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Human Exposure to Imidacloprid from Dogs Treated with Advantage®

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The objective of this investigation was twofold: (1) to determine the transferable residue of imidacloprid in gloves worn while petting experimental household dogs after the application of Advantage® and (2) to determine the imidacloprid residue in the dog's blood. Advantage® contains 9.1% imidacloprid, which controls fleas on dogs for up to 30 days. Imidacloprid produces toxicity by interacting with nicotinic receptors. Advantage® (364 mg imidacloprid/dog) was applied topically to six household dogs. The glove and blood samples were collected at 24 h, 72 h, and then on a weekly basis for 5 weeks post-Advantage® application. The glove samples were collected by petting each dog for 5 minutes while wearing a different glove per dog. The blood samples (5 mL from each dog) were collected into EDTA tubes. The imidacloprid residue was determined in the blood extracts and glove samples using RP-HPLC. The highest levels of imidacloprid residues were detected at the 24-h interval in both glove (254.16 ± 25.49 ppm) and blood (54.06 ± 3.00 ppb) samples. The blood imidacloprid residue was reduced by one third at the 72-h interval (18.73 ± 2.00 ppb) and was not detected after 1 week. Imidacloprid residue in the glove samples decreased approximately one third between each collection interval. The residue of imidacloprid in the glove extract by the fourth week was very low (0.08 ± 0.02 ppm) and not detected by the fifth week. The present findings suggest that following topical application of Advantage®, imidacloprid residue can be detected in the dog's blood for up to 72 h, and transferable residue on the dog's coat can be detected for up to 4 weeks. Repeated chronic exposure to imidacloprid may pose possible health risks to veterinarians, veterinary technologists, dog caretakers, and owners.

Keywords Imidacloprid, Advantage®, Insecticide, Residue, Dogs, Blood Samples

INTRODUCTION

Imidacloprid was first discovered during the 1970s from a chemical group called nitromethylene (Arther et al. 1997). It is a chloronicotinyl insecticide that is registered for use on food crops, tobacco crops, turfs/soils, and termite control in buildings. It is produced by the Bayer Corporation and sold under various brand names such as Premise® (termiticide), Admire® (seed treatment), and Grub-ex™ (grub control). Most recently, its use was expanded to include a 30-day flea preventative for dogs and cats sold as Advantage.®

Imidacloprid is chemically known as 1-[-6chloro-3-pyridinyl) methyl]-N-nitro-2-imidazolidinimine (Lee et al. 2001). It has an empirical formula of $C_9H_{10}ClN_5O_2$ and a molecular weight of 255.7 (Budavari et al. 1996). In the physical form imidacloprid is a clear liquid with slight yellow color and a mild odor (Bayer Corporation 2002). Imidacloprid is readily soluble in organic solvents (methylene chloride, acetonitrile, methanol, etc.) and slightly soluble in water (0.51 g/L).

Advantage® is a topical preparation, which consists of 9.1% active ingredient imidacloprid (an insecticide) and 90.9% of unspecified inert ingredients. This product is applied directly to the skin above the shoulder blades at the base of the skull in dogs (weighing <21 lbs) and cats. For dogs weighing more than 21 lbs, the product is evenly applied to the skin above the shoulder blades at the base of the skull, in addition to the skin below the shoulder blades at the start of the animal's back, again in the

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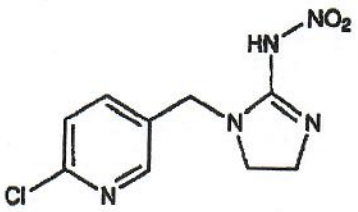

FIG. 1. Chemical structure of imidacloprid (molecular weight 255.7)

middle of the back, and at the base of the tail. Once applied, the Advantage® is absorbed into the sebaceous glands of the skin within 24 h after application, as stated by the Bayer Corporation (2000). Imidacloprid uses the oils produced by the glands to spread over the animal's body.

Imidacloprid acts on targeting the nervous system of insects by irreversibly binding to the nicotinic acetylcholine receptor sites found within ganglia and skeletal muscles (Lu and Kacew 2002). It binds on the postsynaptic membrane competitively and thereby inhibits acetylcholine from binding to the receptors (Anonymous 1998). Acetylcholine is a chemical transmitter that is released from depolarized nerve endings (Frandson and Spurgeon 1992). A build-up of acetylcholine results because it cannot bind to the receptor sites to deliver information and is degraded by acetylcholinesterase. Therefore, hyperexcitation of the ganglia and skeletal muscles occurs, resulting in tremors, seizures, convulsions, and death of the insect. Whether a similar toxicological mechanism exists in the mammalian system is yet to be elucidated.

This investigation was undertaken with two specific objectives: (1) to determine the transferable residue of imidacloprid in gloves worn while petting experimental household dogs after the application of Advantage® and (2) to determine the imidacloprid residue in the dog's blood.

MATERIALS AND METHODS

Animals

A group of six healthy adult household dogs (three males and three females; weighing 60 to 80 lbs) was recruited for the present study. During the course of the entire study, each of the animals was given *ad libitum* food and water.

Chemicals

Imidacloprid (99.5% technically pure) was purchased from ChemService (West Chester, PA). All other chemicals used were high-performance liquid chromatograph (HPLC) or American Chemical Society (ACS) grade purchased from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Fair Lawn, NJ).

Experimental Protocol

The present study was designed for the determination of imidacloprid residue present on the canine coat and in the blood to imitate the natural exposure between veterinary personnel and canine patients. Often when patients enter a veterinary clinic, they are petted or restrained while being treated by two or more employees. This includes the patients who are wounded, bleeding, and in need of urgent care. These patients especially offer exposure to veterinary personnel through contact with their coat and contact with internal tissue fluids and blood.

Samples were collected from each patient in the order that the Advantage® was applied, and the same order was used during each collection interval. Control samples were collected before treatment (day 0), and then Advantage® was topically applied to each dog. Glove and blood samples were collected at 24 h and 72 h, and at 1, 2, 3, 4, and 5 weeks postapplication.

Glove samples were collected by wearing a 100% white cotton glove and petting forward and back and along each dog's side for 5 min as described by Boone et al. (2002) and Jennings et al. (2002). After 5 min of petting the dog, the glove was immediately placed into a clean glass jar with a Teflon lid labeled with the dog's identification number. The gloves were worn to simulate residue exposure to humans that could naturally occur during a physical examination of or while playing with dogs treated with imidacloprid. Then, 5 mL of blood was drawn from the cephalic vein of each dog using a 6-mL syringe and a 22-gauge needle. The blood was transferred into an EDTA (ethylenediaminetetraacetic acid) tube to prevent the blood from clotting. All precautions were taken to prevent any contamination of the samples.

Sample Extraction for Imidacloprid Residue

All blood and glove samples were weighed and extracted in methylene chloride/petroleum ether (1:1). After 30 min, extracts were filtered through a filter paper having sodium sulfate. All samples were analyzed within 24 h of sample collection. Extracts were evaporated to dryness and reconstituted to a required volume just before imidacloprid residue analysis by HPLC.

HPLC Analysis for Imidacloprid Residue

Imidacloprid residue was detected in glove and blood extracts using HPLC (Waters Associates, Inc., Milford, MA) method (Baskaran et al. 1997; Galera et al. 1998) with necessary modifications. HPLC system consisting of a pump (model 510), a dual λ absorbance UV detector (model 2487), a temperature control module, a manual injector (model U6K), and a computer with Millennium^{32} software (version 4.0) was used for the analysis of imidacloprid. HPLC conditions included detection wavelength: 270 nm; column: YMC J'sphere (ODS-H80) 150 × 4.6 mm ID, particle size S-4 μm, 80A; and mobile phase (acetonitrile: water, 30:70) with a flow rate of 1 mL/min. Using various concentrations of imidacloprid (10 ng to 100 ng), a standard curve was constructed, which was used to calculate the residue of imidacloprid in the extracts of gloves and blood. A HPLC chromatogram of imidacloprid is shown in Fig. 2. Imidacloprid peak eluted at 4.335 min.

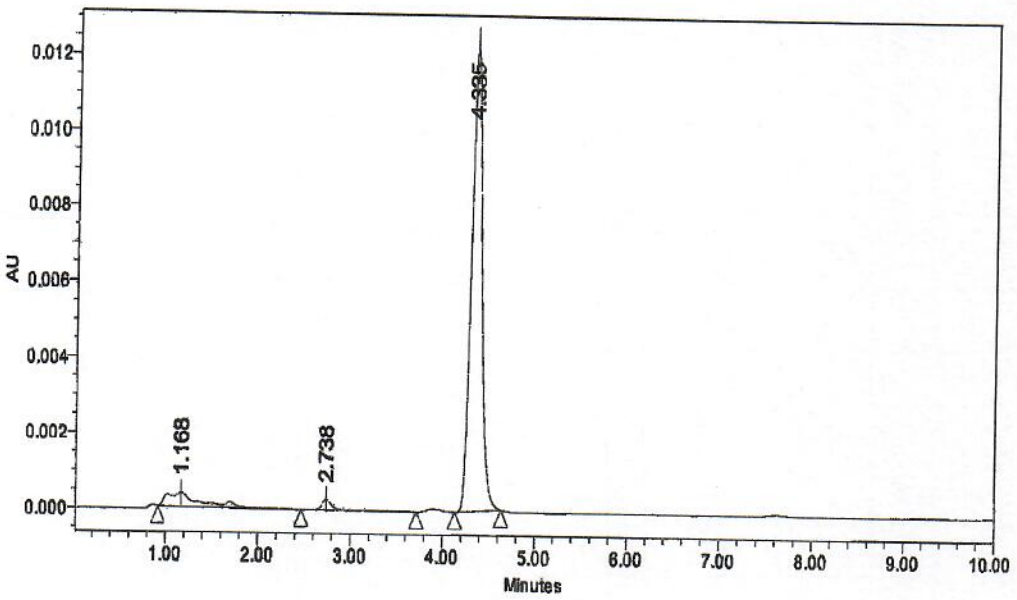

FIG. 2. HPLC chromatogram of imidacloprid (20 ng).

RESULTS AND DISCUSSION

Currently, Frontline® (fipronil), Advantage® (imidacloprid), and Revolution™ (selamectin) are the most commonly used flea killers prescribed for dogs. Each of these insecticides is applied topically, and efficacy lasts for a month against ectoparasites. Product information also indicates that after a few hours of application, the residue is nontransferable. Topical application of Advantage® (9.1% imidacloprid) has been shown to have efficacy against fleas on dogs and cats. The purpose of this study was to determine the transferable residues of imidacloprid found in glove samples from the canine coat and to determine the imidacloprid residues present in canine blood postapplication of Advantage®. This study demonstrates the possibility that significant levels of imidacloprid can be transferred to human skin from coming in contact with dogs treated with Advantage®. The transferable residue found in gloves can provide an estimation of the levels of imidacloprid that veterinarians, veterinary personnel, dog caretakers, and owners can be exposed to from canine patients over a prolonged time period.

The average daily intake (ADI) for imidacloprid is 0.06 mg/kg body weight/day, which is set by the World Health Organization and National Registration Authority (Marshall and Begg 2003). The ADI was set by chronic and carcinogenicity studies in rats with an uncertainty factor of 100 for inter- and intraspecies variation (Marshall and Begg 2003).

Imidacloprid was found to be moderately toxic in acute toxicity studies using the technical grade. The LD_{50} of imidacloprid is 450 mg/kg body weight in rats and 131 mg/kg body weight in mice. Inhalation test using airborne concentration of imidacloprid resulted in mortality to half of the test organisms with an LC_{50} of greater than 69 mg/cubic meter air in the form of an aerosol and an LC_{50} of greater than 5323 mg/cubic meter air in the dust form (Extension Toxicology Network 2003).

The model for this investigation closely followed the guidelines of previous studies using insecticidal exposure assessment (Lu and Fenske 1999). In brief, the protocol included the techniques used earlier for the sample collection of residues from the dog's coat and blood circulation as well as sample and data analyses.

At no time during the study period did the dogs show any signs of toxicity. All the dogs remained healthy in appearance, and daily level of activity was normal throughout the course of this study.

The coat samples were collected using a glove. The samples collected at the 24-h interval ranged from 173.34 to 348.92 ppm, with a mean value of 254.16 ± 25.49 ppm. Imidacloprid residue gradually decreased over the 5-week period. Samples collected at 72 h after Advantage® application indicated a sharp decrease in the residue (101.58 ± 9.86 ppm). The residues detected after 1 week (4.96 ± 0.48 ppm) were very low compared to that observed after 72 h (Fig. 3). A further descending trend in residue elimination of imidacloprid on dog' coat was observed after the second, third, and fourth weeks (2.60 ± 0.13 ppm, 0.51 ± 0.09 ppm, and 0.08 ± 0.02 ppm, respectively). After 5 weeks of Advantage® application, there was no detectable residue of imidacloprid found in the glove samples. This concurs with the information provided on the label of Advantage® that flea control efficacy of imidacloprid lasts for up to 30 days. In a recent study, Jennings et al. (2002) reported a similar elimination pattern for fipronil residue on dog's coat after application of Frontline®.

The blood samples collected at various intervals after application of Advantage, revealed the highest residue of imidacloprid

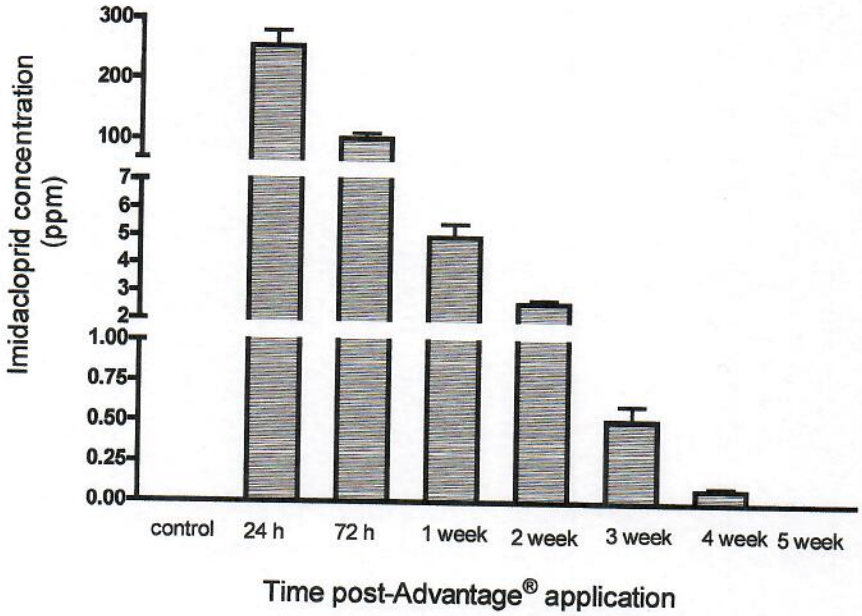

FIG. 3. Imidacloprid residue in gloves (ppm) following a single topical application of Advantage® (364 mg imidacloprid).

at 24 h, which ranged from 37.81 to 89.20 ppb with a mean value of 54.06 ± 3.00 ppb. After 72 h, the imidacloprid residue had decreased approximately to one third (18.73 ± 2.00 ppb). Imidacloprid residue was undetectable in the blood samples collected after 1 week (Fig. 4).

Accurately determining the risk associated with imidacloprid exposure to humans is difficult due to the nature of skin regarding absorption when compared to adsorption to a glove. There is no data currently addressing the effects of Advantage® (imidacloprid) in humans since this product is used on nonfood animals. To evaluate the possible risks of imidacloprid to veterinarians, veterinary technologists, and dog handlers, it is necessary to know the amount of residue absorbed by human skin through contact, the number of patients seen each day treated with Advantage®, and the amount of time spent with each canine patient.

CONCLUSIONS

This study demonstrated that imidacloprid residue is transferable from a dog coat and is present in the dog's blood. Imidacloprid residues present on the dog's coat are in the

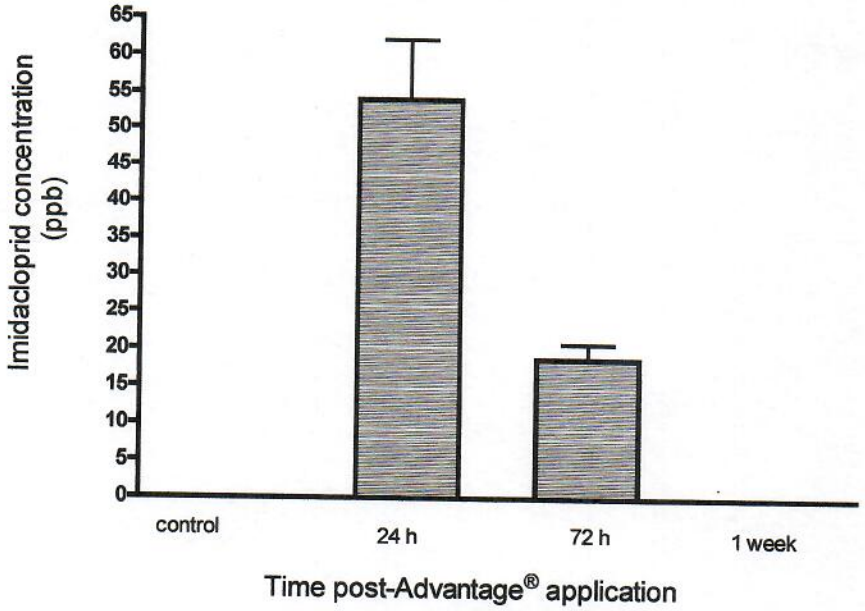

FIG. 4. Imidacloprid residue in dog blood (ppb) following a single topical application of Advantage® (364 mg imidacloprid).

significant amount, and the possibility exists for a daily transfer to human skin through contact with treated dogs. The levels found within the blood circulation are also of importance; however, the transfer that could occur from the blood to humans is more selected in that not every veterinary personnel or dog handler is exposed daily to dog's blood. Therefore, the highest risk period for transfer of imidacloprid residues is during the first 12 to 24 h after Advantage® application, when residue levels for both glove and blood samples are at their peak. The risk of residue exposure from blood seems low due to its rapid elimination from the circulatory system. Furthermore, though the imidacloprid residue persist up to 4 weeks, it may not be significant to pose a serious concern to human health. Thus, it can be concluded that the transferable residue of imidacloprid can be a health concern to veterinarians and their personnel for first few days after Advantage® application on dogs. In future studies, we intend to determine imidacloprid residue in the blood and urine of veterinary personnel who might be chronically exposed to imidacloprid from canine patients.

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