Transferable Residues From Dog Fur and Plasma Cholinesterase Inhibition in Dogs Treated with a Flea Control Dip Containing Chlorpyrifos

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We studied chlorpyrifos, an insecticide present in a commercial dip for treating ectoparasites in dogs, to estimate the amount of transferable residues that children could obtain from their treated pets. Although the chlorpyrifos dip is no longer supported by the manufacturer, the methodology described herein can help determine transferable residues from other flea control insecticide formulations. Twelve dogs of different breeds and weights were dipped using the recommended guidelines with a commercial, nonprescription chlorpyrifos flea dip for 4 consecutive treatments at 3-week intervals (nonshampoo protocol) and another 12 dogs were dipped with shampooing between dips (shampoo protocol). The samples collected at 4 hr and 7, 14, and 21 days after treatment in the nonshampoo protocol averaged 971, 157, 70, and 26 µg chlorpyrifos, respectively; in the shampoo protocol the samples averaged 459, 49, 15, and 10 µg, respectively. The highest single sample was about 7,000 µg collected at 4 hr. The pretreatment specific activities in the plasma of the dogs were about 75 nmol/min/mg protein for butyrylcholinesterase (BChE), and 9 nmol/min/mg protein for acetylcholinesterase (AChE). BChE was inhibited 50–75% throughout the study, and AChE was inhibited 11–18% in the nonshampoo protocol; inhibition was not as great in the shampoo protocol. There was no correlation ($p \leq 0.05$) between length of hair and residues measured that would explain the residue differences among dogs. Transferable residues had largely dissipated during the three weeks after treatment, with the largest decrease occurring during the first week. Greater plasma ChE inhibition was observed at 7 days than at 4 hr, probably reflecting the bioactivation of chlorpyrifos to chlorpyrifos-oxon. Plasma cholinesterase activity did not return to control levels during the 3-week period. The differences between the shampoo and nonshampoo protocols were explained by differences in the techniques of the dip applicators. *Key words*: acetylcholinesterase, butyrylcholinesterase, chlorpyrifos, dog fur, flea control, organophosphate insecticide, pesticide monitoring, transferable residues. *Environ Health Perspect* 109:1109–1114 (2001). [Online 19 October 2001]
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Exposure of children to pesticides is a major health concern. Children could be exposed to pesticides from carpet, house dust, and toys from treated houses, from clothing of parents who are farm workers and pesticide applicators, and from playing outside in treated lawns and gardens (*1*–*5*). One overlooked but important potential source for pesticide exposure to children is pets treated with parasite control products. These products often contain carbamate, pyrethroid, and organophosphate insecticides.

Organophosphate (OP) compounds have been used commonly as insecticides in and around households, on farm and domestic animals, and on agricultural lands. These compounds are used widely in the United States because of their relatively low mammalian toxicity, their short half-lives, and their ease of use. These insecticides have been used residentially for the control of termites, ants, roaches, ticks, fleas, and other insect and arachnid pests. Thus, there is increased opportunity for children to be exposed by multiple routes, and these aggregate exposures could contribute to significant toxicity. Identification of all possible routes of exposure and the quantification of the magnitude of these exposures may contribute to a more accurate calculation of pesticide risk, and thereby decrease the reliance on uncertain default assumptions in risk assessment.

The amount of exposure data to OP insecticides for children is limited. Most of the risk calculations are for adults and may not consider the potentially greater absorption and sensitivity of children, poorer personal hygiene, potentially lower capacity for detoxication, developing organ systems, and a greater body surface area to volume ratio. Human potential exposures to organophosphate compounds have been documented from several sources, such as carpeting and household dust (*2*). Children have also been poisoned by OP insecticides through exposure to contaminated items, such as bed linens, clothing, and burlap sacks (*3*–*5*). However, the amount of exposure to organophosphate compounds from flea and tick control products on pets, such as dogs and cats, has not been documented.

A demographic survey of companion animals by the American Veterinary Medical Association (*6*) indicated that in 1991 approximately 34.6 million households (36.5%) in the United States owned a dog or dogs, a number essentially unchanged from 1987. More households had dogs as pets than other types of animals. There was a mean of 1.52 dogs per dog-owning household, yielding an estimated national population of 52.5 million dogs. Fifty percent of pet-owning households were parental households with children; in comparison, 40% of total households have children. The authors of that study projected that there would be 53.6 million dogs in the United States in 1998 (*7*). These dogs could be a source of exposure to millions of children who live in the same environment and come into direct contact with dogs treated with flea control products. Also, millions of cats and other pets or domestic animals, such as horses or cows, are also treated for insect pests and could serve as additional sources of intermittent insecticide exposure to children. The control of fleas and ticks on pets is a high priority for pet owners. Dips, flea and tick collars, treatment of yards with powders, sprays, and granular forms of insecticides, and the use of sprays and foggers in the home are all insecticide methods used by pet owners to control pests associated with pets.

Chlorpyrifos is one of the most commonly used organophosphate insecticides in the United States. This broad-spectrum insecticide effectively controls a wide variety of insects, primarily as a contact poison, and is used in flea control products such as flea collars, sprays, and, until recently, as a dip. When this study was conducted, chlorpyrifos

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was used in dip products, but this formulation has been withdrawn by the manufacturer.

This study was designed to determine the amount of chlorpyrifos that could be transferred to humans from dogs treated with a flea and tick dip. We quantified transferable residues from cotton gloves, which were used to rub the fur of the back of the dogs for 5 min according to a standard protocol and at set intervals after application of the dip. We investigated the time course of dissipation of these residues after initial treatment, considered the influence of different fur lengths on transferable residues, and studied the effects of bathing the dogs between dips. We also determined the amount of plasma cholinesterase inhibition in the dog as a biomarker of insecticide absorption and persistence within the dog.

We also performed a test (reproducibility test) to determine whether rubbing with a cotton glove removed a significant amount of pesticide from the dog, which would bias subsequent samples. The reproducibility test determined residues after 7 days on two locations after an initial residue test on only one location at 4 hr.

Methods

Chemicals. We obtained the dip, shampoo, and eye drops from a veterinary supply company. The Adams Flea and Tick Dip (chlorpyrifos 3.84%, 2 oz/gal, SmithKline Beecham Animal Health) and the shampoo (Mycodex Pearlescent) were manufactured by SmithKline Beecham (West Chester, PA). The eye drops (Akwa Tears) were manufactured by Akorn, Inc. (Abita Springs, LA). All solvents used for the pre-extraction and extraction of the gloves were pesticide grade. The chlorpyrifos standard was obtained from Chem Service and was greater than 99% pure. All other reagents were purchased from Sigma Chemical Company (St. Louis, MO).

Animals. Care and use of the dogs were in accordance with the *Guide for the Care and Use of Laboratory Animals* (*8*) in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, overseen by J.E. Harkness, University Laboratory Animal Veterinarian, Office of Research, Mississippi State University. The collection of blood, collection of transferable residues, and the dipping of the dogs occurred in rooms in the College of Veterinary Medicine Animal Health Center. The dogs were monitored by the directing veterinarian of the project (J. Tyler) and veterinary students. Dogs were selected from pet owners at the College of Veterinary Medicine, Mississippi State University, and met the following criteria: They had had no known organophosphate compound exposure one month before inception of the project; the dog must weigh > 10 lbs; and the owner must comply with the experimental protocol, including housing the dog at the College of Veterinary Medicine when needed and not using any other organophosphate compound during the study. Dogs used in the study are described in Table 1. Dogs were tested in two groups of six for each protocol because of space and time constraints. The dogs represent many sizes, ages, and inside/outside conditions. There were 12 replicates (individual dogs) for each of the two protocols.

Protocols. We employed two protocols in this study, one not involving shampooing between dippings (nonshampoo protocol) and the second involving shampooing immediately before each dipping (shampoo protocol). We designed the latter protocol to determine whether intermediate shampooing would reduce potential accumulation of residue levels.

Petting. The dogs were rubbed in anterior and posterior directions (back and forth motions) in a firm pattern but not one that would cause discomfort to the dog. The dogs seemed to enjoy the attention and petting. The dogs were petted in a 10 × 4 inch area marked with a readily removable adhesive tape on the back just caudal to the neck for 5 min (fur samples) with 100% cotton gloves that had been precleaned. The gloves were inverted when removed and placed into a clean, labeled glass jar.

Collection of samples. Handlers who dipped the dogs wore eye protection, shoulder-length rubber gloves, and a rubber-coated cotton apron. Dogs were bathed one day before the start of the study and were dipped according to the directions on the product label. Eye drops were placed into the eyes of the dogs before dipping. The dip was diluted to 2 oz/gal and sponged over the dog until the undercoat was wet (approximately 2 min). A fresh dip was prepared for each subsequent dog and dipping; each dog's coat was saturated with the dip, and no dip solution was reused. The dogs were placed in cages and dried with cage dryers on high fan setting with low heat (approximately 2 hr for complete drying).

For the nonshampoo protocol, we collected fur samples before dipping (0 hr), 4 hr after dipping, and at 1, 7, 14, and 21 days after the dip. At 21 days the dogs were sampled (21-day sample), dipped, dried, and sampled 4 hr after the dipping (4-hr sample) and at 7, 14 , and 21 days later. This process was completed 2 more times, so there were four sequential dips per replicate. We collected blood samples in evacuated blood collection tubes with no anticoagulant. Blood samples, taken at the same time as fur samples, were centrifuged after collection and stored at 4°C overnight, and cholinesterase activity was determined within 24 hr after collection.

For the shampoo protocol, we collected the fur samples before dipping (0 hr), at 4 hr

Table 1. Dogs used in the transferable residue and cholinesterase experiments, including breed, weight, fur length, and percent change from average within protocol at 4 hr.

Dog,[a] breed (sex)	Weight (lbs)	Fur length	Percent of average[b]
Nonshampoo protocol			
1 German shepherd (F)	77.2	medium	47.39
2 Labrador x Greyhound (F)	48.5	short	135.74
3 Mix (F)	19.0	long	57.14
4 Labrador x Mix (M)	62.3	long	69.31
5 Boston terrier x Mix (F)	22.5	short	62.99
6 Border collie x Greyhound (F)	44.5	medium	89.62
7 Rottweiler (F)	43.0	short	65.17
8 Labrador (F)	74.3	short	301.77
9 Mix (F)	23.1	long	78.90
10 Rottweiler x Mix (M)	73.3	short	109.74
11 Mix (F)	18.7	short	58.09
12 Rottweiler x Mix (F)	72.5	short	124.15
Shampoo protocol			
13 Rottweiler (F)	107.1	short	199.29
14 Rottweiler (F)	109.5	short	175.32
15 Border collie (M)	55.5	long	22.25
16 Mix (F)	33.3	short	26.13
17 Labrador (F)	73.5	short	47.46
18 Boxer (M)	86.1	short	46.46
19 Mix (M)	48.0	short	167.78
20 Schnauzer x Poodle (F)	13.4	med/long	68.73
21 Spitz x Poodle (M)	13.2	long	52.14
22 Weimaraner (F)	85.0	short	147.64
23 Weimaraner (M)	88.0	short	135.50
24 Husky x Mix (F)	33.9	long	111.30

Abbreviations: F, female; M, male; med, medium; Mix, mixed breed; x, cross breed.
[a]Dogs identified by number. [b]This number represents the percent of the average over four dippings at 4 hr from each protocol shown in Tables 2 and 3. For example, dog 1 averaged 47% of the total average (1,229 µg), or 578 µg over the four dippings at 4 hr.

and 1, 7, 14, and 20 days after dip, and the day after shampooing (21 days). The dogs were shampooed the day before each dipping, fur samples were taken on day 20, and the dogs were shampooed and allowed to dry overnight at home. At 21 days the dogs were sampled (to check for shampoo removal of chlorpyrifos), dipped, dried, and sampled at 4 hr after dip. The sampling was repeated (4 hr and 7, 14, 20 and 21 days) for a total of four dips. Blood samples were collected at the same time points as the fur samples, except a postshampoo blood sample between dips was not taken (day 21).

Reproducibility test. We developed this test to check collection of samples to make sure the decrease seen in the levels of chlorpyrifos over time was caused by the dissipation and not removal by the rubbing; we conducted two tests. In this study, 4 dogs were dipped, dried, and then sampled along the dorsal cervicothoracic spine (back/neck) in a 40-square-inch area at 4 hr as described previously. At 7 days the dogs were again petted along the dorsal cervicothoracic spine (back/neck), the same area as the 4-hr sample. Also at 7 days an additional fur sample was obtained from the fur along the dorsal lumbosacral region (back/tail) in a 40-square-inch area for comparison. If the sample from the dorsal cervicothoracic spine region at 7 days was not significantly different from that of the dorsal lumbosacral region at 7 days, this would indicate that the rubbing did not cause the decrease over time; the 4-hr rubbing had no significant effect on the sample collection at subsequent times.

In the reproducibility test, there was no significant difference (general linear model, least significant difference, $p \leq 0.05$) between the back/neck sample and the back/tail sample at 7 days (back/neck, 93 ± 12 µg; back/tail, 92 ± 10 µg); samples at 4 hr had originally been 1,113 ± 195 µg at the back/neck area.

Determination of chlorpyrifos on gloves. The gloves used for rubbing were 100% cotton. They were washed once with laundry detergent, three times without detergent, pre-extracted for 8 hr with methylene chloride, dried, and stored in glass jars washed and prerinsed three times with water, acetone, and petroleum ether. After sampling, the gloves were extracted with petroleum ether using an Accelerated Solvent Extractor (ASE) by Dionex (Sunnyvale, CA). The operating conditions were heat for 5 min at 100°C and 1,500 psi; static for 2 min; flush 50% of volume; static for 2 minutes; purge with nitrogen for 150 sec; and a final purge for 60 sec. For every 20 samples three additional gloves were spiked with 20 µL hexane, 10 µg chlorpyrifos, or 1,000 µg chlorpyrifos at the time of sampling and stored and extracted with the samples.

During method development, we quantified gloves from dogs and applied various concentrations from 1 to 5,000 µg of chlorpyrifos to different gloves to check for recovery rates and extraction parameters. All spiked gloves yielded 90–100% recovery. After collection, the sampling gloves and the spiked gloves were stored at 4°C until extraction. After extraction by the ASE, the extract was evaporated and transferred to hexane under a nitrogen stream using an N-EVAP (1–3 mL; Organomation Associates, Inc., Northborough, MA), and then was transferred to graduated test tubes, and the volume was adjusted to 10 mL. We analyzed the extract by gas chromatography/electron capture detection using an Rtx-5 Amine column (30 m, 0.53 mm ID, 1.0 µm df; Restek Corporation, Bellefonte, PA). Injector temperature was 280°C; oven temperature was 180–200°C (ramp 2 degrees/min for 5 min); and detector temperature was 325°C. The recovery rate for the spiked gloves throughout the study was 90–100%. The level of detection (LOD) and level of quantitation (LOQ) were 0.6 pg/µL and 2.7 pg/µL for 1 µL injections. The LOD and LOQ were determined by the methods described by Taylor (*9*), and we used spiked gloves.

Cholinesterase assay. The cholinesterase (ChE) assay was a modification of the procedure described in Chambers and Chambers (*10*), which is based on Ellman et al. (*11*). We determined cholinesterase by four different means using various combinations of inhibitors and substrates to investigate plasma enzymes. We diluted the plasma in 0.05 M Tris-HCl buffer, pH 7.4 at 37°C. We used 14 test tubes for each plasma sample. Each test tube contained 0.05 M Tris-HCl buffer, pH 7.4 at 37°C, and the plasma sample. We added eserine sulfate to four of the test tubes to determine noncholinesterase-mediated hydrolysis of acetylthiocholine (ATCh) iodide. To four of the test tubes we added tetraisopropyl pyrophosphoramide (iso-OMPA) to determine nonbutyrylcholinesterase (BChE)-mediated hydrolysis of butyrylthiocholine (BTCh) iodide. We added ATCh iodide (final concentration 1 mM) to seven of the tubes, which were vortexed and returned to a 37°C shaking water bath for 15 min. The seven test tubes comprised 3 for uninhibited assays, 2 with eserine sulfate, and 2 with iso-OMPA. We assayed a parallel set of tubes with BTCh iodide (final concentration 1 mM). We added sodium dodecyl sulfate (SDS) and 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB) (final concentrations of 0.44% and 0.02%, respectively) to each tube to stop the reaction (SDS) and for the chromogen (DTNB) to react with the thiocholine released from ATCh or BTCh hydrolysis; tubes were vortexed and the absorbance was read at 412 nm. We determined acetylcholinesterase (AChE) activity by subtracting the eserine sulfate readings from the iso-OMPA readings in the acetylthiocholine iodide set. We determined total ChE activity by subtracting the eserine sulfate readings from the uninhibited readings in the ATCh set. We determined butyrylcholinesterase activity with ATCh by subtracting the iso-OMPA readings from the uninhibited readings in the ATCh set. We

Table 2. Transferable residues of chlorpyrifos from fur after four consecutive treatments of dogs with flea control dip during the nonshampoo protocol.

Time	Amount on glove (µg)[a]	95% Confidence intervals		Range	
		Lower	Upper	Lower	Upper
Pretreatment	0.1583	0.0697	0.3600	< 0.03	0.73
4 hr	971.18	491.93	1917.34	157	6,999
7 days	157.00	79.52	309.96	4	2,584
14 days	70.11	35.51	138.39	1	2,472
21 days	26.63	13.49	52.57	1	1,469

[a]Geometric means are significantly different at $p \leq 0.05$. Each point is the average of 12 dogs (replicates) over all of the four treatments.

Table 3. Transferable residues of chlorpyrifos from fur after four consecutive treatments of dogs with flea control dips during the shampoo protocol.

Time	Amount on glove (µg)[a]	95% Confidence intervals		Range	
		Lower	Upper	Lower	Upper
Pretreatment	0.1079	0.0560	0.2078	< 0.03	0.97
4 hr	458.78	278.93	754.40	17	2,674
7 days	49.26	29.96	81.02	9	479
14 days	14.86	9.03	24.48	1	190
20 days	9.62	5.84	15.83	1	130
Shampoo 21 days	2.98	1.78	5.00	< 0.03	72

[a]Geometric means are significantly different at $p \leq 0.05$. Each point is the average of 12 dogs (replicates) over all of the four treatments.

determined BChE activity with BTCh by subtracting the iso-OMPA readings from the uninhibited readings in the BTCh set. We determined protein by the method of Lowry et al. (*12*) for standardization.

Statistics. We analyzed data using general linear model analysis of variance (ANOVA) for a randomized complete block design with a control and a 4 × 4 (nonshampoo protocol) or a 4 × 5 (shampoo protocol) factorial arrangement of treatment and time after treatment application. The individual dog was the block; multiple observations at each time point were averaged. We examined the residuals from each ANOVA using frequency histograms and normal probability plots; the data were logarithmically transformed and reanalyzed if the normality assumption appeared to be substantially violated. This was true for the residue data but not for the ChE data. If significant effects were found, we separated the means using the Least Significant Difference Test. We performed calculations on a personal computer using the SAS System for Windows, Version 8.0 (SAS Institute Inc., Cary, NC); all statistical tests used the 0.05 level of significance. There was no interaction between treatment and time for the residue studies. There was an interaction between treatment and time for the 4-hr ChE measurements because of the initial inhibition onset. We determined terminal half-life of dissipation of transferable chlorpyrifos residues from linear regression of each sample from 7 to 21 days.

Results

Table 1 describes the dogs used in this study, including different breeds, weights, and fur length. There was no correlation between transferable residues and fur length at $p \leq 0.05$. The type of fur and amount of undercoat were not quantified. In the nonshampoo protocol, dog #8 had the highest transferable residue, 6,999 µg after dip 1, and in the shampoo protocol, dog #13 had the highest transferable residue, 2,676 µg after dip 4 at 4 hr. Comparable breeds of dogs within each protocol did not exhibit the high residues found with dogs #8 or #13. In neither of the protocols did a single dog exhibit consistently the lowest transferable residue as was seen with the high residues.

In both protocols, the levels of chlorpyrifos dissipated quickly after each of the four dippings. Tables 2 and 3 contain the averaged transferable residues and ChE inhibition of all four dippings of the nonshampoo and shampoo protocols, respectively. Figure 1 shows the amount of transferable residue for each dipping in succession for the nonshampoo and shampoo protocols. For the nonshampoo and shampoo protocols, the terminal half-lives of dissipation of transferable chlorpyrifos residues from 7 to 21 days followed first-order kinetics and were 5.38 days and 5.40 days, respectively.

In both protocols, the plasma ChE was inhibited maximally at 7 days (Tables 2 and 3, average of all four dippings). Figures 2, 3, 4, and 5 show the inhibition of ChE following each of the four dippings for the two protocols. In both protocols, the pretreatment BChE activity was 7- to 10-fold higher than AChE activity (Tables 2 and 3; Figures 2 and 3). The BChE was inhibited to the greatest extent, whereas AChE in both the protocols was minimally inhibited (Tables 2 and 3; Figures 2 and 3). The inhibition of the measured activity of total ChE and BChE (using ATCh) reflects the same pattern of inhibition as BChE using BTCh (Tables 2 and 3; Figures 4 and 5).

Discussion and Conclusions

By designing this experiment to use a variety of breeds of dogs and several people for the treatments and sampling, we anticipated that variability in chlorpyrifos residues from dipping (differences in formulation and applicators), dog physiology (differences in breeds and coats), dog activity (rolling in yard, swimming), and petting (pressure, motion, and skin conditions) would reflect the range of values that pet owners might expect to encounter when using a chlorpyrifos dip in the recommended manner for routine pet flea and tick control. The differences among the dogs, as seen from the percent change in Table 1, demonstrate the differences in amounts of transferable residues that might be expected from flea control products caused by the diversity of the animals and handlers. The differences between the shampoo and nonshampoo experiments probably derived from the differences in fur saturation during dipping and in rubbing pressure among samplers. The two protocols were conducted by two different groups of workers and used 12 different dogs in each protocol.

There was a 73–87% average decrease in transferable residue by 7 days after dipping in both protocols. This rapid decrease was not anticipated in the original protocol design, and subsequent studies should include time points between 4 hr and 7 days to fully understand residue dissipation from dog fur. Within the shampoo protocol there was an average decrease of 32% in chlorpyrifos residues after shampooing, but this did not affect the level of cholinesterase inhibition. Although this percent decrease following shampooing appears large, the amount of transferable chlorpyrifos had declined about 97% 20 days from the initial 4-hr levels. The shampooing was not significant in preventing accumulation of chlorpyrifos or decreasing cholinesterase inhibition because the transferable residue had decreased to almost negligible levels by the end of the time period between approved dippings.

The reproducibility test showed that the decrease in transferable residues is a result of

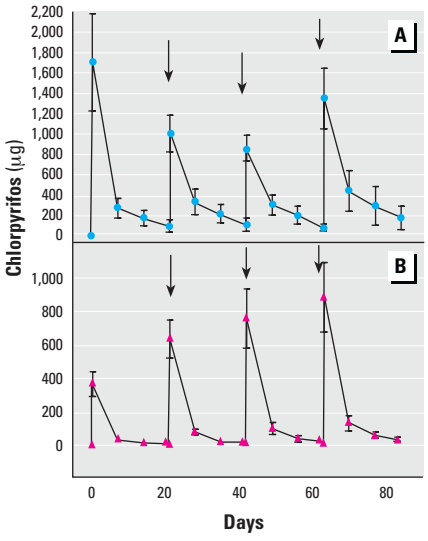

Figure 1. Transferable residues of chlorpyrifos from cotton gloves used in petting dogs for 5 min over a 40-square-inch area. The residues are from 12 dogs treated with four sequential dippings of chlorpyrifos every 21 days (*A*) without shampooing between dips and (*B*) with shampooing between dips. The sample times are 4 hr, 7 days, 14 days, and 21 days after dipping. Arrow represents times of subsequent dippings. The graph represents geometric means with 95% confidence intervals.

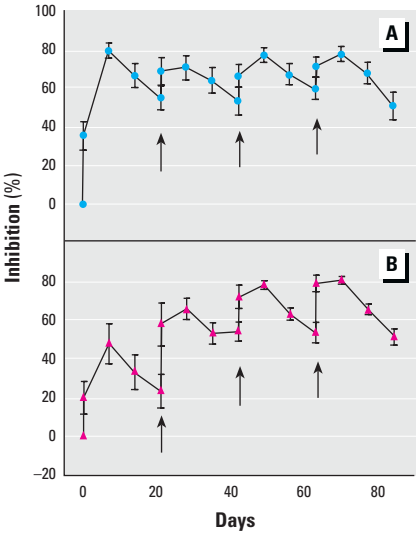

Figure 2. Percent inhibition of BChE (using BTCh as a substrate and iso-OMPA as an inhibitor) from the plasma of 12 dogs treated with four sequential dippings of chlorpyrifos every 21 days (*A*) without shampooing between dips and (*B*) with shampooing between dips. The sample times are 4 hr, 7 days, 14 days, and 21 days after dipping. Arrow represents times of subsequent dippings. The graph represents geometric means with 95% confidence intervals.

decline in the levels by degradation or wear and not the result of removal by the sampling procedure. These data showed that the use of cotton gloves for sampling transferable residues leads to reproducible results and that the gloves do not remove all of the pesticide available for exposure.

Maximum observed inhibition of plasma ChE activities measured in both studies occurred at 7 days and not at 4 hr probably because of the required activation of the parent compound. Maximum inhibition may have occurred at 2, 3, or 4 days, but blood samples at these time points were not taken because of the experimental design. As with the residue monitoring, subsequent studies should monitor the blood ChE between 4 hr and 7 days. The BChE in the plasma gave the highest enzyme activity and would serve as the most effective biomarker of insecticide action and persistence after an exposure to chlorpyrifos flea and tick control in dogs. Measuring AChE would not be a useful indicator of chlorpyrifos exposure in dogs because AChE levels are low and apparently not as sensitive to inhibition as BChE. Measuring BChE using BTCh gave the highest activity and is the most useful of the several ChE measurements performed for determining decreases in ChE levels after exposure to chlorpyrifos. The study demonstrated also that BChE hydrolyzed ATCh better than AChE hydrolyzed BTCh, and was the reason that inhibition of total ChE using ATCh reflects the inhibition pattern of BChE.

The ChE activity in the blood was depressed substantially after the first treatment, recovered incompletely by the time of the next treatment, and became substantially inhibited again with each subsequent treatment. Inhibition was maintained at 60–80% throughout the experiment. These results were surprising. Although the status of the dog was not the goal of the experiments, there is some concern that dogs with this level of inhibition would be vulnerable to additional exposures to anticholinesterases. Although this information would be used to caution dog owners, chlorpyrifos dips are no longer on the market.

The information gained from this study shows that humans are at greatest potential risk of exposure to chlorpyrifos shortly after dipping. Because children spend time with the pets and are potentially more sensitive to exposures than are adults, plausible exposure levels to calculate risk are needed. As an example, calculating from the 4-hr geometric means, if a child played with a dog for 5 min over an 80-square-inch area (twice the surface area sampled in this study), and the transfer was comparable to that transferred to our experimental gloves, the child could be exposed to an average of 0.9–1.9 mg of chlorpyrifos. With an absorption rate of 3% (*13*) the child could have a potential dosage of 0.027–0.057 mg. In a 25-kg (55 lb) child the absorbed dosage could be 0.0011–0.0023 mg/kg. In an 80-kg (175 lb) adult the absorbed dose could be 0.0003–0.0007 mg/kg.

The 21-day repeated exposure no-observed-effect level (NOEL) for chlorpyrifos is 0.03 mg/kg/day and the reference dose (R_fD) is 0.003 mg/kg/day (*14,15*). The numbers calculated above for the potential exposure from a flea dip are in the range of the NOEL and R_fD, but the child would probably not rub the dog with as much pressure as the workers were trained to do, and would probably be cautioned not to play with the dog for the first day after dipping. However, the 5 min data obtained here are believed to be an appropriate surrogate for a much longer handling by a child. Within 1 week the residues declined substantially. At 7 days after the initial dipping using the geometric means, the exposure to the child in this same scenario would decrease to 0.00006–0.0002 mg/kg. Also the numbers calculated from this example are from one time point, and the NOEL and R_fD are calculated for 21 days. By extrapolating the geometric mean data throughout the study on a per day basis, the 21-day exposure would range from 0.005 to 0.012 mg/kg, which is 0.0002 to 0.0006 mg/kg/day. With the worst-case-scenario data (the highest level at each time point independent of individual dog) the dosage would be 0.0077 mg/kg/day. The worst-case-scenario level is below the NOEL but slightly above the R_fD. If time points such as 1, 3, and 5 days were taken to predict more accurately the decline of transferable residues, then the predicted exposure would be lower. Also, the habits of

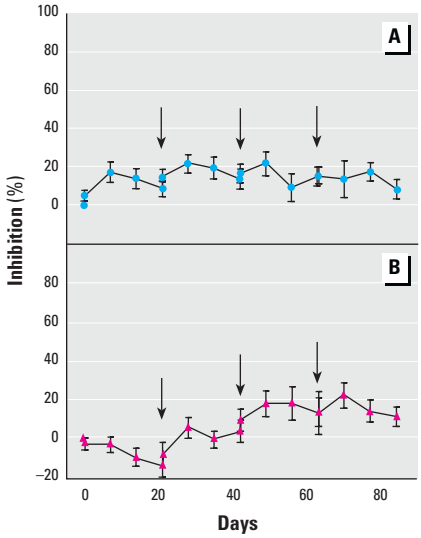

Figure 3. Percent inhibition of AChE (using ATCh as a substrate and eserine sulfate as an inhibitor) from the plasma of 12 dogs treated with four sequential dippings of chlorpyrifos every 21 days (*A*) without shampooing between dips and (*B*) with shampooing between dips. The sample times are 4 hr, 7 days, 14 days, and 21 days after dipping. Arrow represents times of subsequent dippings. The graph represents means with 95% confidence intervals.

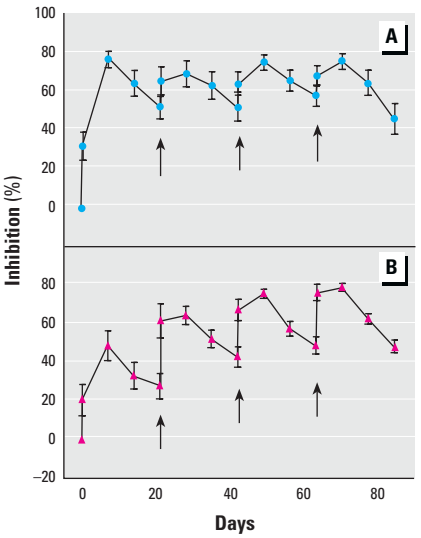

Figure 4. Percent inhibition of BChE (using ATCh as a substrate and iso-OMPA as an inhibitor) from the plasma of 12 dogs treated with four sequential dippings of chlorpyrifos every 21 days (*A*) without shampooing between dips and (*B*) with shampooing between dips. The sample times are 4 hr, 7 days, 14 days, and 21 days after dipping. Arrow represents times of subsequent dippings. The graph represents means with 95% confidence intervals.

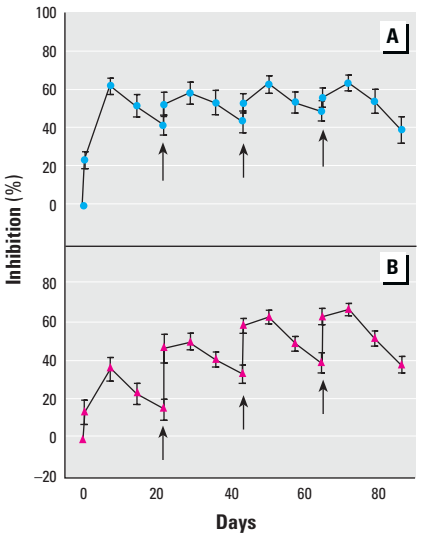

Figure 5. Percent inhibition of total cholinesterase (using ATCh as a substrate and eserine sulfate as an inhibitor) from the plasma of 12 dogs treated with four sequential dippings of chlorpyrifos every 21 days (*A*) without shampooing between dips and (*B*) with shampooing between dips. The sample times are 4 hr, 7 days, 14 days, and 21 days after dipping. Arrow represents times of subsequent dippings. The graph represents means with 95% confidence intervals.

children vary with age, the amount of contact with body areas other than hands and forearms, the amount of clothing, and length of time the child plays with the dog. The absorption potentials vary greatly with the site of exposure from 8.6% to 50% for parathion (*16*). Also, the amount of occlusion, skin damage, and skin binding of the chemical will determine the risk of exposure (*17*).

We chose the use of cotton gloves as a measurement device for potential exposure to increase the reliability and reproducibility over hand washes. Hand wash protocols vary in time, number of washes, and the wash itself (2-propanol, 10% ethanol/water, 10% isopropanol/water, and 95% ethanol/water) (*18*–*22*). Because this was an experimental study and not a field monitoring study, we could not wash one sampler's hands several times over a few hours with a solvent because of skin damage and absorptive problems of the chemical, and we did not wish to expose the samplers to the chemical directly, especially when the treatment was fresh. It has been stated that gloves overestimate hand exposure up to 5 times, but that study did not test for the percent recovery of hand washes (*23*). A test for percent recovery of hand washes would include exposing a person's hand to a known amount of compound, waiting for a specified amount of time, washing the area, and calculating percent recovery. In many published articles, the percent recovery of compounds was not assessed, and exposure could be underestimated by up to 5-fold with chlorpyrifos (*24*) and 3-fold with captan (*25*). The percent recovery and residue removal of chlorpyrifos was not consistent and decreased with decreasing levels of loading (*24*). Also, skin absorption makes standard hand washing procedures inefficient at removing compounds (*17*). If hand washes are to be employed, care must be taken to estimate accurately the amount of residue recovered with standardized methods for removal efficiency. If glove dosimeters are slightly higher than hand washes, the more conservative numbers will help risk management better protect our children. For industry, the glove extractions are a conservative, quick, and relatively inexpensive test.

The data generated by this research are useful in determining the amounts of pesticides that children could be exposed to from pets treated for flea and tick control with a dip formulation. It is unknown how representative these data will be for other types of formulations still on the market; our laboratories are currently assessing transferable insecticides from other dips and flea collars using similar methodology. However, more information is needed regarding behavior of children, including their play time with pets and their hand-to-face and hand-to-mouth patterns. Many more types of insecticidal agents with different formulations and application techniques need investigation. The differences between the length of fur and amount of transferable residues were not significant, but we may need further evaluation of fur composition differences among dogs, i.e., undercoat. The data generated from this study demonstrate that BChE measurements are effective biomarkers of insecticide action and persistence following chlorpyrifos flea and tick control in dogs.

There is no perfect measurement technique for determining the amount of pesticide exposure, nor is there a perfect method for determining risk. Given the lack of information in this area and the large number of pesticides in use, the cotton glove dosimeter model is a quick, reliable, and very useful tool in determining potential pesticide exposure.

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