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Residue of Fipronil, S-Methoprene, and Amitraz in Dog Blood and in Gloves from Topical Certifect® Application: Toxicity and Safety Considerations

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Abstract

Currently, the use of ectoparasiticides on dogs to control ticks and fleas is inevitable, and the safety data for dogs, dog owners, and veterinary personnel are scarce. This investigation was therefore undertaken with two objectives: (1) to determine the residue of fipronil, s-methoprene, and amitraz in dog blood, and (2) to determine the transferable residues of these insecticides to gloves worn while petting experimental household dogs after the topical application of Certifect®. Certifect®(4.28 mL) contains 9.8% fipronil, 8.8% s-methoprene, and 22.1% amitraz. With a combination of these three insecticides, Certifect® kills ticks, fleas and chewing lice of all stages. All three insecticides produce their toxicity by different mechanisms. The blood samples (approximately 3-5 mL from each dog) were collected into EDTA tubes. Gloves were worn for 5 min while petting individual dogs and then collected for pesticide residue analysis. Blood and glove samples were extracted in methylene chloride/petroleum ether (1:1), and the extracts were assayed for residues of fipronil, s-methoprene, and amitraz using GC/MS. Blood analysis revealed the presence of only amitraz (0.42±0.16 µg/mL) 48 hr post-Certifect® application. In gloves, significant residues of fipronil, s-methoprene, and amitraz were detected after 24 hr, with maximum transferable residue at 72 hr (432.16±79.18; 301.84±52.51; and 398.37±112.19 µg/g, respectively). After day 7, the concentrations of these insecticides in glove extracts were about 100 µg/g glove wt. While fipronil and s-methoprene were detected until day 28 (0.87±0.55 and 2.66±1.22 µg/g, respectively), the residue of amitraz was detectable only until day 14 (6.01±1.75 µg/g). In conclusion, Certifect® appears to be safe for dogs and their owners following once a month exposure, but veterinary personnel can be at risk following daily exposure to transferable residues of fipronil, s-methoprene, and amitraz, if not properly protected.

Keywords: Certifect®; Amitraz; Fipronil; S-methoprene; Ectoparasiticide; Larvicide; Tickicide; Transferable Insecticide residue

Introduction

Currently, the use of ectoparasiticides on pets is inevitable because their infestation with ectoparasites is very common. Common ectoparasites include blood sucking ticks, fleas, and lice, which pose major health problems to both animals and humans worldwide because they transmit infectious diseases (such as Lyme disease, Canine Ehrlichiosis, Anaplasmosis, Rocky Mountain spotted fever, Babesiosis, etc). In the past two decades, ectoparasiticides such as organophosphates and carbamates have largely been replaced by pyrethrins and pyrethroids which exert selective toxicity, i.e., they are more toxic to parasites and less toxic to the mammalian species. Additionally, there are many other insecticides from different classes used singly or in combination as preventative or therapeutic measures. Currently, the ectoparasiticide products consist of a combination of various insecticides to provide broad spectrum activity and exert synergistic action to prolong their persistence on the pet's coat. But due to a lack of selective toxicity, these same ectoparasiticides pose health risks to both animals and humans.

Presently, Certifect® is one of the commonly used ectoparasiticide products available on the market. It is a monthly topical spot-on product used to kill adult fleas, flea eggs, flea larvae, ticks of all stages, and chewing lice on dogs eight weeks of age and older. This product is also indicated for the control of sarcoptic mange mites in dogs. Certifect® is a product of Merial Ltd (Duluth, GA, USA) and consists of three insecticides (9.8% fipronil, 8.8% s-methoprene, and 22.1% amitraz).

Fipronil (5-amino - 1 - [2, 6 - dichloro - 4 - trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfonyl]-1H-pyrazole) is a member of a new class of insecticides called phenylpyrazoles. Although the United States Environmental Protection Agency (EPA) has determined fipronil to be safe for use on dogs and cats, with no harm to humans who handle these animals, poisoning cases from accidental exposure or misuse of fipronil may occur in animals [1-3]. In humans, poisoning is mainly due to an accident or a suicidal attempt. The Paris Poison Center in France recorded 81 human cases of fipronil exposure from 1994 to 1999. Of these 81 cases, 57 involved veterinary ectoparasiticides and seven were domestic insecticide preparations [4]. In the United States, 103 cases were identified in 11 states during the period of 2001-2007. The majority (76%) had exposure in a private residence, 37% involved the use of pet-care products, and 26% had work-related exposures [5]. Fipronil and its major metabolite fipronil sulfone produce toxicity in insects and mammals by the same mechanism; however, due to their selective action, toxicity is much more severe in insects than in mammals [6-9]. Fipronil and other phenylpyrazole compounds exert neurotoxicity by blocking the transmission of signals by the inhibitory neurotransmitter γ-aminobutyric acid (GABA). These compounds bind within the chloride channels and consequently inhibit the flux of chloride ions into the nerve cells, resulting in hyperexcitation[1,3].

Amitraz is a triazapentadiene compound, which is a member of the formamidine pesticide family. Amitraz is currently approved in the United States for various applications, including control of ticks, mites, lice and many other pests on dogs. Amitraz poisoning is frequently encountered in dogs and cats due to accidental ingestion of tick collars [10-13]. In humans, poisoning occurs due to the widespread use of amitraz veterinary products [14-18]. Amitraz kills mites, ticks, and other parasites by interfering with their nervous system. The tick's sharp barbed mouth parts become paralyzed and cannot pierce the skin, thereby inhibiting the tick from feeding on dogs. In higher doses, amitraz has been shown to produce toxicity in several animal species by multiple mechanisms [3,19]. Amitraz, by stimulating α2-adrenergic receptors, causes impairment of consciousness, respiratory depression, convulsions, bradycardia, hypotension, hypothermia, and hyperglycemia [20,21]. In addition to being an α2-adrenergic receptors agonist, amitraz is a potent inhibitor of the enzyme monoamine oxidase (MAO), which is responsible for degrading the neurotransmitters norepinephrine and serotonin, resulting in behavioral and neurotoxic effects [22-24]. In a recent study, amitraz evoked a significant decrease in serotonin, noradrenaline, and dopamine levels in brain regions of rats [25]. Amitraz is also known to cause inhibition of prostaglandins [26].

S-methoprene [isopropyl (2E,4E)-11-methoxy-3.7,11-trimethyldodeca-2,4-dienoate], is another active ingredient present in Certifect®. It is an insect growth regulator (IGR), as it interferes with an insect's life cycle and prevents it from reaching maturity or reproducing. The compound is considered to be very safe to animals and humans, since it's oral LD_{50} in rats is reported to be >5000 mg/kg (27).

In a series of experimental studies, we determined the transferable residues of fipronil from Frontline, imidacloprid from Advantage®, selamectin from Revolution™, and etofenprox, s-methoprene, and piperonyl butoxide from Bio Spot Defense topically applied on dogs [28-31]. Each active ingredient depicted a different pattern in terms of transferable residue and its persistence on the dog's coat. The present study determined residues of fipronil, s-methoprene, and amitraz in dog blood, and also in gloves to assess the transferable residues of these insecticides to other pets, dog owners, and veterinary personnel.

Materials and Methods

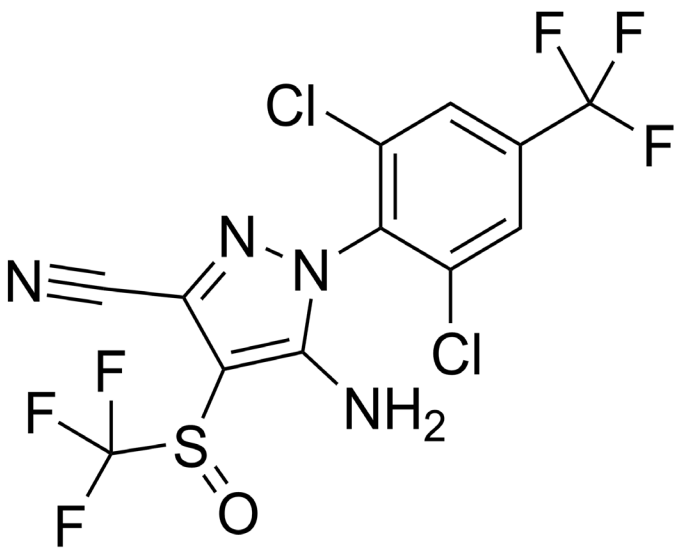
Animals

Six client-owned adult dogs (mixed breed, medium length coat) weighing between 18-28 kg (40-60 pounds) were used in this investigation. These dogs were not treated with any ectoparasiticide(s) for at least six weeks prior to the study. Blood and glove samples were checked for residues of fipronil, amitraz, s-methoprene, or any other commonly used ectoparasiticide, and were found to be negative.

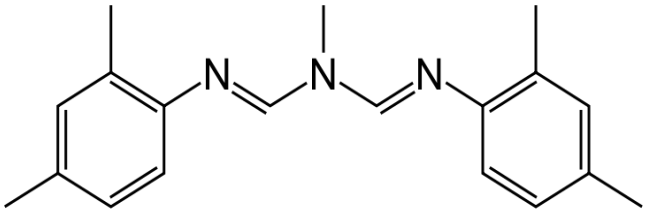
Chemicals

Certifect® for dogs was purchased from Merial Ltd (Duluth, GA, USA). Each pack for a single application on a dog contained 4.28 mL (Fipronil, 9.8%; S-Methoprene, 8.8%; and Amitraz, 22.1%). Technical grade fipronil (98.8%), s-methoprene (99.1%), and amitraz (98.7%) were purchased from Chem Service (West Chester, PA, USA). All other chemicals of highest purity were obtained from Fisher Scientific (Pittsburgh, PA, USA). Chemical structures of fipronil, s-methoprene, and amitraz are shown in Figure 1.

FIPRONIL



AMITRAZ



S-METHOPRENE

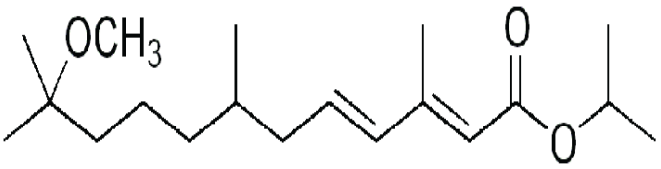


Figure 1. Chemical structure of fipronil, amitraz, and s-methoprene.

Experimental Protocol

Experimental design for this investigation was followed from our recent study conducted with Bio Spot Defense Flea & Tick Spot On® in dogs [31]. Experimental protocol for the present investigation was approved by the Institutional Animal Care and Use Committee (IACUC) of Murray State University. A brief description of experimental protocol is given below.

Certifect® Application:

Certifect® (a product of Merial Ltd, Duluth, GA) was topically applied to each dog directly onto the skin at two places (at the base of the dog's skull and between the shoulder blades, where the dogs could not lick). Following application of the product, the active ingredients redistribute into the skin, with a high concentration going into the sebaceous glands. The sebaceous glands release the drug continuously in the sebum along with other natural oils and waxes [1,28-32]. This allows the drug to coat the hair shaft and skin surface, providing long-lasting protection against fleas, ticks, and mosquitoes (27).

Physical Examination:

Dogs topically treated with Certifect®were evaluated for physical parameters. At specified time intervals, dogs were examined for body weight, behavior, and skin reaction at the Certifect® application site.

Sample Collection for Pesticide Residue Analysis:

Cotton gloves (each weighing about 6 g) used for petting dogs were collected at 0, 24, 48, 72 hr, and 1, 2, 3, and 4 week intervals; and blood samples were collected at 0, 24, 48, 72 hr, and 1 and 2 week intervals, for residue analysis of fipronil, s-methoprene, and amitraz.

Glove sampling included the wipe sampling technique, which consisted of petting the dog forward and back along its back and sides for five minutes while wearing a 100% cotton glove [29-31]. After sampling, the glove was immediately placed in a labeled 800mL glass jar and kept at room temperature until analyzed (<72-96 hr). Blood samples were collected from the cephalic vein using a 6mL syringe with a 22-gauge needle. At each time interval, approximately 3-5mL blood was collected in a EDTA anticoagulant tube and refrigerated until analyzed (<72 hr).

Sample Extraction:

Blood and glove samples were extracted in methylene chloride: petroleum ether (1:1, vol/vol). Extracts were passed through sodium sulfate, evaporated to dryness overnight, and reconstituted in methylene chloride: petroleum ether in a required volume just prior to GC/MS analysis.

GC/MS Analysis:

The active ingredients of Certifect® (fipronil, s-methoprene, and amitraz) were confirmed using an Agilent Gas Chromatograph (GC model 7990A)/ Mass Spectrometer (MS model 5975C) coupled with a computer, and their concentrations were expressed in terms of µg/g (ppm).

The evaporated extract was reconstituted in an appropriate volume of extraction solvents (methylene chloride: petroleum ether, 1:1 on a volume basis) and passed through a Sep-Pak® cartridge (Waters Corp, Milford, MA). One µL of sample extract was injected into the GC. The column used was Ultra II Cross-linked with 5% phenyl methyl siloxane coating and was of the following dimensions (capillary 25 m x 0.52 µm), which was directly connected to the Mass Selective Detector via an interface and heated transfer line. The carrier gas was ultrapure (99.9999%) helium at a flow of 2.3 mL/min, and the injector temperature was 200 ºC. The injector was operated in the splitless mode. A temperature program for the GC-oven was used starting at a temperature of 150 ºC, and then increased to a final temperature of 300º C in 10ºC/min increments. The final temperature was maintained for 1 min. The total duration of each injection run was 16 min, with a solvent delay of 5 min. The transfer line temperature was 280 ºC, and the source temperature was 230 ºC. The instrument was operated in electron ionization mode, and the ion energy was 70eV. Peaks of fipronil, s-methoprene, and amitraz were eluted at 7.822 min, 7.911 min, and 12.008 min, respectively. Sensitivity of the GC/MS for these compounds was in the range of ng, and the limit of detection was in the low µg/g (ppm) range. Total ion chromatogram for fipronil, s-methoprene, and amitraz is shown in Figure 2A. Mass Spectra with characteristic ions for fipronil (77, 213, 255, 367, and 437.9), s-methoprene (73.1, 111, 153.1, 191.2, and 278.1), and amitraz (77, 121.1, 162.1, and 293.1) are presented in Figures 2B, 2C, and 2D, respectively.

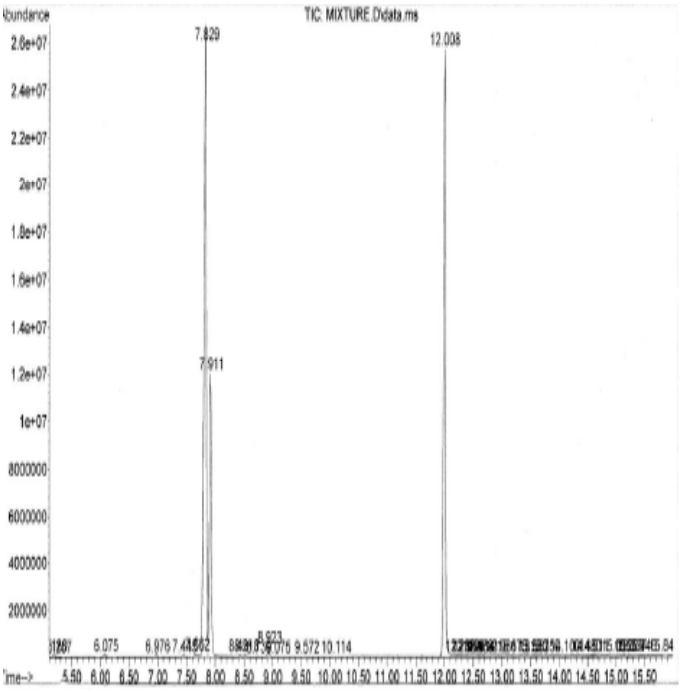


Figure 2A. GC-MS- Total Ion Chromatogram (TIC) of fipronil, s-methoprene, and amitraz (peaks eluted at 7.822, 7.911, and 12.008 min, respectively).

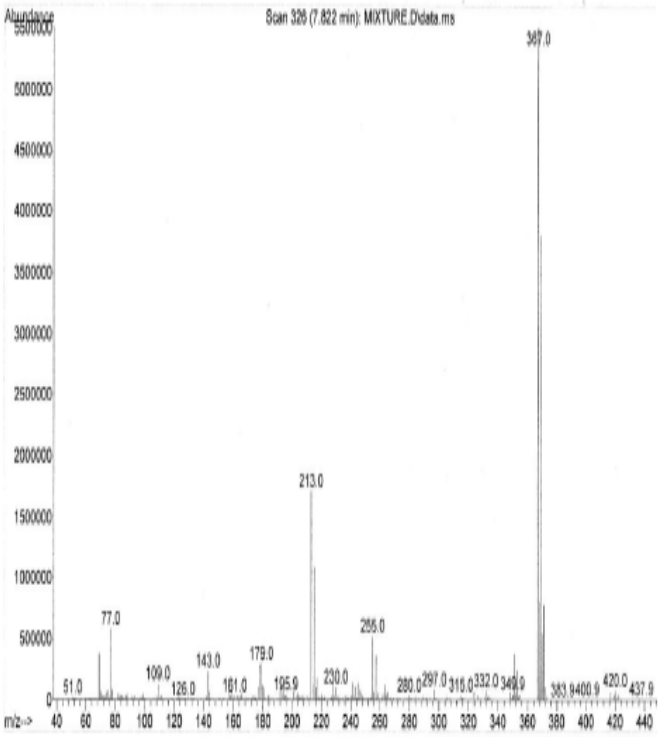


Figure 2B. Mass spectrum of fipronil (Mol. Wt. 437.15) peak eluted at 7.822 min.

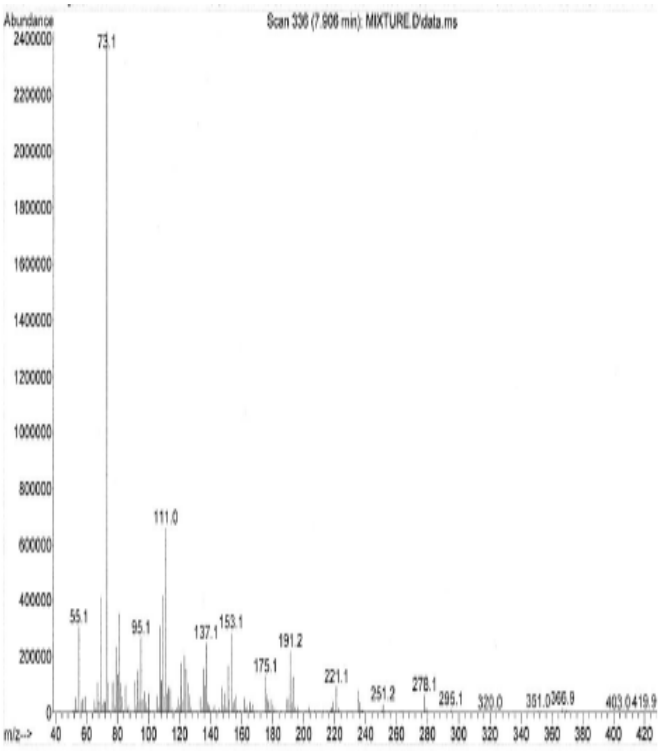


Figure 2C. Mass spectrum of s-methoprene (Mol. Wt. 310.47) peak eluted at 7.906 min.

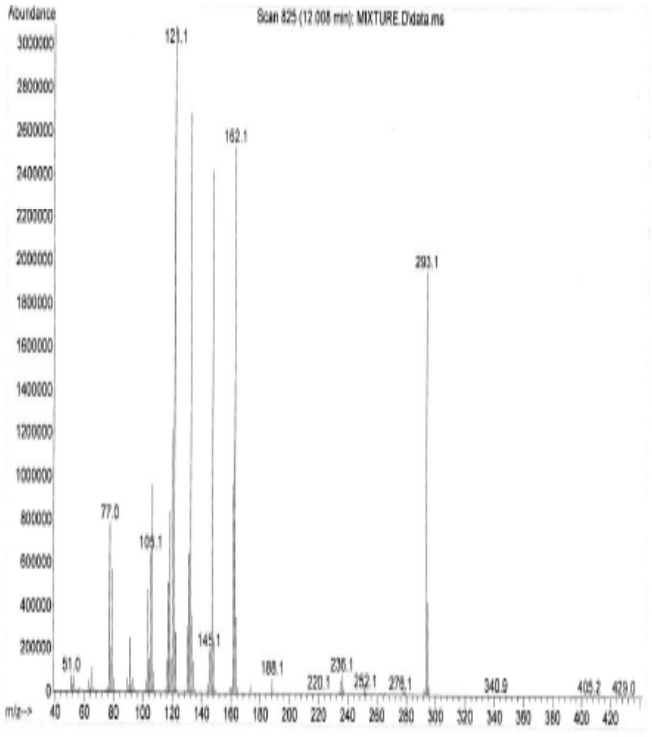


Figure 2D. Mass spectrum of amitraz (Mol. Wt. 293) peak eluted at 12.008 min.

Data analysis

Concentrations of fipronil, s-methoprene, and amitraz determined as µg/g glove or µg/g blood are presented as means ± SEM (n=6).

Results and Discussion

This investigation was undertaken to measure the transferable residue of fipronil, s-methoprene, and amitraz from the dog's coat to gloves worn while petting dogs and also to determine the residue of these insecticides in the dog's blood at various time intervals post-Certifect® application. The study was designed to assess the levels of fipronil, s-methoprene, and amitraz residue present on the canine coat and in the blood that may be transferred onto human skin. Study protocol of this investigation was based on our previous investigations [27-30]. Gloves were used to simulate the condition in which the dog owner and veterinary personnel can be exposed to ectoparasiticides while petting a treated dog. Blood samples were analyzed for the residues of fipronil, s-methoprene, and amitraz to assess the overall exposure of veterinary personnel, as it may vary depending on the kind of practice and the number of patients seen each day.

Following a single exposure to Certifect®, dogs neither exhibited any change in body weight, eating or behavioral habits, nor exhibited any side effects or adverse reaction at the site of product application for up to a period of four weeks, suggesting that the product is well tolerated and safe to use. According to the product label information, dogs treated with Certifect® may show temporary irritation at the site of its application and lethargy. In previous studies, dogs treated with Frontline (fipronil), Advantage® (imidacloprid), Revolution™ (selamectin), or Bio Spot Defense (etofenprox, s-methoprene, and piperonyl butoxide) also did not show any signs of toxicity nor any skin reaction at the site of application [28-31]. But animals and humans have been intoxicated with these products following oral exposure [1,4,12,19,32,33]. Therefore, it is necessary to provide acute toxicity data and to discuss the clinical effects and mechanism of action of fipronil, s-methoprene, and amitraz (active ingredients present in Certifect®) should the dogs be poisoned accidentally or maliciously by oral ingestion of this product.

Topical application of Certifect® has been shown to kill adult fleas, flea eggs, flea larvae, ticks of all stages, and rapidly eliminate chewing lice infestations and it aids in the management of sarcoptic mange mites. Certifect® is restricted for use by a licensed veterinarian, because professional expertise and proper diagnosis are required to determine the safe use of this product.

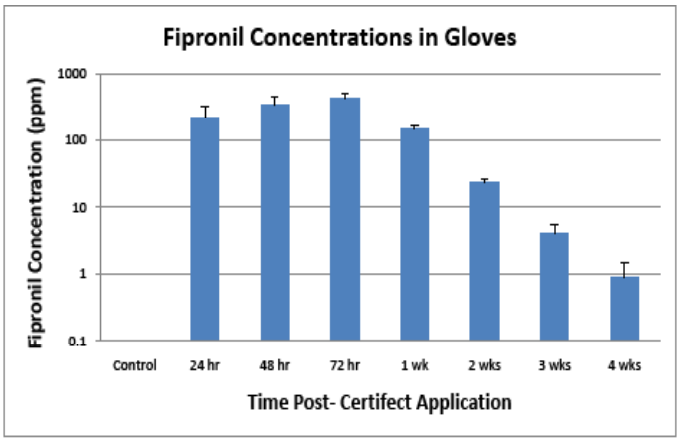


Figure 3A. Concentrations of fipronil (µg/g or ppm) in gloves (Mean±SEM; n=6).

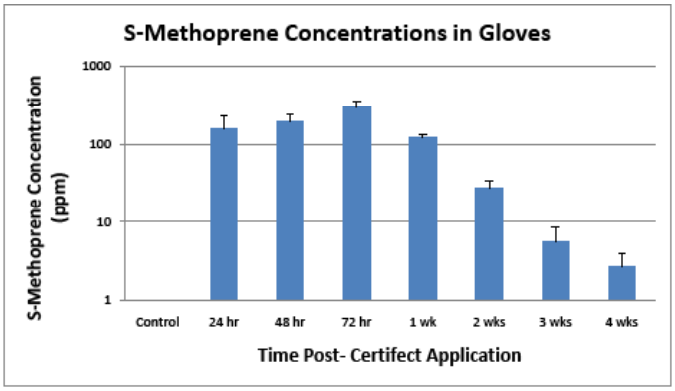


Figure 3B. Concentrations of s-methoprene (µg/g or ppm) in gloves (Mean±SEM; n=6).

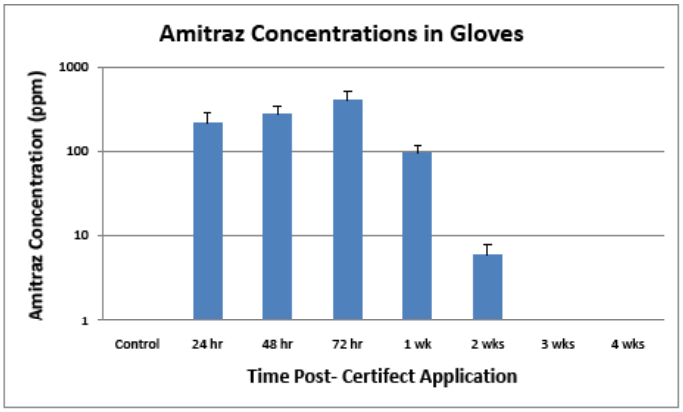


Figure 3C. Concentrations of amitraz (µg/g or ppm) in gloves (Mean±SEM; n=6).

Data of fipronil, s-methoprene, and amitraz residues in glove extracts are shown in Figures 3A, 3B, and 3C, respectively. Appreciable concentrations of fipronil, s-methoprene, and amitraz were detected in glove extracts after 24 hr (217.27±106.17; 158.81±70.43; and 212.37±65.25 µg/g, respectively), with maximum transferable residue at 72 hr (432.16±79.18; 301.84±52.51; and 398.37±112.19 µg/g, respectively) after Certifect® treatment. After 7 days of exposure, the concentrations of these insecticides in glove extracts were 148.16±15.39; 119.42±11.26; and 96.96±17.39 µg/g, correspondingly. While amitraz residue was detectable only until day 14 (6.01±1.75 µg/g glove wt), fipronil and s-methoprene were detectable until 4 weeks (0.87±0.55 and 2.66±1.22 µg/g, respectively).

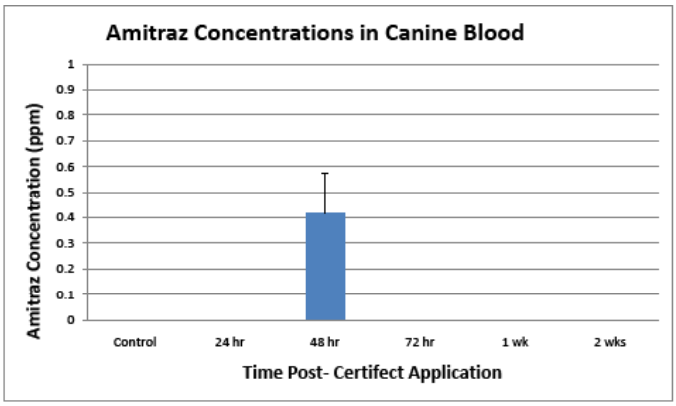


Figure 3D. Concentration of amitraz (µg/g or ppm) in dog blood (Mean±SEM; n=6).

In the present study, blood analysis revealed the presence of a small amount of amitraz (0.42±0.16 µg/g) 48 hr after Certifect® application (Figure 3D). At no time, was residue of either fipronil or s-methoprene detected in the blood of any dog treated with Certifect® (results not shown).

There are numerous reports regarding the toxic effects of fipronil on small animals, birds, and fish, but very little in humans. Poisoning cases of fipronil occur in dogs and cats due to accidental ingestion/licking of fipronil-containing products. The acute studies conducted on rats revealed that fipronil has moderate acute toxicity by oral and inhalation routes. Its LD_{50} was reported to be 97 mg/kg orally and 2000 mg/kg dermally [34]. In rats, signs of toxicity and death were delayed for up to four days after a single dose or repeated oral doses of 75 mg/kg body wt per day for up to five days. WHO recognized fipronil as a Class II moderately hazardous pesticide. Dermal absorption of fipronil in rats is less than 1% after 24 hr and therefore toxicity is considered low. In contrast, it has a moderate dermal toxicity (LD_{50}=354 mg/kg body wt) in rabbits (35). Application of Frontline Top Spot (active ingredient fipronil) has been reported to cause skin irritation and/or hair loss at the site of application. In rats, the major organs affected by acute fipronil toxicity were brain, liver, kidney, and thyroid gland. General toxicity signs included reduced feed consumption, anuria, and hyperexcitability, including tremors, convulsions, seizures, and death [36,37]. Fipronil has been found to be neurotoxic and developmentally neurotoxic in acute and sub-chronic studies and to be carcinogenic in chronic studies conducted in rats and dogs [35,38-40].

The mechanism of fipronil toxicity has been described in our recent publications [1,3]. In brief, fipronil binding blocks the inhibitory action of gamma aminobutyric acid (GABA)-regulated chloride channels, leading to neuronal hyperexcitation, paralysis and death [41,42]. Since fipronil sulfone is rapidly formed *in vivo*, the toxicological effects are likely due to the sulfone metabolite [6,43]. Various studies have shown that fipronil reveals thyroid disrupting properties in rats. Mechanistic investigations in rats suggest that fipronil does not interfere with the incorporation of iodine into thyroxine (T4), but it interferes with the biliary clearance of this hormone. This may trigger an increase in the concentration of thyroid-stimulating hormone by interference with the feedback mechanism and decreased thyroxine concentrations. Interestingly, fipronil treatment (0.5 mg/kg/day, IV for 14 days) had no effect on antipyrine clearance (a marker for hepatic cytochrome activity) in ewes. The difference in rat and sheep for the potential of fipronil as a thyroid disruptor might be related to the difference in the exposure to the toxicant, with the actual exposure to the sulfone metabolite of fipronil being lower in sheep than in rats[44].

S-methoprene is a juvenile hormone (JH) analog which acts as an insecticide growth regulator (IGR) when used as an insecticide. It interferes with the insect life cycle and prevents it from reaching maturity or reproducing. The compound is essentially nontoxic to humans and animals, since its oral LD_{50} in rats is >5000 mg/kg (27). Studies indicated that oral, dermal, or

inhalation exposure to s-methoprene is not likely to cause adverse health effects in humans.

Amitraz exposure is frequently encountered in dogs due to accidental ingestion of the collar, resulting in severe toxicity and sometimes fatal poisoning [10,12,19]. Acute toxicity data of amitraz is available for laboratory animals [19]. Oral LD_{50} values of amitraz for dog, pig, guinea pig, baboon, rat, and mice are reported to be 100, 100, 400, 100, 650, and 1600 mg/kg, respectively. The EPA classifies amitraz as a Class III slightly toxic pesticide. Onset of clinical signs is noted within 30 min to 2 hr after ingestion. Clinical signs of poisoning include GI disturbance, nausea, vomiting, diarrhea, staggering, disorientation, CNS and respiratory depression, bradycardia, hypotension and hypothermia. Biochemical changes include hyperglycemia and elevation of liver enzymes (transaminases) activity. In dogs, following a topical application, amitraz has been shown to increase plasma glucose and decrease insulin release when dogs were dipped at twice the recommended concentration [45]. Oglesby et al [46] observed renal cortical necrosis and hemorrhage in a dog which died from acute renal failure following ingestion of an amitraz-formulated dip. Untreated dogs and cats usually go into a coma and die from respiratory failure. Generally, signs and symptoms of amitraz poisoning subside with full recovery within 24 to 48 hr, while in some cases it may take 7-10 days [47].

Amitraz produces toxicity in mammalian species primarily by stimulating α2-adrenergic receptors (α2-AR), resulting in impairment of consciousness, respiratory depression, convulsions, bradycardia, hypotension, hypothermia, and hyperglycemia. In a recent experimental study, Marafon et al. [48] observed significant declines in heart rate and respiration rate of cats intoxicated with amitraz. Electrocardiography (ECG) on an amitraz-poisoned dog revealed prolonged QT intervals [21]. Amitraz acts centrally to influence blood pressure and heart rate by α2-AR agonism, which causes a reduction in peripheral sympathetic tone [20,49]. In the peripheral vasculature, both α1 and α2-ARs are involved in contributing to the vasopressor action of amitraz, resulting in hypotension. Cullen and Reynoldson [20] suggested that the central α2-AR agonist activity of amitraz is responsible for CNS depression. In addition to being an α2-AR agonist, amitraz is a potent inhibitor of the enzyme monoamine oxidase, which is responsible for degrading the neurotransmitters (norepinephrine and serotonin), resulting in behavioral and neurotoxic effects [22,24]. Amitraz is also known to cause inhibition of prostagalndins [26].

Conclusions

Findings of the present investigation demonstrated that residues of fipronil, s-methoprene, and amitraz can be transferred to humans in a significant amount while petting or handling their dogs. The highest levels of transferable residues of all three insecticides were found at 72 hr post-Certifect® application and this could potentially be the time of highest risk. Amitraz residue was undetectable at week three interval, while fipronil and s-methoprene were detected in small amounts at week four interval, probably due to their rapid elimination from the dog's coat. Following topical application of Certifect®, at no time was fipronil or s-methoprene present in the dog blood, and amitraz was present at 0.42±0.16 ppm level, suggesting that these insecticides have none or very little dermal absorption. Certifect® exposure neither caused an adverse reaction at the site of application nor did it produce any other side effects in any dogs, and therefore it is well tolerated and safe to use. Our future study will determine residues of these insecticides in dog beds, toys, and saliva.

Conflict of Interest Statement

None of the authors of this manuscript have any financial, personal, or other conflict of interest that could have inappropriately influenced the work described.

References

1. Anadon, A.N., and Gupta, R.C. Fipronil. In Veterinary Toxicology: Basic and Clinical Principles. Gupta, R.C. (Editor), Academic Press/Elsevier, Amsterdam. 2012, 604-608.

2. Gill, K.K., and Dumka, V.K. Biochemical alterations induced by oral subchronic exposure to fipronil, fluoride and their combination in buffalo calves. Environ. Toxicol. Pharmacol. 2013, 36: 1113-1119.

3. Gupta, R.C., and Milatovic, D. Insecticides. In Biomarkers in Toxicology. Gupta, R.C. (Editor), Academic Press/Elsevier, Amsterdam. 2014, 389-407.

4. Gasmi, A., Chataigner, D., Gamier, T et al. Toxicity of fiprop nil-containing insecticides. Report of 81 cases from the Paris Poison Center. Vet. Hum. Toxicol. 2001, 43:247.

5. Lee, SJ., Mulay, P., Diebolt-Brown, B et al. Acute illness associated with exposure to fipronil-surveillance data from 11 states in the United States, 2001-2007. Clin Toxicol. 2010, 48: 737-744.

6. Zhao, X., Yeh, J.Z., Salgado, V.L., and Narahashi, T. Sulfone metabolite of fipronil blocks GABA- and glutamate-activated chloride channels in mammalian and insect neurons. Toxicol. Sci. 2005, 84: 401-402.

7. Narahashi T, Zhao X, Ikeda T, Nagata K, and Yeh J Z. Differential actions of insecticides on target sites: basis for selective toxicity. Human Exper Toxicol. 2007, 26: 361-366.

8. Narahashi T, Zhao X, Ikeda T, Salgado V L, and Yeh J Z. Glutamate-activated chloride channels: unique fipronil targets present in insects but not in mammals. Pest Biochem Physiol. 2010, 97: 149-152.

9. Jiang D, Zheng X, Shao G, Ling Z, and Xu H. Discovery of a novel series of phenylpyrazole inner salts based on fipronil as potential dual-target insecticides. J Agr Food Chem. 2014, 62: 3577-3583.

10. Grossman MR, Garvey MS, and Murphy MJ, Amitraz toxicosis associated with ingestion of an acaricide collar in a dog. J Am Vet Med Assoc. 1993, 203: 55-57.

11. Gunaratnam P, Wilkinson G T, and Seawright A A., A study of amitraz toxicity in cats. Aust Vet J. 1993, 60: 278-289.

12. Hugnet C, Buronfusse F, Pineau X, Cadore J L, Lorgue G, and Berney P J. Toxicity and kinetics of amitraz in dogs. Am J Vet Res. 1996, 57: 1506-1510.

13. Andrade S F, Salnchcz O, and Tostes R A, Relato de 5 casos de intoxicacao por amitraz cm cacs c. gatos. Clin Veterinaria. 2004, 53: 38-42.

14. Ertekin V, Alp H, Selimoglu M, and Karacan M. Amitraz poisoning in children: retrospective analysis of 21 cases. J Intl Med Res. 2002, 30: 203-205.

15. Yilmaz H L, and Yildizdas D R, Amitraz poisoning, an emerging problem: Epidemiology, clinical features, management, and preventive strategies. Arch Dis Childhood. 2003, 88: 130-134.

16. Proudfoot, A.T. Poisoning with amitraz. Toxicol Rev. 2003, 22: 71-74.

17. Aăgin H, Calkavur Ö, Uzun H, and Bak M. Amitraz poisoning: clinical findings. Indian Pediatr. 2004, 41: 482-486.

18. Avsarogullari L, Ikizceli I, Sungur M, Sözüer E, Akdur O et al. Acute amitraz poisoning in adults: clinical features, laboratory findings, and management. Clin Toxicol. 2006, 44: 19-23.

19. Gupta R C. Amitraz. In Veterinary Toxicology: Basic and Clinical Principles. Gupta, R.C. (Editor), Academic Press/Elsevier, Amsterdam. 2012, pp. 599-603.

20. Cullen L K, and Reynoldson J A. Central peripheral alpha-adrenoceptor actions of amitraz in the dog. J Vet Pharmacol. Ther. 1990, 13: 86-92.

21. Malmasi A, and Ghaffari S. Electrocardiographic abnormalities in an English bulldog with amitraz toxicity. Comp. Clin. Pathol. 2010, 19: 103-105.

22. Aziz S A, and Knowles C O. Inhibition of monoamine oxidase by the pesticide chlordimeform and related compounds. Nature. 1973, 242: 417-418.

23. Moser V C. Investigations of amitraz neurotoxicity in rats. IV. Assessment of toxicity syndrome using a functional observational battery. Toxicol Sci. 1991, 17: 7-16.

24. Moser V C, and Macphail R C. Investigations of amitraz neurotoxicity in rats. Effects on motor activity and inhibition of monoamine oxidase. Toxicol Sci. 1988, 12: 12-22.

25. Del Pino J, Martinez M A, Castellano V, Ramos E, Martianez-Larranaga M R, and Anadon A. Effects of exposure to amitraz on adrenaline, serotonin, and dopamine levels in brain regions of 30 and 60 days old male rats. Toxicology. 2013, 308: 88-95.

26. Yim G K W, Holsapple M P, Pfister W R, and Hollingworth R M. Prostaglandin synthesis inhibited by formamidine pesticides. Life Sci. 1978, 23: 2509-2516.

27. Environmental Protection Agency (EPA) Methoprine; Pesticide Fact sheet 2001.

28. Jennings K A, Canerdy T D, Keller R J, Atieh B H, Doss R B et al. Human exposure to fipronil from dogs treated with Frontline. Vet Hum Toxicol. 2002, 44: 301-303.

29. Craig M S, Gupta R C, Canerdy T D, and Britton D A. Human exposure to imidacloprid from dogs treated with Advantage®. Toxicol Mechan Methods. 2005, 15: 287-291.

30. Gupta R C, Masthay M B, Canerdy T D, Acosta T M, Provost R J et al. Human exposure to selamectin from dogs treated with Revolution™: Methodological consideration for selamectin isolation. Toxicol. Mechan. Methods. 2005, 15: 317-321.

31. Bland S D, Gupta R C, Lasher M A and Canerdy T D. Safety assessment of etofenprox, s-methoprene, and piperonyl butoxide in dogs topically exposed to Bio Spot defense. J Veter Sci Technol. 2013, 4:6.

32. Gwaltney-Brant S M, DeClementi C, and Gupta R C. Macrocyclic lactone endectocides. In Veterinary Toxicology: Basic and Clinical Principles. Gupta, R.C. (Editor), Academic Press/Elsevier, Amsterdam. 2012, pp.609-619.

33. Ensley S M. Neonicotinoids. In Veterinary Toxicology: Basic and Clinical Principles. Gupta, R.C. (Editor), Academic Press/Elsevier, Amsterdam. 2012, pp.596-598.

34. WHO. Classification of pesticides by hazard. International Program on Chemical Safety. 1999. WHO/IPCS/98.21.

35. Environmental Protection Agency. Office of Prevention, Pesticides and Toxic Substances. Washington, D.C. 90, 1998.

36. Grant D B, Chalmers A, Wolff M, Hoffman H, and Bushbey, D. Fipronil: action at the GABA receptor. Rev. Toxicol. 2: 1998, 147-156.

37. IRIS. Integrated Risk Information System. United States Environmental Protection Agency, 2002.

38. Environmental Protection Agency. New Pesticide Fact Sheet. PB96-181516. epa 737-F-96-005. US EPA Office of Prevention, Pesticides and Toxic Substances. May 1996.

39. Hurley P M, Hill R N, and Whiting R J. Mode of carcinogenoic action of pesticides including thyroid follicular cell tumors in rodents. Environ. Health Perspect. 1998, 106: 437-445.

40. Anonymous. "Fipronil", Pesticide News. 2000, 48, 20.

41. Bobe A, Meallier P, Copper J and Coste C M. Kinetics and mechanisms of abiotic degration of fipronil. J Agri Fd Chem. 1998, 46: 2834-2839.

42. Li P, and Akk G. The insecticide fipronil and its metabolite fipronil sulfone inhibit the rat alpha1 and beta2 gamma21 GABA(A) receptor. Br. J. Pharmcol. 2008, 155: 783-784.

43. Hainzl D, Cole L M, and Casida J E. Mechanism for selective toxicity of fipronil insecticide and its sulfone metabolite and desulfinyl photoproduct. Chem Res Toxicol. 1998, 11: 1529-1535.

44. Laghait J, Gayrard V, Toutain P L, Picard-Hagen N, and Viguie C. Is the mechanism of fipronil-induced thyroid disruption specific of the rat: re-evaluation of fipronil thyroid toxicity in sheep? Toxicol Lett. 2010, 194: 51-57.

45. Hsu W H, and Schaffer D O. Effects of topical application of amitraz on plasma glucose and insulin concentrations in dogs. Am J Vet Res. 1988, 49: 130-131.

46. Oglesby P A, Joubert K E, and Meiring T. Canine renal cortical necrosis and hemorrhage following ingestion of an amitraz-formulated insecticide dip. J S Afr Vet Assoc. 2006, 77: 160-163.

47. Bonsall J L, and Turnbull G J. Extrapolation from safety data to management of poisoning with reference to amitraz and xylene. Human. Toxicol. 1983, 2: 587-592.

48. Marafon C M, Delfim C I G, Valadao C A A, Menotti R, and Andrade S F. Analysis of amitraz in cats by gas chromatography J Vet Pharmacol Ther. 2010, 33: 411-414.

49. Kobinger W. Central alpha-adrenergic system as targets for hypersensitive drugs. Rev Phys Biochem Pharm. 1978, 81: 39-100.