Skin Distribution of Imidacloprid by Microautoradiography After Topical Administration to Beagle Dogs*

Harish Chopade, PhD
David Eigenberg, PhD

Eric Solon, PhD
Paul Strzeminski, BS

Joe Hostetler, DVM

*Funding for this study was provided by Bayer Animal Health, Shawnee Mission, Kansas. A portion of this material was presented at the 2009 World Association for the Advancement of Veterinary Parasitology conference in Calgary, Canada, August 2009, and an abstract of the oral presentation was published in the conference abstract volume (abstract no. CS12.4, pp 28-29). Correspondence should be sent to Dr. Harish Chopade: phone, 913-268-2509; fax, 913-268-2135; e-mail, harish.chopade@bayer.com.

†Terry McNamara’s current affiliation is Exponent, Health & Environmental, 1150 Connecticut Avenue NW, Suite 1100, Washington, DC 20036.

To investigate the cutaneous distribution, localization, and persistence of imidacloprid in dogs, Advantage Topical Solution labeled with carbon 14 (14C) was topically applied as a single treatment at label rates and application pattern based on body weight to two adult beagles. One dog (8.5 kg) received 1.0 mL of the test solution at a single spot in the interscapular area (14 mg active ingredient/kg body weight); the second dog (12.3 kg) was treated with 2.5 mL of the test solution at four sites, each site receiving approximately 0.625 mL, along the dorsal thoracic and lumbar spine area (21 mg active ingredient/kg body weight). Samples of hair, skin surface residue, and skin taken from the application sites and/or distal body regions of the dogs at four intervals between 7 and 56 days after treatment demonstrated the migration of 14C radioactivity from the application sites to distal areas of the canine haircoat and skin. The 14C radioactivity concentrations in the skin biopsy and stratum corneum samples diminished steadily over 56 days after treatment. Microautoradiography of the skin showed focal concentrations of radioactivity in the superficial epidermis, hair follicles, and sebaceous glands. The presence of imidacloprid-derived radioactivity within hair follicles and sebaceous glands and on the skin surface is in good agreement with the reported efficacy of imidacloprid against fleas on dogs and cats for up to 1 month despite posttreatment bathing, shampooing, and/or swimming.
INTRODUCTION

Imidacloprid (1-[(6-chloro-3-pyridinyl)methyl]-N-nitro-2-imidazolidinimine; CAS No. 138261-41-3) is the active ingredient of Advantage (9.1% w/w or 10% w/v imidacloprid) Topical Solution (Bayer AG, Germany). Advantage Topical Solution is used as an efficacious treatment for the elimination and prevention of flea infestations on dogs and cats. The efficacy of spot-on imidacloprid (9.1% w/w) for flea control on cats and dogs has been reported in numerous laboratory studies; the minimum target dosage is 10 mg/kg body weight with monthly application intervals. Imidacloprid is a hydrophobic active ingredient with a low water solubility of 0.57 g/L at 20°C and an octanol/water partition coefficient of 3.7. The efficacy of topical imidacloprid against fleas is only minimally diminished by posttreatment bathing, shampooing, and/or swimming. The physicochemical properties of imidacloprid and its distribution across the canine haircoat and skin (currently thought to be via body oil) are assumed to be responsible for the proven efficacy of Advantage Topical Solution against fleas for 1 month. This study measured the precise distribution and persistence of imidacloprid in canine skin after a single application of Advantage Topical Solution to beagle dogs.

To study imidacloprid distribution in canine skin, it is imperative to understand the basic anatomic structure and functions of canine skin. Canine skin consists of the epidermis, dermis, and subcutaneous layer (or hypodermis). The outermost layer, the epidermis, is further subdivided into strata: the stratum basale (deepest), stratum spinosum, stratum granulosum, stratum lucidum (present only in footpads and the nasal planum and not evaluated in this study), and stratum corneum (most superficial). The functions of canine skin include homeostasis, protection (physical and immune), thermoregulation, sensation, and secretion/excretion. These functions are accomplished and supported by numerous cellular elements and adnexal structures, including keratinocytes, Langerhans cells, CD (cluster of differentiation) cells, mast cells, melanocytes, dermal fibers (collagen, reticulin, and elastin), interstitial ground substance, hair follicles, sebaceous glands, sweat glands, arrector pili muscles, blood vessels, lymph vessels, and nerves.

The objective of this nonclinical, nonguideline laboratory investigation was to demonstrate the localization, distribution, and persistence of radioactivity derived from imidacloprid labeled with carbon 14 (14C) within canine skin and hair after a single topical administration of [14C]imidacloprid solution at a label dose to two adult beagle dogs. For the first dog (study 1), the solution was applied as one spot to the interscapular region, and radioactivity was monitored at days 7 through 56 posttreatment. For the second dog (study 2), the solution was applied as multiple spots (interscapular, dorsal thoracic, and dorsal lumbar regions), and monitoring was performed from days 7 through 28 posttreatment. The mode of application (single or multiple spots) was according to the label for the body weight of the dog. In both studies, microautoradiography of skin samples was used to detect the posttreatment microanatomic location of 14C radioactivity on and within the canine skin.

This investigation was conducted at Toxicology and Environmental Research Laboratories of Bayer CropScience LP, Bayer Research Park, Stilwell, Kansas. This research facility is licensed by the US Department of Agriculture and fully accredited by the Association for Assessment of Accreditation of Laboratory Animal Care International. The study procedures, including care of the animals, were reviewed and approved by Bayer’s Institutional Animal Care and Use Committee before study initia-
tion. The studies were conducted in accordance with the approved study protocols and standard operating procedures and in compliance with the Good Scientific Practices of the research laboratories.

**MATERIALS AND METHODS**

Two studies differing in application sites (one-spot versus four-spot application), sampling sites, and sampling matrices were conducted in adult beagle dogs. According to the Advantage (9.1% w/w imidacloprid) Topical Solution label, adult dogs weighing ≤5 to ≥45 kg can receive from 8.8 to 20 mg imidacloprid/kg body weight based on unit dose application and dose banding.

**Animals and Husbandry**

Two clinically healthy beagle dogs (*Canis familiaris*) from a group of dogs supplied by Marshall BioResources, Inc. (North Rose, NY) were used in this investigation. At study initiation, the male dog (study 1) was 12 months of age and weighed 8.5 kg; the female dog (study 2) was 17 months of age and weighed 12.3 kg. On arrival at the research facility, the dogs were each identified with a unique ear tattoo and individually housed in stainless steel metabolism cages. A 4-week acclimation period preceded the treatment. Tap water and a commercial pelleted diet (2025C Teklad Global 25% Protein Certified Dog Diet, Harlan Laboratories) were available ad libitum. The animal room was maintained at 64°F to 84°F with a 12-hour light/12-hour dark cycle and 30% to 70% relative humidity for the duration of the study.

**Test Substance**

[Methylene-\(^{14}\)C]imidacloprid (Vial C-831A) was synthesized by Bayer CropScience, with a specific activity of 25.3 mCi/mmol or 99.2 µCi/mg (Figure 1).

**Dosing Solutions and Dermal Applications**

**Study 1**

For the treatment of one adult beagle dog (8.5 kg), commercial Advantage Topical Solution 20 for dogs weighing 10 to 20 lb (1.0-mL tube; imidacloprid 9.1% w/w per EPA label; Lot No. KP02XD4, supplied by Bayer Animal Health) was admixed with 2.0 mCi (20 mg) of \(^{14}\)C][imidacloprid. This resulted in the final 1.0-mL dosing solution containing 120 mg of imidacloprid. The specific activity of the dosing solution was calculated at 16.5 µCi/mg (36,630 disintegrations per minute [dpm]/µg). Before dosing, the radiochemical purity of this solution was verified to be >97% by means of a Ramona 90 (Raytest USA, Pittsburgh, PA)
high-performance liquid chromatography (HPLC) radioactivity detector.

Per the Advantage Topical Solution label, the topical dose for dogs weighing between 11 and 20 lb (5 to 9 kg) is 1.0 mL. In this study, hair at the cranial aspect of the interscapular area was parted and the total 1.0-mL dose was directly applied to the underlying skin at one spot per the label with a 1.0-mL tuberculin syringe. The test dog received approximately 233 µCi (14 mg of imidacloprid)/kg body weight. (According to the Advantage Topical Solution label, an 8.5-kg dog would normally be treated with imidacloprid at 11.8 mg/kg body weight.)

**Study 2**

For the treatment of one adult beagle dog (12.3 kg), commercial Advantage Topical Solution 55 for dogs weighing 21 to 55 lb (2.5-mL tube; imidacloprid 9.1% w/w; Lot No. ZY8316-08-2002, supplied by Bayer Animal Health) was admixed with 792 µCi (8 mg) of [14C]imidacloprid. This resulted in the final 2.5-mL dosing solution containing a total of 258 mg of imidacloprid. The specific activity of the dosing solution was calculated at 3,068 µCi/mg (6,811 dpm/µg). Before dosing, the radiochemical purity of this solution was verified at >97% by means of the Ramona HPLC radioactivity detector.

Per the Advantage Topical Solution label, the topical dose for dogs weighing between 21 and 55 lb (9.5 to 25 kg) is 2.5 mL. In this study, hair was parted at four sites along the dorsal thoracic and lumbar spine, and equal treatment volumes (approximately 0.625 mL/site) were applied with a tuberculin syringe directly to the underlying skin at multiple spots per the label. The test dog (12.3 kg body weight) received approximately 61.36 µCi (21 mg of imidacloprid)/kg body weight. (According to the Advantage Topical Solution label, a 12.3-kg dog would normally be treated with imidacloprid at 20.3 mg/kg body weight.)

**CLINICAL OBSERVATIONS**

The dogs were each observed once a day for general health throughout the study period.

**Collection of Hair, Tape Stripping, and Skin Samples**

The collection of hair, tape stripping, and skin samples from the test dogs was performed.

---

**Microautoradiography of the skin showed focal concentrations of 14C radioactivity in the superficial epidermis, hair follicles, and sebaceous glands.**
then collected from each site with a small dermal biopsy punch (0.2-cm diameter, Miltex Inc., York, PA) for radioassay and microautoradiography, and the biopsy sites were closed with tissue adhesive (Vetbond, 3M, St. Paul, MN). The biopsy samples were placed in separate, labeled zip-lock plastic bags, cooled on dry ice for at least 30 minutes, and then stored in a freezer at −70°C until analyzed.

**Study 2**

On study days 7, 14, 21, and 28, the dog was anesthetized and hair was clipped from sites over the lateral scapula, lateral thorax, and lateral hip. The clipped hair was saved in individual zip-lock plastic sample bags for analysis of radioactivity. As in study 1, a 2 × 2 cm area of skin at each clipped site was stripped two times with cellophane adhesive tape to remove surface imidacloprid residue, and the tape stripping samples were saved in a glass scintillation vial for further analysis. Similarly, as described for study 1, three skin biopsy samples were collected for radioassay from each site at each interval. A scalpel was used to obtain an elliptical skin sample (approximately 1 × 2 cm) from each site at each sampling period for microautoradiography. The punch sites were closed with tissue adhesive, and the elliptical incisions were closed with sutures and skin staples. The core biopsy samples were placed in labeled scintillation vials, and the elliptical sections were placed dermis-side down onto filter paper (to promote flattening); all samples were placed into zip-lock plastic bags and cooled on dry ice for at least 30 minutes. Ultimately, all skin samples were transferred and stored in a freezer at −70°C until analyzed.

**Radioassay of Sample Matrices**

Three samples of hair (5 to 15 mg each), both adhesive tape stripping samples (4 cm²; 50 to 200 mg each), and three skin biopsy samples (5 to 15 mg each) were assayed for total radioactivity by combustion to carbon dioxide using a sample oxidizer (Packard, Model 307, Downers Grove, IL). The resulting ¹⁴CO₂ was trapped and measured by a liquid scintillation counter (Beckman, Model LS 6000LL, Irvine, CA). Data were processed using Beckman data reduction software.

**Microautoradiography of Skin Biopsies/Samples**

A single frozen skin sample per interval from study 1 (of the five skin biopsy samples taken per interval, three were used for radioassay as described above and one was retained as a backup at the research facility) and all skin sections (approximately 1 × 2 cm elliptical area) from study 2 were sent via overnight express delivery to Quest Pharmaceutical Services, LLC (Newark, DE), for microautoradiographic analysis. All skin samples were sectioned at 5 to 10 µm at a temperature setting of −20°C to −25°C and thaw-mounted onto glass microscope slides precoated with photographic

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days Posttreatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7</td>
</tr>
<tr>
<td><strong>Interscapular</strong></td>
<td></td>
</tr>
<tr>
<td>Stratum corneum (µg/cm²)</td>
<td>21.2</td>
</tr>
<tr>
<td>Skin biopsy samples (ppm)</td>
<td>99.8</td>
</tr>
<tr>
<td><strong>Lumbar</strong></td>
<td></td>
</tr>
<tr>
<td>Stratum corneum (µg/cm²)</td>
<td>0.1</td>
</tr>
<tr>
<td>Skin biopsy samples (ppm)</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Values are average of three replicate samples combusted and radioassayed at each interval and are presented as imidacloprid-equivalents.

**TABLE 1. [¹⁴C]Imidacloprid-Derived Radioactivity in Stratum Corneum and Skin Biopsy Samples (Study 1)**
emulsion (Kodak NTB; Eastman Kodak, Rochester, NY). Five sets of tissue sections per sample were obtained to select an optimal exposure time. The cryostat sections exposed for 24 hours were deemed acceptable for analysis. Slides were developed using Kodak D-19 Developer and Kodak Fixer (Eastman Kodak).

All tissue sections were stained with hematoxylin and eosin, and the slides were examined and photographed using light microscopy to visualize the localization of [14C]imidacloprid-derived radioactivity at the cellular level.

**RESULTS**

**Clinical Observations**

Both dogs remained clinically healthy throughout the duration of the studies.

**Distribution and Localization of 14C Radioactivity**

**Study 1**

At all four posttreatment sampling periods (days 7, 14, 28, and 56), radioassay of tape stripping and skin biopsy samples confirmed the presence of radioactivity at the application and distal lumbar region sites (Table 1). The results of the tape stripping radioassays are expressed as micrograms of imidacloprid-equivalents per square centimeter of stratum corneum. The results of the skin biopsy samples are expressed as parts per million (ppm; micrograms of imidacloprid-equivalents per gram of sample matrix). As anticipated, the level of radioactivity was much greater at the application site than at the distal lumbar region at each sampling interval, and the concentration of radioactivity decreased steadily with time at both sites.

Microautoradiography of the skin biopsy samples was used to investigate and identify...
the specific posttreatment localization of radioactivity within the layers of canine skin. [14C]Imidacloprid-derived radioactivity, visualized in the form of exposed silver grains, was demonstrated in all skin biopsy samples collected at study days 7, 14, 28, and 56. Dense, dark-silver grainy deposits corresponding to high concentrations of radioactivity were consistently observed in the epidermis, dermis, hair follicles, and sebaceous glands (Figure 2).

**Study 2**

At each posttreatment sampling interval (days 7, 14, 21, and 28), radioactivity was detected in hair, tape stripping, and skin biopsy samples obtained from three body regions (lateral scapula, lateral thorax, and lateral hip) distal to the original application sites (Table 2). The results of the tape stripping radioassays are expressed as micrograms of imidacloprid-equivalents per square centimeter of stratum corneum. The results of the hair and skin biopsy samples are expressed as parts per million (micrograms of imidacloprid-equivalents per gram of sample matrix).

[14C]Imidacloprid-equivalents were demonstrated in all posttreatment hair samples. Between days 7 and 28, the levels of imidacloprid-derived radioactivity present in the hair from the lateral scapula, lateral thorax, and lateral hip sites ranged between 47 and 67, 60 and 85, and 60 and 100 ppm, respectively.

The amounts of 14C radioactivity present on the skin surfaces of the lateral scapula, lateral thorax, and lateral hip were determined by radioassay of the posttreatment tape stripping samples. The levels of imidacloprid-derived radioactivity measured on skin surfaces along the lateral scapula, lateral thorax, and lateral hip fluctuated between 0.1 and 0.65, 0.06 and 0.47, and 0 and 1.0 µg/cm² across the sampling times.

The skin biopsy samples from the scapula, lateral thorax, and lateral hip showed between 0.67 and 10 ppm (µg/g) of [14C]imidacloprid-derived radioactivity, with maximum levels as follows: scapula, 3.6 ppm at day 21; thorax, 10 ppm at day 21; and hip, 2.3 ppm at day 7. At the last sampling interval (day 28), the level of radioactivity in the skin biopsy samples from these sites declined to <0.9 ppm.

Microautoradiography was used as described for the skin samples in study 1 to identify the specific posttreatment localization of radioactivity within the layers of the elliptical skin samples. The presence of [14C]imidacloprid-derived radioactivity, in the form of exposed

### Table 2. Distribution of [14C]Imidacloprid-Derived Radioactivity in Hair, Stratum Corneum, and Skin Biopsy Samples (Study 2)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Days Posttreatment</th>
<th>7</th>
<th>14</th>
<th>21</th>
<th>28</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Scapula</strong></td>
<td>Hair (ppm)</td>
<td>NA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>60</td>
<td>47</td>
<td>67</td>
</tr>
<tr>
<td></td>
<td>Stratum corneum (µg/cm²)</td>
<td>0.60</td>
<td>0.10</td>
<td>0.27</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Skin biopsy (ppm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.90</td>
<td>1.4</td>
<td>3.6</td>
<td>0.78</td>
</tr>
<tr>
<td><strong>Thorax</strong></td>
<td>Hair (ppm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>85</td>
<td>60</td>
<td>68</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>Stratum corneum (µg/cm²)</td>
<td>0.32</td>
<td>0.06</td>
<td>0.42</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>Skin biopsy (ppm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.0</td>
<td>3.4</td>
<td>10</td>
<td>0.67</td>
</tr>
<tr>
<td><strong>Hip</strong></td>
<td>Hair (ppm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79</td>
<td>75</td>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Stratum corneum (µg/cm²)</td>
<td>0.00</td>
<td>0.21</td>
<td>1.0</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>Skin biopsy (ppm)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.3</td>
<td>1.0</td>
<td>2.1</td>
<td>0.88</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are average of three replicate samples combusted and radioassayed at each interval and are presented as imidacloprid-equivalents.  
<sup>b</sup>NA = data not available (sample misplaced)
Figure 3. Microautoradiographs of the radioactivity distributed in the dog’s skin in study 2. (A) Lateral scapula 7 days after topical administration of [14C]imidacloprid. (B) Lateral hip 14 days after topical administration of [14C]imidacloprid. (C) Lateral hip 21 days after topical administration of [14C]imidacloprid. (D) Lateral thorax 21 days after topical administration of [14C]imidacloprid. (E) Lateral hip 28 days after topical administration of [14C]imidacloprid.
silver grains appearing as dark stains, was demonstrated in all skin samples collected at posttreatment days 7, 14, 21 and 28 (Figure 3). In general, radioactivity was diffusely distributed throughout the epidermis and dermis, with focal concentrations found in the superficial epidermis, hair follicles, and sebaceous glands. The intensity of the overall stained pattern diminished slightly with time.

**DISCUSSION**

**Study 1**

A label-recommended dose (1.0 mL) of \([^{14}C]\)imidacloprid was topically applied to the skin of an adult beagle dog (8.5 kg body weight) at a single site as one spot in the interscapular region. At each posttreatment sampling interval (days 7, 14, 28, and 56), the \([^{14}C]\)imidacloprid-derived radioactivity was consistently demonstrated in tape stripping and skin biopsy samples collected from the application site as well as those collected from a distal site in the lumbar region. These results indicate that topically applied imidacloprid migrates from the point of application to skin regions distal to the original treatment site. The results also demonstrated that radiolabeled imidacloprid persisted within the skin and associated adnexa at the application site and at a distal lumbar site for up to 56 days after treatment. The intensity of the overall radioactivity pattern diminished steadily with time.

**Study 2**

Similar to study 1, the study 2 results demonstrated the displacement or migration of \([^{14}C]\)imidacloprid-derived radioactivity from the original application site(s) distally across the skin of the dog. The persistence of \([^{14}C]\)imidacloprid-derived radioactivity was clearly noted in the stratum corneum and the dermis at the lateral scapula, lateral thorax, and lateral hip over 28 days after treatment.

A direct and meaningful comparison of the radioactivity levels found in the various matrices in studies 1 and 2 is not feasible due to the differences in dose rate (14 mg active ingredient/kg body weight in study 1 versus 21 mg active ingredient/kg body weight in study 2) and treatment manner (one-spot application in study 1 versus four-spot application in study 2). These differences were the result of following the label for the test substance (Advantage Topical Solution) appropriate to each dog’s body weight and differences in the sampling sites.

**CONCLUSION**

The cutaneous distribution, localization, and persistence of \([^{14}C]\)imidacloprid-derived radioactivity was studied with radioassay and microautoradiography techniques after a single topical administration at therapeutic dosages to two adult beagle dogs at intervals ranging from 7 to 56 days posttreatment.

\([^{14}C]\)Imidacloprid-derived radioactivity migrated from the application sites (interscapular, dorsal thoracic, and lumbar spine) to areas of skin and haircoat that were distal (dorsal lumbar in study 1) or a little closer (lateral scapula, lateral thorax, and lateral hip in study 2) to the original treatment sites.

In study 1, the concentrations of \(^{14}C\) radioactivity in the skin (ppm) and stratum corneum (µg/cm²) at the application site (interscapular region) and the distal site (lumbar region) decreased steadily over 56 days after treatment.

In study 2, \(^{14}C\) radioactivity in/on the hair (ppm) and stratum corneum (µg/cm²) at the sites (lateral scapula, lateral thorax, lateral lumbar, and lateral hip regions) distal to the application sites was demonstrated at the various intervals up to 28 days after treatment. The \(^{14}C\) radioactivity (ppm) in the skin biopsy samples
from these sites steadily increased for up to 21 days after treatment and then rapidly declined at 28 days after treatment.

Microautoradiography of the skin showed focal concentrations of $^{14}$C radioactivity in the superficial epidermis, hair follicles, and sebaceous glands. The presence of $^{14}$C radioactivity within hair follicles, sebaceous glands, and on the skin surface strongly supports imidacloprid’s efficacy against fleas on dogs and cats despite posttreatment bathing, shampooing, and/or swimming.5

Besides the original intent and the scope of these two studies as stated above, any extrapolation of clinical efficacy beyond the approved 30-day retreatment interval, dose rates, or application modifications cannot be made from the data presented in this study. The exact mechanism and the rates of the absorption and distribution of imidacloprid in canine skin still remain subjects of interest for further investigations.

**REFERENCES**


