

Pharmacokinetics

Advocate combines imidacloprid and moxidectin in a unique topical spot on formulation. The following section summarizes the distribution characteristics of the individual active ingredients in previously-approved formulations, and the favorable kinetic properties of Advocate.

Imidacloprid

Topical

Initial efficacy trials with imidacloprid indicated the optimal results were achieved with a 10% solution at a target dose of 10 mg/kg¹. Further studies conducted with the resulting formulation (Advantage[®]) demonstrated that the compound is spread over the skin surface and throughout the hair coat of dogs and fur of cats within twelve hours after topical application² (Figure 1). These trials were performed by assaying the levels of imidacloprid in hair samples from treated animals from different parts of the body

at different time intervals following application. Due to the greater surface area of larger dogs, application at multiple spots maximizes the translocation and coverage of imidacloprid.

Detailed research has shown that following application, imidacloprid is localized in the water resistant lipid layer of the skin surface and is then spread over the body surface and onto the hair³. Studies using flea cages on cats and similar feeding studies in dogs have demonstrated that imidacloprid acts on adult fleas on contact and not by uptake from blood meals³. Imidacloprid is absorbed through the flea's intersegmental membranes leading to rapid nerve and muscle cell damage and death of the parasite⁴. This rapid killing of fleas on contact is especially advantageous for pets that suffer from flea allergy dermatitis.

The quick distribution of the compound correlates well to the rapid rates of flea elimination on treated pets following initial application of Advantage. Efficacy studies conducted in dogs infested with adult fleas and treated with imidacloprid demonstrated 89% efficacy against existing flea populations at eight hours post-application and 100% efficacy at 12 and



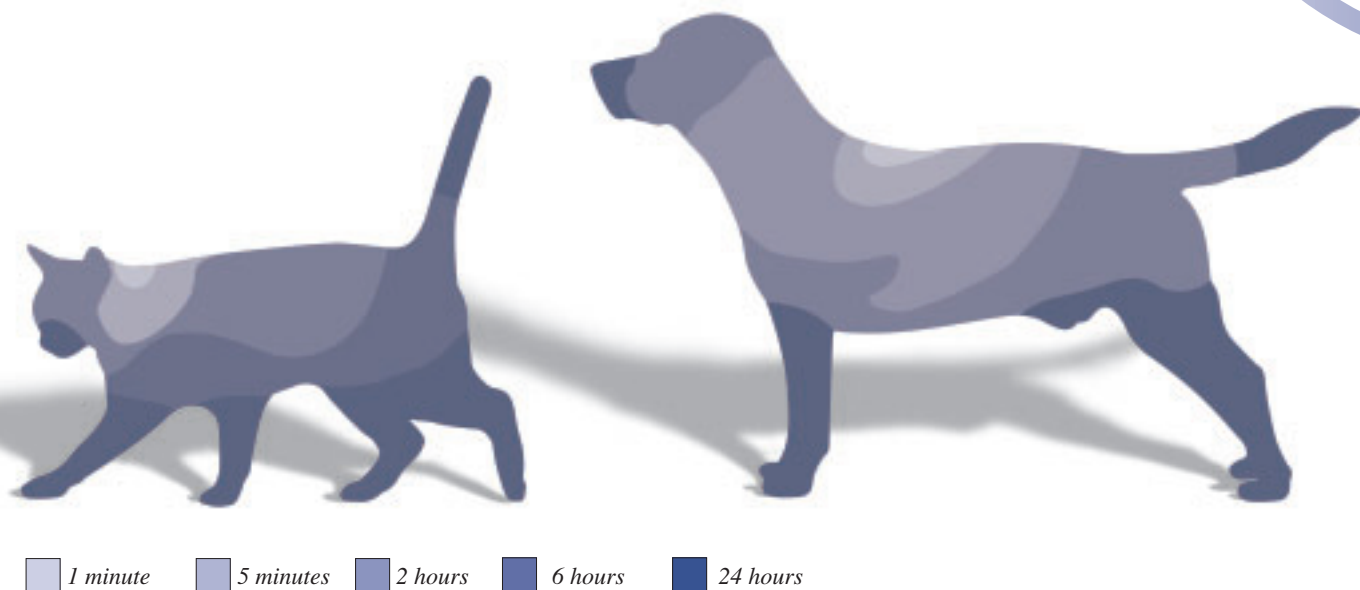


Figure 1: Distribution of imidacloprid after topical application

24 hours following treatment⁵. Additional studies demonstrated efficacy of 98.5% as soon as six hours post-treatment⁶. Once the imidacloprid has spread, the rate of flea kill following weekly infestation is 93.3 to 100% of reinfesting fleas within only 2 hours of exposure on days 7, 14 and 21, and 100% by 8 hours on day 28.

in the feces⁹. The highest tissue levels were found in the fat, with residues depleting over time after treatment, indicating a lack of bioaccumulation. Two primary metabolites of moxidectin have been identified in target animals, however the parent compound accounts for 80 - 95% of levels found in tissues and via excretion.

Moxidectin

Moxidectin is a highly lipophilic compound with a high volume of distribution and longer elimination half-life than other approved macrocyclic lactones. The high lipophilicity of moxidectin – 100 times greater than ivermectin – contributes to its extensive tissue distribution; this likely facilitates deposition in adipose tissue, which may act as a drug reservoir for a prolonged period of time^{7, 8}.

Metabolism studies with radiolabeled moxidectin in rodents and other species (cattle, sheep and horses) indicate that moxidectin is almost exclusively excreted via the bile and then eliminated

Topical

Advocate is the first product for dogs and cats to incorporate moxidectin in a unique topical formulation. Following the convenient spot-on application of Advocate, moxidectin is extensively absorbed through the dermis and widely distributed within the body. Multiple pharmacokinetic studies were conducted in both dogs (moxidectin 2.5%) and cats (moxidectin 1%) to evaluate the transdermal absorption of moxidectin, as well as distribution and elimination characteristics¹⁰. Table 1 lists results of two studies evaluating the pharmacokinetics of moxidectin following a single application of the recommended dose of Advocate in dogs and cats, respectively¹¹. Maximum

serum concentrations are reached in dogs within 4 - 9 days, and in cats by approximately 1 day. These studies also indicated a very high volume of distribution for moxidectin with the topical formulation: approximately 80 l/kg in cats, and approximately 70 l/kg in dogs, based on geometric mean values. The high moxidectin concentrations and long elimination half-life observed with application of Advocate provide prolonged activity against target internal and external parasites (Figures 2 & 3). Additionally, studies evaluating the pharmacokinetic behavior of moxidectin after multiple applications have indicated that steady state serum levels are achieved following 3 monthly treatments of Advocate in cats,

and after approximately 4 - 5 consecutive monthly treatments in dogs¹² (Figure 4). Mean serum concentrations that can be expected under steady state conditions would be approximately 36µg/l in the dog and approximately 9 µg/l in cats.

Dose Determination Studies

The dose-rate and concentration of imidacloprid in the Advocate formulation was adopted from the previously-approved and proven topical product, Advantage. The dose of moxidectin was determined by a number of dose-response studies conducted with different concentrations of moxidectin added to the 10%

Figure 2: Serum levels of moxidectin in dogs following topical application of Advocate (imidacloprid 10%/moxidectin 2.5%)

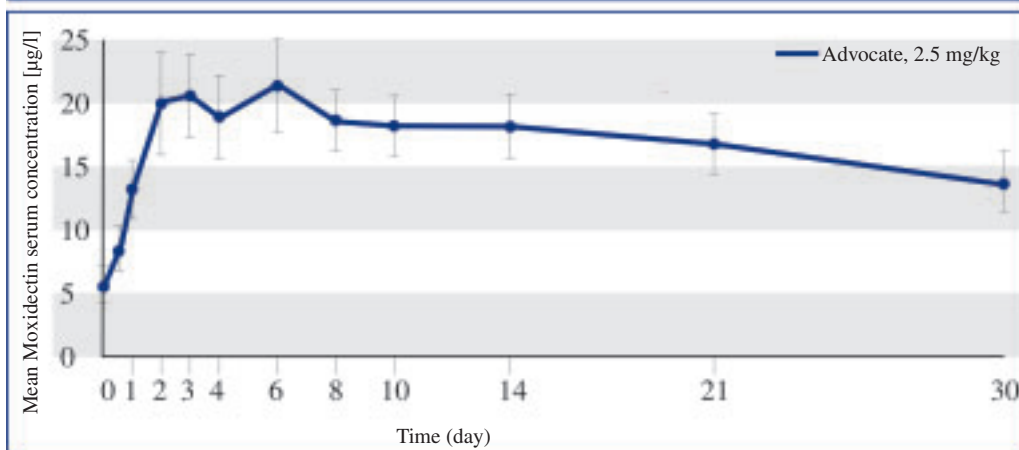


Figure 3: Serum levels of moxidectin in cats following topical application of Advocate (imidacloprid 10%/moxidectin 1%)

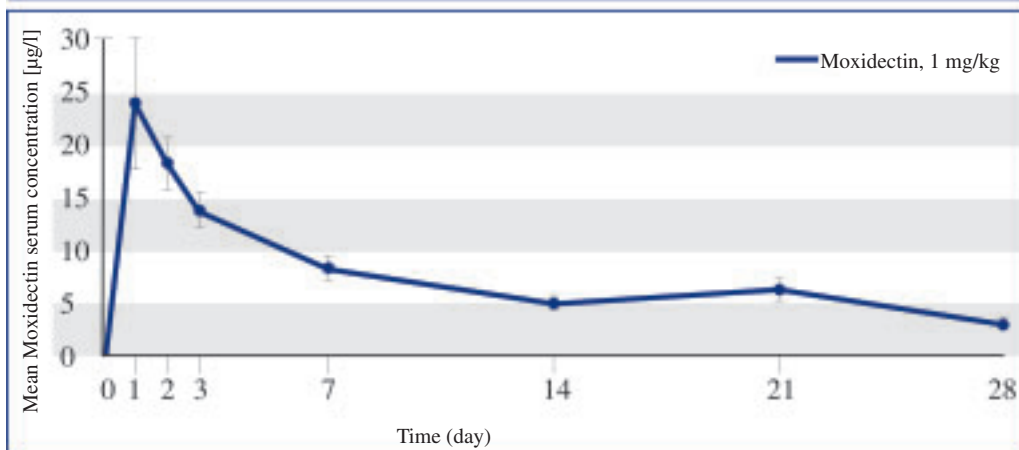


Table 1: Pharmacokinetics of Topical Moxidectin (Advocate)

| | C_{max} ($\mu\text{g/l}$) | T_{max} | $T_{1/2}$ | AUC ($\mu\text{g} \times \text{d/l}$) |
|--------------------------------|-------------------------------|-----------|-----------|---|
| Dog 2.5 mg/kg topically | | | | |
| Moxidectin 2.5% | 15.3 | 9 days | 35 days | 279 |
| Cat 1 mg/kg topically | | | | |
| Moxidectin 1% | 24.5 | 1.4 day | 14.9 days | 208 |

imidacloprid formulation. Because moxidectin is highly effective against larval *Dirofilaria immitis* at low dosages, this was recognized to be a non-dose limiting parasite. Therefore, the initial studies were conducted evaluating *Toxocara canis* and *Ancylostoma caninum*. Based on the results of these studies, a concentration of 2.5% moxidectin was chosen for dogs, at a dosing rate of 0.1ml/kg body weight. This dosing rate, which provides a minimum of 2.5 mg/kg body weight moxidectin, combines a wide margin of safety with exceptional clinical efficacy. Table 2 summarizes the results of the efficacy studies with 2.5% moxidectin for the dose-limiting species¹³.

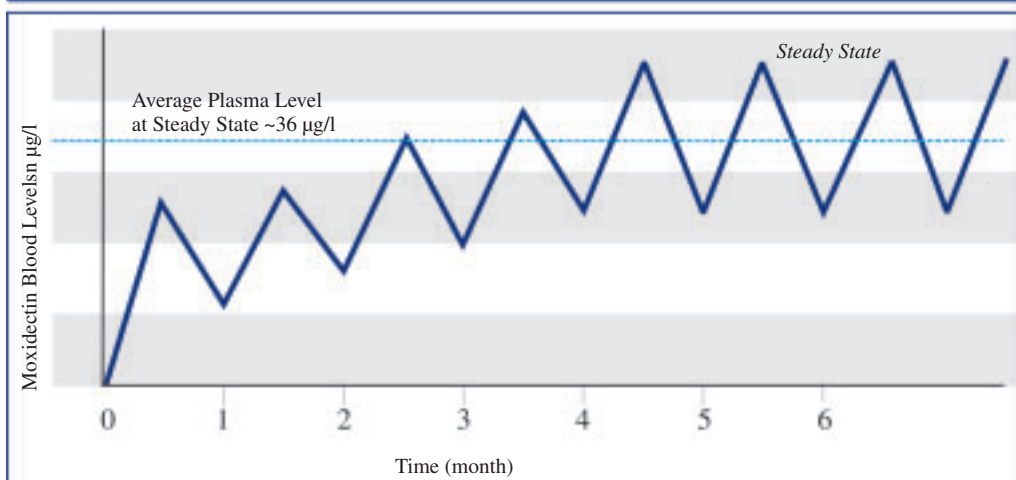
Dose determinations were also conducted for cats, using the dose-limiting parasites *Ancylostoma tubaeforme* and *Toxocara cati*¹⁴. Due to differing species characteristics, a formulation containing

1% moxidectin and providing a minimum dose rate of 1 mg moxidectin per kilogram body weight in cats provided efficacy equal to that seen with the canine formulation (Table 3).

Advocate

Dose Confirmation Studies

Studies with Advocate have shown that the two active ingredients – imidacloprid and moxidectin – do not interact and instead perform as if they were single component formulations. In order to confirm this performance of the combined active ingredients, controlled laboratory and field evaluations were conducted that validated the efficacy of the Advocate formulation for major parasites in dogs

Figure 4: Steady State characteristics of Moxidectin with monthly applications of Advocate

and cats. The following section highlights the results of some of these dose confirmation studies¹⁷.

Fleas

A number of controlled laboratory studies using weekly flea infestations in both dogs and cats were conducted to compare the efficacy of Advocate for fleas to that of imidacloprid topical solution alone. The imidacloprid/moxidectin combination of Advocate for dogs achieved efficacy of > 99% and >95% from day 1 to 35 in the studies¹⁵. In cats, the combination of imidacloprid/moxidectin also achieved very high flea control, with 100% efficacy on Day 1 and > 97% efficacy on day 28, one month following treatment¹⁶. These results demonstrated that the presence of moxidectin does not interfere with the high level of flea control expected with imidacloprid (Table 4).

In addition to the laboratory studies, multi-center clinical studies in veterinary practices in Europe also compared the efficacy of Advocate to that of imidacloprid topical solution (Advantage). Both products produced similar results in dogs and cats, with exceptional flea control for up to 35 days following treatment. These results again confirmed that moxidectin



Figure 4: Cat flea

does not interfere with the excellent activity of imidacloprid when the two actives are combined and Advocate provides a high level of flea control against existing flea burdens and subsequent reinfestations.

Internal Parasites

Dose confirmation studies were conducted in Europe and the United States to demonstrate the efficacy of the canine and feline Advocate formulations for

| <i>Table 2: Advocate Canine Dose Response Studies</i> | | |
|---|---------------------------|-----------------|
| | Percentage worm reduction | |
| | <i>A. caninum</i> | <i>T. canis</i> |
| Imidacloprid 10% Moxidectin 2.5% 0.1 ml/kg | 100 | 98.2 |

| <i>Table 3: Advocate Feline Dose Response Studies</i> | | |
|---|---------------------------|----------------|
| | Percentage worm reduction | |
| | <i>A. tubaeforme</i> | <i>T. cati</i> |
| Imidacloprid 10% Moxidectin 1% 0.1 ml/kg | 100 | 98.4 |

Table 4: Flea Efficacy Confirmation for Advocate in Dogs & Cats

| | % Efficacy (percentage of flea reduction) | | | | |
|-----------------|---|--------|--------|-------|-------|
| | 1 | 7 | Day 14 | 21 | 28 |
| Canine Advocate | 99 | 99-100 | 99-100 | 99 | 97-99 |
| Feline Advocate | 100 | 98 | 98-100 | 89-97 | 97-98 |

Table 5: Advocate Efficacy Confirmation for Endoparasites in Dogs and Cats

| | Adult Nematodes | % Efficacy |
|------------------------|------------------------|------------|
| Canine Advocate | | |
| Ascarids | <i>T. canis</i> | 96 - 100 |
| | <i>T. leonina</i> | 97 - 100 |
| Hookworms | <i>A. caninum</i> | 100 |
| | <i>U. stenocephala</i> | 100 |
| Heartworm | <i>D. immitis</i> | 100 |
| Feline Advocate | | |
| Ascarids | <i>T. cati</i> | 98 - 100 |
| Hookworm | <i>A. tubaeforme</i> | 100 |
| Heartworm | <i>D. immitis</i> | 100 |

treatment of major gastrointestinal nematodes and prevention of heartworm disease. The results of these studies, summarized in Table 5, indicated that the imidacloprid in the Advocate formulation does not interfere with the activity of moxidectin, and that the product is a highly effective anthelmintic against gastrointestinal nematodes and is an effective heartworm prophylactic in dogs and cats at the respected selected dosages.

References

- Hopkins TJ, Kerwick P, Gyr P, Woodley I. Efficacy of imidacloprid to remove and prevent *Ctenocephalides felis* infestations on dogs and cats. *Aust Vet Practit*, 1996, 26(3):150-153.
- Fichtel M. Investigations of the effect of the adulticide imidacloprid against the cat flea *Ctenocephalides felis* on cats and dogs. Dissertation, Free University of Berlin, School of Veterinary Medicine, Berlin, Germany, 1998.
- Mehlhorn H, Mencke N, Hansen O. Effects of imidacloprid on adult and larval stages of the flea *Ctenocephalides felis* after in vivo and in vitro application: a light- and electron-microscopy study. *Parasitol Res*, 1999, 85:625-637.
- Mehlhorn H. Mode of action of imidacloprid and comparison with other insecticides (i.e. fipronil and selamectin) during in vivo and in vitro experiments. *Suppl Compend Contin Educ Pract Vet*, 2000, 22(4A):4-8.
- Cruthers L, Bock E. Evaluation of how quickly imidacloprid kills fleas on dogs. *Suppl Comp Cont Educ Pract Vet*, 1997, 19(5):27.
- Everett R, Cunningham J, Arther R, Bledsoe DL, Mencke N. Comparative evaluation of the speed of flea kill of imidacloprid and selamectin in dogs. *Vet Therap*, 2000, 1:229-234.
- Hennessy DR, Alvinerie MR. Pharmacokinetics of the Macro cyclic Lactones: Conventional Wisdom and New Paradigms. In Vercruysse J, Rew RS (eds.): *Macrocytic Lactones in Antiparasitic Therapy*. CABI Publishing, Wallingford, UK, 2002, p.97-140.
- Vanapalli SR, Hung YP, Fleckstein L, Dzimianski MT, McCall JW. Pharmacokinetics and dose proportionality of oral moxidectin in beagle dogs. *Biopharm. Drug Dispos*. 2002, 23:263-272.
- Rock DW, DeLay RL, Gliddon MJ. Chemistry, Pharmacology and Safety: moxidectin. In Vercruysse J, Rew RS (eds.): *Macrocytic Lactones in Antiparasitic Therapy*. CABI Publishing, Wallingford, UK, 2002, p.75-96.
- 15. Bayer internal research studies, data on file.
- Arther RG, Bowman DD, McCall JW, Hansen O, Young DR. Feline Advantage Heart™ (imidacloprid and moxidectin) Topical Solution as monthly treatment for prevention of heartworm infection (*Dirofilaria immitis*) and control of fleas (*Ctenocephalides felis*) on cats. *Parasitol Res*, 2003, 90:S137-S139.
- Bayer internal research studies, data on file.

Further Reading

- Genchi C, Poglayen G, Kramer LH, Venco L, Agostini A. Efficacy of moxidectin for the prevention of adult heartworm (*Dirofilaria immitis*) infection in dogs. *Parassitologia*, 2001, 43:139-141.
- Krämer F, Mencke N. Imidacloprid. In, *Flea Biology and Control*. Springer-Verlag, Berlin, Germany, 2001, p. 98-106.