A review of Animal Safety Studies for Fipronil in the dog and cat

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

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1 Introduction

The Australian Pesticides and Veterinary Medicines Authority (APVMA) has undertaken a comprehensive review of fipronil in line with reconsideration of the approval and registration of this active constituent in Australia. One aspect of the review concerns animal safety issues, particularly in the target species dogs and cats for which fipronil products are currently registered. The purpose of the current report is to evaluate safety studies in target animals provided by Merial Australia to the APVMA to assist in this review.

Fipronil is used extensively as an agricultural and veterinary pesticide. In Australia, fipronil was first registered as a veterinary pesticide in 1995 as Frontline Spray, with the spot-on form of fipronil (TopSpot) registered from 1996 onwards for dogs, cats, puppies and kittens (Australian Pesticides and Veterinary Medicines Authority (APVMA) website). Fipronil was first registered for animal health in the USA in 1996, consisting of Frontline Spray, containing 0.29% w/w fipronil, and Frontline TopSpot, containing 9.7% fipronil w/w. 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 in Australia.

Fipronil-containing products have proven an effective therapy to control fleas, ticks, biting lice, trombiculid mites and other mites in dogs and cats, although it is not currently registered for mites in Australia. Veterinary Pesticides containing fipronil are marketed for application at monthly intervals for flea control and every two weeks for tick control. (It should be noted, however, that the recommended treatment intervals are as follows. 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.)

2 Methodology of assessment

The data assessed for this safety review were published and unpublished animal safety studies provided by the registrant in support of the safety of fipronil.

The data provided were examined for completeness and scientific validity. All studies were performed under the guidelines of Good Laboratory Practice (GLP). Adequate data collection and recording were observed.

3 Terminology

Acanthosis	A thickening of the germinative layer of the epidermis
ADEs	Adverse Drug Experiences
Alopecia	Hair loss
Epidermatitis	Inflammation of the outer layer of the skin
Erythema	Redness of the skin
FAD	Flea allergy dermatitis
Scurfs	Bran-like desquamation of the epidermis – dandruff
Histopathology	The study of minute changes in diseased tissue

Hyperkeratosis	Hypertrophy (thickening) of the horny layer of the skin
Lesion	Any pathological or traumatic discontinuation of tissue
Parafollicular	Occurring alongside the hair follicles
Parakeratosis	The retention of nuclei in the stratum corneum of skin - normally associated with inflammation
Plasma T_4	Thyroxine (T_4) in plasma is a measure of thyroid function
Pruritus	Scratching and/or biting associated with itchiness or irritation

4 Summary of Animal Safety Studies

4.1. Rhone Merieux - DOG CLI 138– December 1992

Topical spray containing 0.25% fipronil applied to dogs at 1x, 3x and 5x recommended dose for a total of 6 applications each 28 days apart (DOG CLI 138).

A spray formulation of fipronil was applied to 24 Beagle dogs (12 male and 12 female). The dogs were a mean weight of 11.5 kg and 9.3 ± 1.5 months old. The dogs were randomly divided into four groups of 3 males plus 3 females, consisting of: Control (no treatment); Treatment Group 1 = 3 mL/kg (~ 1x recommended dose); Treatment Group 2 = 9 mL/kg (~ 3x recommended dose); Treatment Group 3 = 15 mL/kg (~ 5x recommended dose). Each dog in the treatment groups was treated at the appropriate dose rate for six applications separated by 28 days for each treatment. At the end of the study, there were no significant differences between treated and control animals for body weight, food consumption, rate of vomiting, modification of faeces, clinical examination, rectal temperature, skin fold thickness, haematology, clinical chemistry and plasma T_4 concentrations.

Erythema was found in two dogs, one after treatment at 3 mL/kg and one at 9 mL/kg. This was not thought to be linked to the treatment because subsequent treatments did not exacerbate the lesion. Histopathology samples reported hyperkeratosis, acanthosis and parakeratosis only in the dog receiving fipronil spray at 3 mL/kg.

Reviewer's comments - There were no accompanying pathological reports for this reviewer to verify this information. However, tables of results suggested some incidence of eye irritation and flaky scurfs on the skin. The fine scurfs were only significantly higher when comparing 3 mL/kg group with controls, but not between controls and the other treatment groups or between treatment groups. There could be occasional sensitivity to the treatment which is not dose-related but there is insufficient data to be conclusive.

4.2. Rhone Merieux - PUP CLI 180– July 1993

Topical spray containing 0.25% fipronil applied to nursing puppies at 6 mL/kg for a total of 2 applications 28 days apart (PUP CLI 180).

A spray formulation of fipronil was applied to puppies from three different breeds, Beagles, Spaniels and Griffons. A total of 89 puppies (53 males and 36 females) took part in the study and litter groups were randomly allocated to treatment or control (no treatment) groups. The puppies were a mean weight of 740 g (range 200 to 2250 g) and average age of 16.4 days

(range 2 to 38 days). The puppies had a spray formulation of fipronil applied twice at 28 day intervals. The dose used was 6 mL/kg, which, following manufacturer's recommendations of 3-6 mL/kg, was approximately 0.4 to 5 times the upper recommended dose rate. The study authors concluded that tolerance to the treatment was very good.

Reviewer's comments - A significantly higher number of puppies from the treatment group had irritated eyes (eye discharge) and scurfs in the 24 hour period after the first treatment, although there were no significant differences between control and treatment groups from then onwards. This would suggest some mild and transient reaction to the initial application of the treatment.

4.3. Rhone Merieux - CAT CLI 137– July 1993

Topical spray containing 0.25% fipronil applied to cats at 1x, 3x and 5x recommended dose for a total of 6 applications each 28 days apart (CAT CLI 137).

A spray formulation of fipronil was applied to 12 male cats (European breeds). The cats were a mean weight of 4.7 ± 0.3 kg and a mean age of 8 months. The cats were randomly divided into four groups of 3 cats: Control (no treatment); Treatment Group 1 = 3 mL/kg (~ 1x recommended dose); Treatment Group 2 = 9 mL/kg (~ 3x recommended dose); Treatment Group 3 = 15 mL/kg (~ 5x recommended dose). Each cat in the treatment groups was treated at the appropriate dose rate for six applications separated by 28 days for each treatment.

At the end of the study, there were no significant differences between treated and control animals for body weight, rate of vomiting, modification of faeces, clinical examination, rectal temperature, skin fold thickness, haematology, clinical chemistry and plasma T_4 concentrations. There was a reduction in food consumption in cats receiving treatment at 15 mL/kg, although this was thought to be due to difficulty in acclimatization. Histopathology from skin biopsies from one control cat and the three cats receiving 15 mL/kg revealed no lesions. There were isolated findings of aggression in one cat at 9 mL/kg and two cats at 15 mL/kg displayed nervousness, although these findings were not thought by the study authors to be related to the treatments.

Reviewer's comments – The conclusion that the reduction in food consumption was related to difficulty in acclimatization may not be justified and may be a dose-related and, therefore, treatment related effect. The neurological signs may also be related to treatment and larger numbers of animals in each study group would be necessary to confirm that the nervousness and aggression were not related to treatment.

4.4. Rhone Merieux – CAT Report 94424– September 1994

Topical spray containing 0.25% fipronil applied to juvenile cats at 1x and 5x recommended dose for a total of 3 applications each 28 days apart (CAT 94424).

A spray formulation of fipronil was applied to 28 DOMS cats (14 male and 14 female). The cats were ≤ 8 weeks old. The cats were randomly divided into three groups, consisting of: Control (4 males and 4 females; vehicle only applied at 30 mL/kg); Treatment Group 1 (4 male

and 4 female) = 6 mL/kg (~ 1x recommended dose); Treatment Group 2 (6 male and 6 female) = 30 mL/kg (~ 5x recommended dose). Each cat was treated at the appropriate dose rate (treatment and control) for three applications separated by 28 days. At the end of the study, there were no significant differences between treated and control animals for clinical signs, body weight, food consumption (some minor statistical differences were not considered by the study authors to be biologically significant), haematology, clinical chemistry. There were no reports of localized macroscopic changes and histopathology was not performed in this study.

Reviewer's comments – It is difficult to give a definitive opinion on the safety of a topically applied treatment without histopathology data. However, the reviewer recognizes that suitable representative samples of skin are difficult to collect in a study of this nature and are therefore probably not justifiable to collect from the animals in this study. Overall, the trial was well performed and the topical spray formulation did not appear to induce any adverse effects in the cats participating in this study.

4.5. Rhone Merieux - DOG Report 94423– September 1994

Topical spray containing 0.25% fipronil applied to juvenile dogs at 1x and 5x recommended dose for a total of 3 applications each 28 days apart (DOG 94423).

A spray formulation of fipronil was applied to 28 Beagle puppies (14 males and 14 females). The dogs were ≤ 8 weeks old. The dogs were randomly divided into three groups, consisting of: Control (4 males and 4 females; vehicle only applied at 30 mL/kg); Treatment Group 1 (4 males and 4 females) = 6 mL/kg (~ 1x recommended dose); Treatment Group 2 (6 males and 6 females) = 30 mL/kg (~ 5x recommended dose). Each dog was treated at the appropriate dose rate (treatment and control) for three applications separated by 28 days. At the end of the study, there were no significant differences between treated and control animals for general appearance, behaviour, body weight, food consumption (some minor statistical differences were not considered biologically significant as they were usually increases), haematology, clinical chemistry. There were no reports of localized macroscopic changes and histopathology was not performed in this study.

Reviewer's comments – It is difficult to give a definitive opinion on the safety of a topically applied treatment without histopathology data. However, the reviewer recognizes that suitable representative samples of skin are difficult to collect in a study of this nature and are therefore probably not justifiable to collect from the animals in this study. A similar conclusion can be drawn as to study CAT 94424 in that the study was well performed and the topical formulation did not appear to induce any adverse effects on the dogs participating in this study.

4.6. Rhone Merieux – CAT MRX 24/950746– May 1995

Topical concentrate containing 9.7% fipronil applied to juvenile cats at 1x, 3x and 5x recommended dose for a total of six applications each 28 days apart (CAT MRX 24/950746).

A topical spot-on formulation of fipronil was applied to 40 cross breed domestic cats (20 males and 20 females). The cats weighed between 781 and 1220 g and were approximately 12 weeks old at the start of the study. The cats were randomly divided into five groups, each containing 4

male and 4 female cats: Control 1 (un-dosed); Control 2 (dosed with vehicle only); Treatment Group 1 dosed at 25 mg/kg (~ 1x recommended dose); Treatment Group 2 dosed at 75 mg/kg (~ 3x recommended dose); Treatment Group 3 dosed at 125 mg/kg (~ 5x recommended dose). Each cat was treated at the appropriate dose rate (treatment or control) for six applications separated by 28 days.

At the end of the study, there were no signs of reaction at the site of application. There were also no reported significant differences between treated and control animals for general health, behaviour, body weight change, food consumption, haematology and biochemistry. There were no reports of localized macroscopic changes or histopathology associated with the treatment.

Reviewer's comments – A more detailed examination of the pathology reports revealed that some cats had a prominent number of mast cells in the dermis from the treated site. In male cats, this was only in one cat dosed at 3x and two cats dosed at 5x. In female cats, this condition was seen in two un-dosed controls and three vehicle-only controls, while two of the 3x and four (100%) of the 5x cats had prominent numbers of mast cells in the dermis. There was also one report of alopecia over the treatment site in a male cat in the vehicle-only control group. These results were concerning because there is a possible immunological response to the topical formulation beneath the site of administration. Mast cells are commonly associated with an allergic response and, combined with evidence of alopecia at the site of administration of the vehicle, suggested that a local response to the formulation may have occurred.

4.7. Rhone Merieux – DOG MRX 23/950406– May 1995

Topical concentrate containing 9.7% fipronil applied to juvenile dogs at 1x, 3x and 5x recommended dose for a total of six applications each 28 days apart (DOG MRX 23/950406).

A topical spot-on formulation of fipronil was applied to 40 pure-breed Beagle dogs (20 males and 20 females). The dogs weighed between 2.9 and 5.8 kg and were approximately 10 weeks old at the start of the study. The dogs were randomly divided into five groups, each containing 4 male and 4 female dogs: Control 1 (un-dosed); Control 2 (dosed with vehicle only); Treatment Group 1 dosed at 13.3 mg/kg (~ 1x recommended dose); Treatment Group 2 dosed at 39.9 mg/kg (~ 3x recommended dose); Treatment Group 3 dosed at 66.5 mg/kg (~ 5x recommended dose). Each dog was treated at the appropriate dose rate (treatment or control) for six applications separated by 28 days.

At the end of the study, there were no signs of reaction at the site of application. There were also no reported significant differences between treated and control animals for general health, behaviour, body weight change, food consumption, haematology and biochemistry. There were no reports of localized macroscopic changes or histopathology associated with the treatment.

Reviewer's comments – A more detailed examination of the pathology reports reveals that three histopathological changes were reported by the pathologist: (i) superficial dermal inflammatory cells; (ii) parafollicular inflammatory cells; (iii) parakeratosis. These reports appeared spread evenly over the male dogs, while in the females, one dog exhibited (i) and (ii) and one dog had (ii) in the un-dosed controls, while two dogs in the 1x dose showed condition (ii), three dogs in the 3x group had (i) and at least three dogs in group 5x had one (i) and/or (ii)

– it should be noted that one pathology report is missing from the study. In addition, a number of dogs were reported to be rubbing their necks after treatment. This was one male and two females in the vehicle-only control, three males and one female in the 1x group, one female in the 3x group and three males plus at least 1 female (one report missing) in the 5x group. This is a concern for a topically-applied formulation and suggests that there is some degree of contact irritation. This phenomenon may be related to the vehicle and not the active ingredient, but may cause concern and possibly adverse effect reports, particularly from animals that scratch excessively.

4.8. Rhone Merieux – SAF012 – January 1997

Tolerance of RM1601C/35 (Frontline Spray) at a dose rate of 6 ml/kg in kittens before weaning.

A 0.25% spray formulation (RM1601) was applied to 19 kittens (2-7 days old) in two applications, with a 28 day interval between applications. Since these kittens were not weaned, the Queens were also treated. A further 20 kittens were used as untreated controls, making a total of 39 kittens used in the trial. The dose rate used (6 ml/kg) was at the upper limit of the recommended dose rate (3-6 ml/kg).

There were no significant differences between treated and control animals in any of the parameters measured. There was a slight but non-significant increase in body weight in treated kittens, plus a slight increase in the liver enzyme, ALP. There were no obvious differences between treated and control kittens in clinical health, including evidence of diarrhoea, nasal discharge and ocular signs. Overall, the formulation appeared to be well tolerated and did not induce any obvious adverse effects.

Reviewer's comments – This was a well-performed study in what could be considered a relatively sensitive test group, namely young kittens. It was a concern that all of the animals had a packed cell volume (PCV) below normal and indicated an underlying anaemia within cats provided for this trial. An ideal study design would have selected for animals in perfect health, although the reviewer recognizes that this may not always be feasible in studies of this nature. A further concern was the evidence of erythema in the skin of a number of the kittens which again suggest that overall health was less than optimal. Obviously, the control animals were under the same conditions but it would be expected that fully healthy animals would have been used in this study. However, the test results suggested that the spray formulation of fipronil had no obvious adverse effects in a comparatively sensitive test group, as compared to older animals.

4.9. Merial Limited – CAT PR&D 0023301– September 1999

Topical concentrate containing 10.0% fipronil plus 12% (S)-methoprene applied to juvenile (eight week old) cats at 1x, 3x and 5x maximum recommended dose for two applications each 28 days apart (CAT PR&D 0023301).

A topical spot-on formulation of fipronil plus (S)-methoprene was applied to 48 short haired cats (24 males and 24 females). The male cats weighed between 0.6 and 0.9 kg and had a mean age of 56.2 days at the start of the study. The female cats weighed between 0.5 and 0.9 kg and

had a mean age of 56.0 days at the start of the study. The cats were randomly divided into four groups, each containing 6 male and 6 female cats: Control (un-dosed); Treatment Group 1 dosed at 0.5 mL/cat (~ 1x maximum recommended dose); Treatment Group 2 dosed at 1.5 mL/cat (~ 3x maximum recommended dose); Treatment Group 3 dosed at 2.5 mL/cat (~ 5x maximum recommended dose). Each cat was treated at the appropriate dose rate (treatment or control) for two applications separated by 28 days.

There were no significant differences between control and treatment groups for clinical observations and macroscopic observations at the treatment site for erythema, oedema, alopecia and hair coat condition. Some slight pruritus was noted in one cat in the 3x treatment group and four cats in the 5x treatment group in the 24 hours after initial application, although this was attributed to clumping of the hair coat. Some elevation of rectal temperature was also reported in cats receiving treatment. A frequent report of 'skin flakes' was noted in treated cats compared to controls. A significant increase in plasma urea concentration was noted in the 3x treatment group, compared to controls. This was suggested by the study authors to be unrelated to treatment since this was not found in the other treatment groups. There was no microscopic or histopathological examination of skin performed in these cats.

Reviewer's comments – As above, it is difficult to give a definitive opinion on the safety of a topically applied treatment without histopathology data. However, the reviewer recognizes that suitable representative samples of skin are difficult to collect in a study of this nature and are therefore probably not justifiable to collect from the animals in this study. There appear to be some obvious treatment-related reactions which may not be dose related. Larger numbers of cats in each group may have been used to conclusively demonstrate whether pruritus, skin clumping and 'skin flakes' were related to treatment.

4.10. Merial Limited – DOG PR&D 0020201– June 2000

Topical formulation containing ML-2,095,988 509T (10.0% fipronil plus 9% (S)-methoprene) in adult female dogs during pregnancy and lactation at one and three times the maximum recommended dosage.

A topical spot-on formulation of fipronil plus (S)-methoprene was investigated in 36 adult female dogs, with 12 dogs receiving the formulation at 1x recommended dose rate, 12 dogs receiving 3x recommended dose rate and 12 dogs were used as a control (no treatment). Treatments were applied every 28 days to the two treatment groups from the start of the trial, through mating and during pregnancy and lactation (until pups were about 41-43 days old).

The study report indicated that the fipronil formulation was well tolerated at both dose rates in the dogs. The animals generally appeared healthy throughout the trial. There was a significantly lower number of pups born and number of live pups in the 1x treatment group, which may have been related to the reduced incidence of vaginal discharge in this group, compared to controls, plus the reduced incidence of abnormal faeces in the pups, compared to controls. This difference was not observed with the 3x group and was therefore concluded not to be related to treatment. Importantly, there was a significantly greater incidence of local reaction, which consisted of mild local erythema, for up to 6 hours in both treatment groups. Overall, the treatment appeared to be well tolerated by the bitches at both 1x and 3x dose rates and there did not appear to be any significant effects of treatment on the puppies.

Reviewer's comments – The significant increase in puppy mortality or viability in the 1x treatment group was unexpected, but unlikely to be related to treatment since there were no significant differences in reproductive return between the 3x treatment group and the control animals. It would therefore appear that the treatment had no adverse effects on reproductive indices, such as litter sizes and viability. As a general comment, this particular trial was poorly written up and difficult to follow.

4.11. Merial Limited – CAT PR&D 0020301– June 2000

Topical formulation containing ML-2,095,988 509Q (10.0% fipronil plus 12% (S)-methoprene) in adult female cats (domestic short haired breed) during pregnancy and lactation at one and three times the maximum recommended dosage.

A topical spot-on formulation of fipronil plus (S)-methoprene was investigated in 36 adult female cats, with 12 cats receiving the formulation at 1x recommended dose rate, 12 cats receiving 3x recommended dose rate and 12 cats were used as a control (no treatment). A further 24 male cats were involved in the study for breeding purposes, with two males selected to join each group of three females as a breeding unit. Treatments were applied every 28 days to the two treatment groups from the start of the trial, through mating and during pregnancy and lactation (until kittens were about 42-44 days old).

The study report indicated that the fipronil formulation was well tolerated at both dose rates in the cats and kittens. The animals generally appeared healthy throughout the trial. There were a significantly higher number of kittens born with abnormalities in the control group. There was a significantly higher incidence of flaky skin in both treatment groups, compared to controls. Overall, the treatment appeared to be well tolerated by the Queens at both 1x and 3x dose rates and there did not appear to be any significant effects of treatment on the kittens.

Reviewer's comments – As in some of the other studies included in this review, the treatment induced significant local reaction which, in this trial, consisted of flakey skin. A confounding factor in this study was that all cats appeared to have some underlying skin inflammation and/or irritation. This was not ideal in terms of a ideal study design to investigate the safety of a topically-applied formulation.

4.12. Merial Limited – DOG PR&D 0002101– August 2000

Topical concentrate containing 10.0% fipronil plus 9% (S)-methoprene applied to juvenile (eight week old) dogs at 1x, 3x and 5x maximum recommended dose for six applications each 28 days apart (DOG PR&D 002101).

A topical spot-on formulation of fipronil plus (S)-methoprene was applied to 48 pure-bred Beagle dogs (24 males and 24 females). The dogs weighed between 1.17 and 3.31 kg and had a mean age of 55.4 days at the start of the study. The dogs were randomly divided into four groups, each containing 6 male and 6 female dogs: Control (un-dosed); Treatment Group 1 dosed at 0.133 mL/kg (~ 1x maximum recommended dose); Treatment Group 2 dosed at 0.399 mL/kg (~ 3x maximum recommended dose); Treatment Group 3 dosed at 0.665 mL/kg (~ 5x

maximum recommended dose). Each dog was treated at the appropriate dose rate (treatment or control) for six applications each separated by 28 days.

There were no reported differences between control and treatment animals for clinical observations, rectal temperature, body weight, haematological or clinical chemistry parameters. There were also no reported signs of macroscopic or microscopic changes at the treatment site.

Reviewer's comments – More detailed examination of pathological reports suggested widespread minor focal epidermatitis in many animals (control and treated). Two animals were also reported as having pruritus following treatment. It is always a concern when the test subjects have underlying skin inflammation or irritation prior to undertaking a study of a topical formulation. An ideal study design would use animals without any pre-existing skin conditions to avoid masking any potential effects of a topically-applied formulation.

4.13. Merial Limited –PR&D 0045801– September 2001

Topical formulation containing 10% fipronil and 9% (S)-methoprene in dogs at 1x, 3x and 5x recommended dose rate (FINAL REPORT).

A single topical application of fipronil was applied to 48 Beagle dogs (24 males and 24 females, weighing 2 ± 1.5 kg). The dogs were approximately 8 weeks old (56 ± 7 days) at the commencement of the trial. The dogs were separated into 4 groups of 12 dogs (6 males and 6 females), consisting of a control group (no treatment), and groups of 1x, 3x and 5x recommended dose rate. The trial was well conducted and under the guidelines of Good Laboratory Practice (GLP). There was no effect of the treatment on body weight, although it is expected that young dogs are in a rapid growth phase. There were also no significant differences between control animals and treatment groups in food and milk consumption and haematological and clinical chemistry measurements. There were no obvious localised reactions at the treatment site. Some evidence of focal dermatitis and epidermatitis was reported in treated animals, although this was not statistically different from control animals and was not considered a result of treatment.

Reviewer's comments – The dogs used in this trial could not be considered indicative of the entire population of dogs intended to be treated with fipronil-containing products – they are of one breed, young and with pre-existing evidence of minor skin inflammation (possibly related to environmental factors, including being housed in cages). These animals may be a useful 'sentinel' group, being more likely to exhibit systemic reaction to a topically applied therapy and, as such, may be a reasonable subject for the trial. However, combined with the relatively small numbers in each group, discerning statistical differences in local and skin-related effects following treatment may be difficult.

5 Discussion of Safety Studies

All of the studies presented to this reviewer were, in general, well conceived and performed. The reports were thorough and comprehensive. The studies used a thorough and wide range of

parameters, including haematology and clinical chemistry, to monitor animal health. However, there were several issues in the studies that were of concern and these will be addressed separately.

5.1 Breed

The majority of the animals used in the safety studies were of the same or similar breed. The Beagle was the dog used in all but one study where nursing puppies were the animals used. The cats were more difficult to determine, although it appeared that cross breed and primarily short haired cats were used in the safety studies. As a group, all dogs were considered small and short-haired. The largest animals used had a mean weight of 11.5 kg, while many were ≤ 5 kg. The reviewer recognizes that these conditions are standard for pen trials and are useful to minimize inter-animal variation which, in turn, will minimize the numbers of animals required. This is an acceptable approach to satisfy welfare concerns (reduce, replace, refine) with research studies of this nature. Larger field trials subsequent to satisfactory pen trials would be expected to involve multiple breeds and address any concerns related to inter-breed variation.

5.2 Age

All animals in these safety studies could be considered as young animals. In two studies, animals were approximately 8 to 9 months old, but the rest of the studies were in animals 12 weeks old or less. It may be expected that these may be a higher risk group for medications (Nagle 2006) and the studies have targeted this aspect. However, these are also rapidly growing animals, so any effects on body weight may be not discernable due to the rapid growth rate. Furthermore, the skin is still developing and may be more viable than adult skin and better able to adapt to topically applied medications (the converse may also be true *i.e.* they may be more susceptible to topically applied medications) (Nagle 2006). Most importantly, there were no studies in animals that could indisputably be classified as adults (at least 12 months old).

5.3 Existing skin condition

A large number of the animals in many of the studies had some underlying evidence of histopathology in the skin (e.g. DOG PR&D 002101). This could be attributed to the young age of the animals (Nagle 2006). It could also be attributed to the fact that these animals were (mostly) purpose bred and maintained within cages in groups. However, the pre-existing high incidence of relatively minor dermal and sub-dermal changes, such as focal epidermatitis, increased prominence of mast cells and the presence of superficial dermal inflammatory cells, make it difficult to determine if there are any statistically significant localised effects following topical application of fipronil-containing products.

5.4 Group size

There were relatively few numbers in each treatment group in most of the studies reviewed, although this is acceptable for pen studies of this nature. Statistical analysis can be misleading in studies with limited numbers of animals within each treatment group and I would be more comfortable with an independent statistician to review these studies in light of the comments above to determine if sufficient animals have been used. In particular, the number of reports of pruritus and local evidence of alopecia or histopathology were frequently classified as not related to treatment if they only occurred in one dose range and not others. Larger numbers of animals may indicate whether these incidences are significant. For example, one study reported a

significant increase in plasma urea concentrations in cats from one treatment group, but not others. It was concluded by the study authors that this was not related to treatment because it was not significant in higher dose treatment groups.

5.5 Site reaction

One primary concern regarding the safety of fipronil-containing products was the effect on the site of application following topical application. As mentioned above, this opinion is difficult to verify statistically due to relatively small numbers of animals in each treatment group and an underlying incidence of histopathology, albeit minor, in test animals. It was also evident that local irritation or reaction to treatment may not be dose related and, most importantly, may also be associated with the vehicle and not the active ingredient. It was disappointing that two studies (CAT94424 and DOG94423) did not investigate histopathological changes following application. However, there was one report of alopecia following application of the vehicle (CAT MRX 24/950746), while it was apparent from reported clinical observations that some dogs were pruritic after topical application of the spot-on. This was consistent with at least one study (DOG PR&D 0020201) where the treatment appeared to induce local inflammatory changes over the site of application.

In study DOG MRX 23/950406, a number of dogs were rubbing or scratching the application site after treatment, even in vehicle-only groups. This may be related to general irritation at having a liquid applied to the neck region or could be related to a local reaction to the vehicle and/or active ingredient. The only method to resolve this issue would be to apply a liquid (non vehicle), vehicle or spot-on (at the recommended dose) to a larger number of dogs from different breeds and ages.

In addition to pruritus, there may be some incidence of neurological signs following topical application of fipronil. Three cats displayed nervousness or aggression following administration of fipronil spray, although the report summary suggested that this was not related to treatment. One concern with topical application of any spray is the potential for aerosol production and inadvertent inhalation. Absorption of fipronil into mucosa is higher than through skin and it is possible that the cats may be demonstrating systemic signs of fipronil toxicosis, but the possibility of the cats reacting to the alcohol in the spray or to the mere act of being sprayed cannot be determined from the studies presented to date. Again, greater numbers of animals in the study may confirm or reject this possibility. Neurological signs in dogs from the safety studies appeared limited to anxiety related to pruritus.

Some dogs are naturally predisposed to scratch and application of any liquid may initiate scratching and rubbing of the neck. However, anecdotal evidence from ADE reports (APVMA website) suggested that some dogs resent subsequent treatment with topical fipronil-containing products and associated the spot-on with local irritation. It was also noted that ADEs involving dogs were frequently associated with acute moist dermatitis (“hot spots”). Most importantly, trauma to the skin caused by scratching will remove the stratum corneum, the uppermost layer of the skin. The stratum corneum is the primary barrier to drug (and water) movement through skin and it is conceivable that loss of this protective barrier may lead to systemic absorption of topically applied products.

6 Conclusions

An overall conclusion from the safety studies provided that fipronil-containing products appeared safe when applied topically to dogs and cats. This conclusion was strengthened by the safety studies in young and nursing animals where a higher incidence of ADEs may be expected. Effects on body weight were difficult to determine because all young animals would be expected to normally have a good appetite and gain bodyweight, although this can be compared with negative controls). There does, however, appear to be a possibility of local skin reactions following treatment. Several animals in the safety studies exhibited reactions which included alopecia, irritation or pruritus, scurfs or dandruff appearing and underlying histopathological reactions. It is inadequate to suggest that this was not related to fipronil if a similar effect was not detected in another animal at a higher dose. Effects of fipronil-containing products, particularly at the site of application, may not be dose related and may represent individual sensitivity to active constituent and/or vehicle.

Local skin reactions are unlikely to endanger the overall health of the animal but may be of concern to owners. Alopecia and skin damage would be of particular concern to owners of show animals where hair loss or changes to hair coat is likely to prevent the animal competing. Owners may also be expected to be distressed if animals scratch a lot, particularly if this leads to self-mutilation and dermatitis. It is also possible that ADE reports may include conscious perception of treatment when applied, with some animals showing varying degrees of resentment for subsequent applications.

To confirm or reject a definitive link between fipronil (and/or vehicle) and local skin reactions, it would be necessary to undertake larger trials using animals ranging in breed, gender and age. (Field efficacy trials may be able to provide some supporting information on this issue). In addition, continued assessment of the incidence of local skin reactions in ADE reports as part of pharmacovigilance procedures is warranted.

7 References

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