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Title: **A Test House Study of Pesticides and Pesticide Degradation Products Following an Indoor Application**

Running Title: Pesticides and Degradation Products after an Indoor Pesticide Application.

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Running title: Pesticide Movement and Degradation Following an Indoor Application

Abstract
Pre-existing pesticide degradates are a concern for pesticide biomonitoring studies as exposure to them may result in overestimation of pesticide exposure. The purpose of this research was to determine whether there was significant formation and movement, of pesticide degradates over a five week period in a controlled indoor setting after insecticide application. Movement of the pesticides during the study was also evaluated.

In a simulated crack and crevice application, commercially available formulations of fipronil, propoxur, *cis*/*trans*-permethrin, and cypermethrin were applied to a series of wooden slats affixed to the wall in one room of an unoccupied test house. Floor surface samples were collected through 35 days post-application. Concentrations of the pesticides and the following degradates were determined: 2-iso-propoxyphenol, *cis*/*trans* 3-(2,2-dichlorovinyl)-3-3-dimethyl-(1-cyclopropane) carboxylic acid, 3-phenoxybenzoic acid, fipronil sulfone, fipronil sulfide, and fipronil desulfinyl. Deltamethrin, which had never been applied, and chlorpyrifos, which had been applied several years earlier, and their degradation products, *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid, and, 3,5,6-trichloro-2-pyridinol, respectively, were also measured.

Propoxur was the only insecticide with mass movement away from the application site. There was no measurable formation or movement of the degradates. However, all degradates were present at low levels in the formulated product. These results indicate longitudinal repetitive sampling of indoor degradate levels during short-term studies, is unnecessary.

Keywords: Pyrethroids; Fipronil; Propoxur; Indoor; Degradation.

Exposure to preexisting pesticide degradates may inflate estimates of exposure in biomonitoring studies where these compounds are used as biomarkers. To date there is no published information on formation of pesticide degradates following an indoor application. We found that the study pesticides have low rates of degradation and are unlikely to be a significant factor affecting results of short-term (weeks) biomonitoring studies. Therefore, relatively few indoor samples are needed in order to estimate background levels of degradation products resulting from a recent pesticide application.

1. Introduction

Measurement of pesticide metabolites in human urine (biomarkers) has been used in biomonitoring studies to estimate children's exposure or dose to the parent pesticide (Baker et al., 2000; Heudorf et al., 2004; Macintosh et al., 1999; Riederer et al., 2008). These biomarkers are useful as they may provide data integrating all exposure pathways, thereby offering an alternative to traditional exposure assessments that require concentration measurements of several matrices. However, incomplete understanding of pesticide toxicokinetics and metabolism, in addition to complex exposure scenarios, has made the interpretation of biomarker data challenging (Egeghy et al., 2011; Fensky et al., 2005; Wessels et al., 2003).

Additional uncertainty in biomarker based exposure and dose estimations may result from human contact with exogenous or pre-formed biomarkers present as the result of pesticide degradation in the environment. Immediately following an application, pesticides may begin to degrade through chemical, physical, and biological processes to yield the same compounds that are commonly used as biomarkers. When such pesticide degradates are absorbed by humans and not further metabolized prior to excretion, they are indistinguishable from actual pesticide metabolites found in urine samples. In urinary biomarker studies, this can result in an overestimation of exposure to the pesticide. For example, Timchalk et al. (2007) found that rats dosed orally with chlorpyrifos had urinary metabolite excretion of 3,5,6-trichloro-2-pyridinol (TCP), diethylphosphate (DEP), and diethylthiophosphate (DETP) that accounted for 62%, 40%, and 14% respectively of the administered chlorpyrifos. In contrast, oral dosing with each of the three metabolites resulted in excretion rates of 100% (TCPy), 86% (DEP), and 65% (DETP). Previous research has recognized the potential contribution from degradates to overestimation of pesticide exposure (Zhang et al., 2008) and demonstrated the presence of pesticide degradates in a variety of samples collected indoors (Morgan et al., 2005; Wilson et al., 2003). One study of house dust in residences and child care facilities (Starr et al., 2008) found the relative proportion of degradation products to parent pesticides to be as high as 60% for frequently used, current generation pyrethroid insecticides. However, this study did not determine whether these degradates were formed from a previous indoor pesticide application, or transported from other sources.

Pesticide degradation is facilitated by moisture, sunlight, and microbiota. And, perhaps because indoor usage largely shelters pesticides from these factors, the indoor degradation rate is

assumed to be low. However, because there may be enough breakdown of pesticides to alter biomonitoring based exposure assessments, this presumption needs to be verified. A literature review produced only one study measuring degradate formation following an indoor application of pyrethroid pesticides (Class, 91) and no indoor studies of fipronil degradation were found. In the pyrethroid study, which lasted 48 hours, some degradation of tetramethrin and allethrin was observed but there was no apparent breakdown of cyfluthrin. The literature review also found no data assessing degradate levels in pesticide formulations. It is possible that measureable quantities of degradates are present in finished pesticide products, either from breakdown of the pesticides during storage of the finished formulation or as by-products of manufacturing. If present, they would be co-applied with the parent pesticide and available for uptake.

Because exposure to exogenous degradates may inflate pesticide exposure estimates, it is important for biomonitoring studies to understand the sources, distributions in microenvironments, and levels of human exposure to these chemicals. For longitudinal biomonitoring studies of children it is particularly useful to understand if there is significant formation of these degradates biomarkers in the indoor environment where pesticides are frequently applied and where children spend most of their time (Cohen Hubal et al., 2000).

The purpose of this study was to evaluate the contribution of pesticides recently applied indoors to levels of degradation products in that same environment. Four pesticides were selected based on physical chemical properties in formulations labeled for crack and crevice applications. Cypermethrin, permethrin, propoxur, and fipronilwere applied in the US EPA Indoor Air Quality Test House (Stout II et al., 2007) using simulated crack and crevice applications and each

pesticide formulation was applied according to the manufacturer's specification. Based on each pesticides formulation, method of application, and vapor pressure it was expected that one or more of the pesticides would redistribute away from the application site and so.. floor wipe samples were taken over a five week period following the application in the living room (site of application) and the den (adjacent to the living room). The samples were analyzed to measure the concentrations of the applied pesticides and their common degradates: 2-isopropxyphenol (IPP), *cis*/*trans*-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane -1-carboxylic acid (*cis*/*trans* DCCA), 3-phenoxy benzoic acid (3-PBA), fipronil sulfide, fipronil sulfone, and fipronil desulfinyl. Concentrations of chlorpyrifos and its degradate 3,5,6-trichloro-2-pyridinol (TCP) as well as deltamethrin and its degradate *cis*-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DBCA) were also measured to provide comparative data. Chlorpyrifos had been applied during studies in 1993 (Mason et al., 2000) and 2000 (Stout II and Mason, 2003). Deltamethrin had never been used in the test house. Figure 1 shows the structures of all study analytes and associates each degradate to its respective parent pesticide(s).

This study will help determine the extent to which these common pesticides, when recently applied indoors, move and degrade to become exogenous degradates to which study subjects may be exposed. The results will inform longitudinal biomonitoring studies of these pesticides to help researchers determine the need for additional indoor assessments of degradates after interior residential pesticide applications.

2. Materials and Methods

2.1. Test House

The research house represents an intermediate environment between highly controlled environmental chambers and occupied structures and allows simulated pesticide applications under controlled conditions, while removing interferences from human activity. It is an unoccupied, unfurnished, nine room single story, residential structure with laminate floors throughout. This experiment was conducted between May and June in 2006. The house has an interior volume of 293 m^3 with 122 m^2 of living area. All interior doors were left open during the application and remained open throughout the study, and the furnace fan was set in the off position. After the application the house thermostat was set at 22º C (72º F) for the remainder of the experiment and the furnace fan was returned to the on position. Interior temperature, humidity and air exchange rates were monitored over the duration of the experiment. Average temperatures reported over the 35 day test period were 22.3 °C (standard deviation ±0.62 °C) and a relative humidity of 55.7 °C (standard deviation ±4.19 °C). Air exchange rates were determined using the tracer gas technique (ASHRAE, 1985) and averaged 0.32 ±0.035 air exchanges for the entire house.

2.2. Pesticide Application

Formulated pesticides were purchased from their respective manufactures; Fipronil from Bayer Environmental Science (Montvale, NJ), propoxur from Waterbury Companies Inc. (Waterbury, CT), and cypermethrin and permethrin from FMC Corporation (Philadelphia, PA) and the concentration of each pesticide as purchased is provided in Table 1. All pesticide formulations were analyzed to determine the presence or absence of the relevant degradation products.. All

formulated compounds were obtained through mail order services that provide pesticides to both the general public and professional services. Many of the active ingredients examined in this study are formulated in consumer ready to use products, although it should be noted that since the implementation of this study that propoxur has been deregistered for residential use. These pesticides had not been previously used in or around the test house. Table 1 lists the formulation, amount applied, physical chemical property, and aerobic soil half life of these compounds. Also included are the physical-chemical properties of deltamethrin and chlorpyrifos which were not applied, but measured as part of this study.

Pesticide concentrations of the formulations were assumed to be as listed on the label while the degradate concentrations were determined using LC/MS/MS analysis. All samples were taken inside the US EPA's indoor air quality research house which has been described previously (Mason et al., 2000; Stout II and Mason, 2003).

All four pesticides were mixed and applied following individual product labels and associated literature and delivered as simulated crack and crevice applications. The applications were designed to closely approximate the same type application as performed by professional pest control service. Cypermethrin and permethrin were delivered in a pin-stream using a compressed air sprayer, propoxur as an aerosol from a pressurized spray can, and fipronil as a gel using a syringe. The cracks were created by fixing five separate series of wooden slats onto sheets of wood molding that had been attached to the walls in the living room. Each slat measured 0.5" × 0.75" × 48" (width × height × length) and there was 0.75" between each slat. There were 31 slats in each series and a final crack was created below each of the series by using the floor / wall junction. The manufacturers instructions for preparation of each insecticide were

followed and the pesticides were applied into the 48" crack between the top of each slat, or floor, and the wall. Single pesticides were applied into each crack and each pesticide was applied to an equal number of cracks.

2.3. Sampling Media

Woven cotton wipes (4" × 4"), purchased from M.G. Chemicals (Toronto, Canada) were used to sample all surfaces. The procedures described by Stout II et al. (2009) to pre-treat (solvent clean), store, and take wipe samples of surfaces were used with slight modifications. Before use, the wipes were cleaned with isopropanol (10 hours) then hexane (10 hours) in a high capacity soxhlet extractor and dried in a vacuum oven at 40 °C. Immediately prior to sampling, each wipe was wetted with 6 mL of pesticide grade isopropyl alcohol.

2.4. Pesticide and Degradate Sampling

Eleven surface wipe samples were collected from both the living room and den of the test house prior to pesticide application to determine background levels of chlorpyrifos and the pesticides to be applied (the pesticide degradates and deltamethrin were not included in this analysis). Pesticide concentrations in these samples were measured using Gas Chromatography/Mass Spectrometry (GC/MS). After the pesticide application, study samples were taken from the floor of the living room or the den on days 1, 2, 3, 7, 14, 21, 28, and 35 (n = 5 on days 2, 7, 14,and 28; n = 6 on days 1, 3, and 21; n = 9 on day 35) and processed and analyzed using the PFE and LC/MS/MS methods described above. The den and living rooms was prepared by laying three rows with four distinct sampling squares (delimited with tape) in each row to create a sampling grid on the floor of each room. All surface samples were collected within the individual sampling

squares and each location within the square was sampled once with no over sampling. Some samples were collected in sampling squares that were nearer the application site and hence been in closer proximity to the fallout of larger droplets generated during the application (identified as overspray). . On days 1-14, all samples were taken from the floor of the den. This room was adjacent to the living room, where the application occurred. On day 21, five samples were taken from the living room and one from the den. All samples on day 28 came from the living room. On the final sampling day, eight samples were from the living room, and one was taken from the den. Because no information on indoor decay rates was available for these pesticides, the 35 day duration of the study was based on the half lives of the pesticides in aerobic soils (Mandal and Singh, 2013; USDA, 2008) as shown in Table 1 and the dissipation rate of chlorpyrifos (Mason et al., 2000; Stout II and Mason, 2003) following an indoor application.

2.5. Chemicals used for sample purification and analysis

Acetonitrile, methanol, isopropyl alcohol, and acetone were purchased from Honeywell Burdick & Jackson (Muskegon, MI), ethyl acetate (EtOAc) and hexane from EMD Chemicals (Gibbstown, NJ), anhydrous sodium sulfate (Na_2SO_4) from JT Baker (Phillipsburg, NJ), and hydrochloric acid (HCl) from Fisher Scientific (Fairlawn, NJ). All solutions were prepared using pesticide grade organic solvents.

Calibration, surrogate, and internal standards were acquired from various sources: fipronil, deltamethrin, chlorpyrifos, *cis*-permethrin, and *trans*-permethrin from Absolute Standards (Hamden, CT); fipronil sulfide, fipronil desulfinyl, fipronil sulfone, and cypermethrin from AccuStandard (New Haven, CT); 3-PBA, 4-fluoro-3-phenoxybenzoic acid (4-F-3-PBA),

cis/*trans*-DCCA), $^{13}C_6$ *cis*-permethrin, $^{13}C_6$ *trans*-permethrin, $^{13}C_6$ cypermethrin, D-10 chlorpyrifos, $^{13}C_6$ 3-PBA, and $^{13}C_6$ *trans*-DCCA from Cambridge Isotope Laboratories (Andover, MA); propoxur and propoxur D_3 from CDN Isotopes (Pointe-Claire, Quebec, Canada); and DBCA, IPP, TCP, and fipronil des F3 from EQ Laboratories (Atlanta, GA). *Cis*- and *trans*-permethrin were purchased as individual isomers while cypermethrin was a mixture of four isomers of unknown proportions. DCCA was an isomeric mixture with a proportion of 3:7, *cis*:*trans*. Deltamethrin and DBCA contained the *cis*- isomer only. The purity of *trans*-permethrin was 94% and all other pesticides used as calibration standards were ≥ 98% pure. Excepting TCP, which was 93.5% pure, all degradate calibration standards were also ≥ 98% pure. No adjustment based on the purity of any standard was made when calculating the concentration of any analyte.

2.6. Quality Control / Quality Assurance

All samples were extracted and processed in groups of four. A set of two blank and two spiked wipes (100 ng each analyte and surrogate) were extracted, processed and analyzed with each group. Surrogate standards (100 ng each) were added to each sample wipe prior to extraction while internal standards (50 ng each) were added to the cleaned extracts immediately before analysis. Samples were analyzed in batches of approximately thirty samples (including blanks and spikes) and calibration curves (10 ng – 100 ng/mL) were generated before and after each batch. Sample concentrations were quantified using the initial calibration curve whereas the post batch calibration curve was used only to monitor instrument performance. During each batch, the mid-level standard was rerun after each group. Samples with one or more analyte

concentration outside the calibration range were re-analyzed using a 1 ng – 10 ng/mL or 100 ng – 1000 ng/mL curve as appropriate.

2.7. Standards

Stocks to be used to make calibration, surrogate, and internal standards were prepared by combining and diluting individual standards received from the vendors. Standards used for calibration curves were matrix matched to blank extracted and solvent cleaned wipe media. They were prepared by addition of concentrated stock standards into aliquots of processed blank matrix that was dissolved in methanol:0.01N HCl (8:2, V:V). Concentrations were initially determined using a calibration ranging from 10 to 100 ng/mL, and re-analyzed using a different calibration curve (1 to 10 or 100 to 1000 ng/mL) when sample concentration were outside this range.

2.8. Extraction and Clean-up

A full description of the extraction and clean-up procedures is provided in the Online Supporting Materials. Briefly, Pressurized Fluid Extraction (PFE) was used to extract all analytes from wipe samples; and clean-up was accomplished using a combination of liquid/liquid partitioning and solid phase bonded cartridges. Each sample (consisting of two wipes), was placed in a 33 mL cell and extracted3x with hexane:acetone (25:75, V:V) followed by 2x extractions with methanol. The hexane:acetone and methanol fractions were collected separately but all extractions were done at 75 ^{o}C and 1500 psi. After extraction the solvents containing the extracted analytes were evaporated under reduced pressure and elevated temperature to approximately 2-4 mL and the methanol fraction was solvent exchanged to0.01N HCl and

partitioned three times against equal volumes of EtOAc. Aafter each partition the organic layer was removed and added to the flask containing the hexane:acetone extraction. The combined extract was solvent exchanged into acetonitrile and . cleaned using 500 mg of C_{18}.

Following C_{18} clean-up 200 µL of 0.01N HCl were added to the eluant and the volume was reduced to 100-200 µL under nitrogen at ambient temperature. The internal standards, and methanol, and 0.01N HCl were added to make a 1 mL solution with a methanol:0.01N HCl ratio of 8:2 (V:V), transferred to an autosampler vial and stored at -20 °C until analysis.

2.9. LC/MS/MS Sample Analysis

The process used to optimize the LC and MS/MS conditions for sample analysis are described in the Online Supporting Materials.

Atmospheric Pressure Chemical Ionization (APCI)For sample analysis the mobile phase flow rate was 400uL/min through a C_{18} column (3.0 × 150 mm, 3.5 µm). The mobile phase composition for all analyses was methanol:5 mM ammonium acetate in water. The ratio of organic:aqueous used for the pyrethroids was 98:2 while the organic:aqueous ratio for IPP was 7:3. A gradient was used for the pyrethroid degradates, chlorpyrifos, TCP, fipronil, its degradates, and propoxur. The initial ratios were 6:4 organic:aqueous for three minutes; ramp at 12.5% per minute to 85:15 organic:aqueous and hold for ten minutes. The mobile phase was allowed to equilibrate for five minutes prior to each injection. The identities of all analytes were verified using retention times and transition ion pairs. The mobile phase conditions used for each group provided temporal separation of the *cis-trans* isomers of both permethrin and DCCA. The *cis-trans* isomers of cypermethrin were not resolved.

Forming a charged IPP species required Atmospheric Pressure Chemical Ionization (APCI) but all other analytes were ionized effectively using Electrospray Ionization (ESI). $^{13}C_6$ *trans*-permethrin was used as a surrogate recovery standard for all parent pyrethroids listed. 4-F-3-PBA was used as the surrogate for all degradates, chlorpyrifos, propoxur, and fipronil.Table 1 in the Online Supporting Materials lists all analytes, their retention times, ion pairs, and internal standards and Figure 1 in the Online Supporting Materials shows representative chromatograms of all pesticides and degradates.

2.10. Method Evaluation

The efficiency of the wipes in recovering pesticides from a stainless steel surface has been reported previously (Deziel et al., 2011). In the current study, only the accuracy and precision of sample processing was evaluated. Baseline method recoveries from spiked "clean" wipes were established by spiking pre-cleaned wipes then processing them according to the optimized method. Potential interferences were assessed using pre-cleaned wipes wetted with isoproponyl then wiped across one of three surfaces. The surfaces were: a laboratory bench top, tiled and waxed flooring, and laminate flooring identical to that in the research test house. Although laminate flooring was the only floor surface in the test house, the other two surfaces were used to demonstrate the ability of the cleanup method to remove additional background interferences that could be encountered in the test house.

Each area to be wiped was delimited by a flat aluminum template with an interior area of 929 cm^2. Two sequential wipe samples were taken from each area using an overlapping pattern that covered the entire area inside the template, one time per wipe. Both wipes used for each sampling area were stored together in amber jars at -20^o C until processing.

After wiping, each set of two cotton wipes was spiked with one of three concentrations of pesticides and degradates, then processed and analyzed to evaluate interferences and determine percent recovery. Five replicate samples at each of the three spiking levels were taken from each surface type. Each degradate (including summed *cis*/*trans* DCCA) were spiked at 25, 100, and 500 ng (n=5 each level). Pesticides were spiked at 25, 100, and 1000 ng (n=5 each level). Blank wipes (wipes not spiked after wiping) of each surface were also taken.

2.11. Method Limits of Detection and Quantitation

The method limits of detection (MDL) and quantitation (MQL) are located in Table 2. The MDL and MQL for each analyte were determined using the same preparation, wiping, and spiking procedure as described for the method validation. After wiping the surface, the cotton was spiked with either: 2, 5, 10, or 20 ng of each analyte. Wiping and spiking were repeated four times at all concentrations for each of the three surfaces, resulting in 12 samples per concentration. The samples were processed according to the final extraction and clean-up procedure described above and then analyzed by LC/MS/MS. Each MDL/MQL sample was injected and run on the LC/MS/MS four times. Analyte concentrations in the replicates at each theoretical concentration were determined using a calibration curve ranging from 1-50 ng/mL, then pooled to calculate group standard deviations of the estimated concentrations. The standard deviations were set as the dependent variable while the theoretical concentrations were independent. Least squares regression was used to estimate the y-intercept. The intercept of the regression equation was defined as S_0 with the MDL approximated by $3 \times S_0$, and the MQL approximated by $10 \times S_0$ (Taylor, 1987).

2.12. Data Analysis

Concentrations of pesticides from pre-application samples were compared with day 1 post-application samples using Student's T-tests. Least squares regression was used to evaluate changes in pesticide and degradate concentrations. The data were analyzed after pooling data from both the den and living room and also for each room individually.

3. Results and Discussion

3.1. Method Validation, Limits of Detection and Quantitation

The woven cotton wipes used in this study were pre-cleaned to ensure they were free of any analytes of interest and to remove residual oils and other potential interferences. Hexane:acetone (25:75, V:V) was sufficient to extract the pyrethroids but a subsequent extraction using methanol was required to improve recovery of the more polar pesticides and the degradates. Despite pre-cleaning, extracts of blank wipes contained interfering residues, especially in the methanol extract. These substances were largely removed from that extract by partitioning the analytes into EtOAc after solvent exchange of the methanol extract into pH 3 water. Further removal of co-extracted wipe material was achieved with C_{18} sorbent during the Solid Phase Extraction (SPE) step.

The mean percent recoveries of the analytes from clean spiked wipes are located in Table 2. Comparison of the recovery of the pesticides and their degradates from these wipes shows similar efficiencies of approximately 89-100% for both groups, excepting the degradates *trans*-DCCA, fipronil sulfide, fipronil sulfone, and IPP which ranged from 72% to 84%. As a group

the mean relative standard deviation (RSD) of the degradates was 5% higher than the pesticides and included four analytes with RSD's $\geq$ 20%.

The recovery from the clean spiked wipes demonstrates the method performance without any background interferences acquired by solubilizing materials during surface wiping with isopropanol soaked wipes. The greater solubility of the co-extracted material in a more polar solvent (methanol) would more likely affect the more polar degradates than the parent pesticides during processing and analysis and therefore may partially explain the lower recovery and higher RSDs of some of the degradates.

The percent recoveries from wipes spiked after surface wiping are also presented in Table 2. Wipe blanks for each surface indicated no contamination occurred during wiping or sample processing. For the majority of pesticides and degradates there was no statistically significant difference in percent recovery. Of the 11 (of 54 comparisons) instances where the difference was significant ($p \leq 0.05$), six were from the laboratory floor surface wipes which was heavily waxed and dirtier than the other two test surfaces. No analytes from any surface with isotopically matched internal standards had mean recoveries that were statistically different than that of the associated clean wipe. This indicates that observed recovery differences were likely due to ion suppression or enhancement by matrix contaminants, rather than actual disparities in extraction and processing efficiencies of the floor wipes compared to clean wipes. The differences were most pronounced with the fipronil degradates and IPP which suggests alternative internal standards for these analytes would be useful. For the purposes of this study, all recoveries were within the data quality objectives of the study.

The result of the MDL and MQL calculations are in Table 2. The detection limits ranged from 0.1 ng/sample (DBCA and fipronil sulfide) to 4.2 ng/sample (IPP) and all quantitation limits were ≤ 10 ng/sample except IPP (14 ng/sample). Several blank laboratory floor wipe samples taken during the MDL/MQL determinations showed trace level concentrations of fipronil, and fipronil sulfide and higher concentrations of all pyrethroids. Therefore, laboratory floor wipe results were not used in the MDL/MQL calculation of these analytes. As stated earlier, none of the blank floor wipes taken earlier during method validation, had measurable concentrations of any of these analytes.

3.2. Test House Pesticide Concentrations (pre-application)

The mean pre-application concentrations (± SD) of the applied pesticides and chlorpyrifos are located in Table 3. Of the 22 pre-application wipes, *cis*-permethrin and *trans*-permethrin were found in all samples, chlorpyrifos in 20 , fipronil in 19, and propoxur in 10. T test comparisons between the den and living room showed no significant differences ($p \leq 0.05$) for any pesticide except propoxur which was not detected in any living room samples. Cypermethrin was not detected in any pre-application samples and deltamethrin concentrations were not measured.

The presence of low levels of chlorpyrifos in the pre-application samples was expected as it had been applied in an earlier study, but there was no record indicating previous use of cypermethrin, *cis*/*trans*-permethrin, propoxur, or fipronil in or around the test house. Although propoxur was not detected in the living room, this implies movement of the pesticides into the test house from external sources. Also supportive of an external source is the high frequency with which the pesticides were detected (excepting propoxur) in both living room and den. Given the

hydrophobic nature and low vapor pressures of cypermethrin, *cis*/*trans*-permethrin, and fipronil (Table 1), it is likely that these pesticides were primarily particle bound as they entered the house. The relatively high vapor pressure of propoxur, along with its lower partition coefficient, suggests that it was more likely transported as a vapor. Considering it's physical chemical properties, propoxur would have been the pesticide most likely to be found at similar concentrations in both rooms and the explanation as to why it was detected in only one room of the house pre-application, is not clear. Measurable background of these pesticides was not unexpected as previous research has found them in indoor dust samples from residences with no reported pesticide use. For example, in a population based study which included propoxur and permethrin, Colt et al. (2004) detected the insecticides in 73% (propoxur) and 59% (permethrin) of indoor dust collected from homes where pesticides were not used. It is possible that residences serve as an indoor sink as suggested by Mahler et al. (Mahler et al., 2009) in a study of fipronil in indoor and outdoor dust where they found fipronil in all house dust samples taken from homes with no history of fipronil use and reported median indoor dust concentrations of fipronil to be 15 times higher than that of outdoor dust. In a national survey of the United States housing, Stout II et al. (2009), detected *cis*- and *trans*-permethrin in almost 90% of the samples, while cypermethrin, fipronil, and deltamethrin were found in 46%, 40%, and 27% of homes respectively. While pesticide usage information from that study was not reported, the results demonstrate the ubiquity of some of these compounds in residences.

3.3. Test House Pesticide Concentrations (Post-application)

The concentration means and standard deviations of all study pesticides (den and living room combined), at each time point can be found in Table 3. In Table 2 of the Online Supporting

Materials, pesticide concentrations in the individual rooms are provided. All samples (n = 47) collected during the study had measurable concentrations of all applied pesticides, with the exception of a single sample where fipronil was not detected. Although not applied, chlorpyrifos and deltamethrin were present in 46 and 45 of the post-application study samples, respectively. On day 35, five of the nine samples had concentrations of propoxur, cypermethrin, and *cis/trans*-permethrin that were orders of magnitude higher than any other sample. These sample concentrations, taken from surfaces in the living room that were near the site of pesticide application, almost certainly resulted from overspray rather than movement of the pesticides in the vapor phase. Therefore, they are presented separately in Table 3 and were not used for statistical analysis of concentration changes over time.

All post application day 1 samples were taken in the den and T-test comparisons of pre-application concentrations in the den with those of day 1 showed that mean propoxur concentrations were significantly ($p \leq 0.05$) higher on day 1. No differences observed for *cis*- and *trans*-permethrin, or fipronil. Although present in all day 1 samples, pre-application- day 1 cypermethrin concentrations were not compared as it was not detected in any pre-application samples.

T-test comparison of pre-application and day 1 chlorpyrifos concentrations showed no significant difference ($p > 0.05$). Deltamethrin was not included in the pre-application analysis and therefore no comparative data were available.

As all day 1 samples were taken in the den, the high propoxur concentrations seen at this time point show significant movement this pesticide away from the application site in the living room.

The similarities between the pre- and day 1 sample mean concentrations of *cis*-permethrin, *trans*-permethrin, and fipronil were not surprising as these pesticides all have low vapor pressure and would not be expected to move rapidly from the point of application. Because cypermethrin also has a low vapor pressure and was not found in the pre-application samples, its presence in all day 1 samples was unexpected. The reason for this is not clear as GC/MS analysis used to screen the pre-application samples has a calculated cypermethrin MDL similar to that of the LC/MS/MS method used to analyze the post-application samples.

Using data pooled data from the den and living room, days 1-35 (Table 3), least squares regression of the applied pesticides concentrations showed that only propoxur ($p = 0.001$) and cypermethrin ($p = 0.02$) had slopes that were statistically significant. While the concentration of cypermethrin increased with time, propoxur decreased. Fipronil, *cis*-permethrin, and *trans*-permethrin did not show a linear trend across the entire 5 weeks of the study. Regression analysis of chlorpyrifos and deltamethrin (pesticides not applied) showed no statistical trends ($p > 0.05$) for either pesticide over the 5 weeks of the study.

Results obtained by room (living room and den, Table 2 of the Online Supporting Materials) showed a significant decrease in concentration over time for fipronil ($p = 0.002$) in the living room, while propoxur concentrations decreased ($p = 0.002$) in both the living room ($p = 0.002$) and den ($p = 0.002$). *Trans*-permethrin concentrations increased over time in living room ($p = 0.02$) but showed no trend in the den. Cypermethrin and *cis*-permethrin results were similar to *trans*-permethrin but the changes in living room concentrations did not achieve statistical

significance ($p \leq 0.05$). Concentrations of chlorpyrifos and deltamethrin did not change during the course of the study.

Considering data using combined living room and den results, as well as and that where the rooms were analyzed individually, there was little evidence of mass movement of the less volatile pesticides away from the application site in the living room. Although pooled room data indicate that floor surface concentrations of cypermethrin increased slightly over time, the data from individual rooms shows that den concentrations did not change. In contrast, in the living room, the concentration increase over time approached significance. This between room difference was also observed in the results for *cis*- and *trans*-permethrin where the change in living room *trans*-permethrin concentrations was statistically significant. The between room difference is probably explained by disturbance of the pyrethroid overspray in the living room (Table 3) created during repeated sample collection. This may have caused by adherence of the pyrethroids to shoes, or resuspension of settled dust with higher concentrations of sorbed pyrethroids. Fipronil which had the lowest vapor pressure and was applied as a gel bait rather than an aerosol had no overspray and actually showed a concentration decrease in the living room as the study progressed.pesticides were sorbed to dust particles during application and resuspended as a result of the human activity, and then settled during the study period. . .

There was an obvious difference between the behavior of propoxur and the other pesticides, which were all more lipophilic and had much lower vapor pressures. In the living room, den, and in the combined data set propoxur concentrations decreased over time. This is consistent with the expected behavior of a semivolatile compound that is rapidly redistributed throughout

the structure then gradually removed diffusion into porous surfaces or air exchange (Weschler and Nazaroff, 2008). Because the study ended before the measured propoxur concentrations stabilized, it is not possible to know whether it would have returned to the background levels seen in the pre-application samples. The continued presence of chlorpyrifos (VP = 2.7 mPa) in the test house several years after application (Mason et al., 2000; Stout II et al., 2003) argues for the longevity of semivolatile pesticides, although it is possible that its presence was in part due to transport into the house from remote locations.

3.4. Test House Pesticide Degradation Products

All pesticide formulations contained low but measureable levels of their respective degradation products. Therefore the degradates were co-applied with the pesticides. The concentrations of degradates in each formulation, expressed as a percentage of their active ingredients, are provided in Table 4. The relative concentrations of each degradate was less than 1% of it's parent pesticide. Given the rapid metabolism of cypermethrin and cis-/trans-permethrin (Starr et al., 2012) and fipronil (Lacroix et al., 2010) in rat blood, these degradates, while measurable, would probably not affect pesticide exposure estimates derived from biomonitoring data.

The means and standard deviations of the degradate concentrations (den and living room pooled) during the study are in Table 3 and data for the individual rooms are located in Table 2 of the Online Supporting Material . Due to the presence of degradates in the formulations In Table 3, the day 35 results of the pesticide degradates with obvious pesticide overspray of have been separated from other results. Similarly to the analysis of the pesticides, these overspray results were not used for statistical comparison of concentration across time.

Of the degradation products associated with applied pesticides, IPP, fipronil desulfinyl, and fipronil sulfide were not found in any sample while fipronil sulfone was found in 44 of the 47 samples. Prior to day 35, 3-PBA was detected in only one sample while *cis*-DCCA and *trans*-DCCA were detected in 8 and 33 samples respectively. On day 35, 3-PBA and *trans*-DCCA were present in all 9 samples while *cis*-DCCA was found in 8 of 9 samples. Of the degradates of the non-applied pesticides, DBCA was not detected in any sample whereas TCP was present in all study samples. The concentrations of degradates, when detected, was between one and two orders of magnitude less than the pyrethroid pesticides. In a study by Starr et al. (2008) of selected indoor house dust samples that were known to contain pyrethroids, degradates concentrations were also one to two orders of magnitude less than the parent pyrethroids.

Due to the low frequency of detection, regression analysis of 3-PBA was not done. This analysis of *cis*-DCCA and fipronil sulfone showed a significant increase for both ($p \leq 0.05$) over the course of the study for the den and pooled rooms. In contrast, *trans*-DCCA living room concentrations actually decreased ($p \leq 0.05$) while no change was seen in the den or pooled data.

Whether pooled data (Table 3) or data from individual rooms (Table 2 Online Supporting Materials) were used,there was little evidence of pyrethroid degradate formation. Considering the pooled data, concentrations remained at either consistently low levels or were below the detection limit from day 1 – day 28, and, as mentironed previously, the high concentration of the pyrethroid degradates in several day 35 samples was likely related to co-application with the parent compound, rather than degradation of the applied pesticides. Comparison of measured parent pyrethroid / degradate ratios on day 35 (including overspray samples) with expected

ratiosbased on the ratio of parent to degradate in the formulationsshowed there was no difference between the measured and expected ratios.. IPP was not detected in any day 35 samples but a similar calculation for IPP co-applied with propoxur shows that the calculated concentrations of applied IPP would have been below the detection limit for that analyte.

Mahler et al. (2009) did not measure fipronil sulfone but found the ratio of fipronil to fipronil sulfide and fipronil desulfinyl in indoor dust to be 0.04:1 and 0.07:1 respectively. In this study, fipronil sulfide and fipronil desulfinyl wer not detected but fipronil sulfone was present throughout the study at concentrations approximately equal to the parent pesticide. Although the increase in fipronil sulfone was statistically significant for the den and pooled room data, the change was small and likely due to the single day 35 den sample having a relatively high (13 pg/cm^2) concentration compared to the living room results (4 ± 4 pg/cm^2).. Fipronil sulfone was the most concentrated degradate in the fipronil formulation, however, fipronil was applied as a gel and therefore it is likely that the source of fipronil sulfone, (as well as fipronil), was from an external source, and not the study pesticide application. Due to the varied uses of fipronil it is not possible to isolate the source of the parent or degradate. However, sulfone is an oxidative product whereas the sulfide is a reduction degradate and desulfinyl is a photodegradate which suggests the sulfone was produced by degradation of fipronil under aerobic conditions and the fipronil was not exposed to sunlight (Bobé et al., 1998). Interestingly, all degradates are more stable in saturated systems than the parent compound (Lin et al., 2009) and at least as toxic as fipronil to tested aquatic species (Schlenk et al., 2001) .

The high ratio of TCP to chlorpyrifos observed throughout the study (Table 3) is likely a result of a gradual breakdown of the chlorpyrifos applied years earlier in two previous studies (Mason et al., 2000; Stout II and Mason, 2003) although transport of both compounds into the test house from a remote source cannot be excluded. Whatever the source, the high proportion of TCP in environmental media would likely result in an overestimation of exposure to chlorpyrifos if the excreted metabolite alone were used to estimate exposure (Timchalk et al., 2007). While, this issue has been noted in previous studies of chlorpyrifos (Krieger et al., 2001; Morgan et al., 2005), the results of this research suggest that background levels of the pesticide degradates included in this research are relatively stable over a five week period and that the exposure to TCP from contact with hard surfaces may be estimated using relatively few samples.

3.5. Study Limitations

Several limitations of this study are worth noting. The research house serves as an intermediate between large chambers and occupied homes. The research house is unoccupied and the activity is highly restricted during experiments. In a "real world" field based experiment physical activity of occupants as would be expected to provide a significant contribution to movement of non-volatile pesticides. This study was designed to remove the human activity factor so that the movement of residues would be based predominantly on their physical chemical properties and movement in air as an airborne residue or associated with a particle. Although it is likely that some physical transport of resides from the point of application were associated with the activities of the research team in the house.

The study duration was extended over previous pesticides studies in the research house to 35 days (Mason et al., 2000; Stout II and Mason, 2003). Stout II and Mason (2003) have shown that, for chlorpyrifos, measured surface concentrations typically reach maximal levels at day 1 and decline rapidly over 21 days. Since pesticides with differing vapor pressure were selected for inclusion in this study it was uncertain, particularly for the low vapor pressure pyrethroids, what might be an appropriate study duration. The 35 day end point was selected based on aerobic soil half lives of the pesticides and prior research on dissipation rate of chlorpyrifos (Mason et al., 2000; Stout II and Mason, 2003). Since there appeared to be little evidence of degradate formation over the 7 weeks of this study, these guides clearly overestimate indoor degradation rates. Future research efforts of this type will likely need to be conducted over a longer time frame.

Permethrin, fipronil, and propoxur residues were measured at variable concentration as background during the pre-application sampling. Permethrin, as well the other pesticides, exist at low levels in the environment and in the U.S housing stock (Stout II, et al., 2009). The background residues measured in this study presented an additional challenge and introduced some uncertainty to the interpretation of the trends for *cis*- / *trans*-permethrin and fipronil. In addition, studies have shown that residues may be introduced to homes through a physical process know as track-in (Nishioka et al., 1997). The exterior of the research facility receives no out door applications and the ingress and egress to the facility is monitored and negligible relative to similar occupied dwellings. It is doubtful that track-in significantly contributed to residues and decay products in this study.

When the pesticides have recently been applied indoors, their rate of degradation is low and unlikely to be a significant factor affecting results of short-term (weeks) biomonitoring studies. Therefore, relatively few indoor samples would be needed in order to estimate background levels of degradation products resulting from a recent pesticide application.

4. Conclusions

In this study, there was some evidence of statistically significant indoor movement of the recently applied pyrethroids and fipronil. However, the mass was small and not practically important in describing the indoor behavior of these pesticides after application. In contrast, propoxur, a semi-volatile pesticide, was readily transported away from the application site to flooring in both the den and living room. The propoxur concentration increase over a one week period in both den and living room, then gradually decreased during the remainder of the study period.

There appeared to be no significant formation of the degradation products of any applied pesticides due to breakdown of the parent pesticide after application. Even though the applied pesticides contained low amounts of the degradation products, these degradates are unlikely to impact pesticide exposure / dose estimated from urinary biomarkers. .

When the pesticides have recently been applied indoors, their rate of degradation is low and unlikely to be a significant factor affecting results of short-term (weeks) biomonitoring studies. Therefore, relatively few indoor samples would be needed in order to estimate background levels of degradation products resulting from a recent pesticide application.

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Baker, S.E., Barr, D.B., Driskell, W.J., Beeson, M.D., Needham, L.L. (2000). Quantification of selected pesticide metabolites in human urine using isotope dilution high-performance liquid chromatography / tandem mass spectrometry. *J Expo Anal Environ Epidemiol*. 10, 789-798.

Bobé, A., Cooper, J., Coste, C., and Muller, M. (1998). Behavior of fipronil in soil under Sahelian plain conditions. *Pestic Sci*. 52, 275-281.

Class, T, (1991). Determination of pyrethroids and their degradation products in indoor air and on surfaces by HRGC-ECD and HRGC-MS(NCI). *HRC*. 14, 446-450.

Cohen Hubal, E., Sheldon, L., Burke, J., McCurdy, T., Berry, M., Rigas, M., Zartarian, V., and Freeman, N. (2000). Children's exposure assessment: A review of factors influencing children's exposure, and the data available to characterize and assess that exposure. *Environ Health Perspect*. 108, 475-486.

Colt, J.S., Lubin, J., Camann, D., Davis, S., Cerhan, J., Severson, R.K., Cozen, W., Hartge, P. (2004). Comparison of pesticide levels in carpet dust and self-reported pest treatment practices in four US sites. *J Expo Anal Environ Epidemiol*. 14, 74-83.

Deziel, N.C., Viet, S.M., Rogers, J.W., Camann, D.E., Marker, D.A., Heikkinen, M.S.A., Yau, A.Y., Stout II, D.M., Dellarco, M. (2011). Comparison of wipe materials and wetting agents for pesticide residue collection from hard surfaces. *Sci Total Environ*. 409, 4442-4448.

Egeghy, P.P., Cohen Hubel, E.A., Tulve, N.S., Melnyk, L.J., Morgan, M.K., Fortmann, R.C., Sheldon, L.S. (2011). Review of pesticide urinary biomarker measurements from selected US EPA children's observational exposure studies. *Int J Environ Res Public Health*. 8, 1717-1754.

Fensky, R.A., Bradman, A., Whyatt, R.M., Wolff, M.S., Barr, D.B. (2005). Lessons learned for the assessment oof children's pesticide exposure: Critical sampling and analytical issues for future studies. *Environ Health Perspect. 113*, 1455-1462.

Heudorf, U., Angerer, J., Drexler, H. (2004). Current internal exposure to pesticides in children and adolescents in Germany: urinary levels of metabolites of pyrethroid and organophosphorus insecticides. *Int Arch Occup Environ Health*. 77, 67-72.

Krieger, R.I., Bernard, C.E., Dinoff, T.M., Ross, J.H., Williams, R.L. (2001). Biomonitoring of persons exposed to insecticides used in residences. *Ann Occup Hyg*. 45, (1001), S143-S153.

Lacroix, M.C., Puel, S., Toutain, P.L., Viguie, C. (2010). Quantification of fipronil and its metabolite fipronil sulfone in rat plasma over a wide range of concentrations by LC/UV/MS. *J Chromatogr B Analyt Technol Biomed Life Sci*. 878, 1934-1938.

Lin, K., Haver, D., Oki, L., Gan, J. (2009). Persistence and sorption of fipronil degradates in urban stream sediments. *Environ Toxicol Chem*. 28, (7), 1462-1468.

Macintosh, D.L., Needham, L.L., Hammerstrom, K.A., Ryan, P.B. (1999). A longitudinal investigation of selected pesticide metabolites in urine. *J Expos Anal Environ Epidemiol*. 9, 494-501.

Mahler, B.J., Van Metre, P.C., Wilson, J.T., Musgrove, M. (2009). Fipronil and its degradates in indoor and outdoor dust. *Environ Sci Technol*. 43, 5665-5670.

Mandal, K. and Singh, B, (2013). Persistence of fipronil and its metabolites in sandy loam and clay loam soils under laboratory conditions. *Chemosphere*. (In Press),

Mason, M.A., Sheldon, L.S., Stout II, D.M. (2000). The distribution of chlorpyrifos in air, carpeting, and dust and its reemission from carpeting following the use of total release aerosols in an indoor air quality test house. Proceedings of the Symposium on Engineering Solutions to Indoor Air Quality Problems. 92-102.

Morgan, M.K., Sheldon, L.S., Croghan, C.W., Jones, P.A., Robertson, G.L., Chaung, J.C., Wilson, N.K., Lyu, C.W. (2005). Exposure of preschool children to chlorpyrifos and its degradation product 3,5,6-trichloro-2-pyridinol in their everyday environments. *J Expo Anal Environ Epidemiol*. 15, 297-309.

Nishioka, M.G., Burkolder, H.M., Brinkman, M.C., Gordon, S.M., Lewis, R.G. (1997). Simulation of track-in pesticides from turf to home: comparisons of dislodgeable turf residues with carpet dust and carpet surface residues. Report No. EPA/600/R-97/108, United States Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, NC.

Riederer, A.M., Bartell, S.M., Barr, D.B., Ryan, P.B. (2008). Diet and nondiet predictors of urinary 3-phenoxybenzoic acid an NHANES 1999-2002. *Environ Health Perspect*. 116, (8), 1015-1022.

Schlenk, D., Huggett, D.B., Allgood, J., Bennett, E., Rimoldi, J., Beeler, A.B., Block, D., Holder, A.W., Hovinga, R., Bedient, P. (2001). Toxicity of fipronil and its degradation productsto Procambarus sp.: Field and laboratory studies. *Arch. Environ Contam Toxicol*. 41, 325-332.

Starr, J.M., Scollon,. E.J., Hughes, M.F., Ross, D.G., Graham, S.E., Crofton, K.M., Wolansky, M.J., DeVito, M.J., Tornero-Velez, R. (2012). Environmentally-relevant mixtures in cumulative assessments: an acute study of toxicokinetics and effects on motor activity in rats exposed to a mixture of pyrethroids. *Toxicol Sci*. 130, (2),309-318.

Starr, J.M., Graham, S.E., Stout II, D.M., Andrews, K., Nishioka, K. (2008). Pyrethroid pesticides and their metabolites in vacuum cleaner dust collected from homes and day-care centers. *Environ Res*. 108, (3), 271-279.

Stout II, D.M., Bradham, K.D., Egeghy, P.P., Jones, P.A., Croghan, C.W., Ashley, P.A., Friedman, W., Brinkman, M.C., Nishioka, M.G., Cox, D.C. (2009). American healthy homes survey: A national study of residential pesticides measured from floor wipes. *Environ Sci Technol*. 43, (12), 4294-4300.

Stout II, D.M., Bradham, K.D., Highsmith, V.R., Croghan, C.W., Jones, P.A., Friedman, W., Pinzer, E.A., Cox, D., Dewalt, G. (2007). American healthy homes survey (AHHS): A national study of residential pesticides measured from floor wipes. American Chemical Society, 233rd National Conference. Chicago, IL. March 28.

Stout II, D.M.,Mason, M.A. (2003). The distribution of chlorpyrifos following a crack and crevice type application in the US EPA Indoor Air Quality research House. *Atmos Environ*. 37, 5539-5549.

Taylor, J. K. (1987). Quality Assurance of Chemical Measurements. Lewis Publishers, New York.

Timchalk, C., Busby, A., Campbell, J.A., Needham, L.L., Barr, D.B. (2007). Comparative pharmacokinetics of the organophosphorus insecticide chlorpyrifos and its major metabolites diethylphosphate, diethylthiophosphate and 3,5,6-trichloro-2-pyridinol in the rat. *Toxicology*. 237, 145-157.

USDA, (2008). ARS Pesticide Properties Database. U.S. Department of Agriculture.

Wessels, D., Barr, D.B., Mendola, P. (2003). Use of biomarkers to indicate exposure of children to organophosphate pesticides: implications for a longitudinal study of children's environmental health. *Environ Health Perspect*. 111, (16), 1939-1946.

Weschler, C.J., Nazaroff, W.W. (2008). Semivolatile organic compounds in indoor environments. *Atmos Environ*. 42, 9018-9040.

Wilson, N.K., Chuang, J.C., Lyu, C., Menton, R., Morgan, M.K. (2003). Aggregate exposures of nine preschool children to persistent organic pollutants at day care and at home. *J Expo Anal Environ Epidemiol*. 13, 187-202.

Zhang, X., Driver, J.H., Li, Y., Ross, J.H., Krieger, R.I. (2008). Diaalkylphosphates (DAPS) in fruits and vegetables may confound biomonitoring in organophosphorous insecticide exposure and Risk assessment. *J Agric Food Chem*. 56, 10638-10645.

Pesticide	Formulation	Concentration[a] (gm/liter)	Theoretical Mass Applied (g)	Vapor Pressure (mPa) [b,c]	Log P[b]	Soil Aerobic $T_{1/2}$ (days)
cypermethrin	emulsifiable concentrate	1.87	0.45	2.3×10^{-4}	6.6	6-60[d]
permethrin	emulsifiable concentrate	4.79	1.3	7.0×10^{-2}	6.1	4-40[d]
propoxur	aerosol	1.05	1.6	13.0×10^{-1}	1.6	80-210[d]
fipronil	bait	0.01	0.003	3.7×10^{-4}	4.0	30-33[f]
chlorpyrifos	NA	NA	NA	27×10^{-1}	4.7	NA
deltamethrin	NA	NA	NA	1.2×10^{-5}	4.6	NA

a. Chlorpyrifos and deltamethrin were not applied in this study.
b. The Pesticide Manual (Tomlin, C.D.S., Editor)
c. Vapor pressure of cypermethrin, permethrin, and propoxur at 20 °C; fipronil, chlorpyrifos, and deltamethrin at 25 °C.
d. USDA. ARS Pesticide Properties Database. 2008.
f. Mandal & Singh (2013).

	Percent Recovery[a]				MDL / MQL
Analyte	Clean wipes (n = 15)	Counter wipes (n = 15)	Tiled, waxed floor wipes (n = 15)	Test house floor wipes (n = 15)	(ng/sample) (n = 12)
Pesticides					
chlorpyrifos	93 ± 10	101 ± 11	84 ± 13	93 ± 9	0.8 / 2.8
cypermethrin	92 ± 14	89 ± 10	88 ± 7	94 ± 14	2.9 / 9.8
deltamethrin	91 ± 14	81[b] ± 11	103[b] ± 9	88 ± 13	2.9 / 9.8
cis-permethrin	105 ± 9	102 ± 13	109 ± 8	101 ± 10	1.8 / 6.1
trans-permethrin	95 ± 15	95 ± 10	85[b] ± 10	93 ± 11	2.9 / 9.6

propoxur	94 ± 11	100 ± 14	102 ± 16	93 ± 11	2.8 / 9.2
fipronil	89 ± 9	78 ± 19	72[b] ± 13	80 ± 16	0.4 / 1.5
Degradates					
3-PBA	93 ± 19	102 ± 25	97 ± 26	91 ± 19	0.4 / 1.4
DBCA	95 ± 13	108[b] ± 14	100 ± 14	97 ± 17	0.1 / 0.5
cis-DCCA	91 ± 22	80 ± 13	80 ± 13	81 ± 8	0.4 / 1.2
trans-DCCA	72 ± 13	71 ± 10	72 ± 12	80[b] ± 7	1.7 / 5.6
fipronil sulfone	77 ± 18	81 ± 22	74 ± 19	80 ± 14	0.2 / 0.8
fipronil sulfide	83 ± 8	75 ± 15	64[b] ± 11	77 ± 17	0.1 / 0.3
fipronil desulfinyl	89 ± 15	72[b] ± 18	53[b] ± 14	81 ± 16	0.4 / 1.4
IPP	84 ± 18	75 ± 14	80 ± 15	62[b] ± 14	4.2 / 14
TCP	91 ± 12	90 ± 14	104[b] ± 19	93 ± 14	0.9 / 2.8
Surrogates					
trans-permethrin C13	93 ± 15	90 ± 10	77 ± 7	89 ± 11	NA
4-F-3-PBA	87 ± 11	91 ± 14	94 ± 15	87 ± 11	NA

a. Pooled mean percent recovery ± 1 standard deviation of cotton wipes spiked at 1 of 3 analyte concentrations.

b. Mean was significantly different ($p < 0.05$) than clean wipe.

		Number of Days After Pesticide Application								
	Pre-Application	**1**	**2**	**3**	**7**	**14**	**21**	**28**	**35**	**35 (overspray)**
Pesticides Applied	Concentration[a] (pg/cm^2)									
cypermethrin	ND[b]	15 ± 11 (6)	17 ± 7 (5)	17 ± 7 (6)	24 ± 13 (5)	33 ± 23 (5)	28 ± 11 (5)	29 ± 10 (6)	80 ± 81 (4)	29055 ± [illegible]
cis-permethrin	40 ± 28 (22)[c]	40 ± 18 (6)	31 ± 2 (5)	34 ± 13 (6)	70 ± 50 (5)	53 ± 23 (5)	35 ± 16 (5)	41 ± 9 (6)	72 ± 26 (4)	19412 ± [illegible]
trans- permethrin	45 ± 23 (22)	37 ± 16 (6)	35 ± 10 (5)	35 ± 12 (6)	72 ± 49 (5)	58 ± 29 (5)	34 ± 15 (5)	44 ± 9 (6)	89 ± 44 (4)	26394 ± [illegible]
fipronil	7 ± 7 (19)	3 ± 2 (5)	11 ± 7 (5)	8 ± 7 (6)	10 ± 2 (5)	16 ± 9 (5)	16 ± 5 (5)	12 ± 8 (6)	3 ± 2 (4)	
propoxur	2 ± 2 (10)	213 ± 103 (6)	290 ± 20 (5)	281 ± 121 (6)	282 ± 102 (5)	198 ± 56 (5)	144 ± 58 (5)	119 ± 31 (6)	98 ± 21 (4)	1213 ± [illegible]
Degradates of Applied Pesticides										
3-PBA	NA[d]	ND	ND	ND	1 ± 1 (1)	ND	ND	ND	2 ± 0 (4)	41 ± 0 (5)
cis- DCCA	NA	ND	0 ± 1 (1)	0 ± 0 (1)	0 ± 1 (1)	1 ± 1(2)	0 ± 1 (1)	0 ± 1 (2)	1 ± 1 (3)	11 ± 6 (5)
trans- DCCA	NA	1 ± 1 (3)	2 ± 0 (4)	2 ± 2 (2)	3 ± 1 (4)	2 ± 1(4)	4 ± 3 (5)	2 ± 1 (3)	2 ± 0 (4)	24 ± 12 (5)
IPP	NA	ND	ND	ND	ND	ND	ND	ND	ND (4)	ND (5)
fipronil desulfinyl	NA	ND	ND	ND	ND	ND	ND	ND	ND	-
fipronil sulfone	NA	4 ± 3 (5)	5 ± 2 (5)	5 ± 3 (6)	4 ± 3 (4)	6 ± 2 (5)	7 ± 4 (5)	6 ± 4 (6)	5 ± 5 (8)	-
fipronil sulfide	NA	ND	ND	ND	ND	ND	ND	ND	ND	-
										-
Pesticides Not Applied										
chlorpyrifos	4 ± 3 (27)	5 ± 2 (6)	4 ± 1 (5)	4 ± 1 (6)	6 ± 1 (5)	5 ± 1 (5)	4 ± 1 (5)	3 ± 1 (6)	4 ± 1 (9)	-
deltamethrin	NA	11 ± 4 (6)	12 ± 4 (5)	10 ± 4 (6)	11 ± 4 (5)	10 ± 4 (5)	7 ± 4 (5)	7 ± 2 (6)	12 ± 6 (9)	-

Degradates of Pesticides Not Applied										
TCP	NA	30 ± 12 (6)	24 ± 2 (5)	9 ± 10 (6)	24 ± 5 (5)	29 ± 13 (5)	21 ± 3 (5)	23 ± 5 (6)	30 ± 8 (9)	-
DBCA	NA	ND	ND	ND	ND	ND	ND	ND	ND	-

a. n = 22 samples Pre-Application. n=5 samples days 2, 7, 14, and 28. n = 6 samples days 1, 3, and 21. N = 9 samples day 35 (results for pesticides and degradates of day 35 samples with pesticide overspray of permethrin, cypermethrin and propoxur presented separately).
b. ND = Analyte not detected in any sample.
c. (n) = number of samples where analyte detected
d. NA = No samples taken.

	cypermethrin	permethrin	fipronil	propoxur
3-PBA	0.14	0.08	-	-
cis-DCCA	0.22	0.05	-	-
trans-DCCA	0.26	0.08	-	-
fipronil sulfide	-	-	0.32	-
fipronil sulfone	-	-	0.73	-
fipronil desulfinyl	-	-	0.01	-
IPP	-	-	-	0.27