

## Safety Studies



Advocate for dogs and cats is a low volume topically applied product containing two active ingredients – imidacloprid and moxidectin – both displaying excellent safety profiles. The safety studies for these individual active ingredients are highlighted in this section, followed by an overview of the specific safety studies conducted with Advocate.

### *Imidacloprid*

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#### **Puppies and Kittens**

Over the past decade, imidacloprid has been extensively tested for both efficacy and safety<sup>1</sup> and has demonstrated a favorable safety profile in the field. First introduced as a novel topical flea control product in 1996, imidacloprid has been widely used throughout the world in dogs and cats of all ages.

Target animal safety studies to establish safety of imidacloprid 10% spot-on (Advantage) in 10 day old puppies (weighing less than 1 kg) were conducted with dermal application of 200 mg imidacloprid/kg b.w., which is 20 times the recommended dosage. Even at such elevated dosages, no adverse events were observed in these young puppies. The effect of repeated dosing in young growing dogs was tested by dermal application of 10 mg/kg b.w. (recommended dose) or 50 mg/kg (five times the recommended dose) at 7 day intervals for eight consecutive weeks. The repeated weekly treatments at both the recommended and elevated doses were tolerated by all puppies, starting as young as eight weeks of age.

In elevated dosing studies in 12 - 13 week-old kittens, dermal application of 80 mg imidacloprid/kg b.w. (eight times the recommended dosage) did not result in untoward clinical signs. Additionally, treatment with 240 mg imidacloprid/kg b.w. in 6 - 10 week-old kittens did not cause adverse effects. Single treatment of 80 mg/kg (eight-fold the recommended dosage of imidacloprid) was tolerated by weaned kittens without clinical signs, either local or systemic; the minimum weight of treated kittens was 1.1 kg. To assess possible effects of repeated overdosing in 6-week-old kitten, dermal application of 180 - 239 mg imidacloprid was done two times at two week intervals. This repeated treatment also did not cause any side effects in these young kittens (Table 1).

These studies demonstrated that dosages as high as 20 and 24 times the recommended label dosage in puppies and kittens, respectively, did not cause adverse effects, underscoring the safety of imidacloprid in pets. The combination of potent flea activity and favorable tolerability in very young animals has made imidacloprid an excellent flea control product for puppies and kittens.

## Adult Dogs and Cats

Required target animal safety studies in adult dogs were conducted with the application of ten-fold the recommended dose, 100 mg imidacloprid/kg b.w. This treatment was tolerated without clinical signs occurring during a 72 hours post-treatment observation period. Additionally, dermal application of 200 mg imidacloprid/kg b.w. (20-times recommended dose) did not cause any side effects in treated dogs. The effect of repeated overdosing was tested by daily dermal application of 50 mg imidacloprid/kg b.w. (5x) to dogs for three consecutive days, and also did not result in treatment-related side effects.

Safety studies in adult cats with a single treatment of ten-fold the recommended dose (100 mg imidacloprid/kg b.w.) indicated elevated dosing was tolerated without clinical signs. The effect of repeated overdosing was tested in adult cats using 50 mg imidacloprid/kg b.w., five times the recommended dose, for three consecutive days. This repeated treatment at an elevated dosing rate did not cause any side effects.

**Table 1: Imidacloprid Safety Studies in Puppies & Kittens**

	Age	Dosage	Interval	Results
<b>Puppies</b>	10 days	200 mg/kg (20x)	Once	No effects
	8 weeks	10 mg/kg (1x) or	Every 7 days for 8 weeks	No effects
		50 mg/kg (5x)		
<b>Kittens</b>	6 weeks	180 - 236 mg/kg (18-24x)	Twice	No effects
	6 - 10 weeks	240 mg/kg (24x)	Once	No effects
	12 - 13 wks	80 mg/kg (8x)	Once	No effects

## Reproductive & Developmental Safety

### Reproductive toxicology and teratogenicity

The potential of imidacloprid for reproductive toxicity has been evaluated in standard reproductive studies in rats and rabbits<sup>2</sup>. These studies indicated that there is no evidence that imidacloprid causes primary embryotoxicity or teratogenic effects. The No Observable Effect Level (NOEL) for maternal and fetal toxicity in gestating rabbits given imidacloprid by oral gavage was 8 mg/kg b.w./day and 24 mg/kg b.w./day, respectively. In rats, the respective maternal and fetal NOELs with oral administration were 10 mg/kg b.w./day and 30 mg/kg b.w./day. No primary toxic effects on reproduction were discovered in a 2-generation rat study which resulted in NOELs for parent animals of 12.5/6.7 mg/kg b.w./day (males/females) and a NOEL for reproduction of 12.5 mg/kg b.w./day. These studies indicated a high level of oral tolerance for imidacloprid and absence of reproductive and teratogenic potential with normal exposure.

### Mutagenicity and genotoxicity

Tests used to exclude any potential mutagenic and genotoxic effects of imidacloprid ranged from bacteria and isolated mammalian cells to mammals in vivo<sup>2</sup>. Included in these studies were tests for point mutations, chromosomal aberrations and for DNA damage. The majority of tests with imidacloprid were negative, in contrast to the expected responses obtained with respective positive control substances. From these studies it was concluded that imidacloprid possesses neither mutagenic nor genotoxic potential. Additionally, long term feeding studies in rats and mice have demonstrated the lack of carcinogenic potential for imidacloprid.

## Pregnancy and Lactation

Dermal application of pregnant bitches with 10 to 30 mg imidacloprid/kg b.w. (up to three times the recommended dose) twice, at two to four weeks apart did not cause any side effects during various stages of pregnancy<sup>3</sup>. Dermal application to lactating bitches of doses up to 30 mg/kg b.w. twice, three to four weeks apart, as well as treatments at a rate of 20 mg imidacloprid/kg b.w. four weeks apart did not cause any side effects at various stages of lactation.

Topical application of 40 mg/kg b.w. (four times the recommended dose) twice during gestations and three times during lactation did not cause any side effects in any of the treated queens nor their kittens<sup>3</sup>.

## *Moxidectin*

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### Acute Safety

Acute oral tolerance was evaluated in 8 week old collie puppies given 30 µg/kg body weight moxidectin (10x the oral heartworm prophylaxis dosage) daily for 12 days. Results of the study indicated moxidectin was safe at up to 10 times the recommended dose in 8 week old puppies. Additionally, a study was conducted to evaluate the safety of moxidectin in ivermectin-sensitive Collies. These dogs were treated orally with 30, 60, and 90 µg/kg body weight (10x, 20x and 30x) of moxidectin. No signs

were evident in any ivermectin-sensitive dog at doses up to 30 times the recommended oral dose for heartworm prevention, suggesting a larger therapeutic index than other approved compounds. In another safety study, dogs with patent heartworm infections (*D. immitis*) were shown to have no adverse effects when treated at 1 and 5 times the recommended dose level (3 µg/kg and 15 µg/kg) for three consecutive monthly intervals<sup>4</sup>.

### Chronic Safety

Oral moxidectin was fed to dogs daily for a period of one year to determine chronic tolerance<sup>5</sup>. Levels of 0, 250, 500, or 1130 µg/kg/day were given in food and all animals observed for clinical signs, food intake, changes in body weight, hematology, serum chemistry, and urinalysis. No treatment-related clinical effects were observed at any dosing level (up to 300 times the recommended monthly dose) during the course of the study.

Long term feeding studies in laboratory animals have confirmed that moxidectin does not possess a carcinogenic potential<sup>5</sup>.

### Reproductive Safety

Studies were conducted in male dogs to evaluate the effects of oral moxidectin administration on male fertility, general reproductive performance and pre- and post-natal effects on sired litters, as compared to control (placebo-treated) dogs. There were no adverse effects with moxidectin at 3 times the recommended dose level for heartworm prophylaxis on fertility, reproductive performance, or on offspring of treated dogs. The safety of orally administered moxidectin was eval-

uated in breeding, pregnant and lactating dogs treated at 9 µg/kg b.w. in comparison to placebo, beginning prior to breeding and during gestation and lactation. There were no adverse effects on fertility, reproductive performance, or on offspring of female dogs treated with 3 times the recommended dose level of moxidectin until day 42 of lactation<sup>4</sup>.

## Advocate

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### Treatment of Puppies and Kittens

Target animal safety studies in puppies and kittens were conducted to evaluate the safety of repeated dermal application of Advocate. Puppies from 6.5 - 7 weeks of age and kittens from 8 - 9 weeks of age were treated with Advocate at one, three or five times the recommended label dose at two-weekly intervals (two times the normal frequency of application) for a total of six treatments. Treatment was tolerated in all groups without serious side effects, even at the highest elevated dosage<sup>6</sup>.

### Safety in Ivermectin-sensitive Breeds

Studies have been performed to demonstrate the safety of Advocate when applied to ivermectin-sensitive collies<sup>7</sup>. Ivermectin-sensitive collies were treated topically with either three or five-fold the recommended label dose, or placebo, for three consecutive treatments at 28 day

intervals. The maximum dose rate tested represented an 8 to 13-fold increase above the standard 2.5 mg/kg moxidectin dose. Final observations of the dogs were made on day 98, which was 14 days after the final treatment application. There were no clinical abnormalities or adverse reactions observed in any of the dogs. The results indicated that Advocate was safe when applied at up to five times the maximum recommended dose to Collies that had tested positive for sensitivity to ivermectin. Additionally, controlled clinical field studies were conducted with Advocate that included Collie type breeds. None of the dogs in these studies demonstrated signs of adverse effects related to macrocyclic lactone sensitivity.

Oral administration of 10% of the recommended topical dose produced no adverse effects in ivermectin-sensitive Collie dogs. However, administration of 40% of the unit dose orally resulted in severe neurological signs. Due to the higher concentrations of moxidectin in Advocate (in relation to oral products), oral ingestion of Advocate should be avoided in ivermectin-sensitive breeds.

### Safety in Dogs with Patent Heartworm Infection

In order to establish the safety of Advocate when administered to dogs with a patent heartworm infection, studies were conducted in both artificially and naturally infected dogs<sup>8</sup>. Infected dogs were dosed with Advocate at the recommended labeled dose or five-fold the recommended dose rate at 14 day intervals for three consecutive treatments. No treatment-related adverse effects were recorded in any of the heartworm positive dogs treated with Advocate, even at doses up to five times the recommended dose rate. Based on these findings, it was con-

cluded that the treatment of heartworm positive dogs with Advocate is safe. In the interest of good clinical practice, it is recommended to test dogs for heartworm infection prior to treatment.

### General Dermal Safety

Placebo-controlled target animal safety studies in both cats and dogs were conducted to assess the general dermal tolerance of Advocate<sup>9</sup>. Cats of four to five months of age and dogs of seven to eight months of age were treated topically with 10 times the recommended dose of Advocate at one application, or with placebo. No adverse clinical signs or

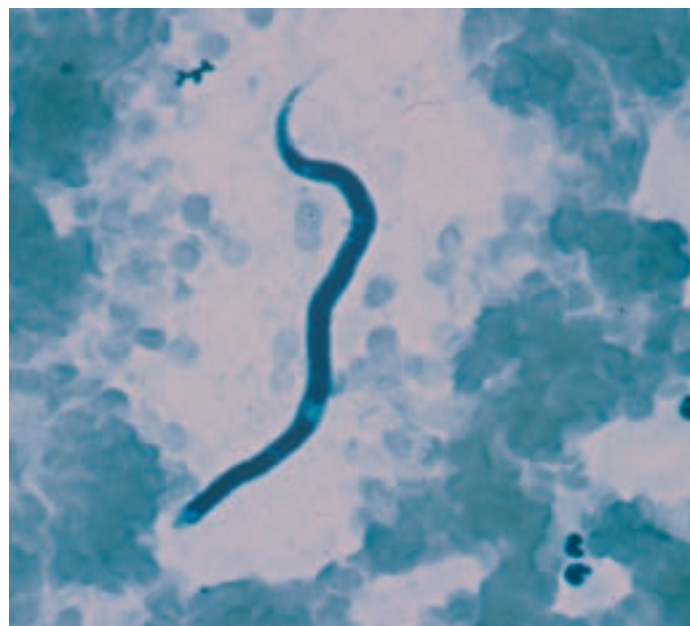


Figure 1: *Microfilaria of D. immitis* evident on a blood smear

significant effects were noted in any of the groups in both the cat and dog studies. It was concluded that the Advocate formulation is well-tolerated at up to 10 times the maximum recommended dermal application rate.

## Oral Ingestion Tolerance

Oral single dose studies with Advocate in adult cats and dogs demonstrated that oral administration of the recommended single dermal dose only caused transient and mild side effects (vomiting and salivation in cats; vomiting, and anorexia in some dogs and transient neurological effects in one dog)<sup>10</sup>. Side effects of a serious nature were not encountered. However, oral ingestion after dermal overdosing could be potentially harmful to kittens. Advocate is intended for dermal application only and pet owners should be instructed accordingly.

## General Safety

Studies with Advocate have not been conducted in pregnant and lactating dogs or cats. Laboratory studies in rabbits and rats with imidacloprid and moxi-

dectin individually have not shown any evidence of teratogenic, fetotoxic or maternotoxic effects. Due to the lack of reproductive effects of the individual active ingredients, it is unlikely that reproductive performance would be compromised in the target species of animals that are treated dermally with Advocate.

Pet owners and Veterinary Professionals are not at any significant risk when treating species of animals with Advocate. The results of the range of mutagenicity studies do not indicate a mutagenic potential and no results suggestive of any carcinogenic effects were obtained in the repeated dose studies.

Tests conducted in rabbits indicate that Advocate can be regarded as non-irritating to the skin, however, the formulation is considered an irritant to the eye.

### References

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### Further Reading

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