et al., 2004). For example, the selectivity of fipronil for GABA chloride channels in the cockroach is 59 times greater than in the rat (Zhao et al., 2003). In addition, an estimation of the median lethal dose (LD_{50}) in rats of 91 mg/kg is far greater than insects (corn rootworm) at 0.07 mg/kg (Zhao et al., 2003) or houseflies at 0.13 mg/kg (Hainzl and Casida, 1996). Thus, the sensitivity of insects to fipronil is 700- to 1300- fold higher than that of rats (Zhao et al., 2004).

4 Metabolism of fipronil

The major metabolite of fipronil (Figure 1) in vertebrates and invertebrates appears to be fipronil sulfone (Hainzl and Casida, 1996; Hainzl et al., 1998). On plants and in soils, fipronil undergoes a photoextrusion reaction, yielding a desulfinyl derivative (Hainzl and Casida, 1996). There have been limited studies of the degradation of fipronil on the surface (skin) of domestic species,

particularly dogs and cats. It has been reported that basic conditions (pH > 7) and increased temperatures will induce hydrolysis of fipronil (Ramesh and Balasubramanian, 1999), conditions that may occur on the skin surface of mammals.

Figure 1. Fipronil and its major metabolite (sulfone) and photoproduct (desulfinyl)



The selective toxicity of fipronil will therefore depend on the relative rate of conversion to the more persistent and less selective sulfone metabolite and desulfinyl photoproduct (Hainzl et al., 1998). The desulfinyl photoproduct, in particular, has a 10 fold greater selectivity for mammalian GABA chloride channels than the parent compound (Hainzl and Casida, 1996). No directly applicable data are available on the influence of degradation products on the toxicity of Fipronil applied to target animals.

5 Published studies investigating the efficacy of fipronil-containing products

It is beyond the scope of this review to investigate the efficacy of fipronil-containing products in the dog and cat. However, some of the published studies of efficacy may provide "field trial-type" information where the product is applied under different conditions to different animal breeds, while laboratory-based efficacy studies are usually under strictly controlled conditions and typically use a single breed (e.g. Beagle for dog studies). This was the case for three studies (Dryden et al., 2000; Medleau et al., 2002; Medleau et al., 2003) where spot-on concentrate was used in client-owned dogs and cats. It was difficult to assess local reactions in these studies because many of the animals had pre-existing dermatitis due to flea infestation and treatment with fipronil resolved this condition due to eliminating fleas. This confounding factor was also present in other published studies of fipronil use for flea control (Hutchinson et al., 1998; Ritzhaupt et al., 2000b, a; Cadiergues et al., 2001; Jacobs et al., 2001; Mehlhorn et al., 2001; Moyses and Gfeller, 2001) and, while good efficacy was found, some local reaction may occur in some animals, based on the target animal safety studies conducted for registration and post-registration ADEs.

Fipronil-containing products have also been shown to provide effective therapy for biting lice (Pollmeier et al., 2002; Pollmeier et al., 2004), trombiculid mites (Nuttall et al., 1998) and other mites (Curtis, 1996; Bordeau and Hubert, 2000; Curtis, 2004) in dogs and cats. Treatment with fipronil was reported to be effective in these studies but, again, it is difficult to determine if fipronil

was associated with any topical reaction due to pre-existing skin conditions caused by the parasite. Topical application of fipronil also appears to control ticks, particularly the brown dog tick (Estrada-Pena and Ascher, 1999; Young et al., 2003) and certain species of *Ixodes* (Endris et al., 2000). Frontline Spray® (0.25 % fipronil) also appeared effective against *Ixodes holocyclus* in dogs when sprayed directly onto the tick (Searle et al., 1995) or applied to the entire dog at recommended dose rates (Atwell et al., 1996).

6 Adverse drug experience reports for fipronil in dogs and cats

Adverse drug experiences (ADEs) are a difficult set of data to define because they may not represent the entire picture and cannot be used to calculate an overall incidence because the number of un-reported ADEs is always unknown. They can also be inconclusive due to any number of confounding factors, such as concurrent drug administration, underlying disease processes (diagnosed or sub-clinical) and the variable ability of pet owners and veterinarians to recognize an ADE. Two major benefits in the reporting of ADEs are that an increased frequency of reporting may highlight previously unknown problems with a newly registered pharmaceutical as part of an ongoing pharmacovigilance process and, secondly, the reporting of ADEs may also amplify non-significant clinical observations noted in smaller clinical trials.

6.1 ADEs in Australia

The information for ADEs in Australia was supplied from the APVMA database (1996 – 2003). In the following tables, ADEs were grouped into broad categories of clinical signs for convenience and to concentrate an understanding of specific problems associated with fipronil use in the dog and cat.

Table 1: ADEs reported for use of fipronil-containing products in cats in Australia

ADE	Cats		
	Frontline Top Spot	Frontline Plus*	Frontline Spray
Alopecia ± hair colour change	23	14	0
Alopecia ± pruritus ± erythema	46	26	1
Neurological	23	9	2
Gastrointestinal	3	0	1

* a spot on formulation containing fipronil and (S)-methoprene

The most frequently reported ADEs in the cat was for hair loss (alopecia), with or without associated pruritus and erythema. This primarily occurred at or around the application site. Many signs observed could be those of local irritation or contact-type dermatitis. The signs classed as neurological included inappetence, lethargy and salivating. Some animals were reported as distressed and/or displaying intense pruritus – these are difficult to separate from behavioural responses to intense local skin reactions. Gastrointestinal signs that were reported were primarily vomiting.

It was noted that some of the ADEs reported for Frontline Plus included comment from owners that Top Spot had been previously used on the animal with no adverse effects. This reviewer acknowledges that the number of reported ADEs for a newly released product increases and peaks in the first two years after release, the so-called 'Weber Effect' (Wallenstein and Fife, 2001; Hartnell and Wilson, 2004). As such, it is difficult to comment on the relevance of prior use on fipronil-containing formulations on subsequent adverse effects. It was also noted that the two reports of neurological signs following the use of the spray may have been related to placing the treated (wet) animal into an enclosed space. The fumes arising from the fipronil spray on the fur may have created an inadvertent inhalation dose of fipronil, although alcohol as the carrier agent in the spray may also be implicated. It was noted that the label for the spray formulation contained a warning to treat the pet either outside or in a well-ventilated room, which should avoid this potential ADE.

Table 1: ADEs reported for use of fipronil-containing products in dogs in Australia

ADE	**Dogs**		
	Top Spot	Plus	Spray
Skin reaction	156	57	23
Neurological	23	15	0
Gastrointestinal	9	5	0
Ocular	0	0	1

Similar to for cats, skin reactions were the highest reported ADEs for fipronil-containing products in dogs. There did not appear as many obvious reports of alopecia alone, with frequent pruritus and erythema associated with the area of application. Up to half of the skin reactions were quite severe and acute moist dermatitis ("hot spot") was reported. This is possibly secondary to self trauma. It was noted that self trauma or primary skin reactions may reduce the integrity of the stratum corneum and increase the systemic absorption of fipronil (Roberts et al., 2002). Many of the skin reactions occurred immediately or soon after application, establishing a direct link between fipronil and skin irritation. There were some reports of dogs avoiding subsequent application of Top Spot which may indicate skin irritation on topical application. Neurological clinical signs included ataxia, lethargy and two instance of biting or aggression. Gastrointestinal signs included vomiting and diarrhoea. It is possible that gastrointestinal problems were induced following ingestion of the concentrate (after chewing the application tube); whether or not these arose from specific direct irritation of gastrointestinal mucosa or a reaction to fipronil *per se* has not been established.

6.1.1 Off label use of fipronil

A number of reports of ADEs following "off-label" use were also noted, particularly in the rabbit. There was only one published report (Webster, 1999) of this potential problem in using fipronil in rabbits and this was cited in many instances throughout the literature. However, there were ADE reports of 32 rabbits dying following application of fipronil concentrate or spray, with 13 animals recovering. All displayed neurological signs of severe lethargy, depression and inappetence. There was also one ADE of nine guinea pigs exhibiting ADEs, with six deaths occurring after displaying neurological signs.

6.1.2 Efficacy issues

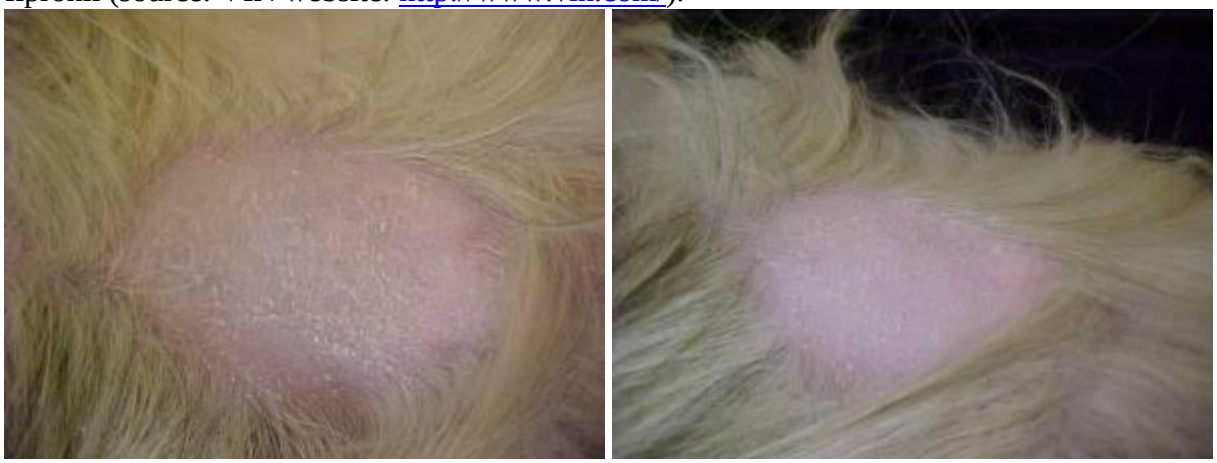
It is beyond the scope of this review to examine efficacy of fipronil containing products. However, it would be impossible to fully consider ADEs without some mention of efficacy since a substantial number of the ADEs reported were related to lack of efficacy, particularly against paralysis ticks. There were 15 reports of fipronil spray not killing ticks and at least 25 reports of live ticks found on dogs following application of fipronil concentrate. Several of these animals subsequently died as a result of tick paralysis.

The 'ADEs' reported for animals concurrently suffering from tick paralysis appear to result primarily from the tick envenomation. These clinical signs include depression, paralysis or paresis (weakness), respiratory depression, vomiting and death. This reviewer has had extensive experience in diagnosis and treatment of tick paralysis and it would appear that the majority, if not all, the clinical signs reported as ADEs with concurrent attachment of *I. holocyclus* probably resulted from tick venom and not a reaction to fipronil. As such, these were not considered by this reviewer as ADEs and were not included in the above tables. It should be noted that the label claim is for **control** of ticks, not **prevention of attachment** and the label contains a warning to this effect.

6.2 ADEs in USA

A search of the Veterinary Information Network (VIN) website (a restricted information network for veterinarians and veterinary specialists (http://www.vin.com/), with chat rooms to discuss specific themes, such as accessed for this report) revealed several ADEs which were consistent with ADEs reported in Australia. These included alopecia, change of coat colour, excoriations and neurological clinical signs. Substantial anecdotal reports of alopecia related to the topical site of administration of the fipronil-containing product, particularly to cats. Two photographs were also posted on the VIN website illustrating the alopecia that has been reported and these have been attached below (Figure 2).

Figure 2 a & b: Local alopecia reported (non-peer reviewed) following topical application of fipronil (source: VIN website: http://www.vin.com/).



6.3 Global ADE reports

The applicant has presented a comprehensive summary of suspected ADEs to fipronil in animals reported globally for a four year period between 2000 and 2003. The reporting incidence of ADEs in the cat and dog was low (<0.001%) for spray and spot-on (and spot-on plus) products. This would appear within reasonable acceptable limits for this extensively used group of products. The reporting incidence was calculated on the number of doses sold. Of the 4534 ADEs reported, 2847 may potentially be considered as consistent with hypersensitivity, with cutaneous reactions (86.3%) forming the greater majority of the latter. This reviewer agreed with the applicant that the global incidence of ADEs to fipronil in dogs and cats is not common.

The total incidence within Australia (<0.0024%) was 2.7 times higher than globally reported ADEs. Similarly, the majority of ADEs reported in target species in Australia were related to cutaneous signs (75.6%). While still low, it is concerning that the incidence of ADE reports is higher in Australia and the applicant did not offer any reason for this disparity, although the low reporting incidence and variability of ADEs may account for this difference. Of greater concern to this reviewer is that the applicant suggested that signs of skin irritation were unremarkable because studies in laboratory species have demonstrated that fipronil has the potential to induce mild to moderate cutaneous irritancy. It is realized that cutaneous reactions are unlikely to be life-threatening but can be of major concern to owners, particularly if they are not advised in the labelling that skin irritation and/or damage can occur. This would be particularly likely in breeders and trainers where external appearance and a healthy skin are essential, while alopecia or skin damage may preclude an animal from competition or create an impression of poor health care.

The applicant did not include ADEs reported involving paralysis ticks in the overall ADEs reported for fipronil in Australia. This appears justified because it is difficult to separate the clinical signs of tick paralysis from any possible neurological clinical signs that may result from fipronil toxicosis. It is the opinion of this reviewer that neurological signs described in the ADE reports are consistent with tick paralysis alone and these ADE reports are more accurately termed ‘lack of efficacy’ reports.

7 Summary and interpretations

7.1 Oral toxicity

The mechanism of action of fipronil is blockage of glutamate-gated GABA chloride channels and, since this is primarily an inhibitory mechanism, will result in uncontrolled excitability in pathways controlled by this mechanism. It could therefore be expected that the signs of fipronil efficacy in the control of insect pests, and toxicity in mammals, will involve neuronal excitation, such as irritability, aggression, in-coordination, convulsions and death. The specificity of fipronil for insect GABA chloride channels, some 700 to 1300 times greater than mammals, permits the selective control of invertebrate ectoparasitic hosts on the target species, dogs and cats (Zhao et al., 2003; Zhao et al., 2004).

Despite this large apparent safety margin, there remains a potential for toxicity in target species due to a range of factors, including abnormal intake (ingestion or inhalation), variable receptor sensitivity to fipronil (individual variation) and the effects of disease, including systemic and/or cutaneous ailments, which may predispose to toxic effects. Toxicity of fipronil appears to be dose-related, primarily manifested as gastrointestinal and/or neurological signs (Bloomquist, 2003; Zhao et al., 2003).

The primary initial studies that investigated the oral toxicity of fipronil examined administration of fipronil to laboratory animal species, as part of the human toxicological studies and not target or non-target animal safety. The results of the toxicological studies are used to determine the hazard profile of the product for human exposure, to set first aid and safety directions, and to set acceptable daily intakes and maximum residue limits when the products are used on food commodities.

Following oral administration of fipronil at single doses of 25-200 mg/kg bodyweight (mice), 25-200 mg/kg bodyweight (rats) or daily doses of 75 mg/kg bodyweight for 5 days in rats, acute toxicity studies showed a dose-dependent appearance of neurological signs, including tremors, gait abnormalities and seizures in laboratory animals (Gardner, 1988a; Mondot and Dange, 1995; Ray, 1997). Some bioaccumulation of the test material may occur in laboratory species (Ray, 1997), although this is unlikely to be a potential risk to target animals when used according to label directions.

Short term oral toxicity studies in rats and mice dosed daily with fipronil at levels up to 55 mg/kg bodyweight for 4 weeks, up to and over 22 mg/kg bodyweight for 6 weeks, or up to 24 mg/kg for 13 weeks also revealed dose-dependent toxicity of orally administered fipronil, although obvious signs of gastrointestinal (inappetence and weight loss) and neurological signs (depression, lethargy and/or death) did not occur until higher dose rates with mortality prominent at the highest doses administered (Broadmeadow, 1991; Holmes, 1990; Holmes, 1991a; Peters et al., 1990). The liver and thyroid were the main organs undergoing histological changes in response to short term (4-13 wks) fipronil dosing and, as this did produce overt clinical signs at lower dose rates (< 5 mg/kg/d), a NOAEL could not be determined.

In Beagle dogs, short term oral toxicity studies also reported toxic effects at the higher dose rates used, particularly 10 mg/kg/d for 13 weeks where neurological and gastrointestinal signs were observed (Holmes, 1991b). The NOAEL was 0.5 mg/kg/day. Oral administration of fipronil for one year revealed neurological signs when higher dose rates were administered (> 1 mg/kg/d) and a NOAEL of between 0.2-0.3 mg/kg/d by oral administration was calculated for the dog (Holmes, 1992, 1993). Since one female dog did exhibit possible neurological signs (over-activity) when dosed at 0.2 mg/kg/d, a “safe” daily dose of fipronil may be less than this suggested NOAEL.

7.2 Neurotoxicity

The potential for fipronil to induce specific neurotoxicity in target and non-target species has also been reported. Acute neurotoxicity studies in the rat following single oral administration of fipronil at levels up to 25 mg/kg bodyweight reported a NOAEL of 2.5 mg/kg with clinical signs reverting to normal by 14 days (Hughes, 1997). A second study with single oral administration of fipronil at

levels up to 50 mg/kg bodyweight reported a NOAEL of 0.5 mg/kg, based on histopathology and clinical signs up to 16 days after treatment (Gill et al., 1993), while a longer study (fipronil administered orally at levels up to 11 mg/kg bodyweight for 13 weeks) found a NOAEL of 0.3 mg/kg for neurotoxicity in the rat (Driscoll and Hurley, 1993). A limited study in the dog reported that 20 mg/kg/d orally induced neurotoxicity from 5-13 days after commencement of treatment and food consumption decreased from 1-2 days after commencement of treatment (Holmes, 1991b). These signs resolved within 12 days of cessation of treatment and it was concluded that clinical signs resulted from systemic pharmacological modulation which disappeared as the fipronil was eliminated from the body.

Recommended application intervals for fipronil spot ons are as follows: Spray: up to 12 wks for fleas on dogs and 8 wks for fleas on cats; FAD apply monthly; 3 wks for paralysis ticks, 4 wks for brown dog tick. Dog spot ons: monthly for fleas, FAD and brown dog tick; 2 wkly for paralysis tick. Cat spot-ons: monthly for fleas and FAD; note NO claim for paralysis tick. The dose rate equates to a possible 13.4 mg fipronil/kg bodyweight for spot-on products and 15.0 mg fipronil/kg bodyweight for fipronil spray, assuming application to the lowest bodyweight in the dosing range, at each dosing interval. The potential for systemic toxicity will depend on the bioavailability of active ingredient, either via ingestion or transdermal penetration. This review was particularly concerned with safety of fipronil under normal conditions of use (*i.e.* topical application). However, ingestion of fipronil concentrate (packaged to dispense 13.4 mg/kg per tube) may approach acute toxic doses (75 mg/kg in mice), particularly if the entire contents (3-6 tubes) of a package are ingested (Mondot and Dange, 1995). This would not be considered normal use and would fall under inadvertent toxicity. Some ingestion may also occur following licking and grooming of the site of application, despite the relatively low accessibility of this site (normally the back of the neck).

Significant toxicity, particularly neurological signs, was reported in dogs after daily oral administration of fipronil at 10 mg/kg/d for 13 weeks, while inappetence and poor condition was noted at 2 mg/kg/d (Holmes, 1991b). Since total absorption of radiolabelled fipronil applied topically to rats was less than 1% over 24 hours, it would appear highly unlikely that application of a single dose of the commercially-available fipronil formulations would approach significantly toxic doses (2+ mg/kg/d), even in the cat (assuming similar toxicological effects) with more fastidious grooming habits. Yet a closer inspection of some of the ADEs reported for cats under 'neurological' signs were listed as salivation/drooling and mild inappetence which may be secondary to grooming the site of application. It is difficult to differentiate, particularly in the cat, if these reactions are a specific toxicosis or a local reaction of the oral mucosa to an ingested foreign substance.

7.3 Endocrine disruption

The effects of fipronil on endocrine function were studied in the rat since some disruption of endocrine function is possible (Bloomquist, 1996; Colborn, 1998; Davis et al., 1996; Davis et al., 2000; McCarthy, 1995). Pregnancy rates were reduced at high doses (280 mg/kg) of fipronil (Frontline Top-Spot) applied as a single dose topically to rats, while hormonal changes were found at lower doses (70 mg/kg) (Ohi et al., 2004). These doses are substantially higher than what may be expected following topical application and appear to suggest that reproductive and endocrine

function would be unlikely to be disrupted with normal use of fipronil-containing products. Studies in the dog (DOG PR&D 0020201; Godin and Alva, 2000b) and cat (CAT PR&D 0020301; Godin and Alva, 2000a) have shown that fipronil can be applied safely to these animals during pregnancy and lactation, with no adverse effects to either parent or offspring. Similarly, in trials with weanlings and juveniles, there were no significant adverse effects observed in this relatively vulnerable group and therefore fipronil could be considered safe to use in younger animals, including weanlings.

7.4 Skin Absorption

Fipronil products registered for use in dogs and cats are formulated for topical application. The relative proportion of radiolabelled fipronil that is absorbed through the skin appeared to decrease with increasing dose rate (Cheng, 1996), which suggested that pathways of transdermal absorption may be saturable. The bioavailability of topically-applied fipronil is generally accepted as ≤ 5% (Brayden, 2003) due to limited permeability through the stratum corneum (Birckel et al., 1996; Cochet et al., 1997). *In vivo* studies in the rat showed that absorption of a suspension of radiolabelled fipronil decreased with increasing dose and that absorption was saturated when 48.5 mg/rat (194.0 mg/kg if the rat weighed 250 g) was applied. The percent of the applied dose that had penetrated the different membranes also varied with species and time, with relatively less penetrating after application of a 4.0 g/L formulation, compared to 0.2 g/L. It was reported that less than 1% of the applied dose was absorbed, but up to 3.3% (following application of 8.35 mg/rat) was found left on the skin after the first 24 h (Cheng, 1995). This equates to 0.13 mg/kg (1%) absorbed following a single topical administration to dogs at recommended dose rates and 0.43 mg/kg (3.3%) remaining on the skin. These results agreed with studies using radiolabelled fipronil *in vivo* to demonstrate that percutaneous passage of fipronil was low (Cochet et al., 1997), similar to the cat (Birckel et al., 1996).

A NOAEL for oral toxicity of fipronil in the dog of 0.2 mg/kg/d (calculated from daily oral administration for one year (Holmes 1992, 1993)) is substantially higher than a possible 0.13 mg/kg (or 0.15 mg/kg of spray formulation) every two to four weeks. This level of exposure is also considerably less than the dermal LD_{50} for fipronil applied in distilled water to rats (> 2000 mg/kg in both males and females), while in rabbits the dermal LD_{50} for test material moistened with corn oil was 354 mg/kg for the two sexes combined. Neither clinical signs of toxicity nor deaths were seen in rats. In rabbits, fipronil induced deaths and one or more clinical signs of toxicity including convulsions, sluggishness, salivation, spasms, tremors, hyperactivity, diarrhoea, emaciation, and perioral and perinasal red discolouration in all groups except that at the lowest dose (100 mg/kg). Delays in the appearance of signs of toxicity and death were noted at all doses except the lowest. In particular, convulsions were not observed until days 3-9 after treatment, and some animals did not die until days 11-14 (Gardner, 1988b; Myers and Christopher, 1992).

It would appear, in the limited number of dogs used in these studies (Birckel et al., 1996; Cochet et al., 1997), that fipronil, applied at recommended dose rates and dose intervals, would be highly unlikely to be absorbed in sufficient quantities to induce signs of systemic toxicosis (*i.e.* neurological and gastrointestinal signs) in the normal dog and cat and, importantly, suggests that topical application of marketed formulations of fipronil-containing products is safe in the dog and the cat.

7.4.1 Transdermal penetration of fipronil and/or metabolites

Topically-applied fipronil is sequestered by sebaceous glands and is gradually released over a two month period (Dryden et al., 2000). The bioavailability of topically-applied fipronil is generally accepted as ≤ 5% dogs and cats (Brayden, 2003) due to limited permeability through the stratum corneum (Birckel et al., 1996; Cochet et al., 1997).

Limited information is available regarding transdermal movement of fipronil and/or metabolites through dogs and cats. Radiolabelled (^{14}C) fipronil to dogs was investigated using auto-historadiography for up to 56 days after topical application. The radioactivity was found to spread down to the lumbar region and penetrate into the stratum corneum, pilo-sebaceous units and the viable epidermis. No radioactivity was found in the dermal of hypodermal layers which was suggested to represent low percutaneous passage of fipronil in the dog (Cochet et al., 1997). A similar study in the cat also found that topically applied fipronil has low percutaneous penetration and was primarily found in the stratum corneum and sebaceous glands (Birckel et al., 1996).

7.5 Toxicity in cats

This reviewer was unable to find information relating to fipronil toxicity and toxicological studies specifically in the cat, although the toxicological studies were conducted for human safety and these are not usually conducted in cats. It is noteworthy, however, that obvious species differences have been found in transdermal penetration of fipronil (*i.e.* rabbit vs rat) (Birckel et al., 1996) and further studies are strongly recommended to quantify fipronil movement through feline skin. *In vitro* studies in human, rabbit and rat skin showed that the extent of penetration increased with time across species (Walters and Brain, 1990). It was interesting that fipronil appeared to penetrate rabbit skin up to a 10 fold order of magnitude higher than rat skin and this may be a predisposing factor to toxicity in this species. It was also noted that there were no *in vitro* studies in either dog or cat skin, despite the reported species differences in transdermal fipronil penetration.

7.6 Toxicity in young animals

An additional factor that was apparent in reported toxicological studies relating to topical application of fipronil is that generally young healthy animals, or skin from healthy animals for *in vitro* studies, were used. A survey of published studies relating to efficacy of fipronil-containing products reveals that it is applied to animals to control ectoparasites which are often associated with skin damage, such as flea allergy dermatitis (Hutchinson et al., 1998; Cadiergues et al., 2001; Medleau et al., 2002; Medleau et al., 2003). Damage to the epidermis, particularly the stratum corneum, will dramatically reduce the barrier function of skin (Roberts et al., 2002), which may then permit significantly greater amount of active ingredient and/or vehicle through to the systemic circulation. The potential toxicity of fipronil-containing products applied to damaged skin has not, to this reviewers knowledge, been specifically assessed in any species, most importantly the target species.

7.7 Toxicity of metabolites

Specific toxicity resulting from metabolites of fipronil is difficult to determine because it is uncertain how much degradation of the parent compound will occur when applied to animal skin. Basic conditions (pH > 7) and increased temperatures will induce hydrolysis of fipronil (Ramesh and Balasubramanian, 1999) and these conditions may occur on the skin surface of mammals. The influence of sunlight to induce degradation of fipronil to the desulfinyl derivative (Hainzl and Casida, 1996) following topical application is also uncertain. However, since transdermal passage of radiolabelled fipronil (which will include any metabolites formed) is less that 5%, it is reasonable to assume that systemic penetration of any metabolites formed would be less than this. Furthermore, the recovery of topically applied radiolabelled fipronil desulfinyl (in 1% carboxymethylcellulose) was 93-103%, with the majority of this (90-102%) being present in the skin wash, suggesting that much of an applied dose resides on or within the skin (Cheng, 1996). It would therefore be unlikely that any significant toxicity or specific neurotoxicity would result from degradation of fipronil after topical administration, despite the reported 10 fold greater selectivity for mammalian GABA chloride channels than the parent compound (Hainzl and Casida, 1996). It was noted, however, that insufficient information exists concerning the degradation of fipronil *in vivo* and the potential toxicity of metabolites to the target species.

7.8 Adverse Drug Experiences

Cutaneous skin reactions appear to be the most common ADE reported for fipronil in dogs and cats both globally and in Australia. Fipronil moistened with corn oil caused slight dermal irritation to New Zealand white rabbits after a 4 hour application to intact skin, yet this did not occur if fipronil was moistened with water (Liggett, 1988a; Myers and Christopher, 1993). Furthermore, it was reported in the toxicology studies conducted in guinea pigs that fipronil (10% in propylene glycol) was a mild or weak skin sensitizer, using the method of Magnussen and Kligman (Johnson, 1993). However, using the Buehler method, fipronil (30% w/v in paraffin oil) produced no sign of dermal sensitization (Smith, 1990). It cannot be discounted that the vehicle may contribute to the dermal response to fipronil-containing products, either directly or related to concentration of active ingredient in the vehicle (the maximum flux (J_{max}) or driving force for transdermal drug penetration is dependent on concentration, and therefore solubility, of the drug in the vehicle (Roberts et al., 2002)). No signs of dermal irritation were seen in rabbits when the metabolite, fipronil sulphone, was applied to the skin at a single dose of 0.5 mg moistened with distilled water for 4 hours (Liggett, 1988b).

Indications from published and anecdotal reports suggest that fipronil-containing products can induce cutaneous reactions in some dogs and cats. These reactions, ranging from alopecia to acute moist dermatitis, are unlikely to cause serious signs of disease in affected animals, but may cause distress to owners and the affected animals. This would be particularly so in breeders and owners of show or competition animals where perturbations in the skin may preclude entering in competition. A further consideration for cutaneous reactions is that damage to the skin, either directly or secondary to pruritis and self-trauma, may predispose the animal to enhanced systemic absorption of fipronil as the barrier function of the skin is compromised. Fipronil resides within the openings of appendages (hair follicles and possibly sweat glands) and damage or removal of follicles and, particularly, the stratum corneum (outermost skin layer), removes the primary barrier

to drug movement through skin. A significantly greater potential for increased systemic concentrations of fipronil and its metabolites is possible if fipronil-containing products are applied to skin damaged in any way. However, the still relatively low percutaneous penetration and wide margin of safety are consistent with no reports of toxicity attributable to enhanced passage of fipronil-containing products through damaged or inflamed skin.

Neurological signs, including inappetance, lethargy and salivation, were the second most frequent ADE reported in cats and dogs within Australia. This is a difficult category to evaluate because of overlap between true neurological signs and animals exhibiting excitation or anxiety secondary to fipronil application. For example, some dogs are reported to resent topical application of fipronil-containing products, while other may show apparent neurological signs (e.g. shaking, anxiety, intense scratching) if a local skin reaction is upsetting the animal. Similarly, cats are known to froth from the mouth and become distressed if certain foreign substances contact oral mucosa which can readily occur if the cat ingests recently applied fipronil during grooming. Close examination of ADE reports suggest that many 'neurological' signs were probably local reactions because (i) the classic neurological signs of fipronil toxicity, including fitting, hypo-activity, ataxia, tremors and lack of vision, were not reported; (ii) many of the reactions occurred soon after topical application and true neurological signs were reported to appear after a variable period of time (up to three days in rabbits) as the drug penetrated through the skin and into the systemic circulation. However, there were some reports of ADEs that do appear to be true neurological signs, particularly animals exhibiting aggression and marked in-coordination. Some guidelines on reporting ADEs for fipronil may be useful in future to assist distinction between specific clinical signs. For example, failure to prevent paralysis tick attachment is not a neurological sign per se.

A further problem with evaluation of neurological ADE reports in Australia is that many of the reports were associated with concurrent effects of paralysis tick. There is some overlap between the clinical signs observed in both fipronil toxicity and tick paralysis, particularly hypo-activity, hind leg splay and increased respiratory effort. It is the strong opinion of this reviewer that the majority, if not all, neurological signs reported as ADEs when the animal was concurrently suffering from tick paralysis were related to tick venom and not fipronil. The reports of ADE are therefore best considered as lack of efficacy in controlling the tick.

8 Conclusions

A close examination of the published and unpublished information concerning fipronil use in the dog and cat would suggest that fipronil-containing products are generally safe to use in the healthy target species at recommended dose rates and route of administration. Extensive studies in target and non-target species show that NOAEL figures, calculated from daily oral administration of fipronil, were significantly higher than would normally be administered topically on a two- to four-weekly basis. Information supplied by the applicant on global and Australian ADE reports shows a particularly low incidence of problems encountered in the target species, dogs and cats, when used according to manufacturer's recommendations. There were, however, several areas of concern in the use of fipronil-containing products that should be addressed by the applicant either by changes to product labelling or in advice to veterinarians when dispensing this product:

- Individual variation in response to fipronil is to be expected and some differences in tolerances or reactions to fipronil should preclude use of fipronil-containing products in that individual. This was evident when some animals reacted to Frontline Plus yet not with prior use of Frontline® Top Spot. The extent and incidence of individual variation in response to fipronil-containing products could not have been predicted from the published and unpublished studies in dogs because a single breed (Beagles) was used and this may not be representative of all breeds. More importantly, a single breed of dog may also not be representative of transdermal drug movement in all breeds. However, it's also noted that the reporting incidence is very low, so the chances of seeing a response in the studies may also be expected to be low.

- There was no information or studies into transdermal movement of fipronil through non-normal skin, such as occurs during flea allergy dermatitis or secondary to pruritus. This may contribute to the incidence of 'individual variation' or unexpected toxicity to fipronil. It is recommended that care be taken when administering fipronil to severely excoriated skin and it would be considered good veterinary practice to treat damaged skin conventionally and avoid application of topical anti-parasiticides to the damaged regions.

- While it is appreciated that the global and Australian incidence of cutaneous ADEs is low, there appears to be a real and characteristic reaction to fipronil-containing products in certain animals (i.e. alopecia, pruritus and erythema). This should be noted as a possible adverse reaction in product labelling, particularly for show or competition animals.

- The LD_{50} for fipronil by the inhalation route is substantially lower than for oral administration (Cracknell, 1991; Gardner, 1988a; Mondot and Dange, 1995; Nachreiner, 1995). It is strongly recommended that some warning to avoid placing animals that have been treated with the spray formulation into enclosed spaces until the coat is dry. Inhalation of fipronil fumes in an enclosed space could readily contribute to toxicological effects, although the alcohol base for the spray may also be implicated in adverse effects. It is noted that the label contains a warning to treat the pet outside or in a well-ventilated room.

- It is recommended to use fipronil-containing products according to the label directions. This particularly applies to non-target species, such as rabbits and guinea pigs. In fact, it is strongly recommended NOT to use fipronil-containing products in rabbits or guinea pigs. It is noted that since 1993, all Frontline Spray and Frontline Plus products now carry label warnings against using the products in rabbits: "DO NOT USE IN RABBITS". However, it is also noted that The Frontline Top Spot products do not have this label warning.

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