

## Safety Evaluation of Permethrin and Indoxacarb in Dogs Topically Exposed to Activyl® Tick Plus

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### Abstract

The use of ectoparasiticides on pets is incredibly important since pets are commonly infested with fleas, ticks, and other external parasites. Unfortunately, there is very little data on the safety of these products for dogs, their owners, and veterinary personnel who come into contact with the animals on a daily basis. Therefore, this investigation was undertaken to determine the residue of permethrin and indoxacarb in the dog's blood and to determine the transferable residues of these insecticides to gloves worn while petting six adult dogs after the topical application of Activyl® Tick Plus. Samples were collected on days 0, 1, 2, 3, 7, 14, 21, 28, and 35. At these time intervals, the dogs also underwent physical examination. The blood samples (approximately 4-5 ml) were collected into EDTA tubes. The glove samples were obtained by using 100% cotton gloves and petting each dog for 5 minutes, using a new glove each time. Blood and glove samples were extracted in methylene chloride:petroleum ether (1:1), and the extracts were assayed for residues of permethrin and indoxacarb using GC/MS. Blood analysis did not reveal the presence of permethrin or indoxacarb at any time during the investigation. In the gloves, the highest concentrations of permethrin and indoxacarb were determined at 24 hr ( $819.80 \pm 253.22$ ;  $90.80 \pm 35.16$   $\mu\text{g/g}$ , respectively). Residues of both compounds were found in significant concentrations in the gloves until day 7 ( $174.85 \pm 46.98$ ;  $7.63 \pm 2.83$   $\mu\text{g/g}$ , respectively). Permethrin residue was found in the gloves in detectable amounts until day 35 ( $28.12 \pm 11.59$   $\mu\text{g/g}$ ). Indoxacarb residue was found in the gloves in insignificant amounts until day 21 ( $0.65 \pm 0.45$   $\mu\text{g/g}$ ). In conclusion, Activyl® Tick Plus appears to be safe for dogs, as no adverse reactions occurred and residue was never found in the blood. Owners and veterinary personnel can be exposed to significant levels of permethrin and indoxacarb following daily exposure if proper precautions are not taken.

**Keywords:** Indoxacarb; Permethrin; Pyrethroids; Ectoparasiticide; Pesticide; Dog safety; Activyl® Tick Plus; Ectoparasiticide's safety; Human exposure

### Introduction

Infestation of pets with ectoparasites, such as fleas, ticks, lice, mites, and mosquitoes, is very common. This can pose major health problems to animals and humans and is a global concern. These ectoparasites are vectors, transmitting diseases between animals and humans. Mosquitoes can spread malaria, heartworms, and West Nile Virus. Ticks can spread Lyme disease, Babesiosis, Rocky Mountain Spotted Fever, Anaplasmosis, and Ehrlichiosis. Ticks can also cause tick paralysis due to toxins secreted by engorged females during feeding and can be fatal if the respiratory or cardiac systems become paralyzed. Fleas can spread tapeworms and Rickettsia. Many ectoparasites can also cause severe pruritus, which can lead to skin disorders. Many times, if the ectoparasite infestation is large enough, it can also result in anemia. Therefore, the use of ectoparasiticides on pets is crucial and inevitable.

Currently, pyrethrins and pyrethroids are more widely used than other classes of ectoparasiticides (organophosphates, organochlorines, carbamates, etc.) because they are considered to be safe for animals and humans due to their selective toxicity, i.e., more toxic to insects and less toxic to mammals [1,2]. While the targets of the drugs are similar in mammals and insects, mammals are less susceptible because their targets are less sensitive, they have a faster metabolic clearance rate due to their larger size, and higher body temperature [2-4]. Some products contain only one insecticide, while others contain multiple insecticides to provide broad spectrum activity. Not all classes of insecticides have the selective toxicity that pyrethrins and pyrethroids do and they can pose health risks to animals and humans alike. Due to the lack of safety data, concerns have been raised for their safe use in dogs and the amount of transferable residue passed to those that handle the dogs on a daily basis.

Activyl® Tick Plus is an over the counter ectoparasiticide product currently on the market applied topically once a month to kill and repel adult fleas, flea eggs, flea larvae, and all stages of ticks. The product consists of two active ingredients: 1) Permethrin [3-phenoxybenzyl ( $\pm$ )cis/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylate], 42.50%; and 2) Indoxacarb [(S)-methyl 7-chloro-2,5-dihydro-2-[[methoxycarbonyl [4-trifluoromethoxy] phenyl]amino]-carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a(3H)-carboxylate], 13.01% [5]. Chemical structures of permethrin and indoxacarb are shown in Figure 1.

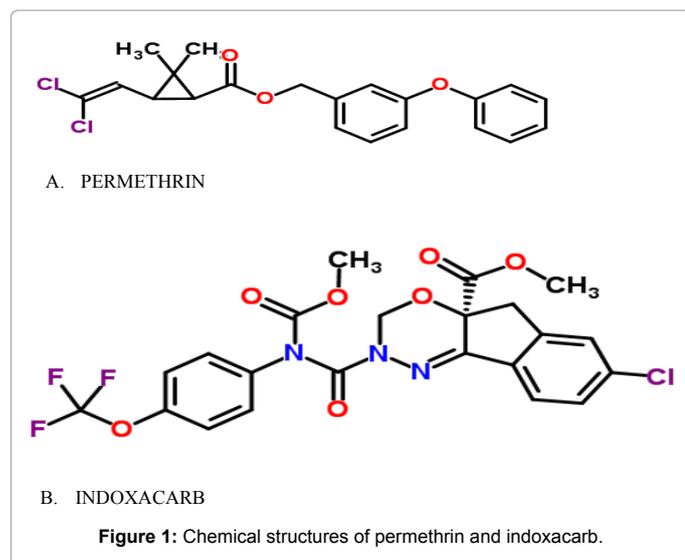
According to the Activyl® Tick Plus website, the only possible side effect listed is individual sensitivities. It is only to be used on dogs 8 weeks of age and older weighing at least 4 pounds [5]. The label states the product is safe once dry but there are still some concerns regarding the safety of the product on dogs as well as the amount of transferable residue to humans that come into contact with dogs that have been treated with Activyl® Tick Plus. This is especially important to veterinarians and veterinary technologists since they come into contact with animals every day that have been treated with this product. Due

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to this concern, the present study determined the levels of permethrin and indoxacarb transferable residue from the dog's coat and in blood in order to assess the exposure to veterinary personnel coming into contact with dogs treated with Activyl® Tick Plus.

Permethrin is in the pyrethroid class of insecticides. Pyrethrins come from the flower heads of *Chrysanthemum cinerariaefolium*. Pyrethroids are synthetic pyrethrins, which are used as insecticides on animals, homes, gardens, etc [2,6]. Permethrin was developed to provide prolonged efficacy because it is more stable than natural pyrethrins, which were found to break down rapidly when exposed to air, light, and heat [2,7,8]. Permethrin is a broad spectrum insecticide found in many sprays, dusts, spot-on applications, etc. It kills and repels ectoparasites, flies, cockroaches, and termites [9]. Permethrin acts on the nervous system of organisms and affects the sodium channels, causing the disruption of the function of neurons [10].

Indoxacarb is a relatively new oxadiazine insecticide. It has been used against lepidopteran pests but has recently been approved for use against fleas in dogs and cats. Indoxacarb exists as two enantiomers but only the S enantiomer has insecticidal activity [11]. The flea lands on the animal and ingests the indoxacarb, and through bioactivation the metabolite formed is highly toxic to insects. The parasites become paralyzed and unable to feed [5,12]. Indoxacarb exerts its insecticidal action at voltage-dependent sodium channels [13].

Both permethrin and indoxacarb have been recognized safe by the Environmental Protection Agency (EPA). The EPA is responsible under the Federal Insecticide, Fungicide, and Rodenticide Act and the Food Quality Protection Act for regulating pesticides that have public health uses and guaranteeing that these products do not pose unreasonable risks to humans, animals, and the environment [14]. Before the EPA can approve a pesticide, limits must be set on how the pesticide may be used, how often it may be used, and what protective clothing or equipment must be used. These limits are put in place to protect public health and the environment [15]. Currently, there is no reported data regarding the levels of permethrin and indoxacarb that humans may be exposed to from coming in contact with dogs treated with Activyl® Tick Plus.

Pesticides pose threats to humans as their widespread use has caused health problems and fatalities around the world due to occupational

hazard and accidental or intentional poisonings [16,17]. More than one million people are poisoned and hundreds of thousands die each year. In previous studies, we reported transferable residues of fipronil from Frontline™, imidacloprid from Advantage®, selamectin from Revolution™, etofenprox, s-methoprene, and piperonyl butoxide from Bio Spot Defense Flea and Tick Spot On®, and fipronil, s-methoprene, and amitraz from Certifect to humans from dogs topically treated with these products [18-22]. Each active ingredient depicted a different profile in terms of its dermal absorption, and persistence on the dog's coat and residue transferability. The present study assessed the toxicity and safety of Activyl® in dogs, as well as assessed the potential of human health exposure by determining the residue of permethrin and indoxacarb in the dog's blood and in gloves worn while petting the dogs.

## Materials and Methods

### Animals

Six healthy adult dogs of mixed breeds (medium length coat), weighing between 22-44 pounds, were used in this investigation. The dogs were not treated with any ectoparasiticide(s) for at least two months prior to this study. Prior to this investigation, glove and blood samples were checked for residues of permethrin and indoxacarb, and were found to be negative.

### Chemicals

Activyl® Tick Plus, containing permethrin and indoxacarb, was purchased from Merck Animal Health (Summit, NJ, USA). Each pack for a single application on a dog contained 2.0 mL (permethrin, 42.5%; and indoxacarb, 13.01%). Technical grade permethrin (99.5%) and indoxacarb (98%) standards were purchased from Chem Service (West Chester, PA, USA). All other chemicals of highest purity were obtained from Fisher Scientific (Pittsburgh, PA, USA).

### Experimental design

Experimental design for this investigation was followed from the study that was recently conducted with Bio Spot Defense Flea & Tick Spot On® in dogs [21]. A brief description of experimental protocol is given below.

**Activyl® Tick Plus application:** Activyl® Tick Plus (a product of Merck Animal Health, Summit, NJ) was topically applied to each dog directly onto the skin between the shoulder blades. 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 drugs continuously in the sebum along with other natural oils and waxes. This allows the drugs to coat the hair shaft and skin surface, providing long lasting protection against fleas and ticks [18-22]. To ensure that the necessary oils were present for distribution, the dogs did not receive a bath 3 days prior to or after the application of Activyl® Tick Plus.

**Physical examination:** Dogs topically treated with Activyl® Tick Plus were evaluated for physical parameters and skin reactions at the Activyl® Tick Plus application site.

**Sample collection for pesticide residue analysis:** Cotton gloves used for petting dogs were collected at days 0, 1, 2, 3, 7, 14, 21, 28, and 35; and blood samples were collected at days 0, 1, 2, 3, and 7 for residue analysis of permethrin and indoxacarb.

Glove sampling included the wipe sampling technique, which consisted of petting the dog forward and back along its back and sides,

while avoiding the application site, for five minutes while wearing a 100% cotton glove [18-22]. This was done to simulate the exposure that owners, veterinarians, veterinary technologists, and others that handle the dogs might be subjected to. After sampling, the glove was immediately placed in a labeled 473 mL (one pint) glass jar and kept at room temperature until analyzed (<24-48 hr). Blood samples were collected from the cephalic vein using a 6 mL syringe with a 22-gauge needle. At each time interval, approximately 3-5 mL blood was collected in a labeled EDTA anticoagulant tube and refrigerated until analyzed (<24-48 hr).

### Sample extraction

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

### GC/MS analysis

Both active ingredients present in Activyl® Tick Plus (permethrin and indoxacarb) were confirmed using an Agilent Gas Chromatograph (GC model 7890A)/Mass Spectrometer (MS model 5975C) coupled with a computer, and their concentrations were expressed in terms of  $\mu\text{g/g}$  (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  $\mu\text{L}$  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  $\mu\text{m}$ ), 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 °C. The injector was operated in the splitless mode. A temperature program for the GC-oven was used starting at a temperature of 100 °C, and then increased to a final temperature of 300 °C in 20 °C/min increments. The final temperature was maintained for 5 min. The total duration of each injection run was 16 min, with a solvent delay of 5 min. The transfer line temperature was 280°C, and the source temperature was 230 °C. The instrument was operated in electron ionization mode, and the ion energy was 70 eV. Two peaks of permethrin (cis and trans isomers) were eluted at 10.106 min and 10.168 min. The peak of indoxacarb eluted at 11.789 min. Sensitivity of the GC/MS for these compounds was in the range of ng, and the limit of detection was in the low  $\mu\text{g/g}$  (ppm) range. The total ion chromatogram (TIC) for permethrin is shown in Figure 2, and mass spectrum with characteristic ions for permethrin (91, 127, 163, 188.1, and 390.1) is shown in Figure 3. The TIC for indoxacarb is shown in depicted in Figure 4, and mass spectrum with characteristic ions for indoxacarb (59, 150, 218, 264, and 527.1) is depicted in Figure 5.

### Results

After a single application of Activyl® Tick Plus and exposure to the active ingredients permethrin and indoxacarb, none of the dogs exhibited any adverse effects from the product nor did they exhibit any skin reactions at the site of application. This suggests that the product is safe to use. The product label only lists individual sensitivities as a possible side effect [5].

Blood analysis revealed no residues of either permethrin or indoxacarb at any time during this investigation. Residues of permethrin and indoxacarb transferred from the canine hair coat

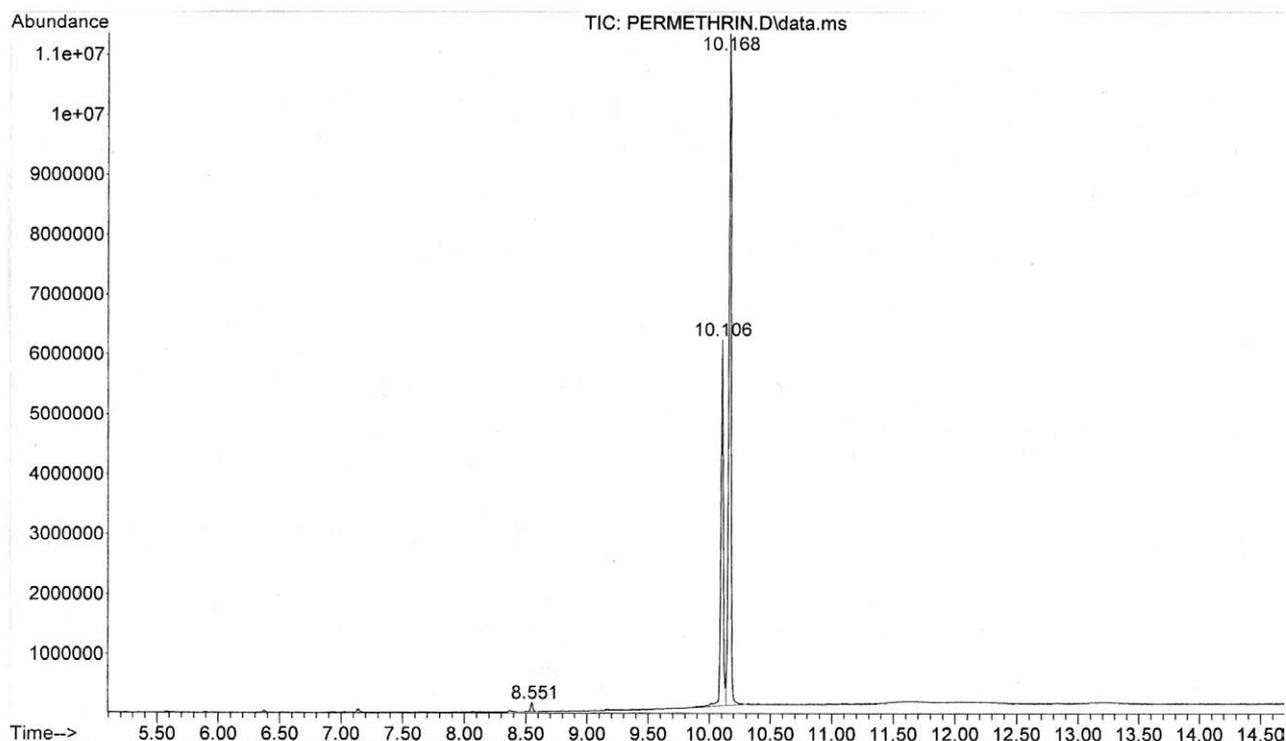
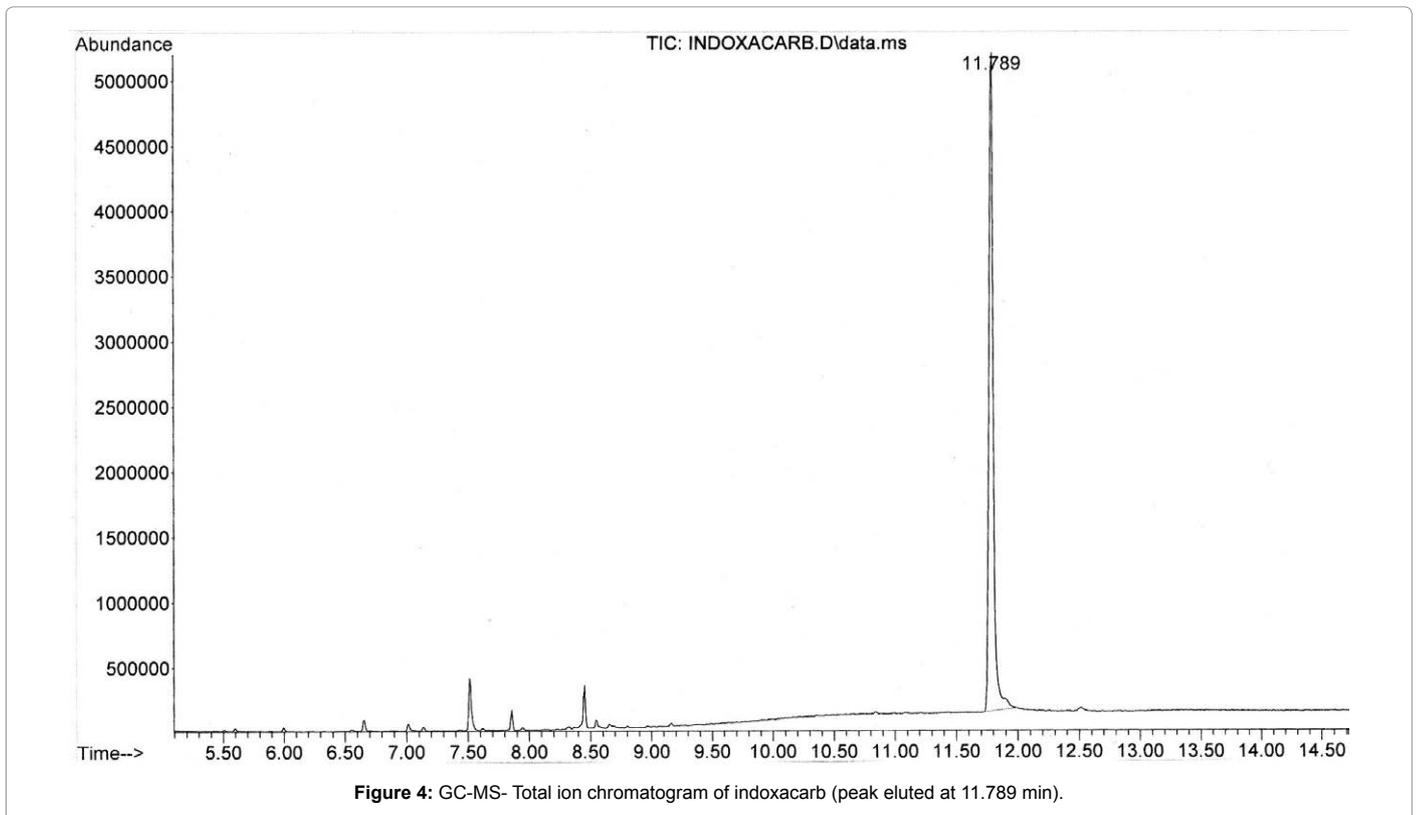
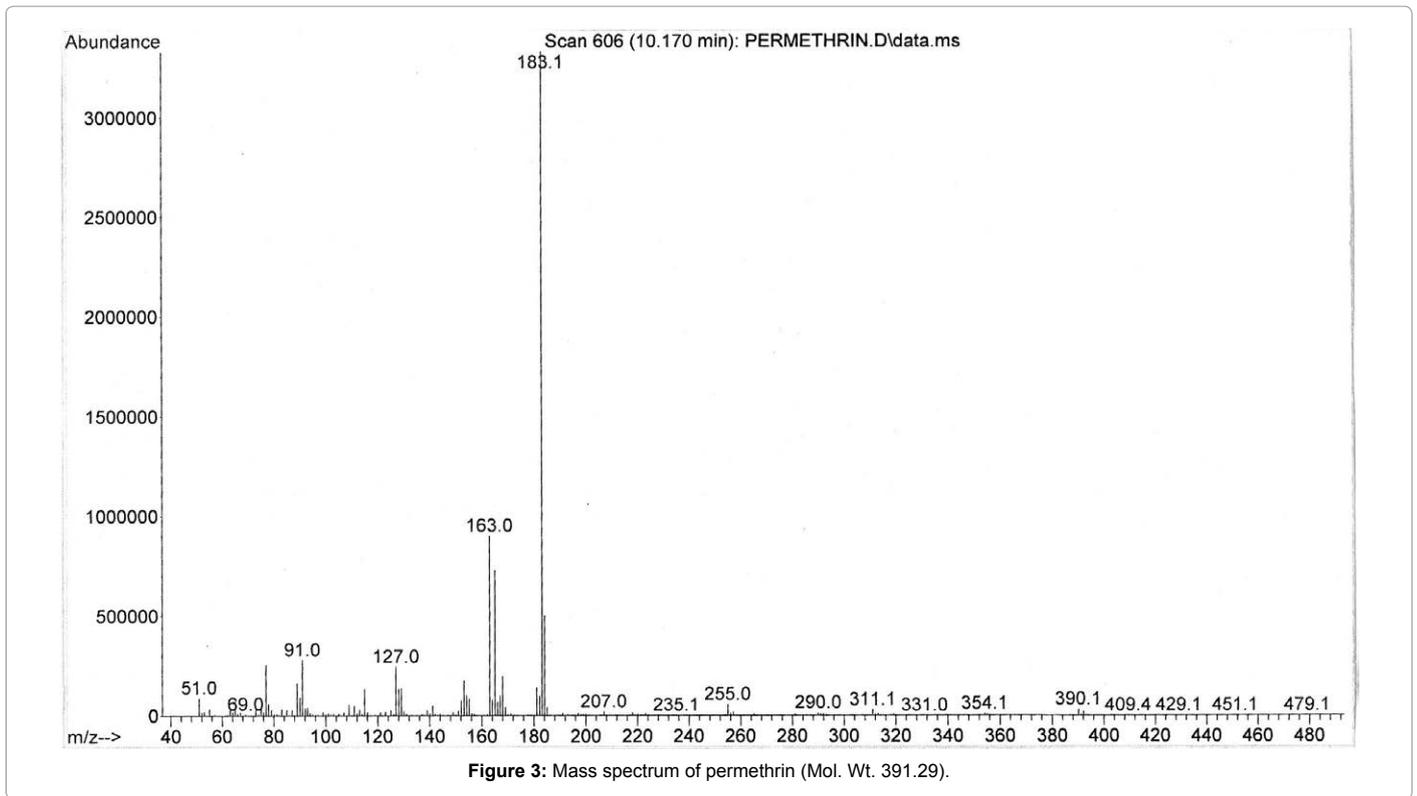


Figure 2: GC-MS-Total ion chromatogram of permethrin (peaks eluted at 10.106 min and 10.168 min, respectively).



were measured in cotton gloves at various time intervals using GC/MS, and data are presented in Figures 6 and 7, respectively. The highest concentrations of both permethrin and indoxacarb were

found at 24 hr post-application ( $819.80 \pm 253.22$ ;  $90.80 \pm 35.16$   $\mu\text{g/g}$ , respectively). Significant concentrations of permethrin and indoxacarb were found in the gloves until day 7 ( $174.85 \pm 46.98$ ;  $7.63 \pm 2.83$

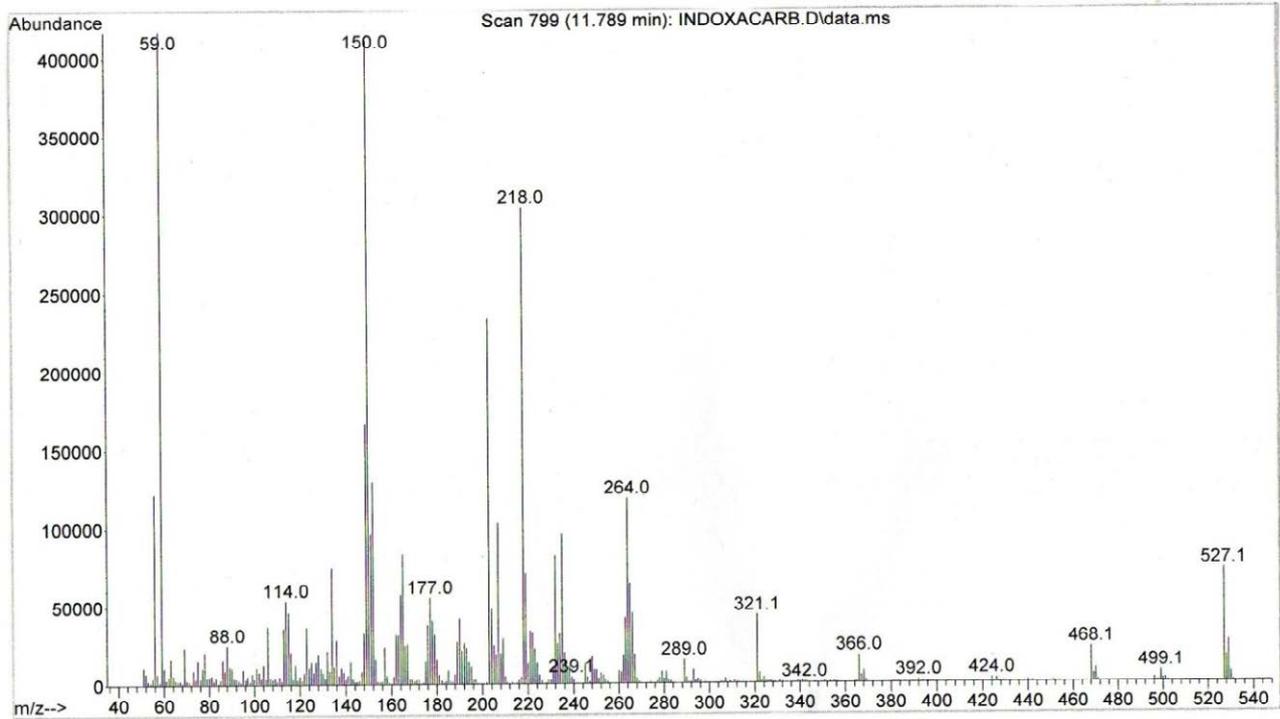


Figure 5: Mass spectrum of indoxacarb (Mol. Wt. 527.84).

### Permethrin Concentration in Gloves

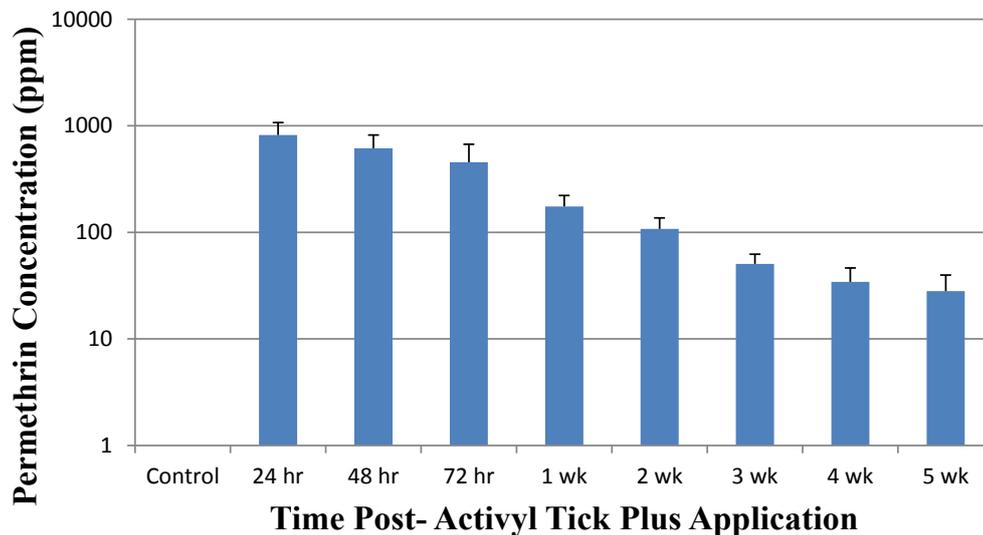


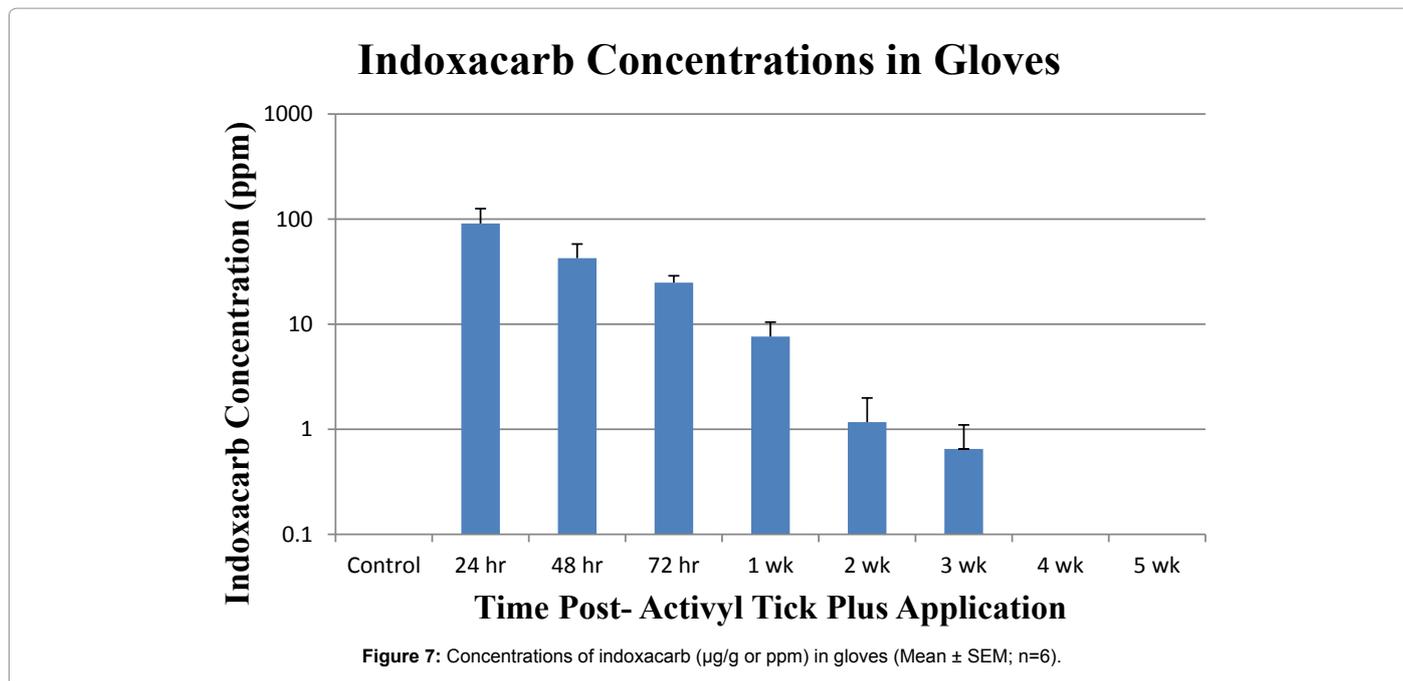
Figure 6: Concentrations of permethrin ( $\mu\text{g/g}$  or ppm) in gloves (Mean  $\pm$  SEM; n=6).

$\mu\text{g/g}$ , respectively). Permethrin residue continued to be detected in the gloves at insignificant concentrations until day 35 ( $28.12 \pm 11.59 \mu\text{g/g}$ ). Indoxacarb residue was detected in the gloves at insignificant concentrations until day 21 ( $0.65 \pm 0.45 \mu\text{g/g}$ ).

### Discussion

The present investigation was undertaken to address concerns

regarding the safety of permethrin and indoxacarb to dogs and the risk of human exposure from transferable residues of these insecticides from dogs topically exposed with Activyl® Tick Plus. While there is a vast amount of literature on the toxicity of permethrin and indoxacarb in laboratory animals, there appears to be little data regarding toxicity in dogs and no data to support the safety of these insecticides in humans coming into contact with the ectoparasiticide-exposed dogs.



Therefore, this study was undertaken to measure the transferable residue of permethrin and indoxacarb that veterinary personnel and owners might be exposed to from the coat and blood of dogs treated with Activyl® Tick Plus. Overall, the exposure of veterinary personnel to permethrin and indoxacarb may depend on the type of practice and the number of patients that are seen daily.

In the present investigation, at no time did dogs show any signs of adverse effects such as skin reactions or behavioral changes. The lack of permethrin and indoxacarb residue in blood ascertained insignificant dermal absorption of these insecticides from topical application of Activyl® Tick Plus on dogs. Residues of permethrin and indoxacarb on dog coat appear to be within safe limits after a single treatment with Activyl® Tick Plus. In previous studies also, dogs treated with Frontline™ (fipronil), Revolution™ (selamectin), Advantage® (imidacloprid), Bio Spot Defense Flea and Tick Spot On® (etofenprox, s-methoprene, and piperonyl butoxide), and Certifect® (fipronil, s-methoprene, and amitraz) showed no signs of toxicity or skin reactions [18-22].

Poisonings in dogs and cats due to permethrin or indoxacarb are most likely because of accidental or intentional ingestion. In clinical practice, poisoned dogs and cats are often suspected for permethrin and indoxacarb, and we realize that some discussion on the toxicological profile of permethrin and indoxacarb is needed in this paper.

According to a recent nomenclature, pyrethroids are of two types and produce two syndromes through multiple mechanisms of action. Type I pyrethroids (lack  $\alpha$ -cyano moiety) cause repetitive activity of nerve fibers, resulting in hypersensitivity, tremors, and production of 'T' syndrome. Type II pyrethroids by having a  $\alpha$ -cyano moiety exert choreoathetosis and salivation and produce 'CS' syndrome [2,11,23,24]. Type I syndrome involves action in the CNS and PNS, while type II syndrome involves primarily the CNS. Pyrethroids exert neurotoxicity by interfering primarily with sodium channels, but they also affect the chloride and calcium channels in neurons [25-29]. These pyrethroids slow the opening and closing of the sodium channels, which results in excitation of the cells [25,30,31]. This causes prolonged sodium permeability of the neuronal membrane [32]. Type I pyrethroids

prolong channel opening long enough to cause repetitive firing of action potentials, while type II hold the channels open for such a long time that the membrane potential becomes depolarized [2,25].

Permethrin is a Type I pyrethroid composed of a mixture of disproportional *cis*- and *trans*-isomers (25-40%, and 60-75%, respectively). The low mammalian toxicity of permethrin appears to be due to a greater content of *trans*-permethrin isomer, as in general *trans*-pyrethroids are less toxic than *cis* pyrethroids. The acute oral LD<sub>50</sub> value ranges from 430-8900 mg/kg body weight, and is usually influenced by factors such as isomer ratio, age, sex, and vehicle. The acute oral LD<sub>50</sub> value for permethrin in rats and mice is reported to be 2000 mg/kg and 540-2690 mg/kg, respectively. The acute dermal LD<sub>50</sub> value for permethrin in rabbits and rats is reported to be >2000 mg/kg and >5000 mg/kg, respectively [2,8,9,10,24]. Permethrin did not produce skin irritation, but did produce mild eye irritation in rabbits [10]. While permethrin is known to have a low mammalian toxicity, there have still been reports of poisonings in dogs and cats involving pyrethroids. Toxic reactions in dogs and rats are similar for type I and type II pyrethroids, and may include salivation, hyperexcitability, tremors, seizures, and loss of coordination [2,24]. Death in dogs due to pyrethroid poisoning is very rare. On the other hand, cats are very sensitive to pyrethroids and exposure can be fatal [33,34]. Symptoms of systemic pyrethroid poisoning in humans include fatigue, dizziness, headache, anorexia, and nausea. In more severe cases, symptoms can include disturbance of consciousness, coma, and convulsions [35-37]. Many pyrethrins and pyrethroids can produce cardiotoxic, neurobehavioral, reproductive, developmental, and endocrine disruptive effects in animals and humans [2,38-41]. The prognosis for pyrethroid toxicity is good because of the low toxicity. Currently, there are no specific antidotes for pyrethroid toxicity. Animals and humans must be treated symptomatically.

The toxicokinetic data and susceptibility to pyrethroid toxicity greatly vary due to factors, such as species, age, and route of administration [2,42,43]. Following dermal application, the absorption of pyrethrins/pyrethroids in mammals through the skin is

approximately 2%, but following oral administration the absorption rate appears to be 40-60% [2,27,43]. Pyrethroids are lipophilic, and as a result they distribute to tissues with high lipid content. They are rapidly hydrolyzed in the gastrointestinal tract and can be metabolized by oxidases and esterases, resulting in water soluble metabolites [2]. Permethrin metabolism proceeds via carboxylesterases rather than oxidative metabolism routes. Following the oral administration of permethrin (1.6-4.8 mg/kg) in rats, its bioavailability was 60.69%. Residue was detected in the liver (<25-55 ppb), fat (<25-618 ppb), brain (<25 ppb), and blood (49.46 µg/mL) [44,45]. Phase I metabolites of permethrin include OH-permethrin, *c*-,*t*-Cl<sub>2</sub>CA, *c*-,*t*-OH-methyl-Cl<sub>2</sub>CA, *m*-phenoxybenzyl alcohol, *m*-phenoxybenzoic acid, 4-OH-*m*-phenoxybenzoic acid, and *c*-OH-methyl-Cl<sub>2</sub>CA lactone. Phase II metabolites include glucuronide, glycine, and sulfate conjugates of hydrolysis and oxidation products [27,46]. Permethrin is eliminated as 1R, *cis* (37-39%) and 1R, *trans* (70-71%) in the urine; and 1R, *cis* (31%) and 1R, *trans* (8-13%) in the feces [44,45]. It is eliminated as a hydrolytic product of the parent ester. *Trans*-permethrin hydrolyzes more rapidly than *cis*-permethrin [41]. The slower hydrolytic metabolism rate of the 1R, *cis* isomer has shown longer residence in the system than 1R, *trans* isomer, which is why it exhibits a higher toxicity [47].

Indoxacarb is in the oxadiazine class and is a non-systemic synthetic insecticide. It exerts its action at voltage-dependent sodium channels by blocking the flow of sodium ions into nerve cells. The mechanism of action is similar to that of local anesthetics, anticonvulsants, and antiarrhythmics [48-50]. Using the whole-cell patch clamp technique in rat embryonic cerebral cortical neurons in primary culture, Zhao et al. [51] reported that indoxacarb has potent modulating actions on nicotinic acetylcholine receptors (nAChRs). Their findings suggested that the nAChRs could be one of the primary target sites of this insecticide in mammals. Indoxacarb has been found to have low to moderate acute toxicity. Studies have been done using DPX-MP062 which has mixture of two isomers (75% S and 25% R enantiomers). The acute oral LD<sub>50</sub> values for indoxacarb in male and female rats are reported to be 1730 mg/kg and 268 mg/kg, respectively. The acute dermal LD<sub>50</sub> value for indoxacarb in rats is >5000 mg/kg. An acute inhalation study was done using a racemic mixture of isomers, known as DPX-JW062. The LC<sub>50</sub> value in rats was reported to be >5.5 mg/L [11,49,52]. DPX-JW062 is a less effective insecticide than DPX-MP062 due to lower levels of the active isomer (DPX-KN128) present in the compound [53]. Indoxacarb did not produce skin irritation, but did produce moderate eye irritation in rabbits. In rats and dogs, clinical signs of toxicity included ataxia, immobility, lethargy, and tremors. There were also several hematological alterations, including Heinz bodies within erythrocytes, increases in neutrophil and lymphocyte counts, decreases in erythrocytes, increases in mean corpuscular volume, and bone marrow hyperplasia [11,54]. Humans that have ingested indoxacarb may develop methemoglobinemia, requiring methylene blue treatment and supportive care [55-58]. Clinical signs include cyanosis, chocolate brown mucous membranes, dyspnea, lethargy, and coma. Indoxacarb is a relatively new insecticide and as of the time of this study, there had been no reports of any adverse effects in humans occupationally exposed to indoxacarb. Of course, indoxacarb has been involved in suicidal intent [56]. Indoxacarb has not been shown to produce carcinogenic, mutagenic, developmental, or reproductive effects in animals or humans [52,59]. Other than methylene blue, there is no specific antidote for indoxacarb toxicity. Therefore, treatments for adverse effects are symptomatic and supportive.

Published reports indicate that the toxicokinetics and metabolism of *s*-indoxacarb and other isomers are similar [13,60]. There appears

to be a lack of data regarding dermal absorption, but following oral administration of indoxacarb (5 mg/kg or 150 mg/kg) in rats, absorption was estimated to be 70-80% and 8-14%, respectively. Radiolabeled indoxacarb was predominately found in the erythrocytes and the fat [60]. When the indoxacarb was radiolabeled in the indanone group, the half-life was 45-59 hr, but when it was radiolabeled in the trifluoromethoxy phenyl group, the half-life was 92 hr and 114 hr for males and females, respectively. The distribution of radioactive indoxacarb in rats given indanone labeled compound as a single oral dose (5 mg/kg) was 37-41% in urine, 44% in feces, and 3.4-7.8% in tissues. Those that were given indoxacarb with a trifluoromethoxy phenyl label as a single oral dose (5 mg/kg) showed a distribution of radioactivity 47-55% in urine, 27-30% in feces, and 10-17% in tissues. Studies show that females metabolize indoxacarb more slowly than males and produce almost ten times the amount of the toxic metabolite, IN-JT333 [60]. Along with IN-JT333, a range of other metabolites are formed and converted into sulfate and other conjugates. Substantial amounts were eliminated in the feces and urine, the amount depending on the position of the label. Biliary excretion of indoxacarb (for both labeled compounds) at 5 mg/kg in males and females was 23% and 17%, respectively. At 150 mg/kg, excretion was 6.4% and 1.8% in males and females, respectively [11,60]. The low level of neurotoxicity of indoxacarb in mammals is due to its insufficient bioactivation and rapid metabolic degradation compared to insects.

## Conclusions

This investigation was undertaken to assess the safety of Activyl® Tick Plus in dogs and to measure the transferable residues of permethrin and indoxacarb to humans. At no time, did any of the dogs exhibit any adverse effects from the product nor did they exhibit any skin reactions at the site of application, suggesting that Activyl® Tick Plus was safe for topical application. Findings revealed the persistence of permethrin and indoxacarb residues on the dog's coat in significant amounts that can be transferred to humans that come into contact with the animal. The levels of permethrin and indoxacarb on the dog's coat were highest 24 hr post-application, making this the time period that humans have the highest risk of exposure. Residues of both ingredients were found in significant concentrations until day 7. After day 7, the concentrations rapidly declined. Permethrin was detected in insignificant amounts until day 35, while indoxacarb until day 21. Following topical application of Activyl® Tick Plus, blood analysis did not reveal the presence of permethrin or indoxacarb at any time during the investigation. However, if proper precautions are not taken, those that come into contact with the dog such as owners, veterinarians, and veterinary technologists can be exposed to significant levels of permethrin and indoxacarb.

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