Application of a permethrin immunosorbent assay method to residential soil and dust samples

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ABSTRACT

A low-cost, high throughput bioanalytical screening method was developed for monitoring cis/trans- permethrin in dust and soil samples. The method consisted of a simple sample preparation procedure [sonication with dichloromethane followed by a solvent exchange into methanol:water (1:1)] with bioanalytical detection using a magnetic particle enzyme-linked immunosorbent assay (ELISA). Quantitative recoveries (83-

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126%) of cis/trans-permethrin were obtained for spiked soil and dust samples. The percent difference of duplicate ELISA analyses was within ±20% for standards and ±35% for samples. Similar sample preparation procedures were used for the conventional gas chromatography/mass spectrometry (GC/MS) analysis except that additional cleanup steps were required. Recoveries of cis/trans-permethrin ranged from 81 to 108% for spiked soil and dust samples by GC/MS. The ELISA-derived permethrin concentrations were highly correlated with the GC/MS-derived sum of cis/trans-permethrin concentrations with a correlation coefficient (r) of 0.986. The ELISA method provided a rapid qualitative screen for cis/trans-permethrin in soil and dust while providing a higher sample throughput with a lower cost as compared to the GC/MS method. The ELISA can be applied as a complementary, low-cost screening tool to prioritize and rank samples prior to instrumental analysis for exposure studies.

Keywords: human exposure, cis-permethrin, trans-permethrin, immunoassay, ELISA, soil, dust

INTRODUCTION

Pyrethroid insecticides are synthetic analogues of pyrethrum found in chrysanthemum flowers.[1] Pyrethrum has seldom been used in agriculture because of its high cost and instability in sunlight. Pyrethroids are the result of modifying the chemical structures of the natural pyrethrins to confer stability while maintaining insecticidal activity. The synthetic pyrethrins are very stable in sunlight and are generally effective against most agricultural pests at low application rates (0.11 to 0.23 kg ha^{-1}). They can be persistent indoors, have low volatility, and tend to adsorb onto materials (i.e., carpets, fabrics and dust). Pyrethroids have found widespread application for agricultural, institutional, domestic and veterinary uses. Common trade names for permethrin include Ambush, BW-21-Z, Cellutec, Dragnet, Ectiban, Eksmin, Exmin, FMC 33297, Indothrin, Kafil, Kestrel, NRDC 143, Pounce, PP 557, Pramex, Qamlin, and Torpedo. There is an increasing trend in the usage of these compounds since the federally mandated phase-outs of most residential uses of organophosphates, particularly chlorpyrifos and diazinon.[2, 3] The pyrethroids are frequently used around food preparations and on pets due to their fast knockdown capability, high insecticidal activity and presumed low mammalian toxicity. However, pyrethroids are neurotoxins and recent studies have shown that neonatal and adult exposures may cause developmental neurotoxic and immunotoxic effects.[4-8] Pyrethroids have also been shown to pose risks to non-target insects and aquatic organisms.[9] The widespread and accelerating use of pyrethroids may increase exposures through occupational and domestic routes as well as dietary intake. The greater risk of exposure and the toxic effects to non-target species indicates prudent environmental monitoring is warranted.

Exposure to pyrethroids may occur through inhalation, dermal absorption, or ingestion, with dietary ingestion as typically the major route of exposure.[10-13] Permethrin, a mixture of cis- and trans-isomers, is a commonly used pyrethroid, and has been identified in various environmental and personal samples.[13-15] In a recent exposure study,[13] cis/trans-permethrin was detected in samples of air, dust, soil, food and dermal wipes, while

the generic pyrethroid metabolite, 3-phenoxybenzoic acid (3-PBA), was found in human urine study samples. Conventional analytical methods for measuring cis/trans-permethrin in soil and dust samples are time-consuming and costly. Samples typically undergo extraction and cleanup, with analysis of the final fraction by gas chromatography/mass spectrometry (GC/MS) or GC using flame ionization or electron capture detection. Environmental monitoring and exposure studies can be hampered by high analytical costs due to the large number of samples often generated in these studies. High-throughput screening methods can increase the amount of information available concerning the source and/or concentration of contaminants of concern. Rapid and cost effective screening methods, as well as, efficient high sample throughput methods are needed to support large-scale environmental monitoring and exposure field studies. Immunoassay techniques with their ease of use, high sample throughput and lower costs may facilitate such studies. Methods such as the enzyme-linked immunosorbent assay (ELISA) have proven useful for monitoring small molecular (<1000 Daltons) pollutants through the environment.[16-18] Immunoassay method performance data have been reported for real-world samples for monitoring pollutants of exposure interest.[19, 20]

Previously, a monoclonal anti-permethrin antibody (Py-1) was applied to the determination of permethrin in meat and grain using a 96-microwell ELISA format.[21, 22] The permethrin ELISA assay linear response range was 50 to 500 ng/mL with a detection limit of 150 ng/mL. Presented here is the evaluation of an ELISA analysis using the Py-1 antibodies in a magnetic particle format for screening soil and dust samples. Extraction procedures were optimized for real-world soil and dust samples from an exposure field study. Solid phase extraction (SPE) cleanup for sample extracts was required for GC/MS, but not for ELISA. The ELISA and GC/MS data were compared to determine the suitability of the ELISA method as a monitoring tool for permethrin in residential soil and dust at low ng g^{-1}levels.

MATERIALAS and METHODS

Chemicals and Materials

The standards cis-permethrin, trans-permethrin, diazinon-d_{10} were purchased from Sigma (St Louis, MO, USA). The $^{13}C_6$-labeled cis- and trans-permethrin standards were from Cambridge Isotope Laboratories (Andover, MA, USA). Solvents (dichloromethane (DCM), methyl tert-butyl ether (MTBE), ethyl ether (EE), n-hexane, and methanol) used in extraction and/or cleanup procedures were distilled-in-glass grade and obtained from VWR (West Chester, PA, USA). Extrelut and Bakerbond SPE Florisil cartridges were also purchased from VWR. Magnetic particle permethrin ELISA test kits were obtained from Abraxis (Warminster, PA, USA). The GC column, RTX 5 MS fused silica capillary column (60-m x 0.25-mm ID, 0.25 μm film thickness) was purchased from Restek Corporation (Bellefonte, PA, USA).

Evaluation of Extraction Methods

Yard soil and floor dust samples from an observational field study were used for evaluating sample extraction procedures for ELISA and GC/MS.[23] Indoor floor dust samples were collected with an HVS3 vacuum sampler from various residential dwellings using an ASTM standard procedure.[24] The yard soil samples were collected from the top 1-2 cm over an area of 0.1 m^2 from the backyard of participating residences where children spent most of their time playing. The extraction efficiency of DCM, MTBE, and 10% EE in n-hexane using sonication was determined for the soil and dust samples. Samples were mixed thoroughly and different aliquots were removed for spiking to determine extraction efficiency. Samples were prepared by fortifying with a known amount of a cis/trans-permethrin (1:1) mixture onto the collected soil and dust samples for ELISA analysis. Known amounts of a (1:1) mixture of both unlabeled and $^{13}C_6$-labeled cis/trans-permethrin were spiked onto the samples for GC/MS analysis. Aliquots (0.5 to 5 g) of each sample were extracted with 2 x 10 mL of DCM by

sonication (2 x 15 min). Soil samples containing excess moisture were mixed with Extrelut (1 to 2 g) prior to sonication. The same extraction procedures were used for the samples with two other solvents: MTBE and 10% EE in n-hexane. A simple shaking method was also evaluated. Aliquots of randomly selected soil samples were extracted with 20 mL of methanol using an orbital mechanical shaker at 55 rpm for 1 hr. Longer shaking times up to 16 h were also evaluated as quantitative recoveries were not achieved after one hour.

Sample Preparation

A total of 50 non-spiked samples (14 soil and 36 dust) from the observational field study[23] were extracted with DCM as described above and used in the ELISA method performance evaluation. The ELISA analysis simply required a solvent exchange into methanol from the DCM extract. The methanol extract was diluted with an equal amount of reagent water prior to ELISA. The final assay solvent was methanol:water (1:1) which was used for further dilutions for sample reanalysis when the ELISA results were outside the calibration range. Prior to GC/MS analysis, the DCM extract was solvent exchanged into n-hexane, followed by a Florisil SPE column clean-up using 12 mL of 15% EE in n-hexane and 6 mL of DCM. The combined eluates were concentrated to 1 mL, spiked with a known amount of diazinon-d_{10}, internal standard (IS), and transferred into a 1.8 ml GC vial to await analysis. The measured amounts of cis/trans permethrin were based on the comparative ratios of the signal of the target analytes, to the constant amount of IS (diazinon-d_{10}) added to the sample and calibration standards.

Sample Analysis

The ELISA analysis was performed using a magnetic particle permethrin ELISA. A solution of 41% cis-permethrin and 59% trans-permethrin was used as a calibrant and as a control. An aliquot (250 µL) of either a

131 calibration solution (0, 0.75, 2.5, 5.0, and 15 ng mL^{-1}), a control solution (3.0 ng mL^{-1}), or diluted sample
132 extract was carefully placed in the bottom of individually labeled test tubes. The test tubes were secured to the
133 rack of a magnetic separation system. An aliquot (500 µL) of the anti-permethrin antibody coupled to
134 paramagnetic particles was added to the inside wall of each tube and allowed to flow to the bottom. This
135 solution was mixed using a Vortex mixer (Scientific Industries, Bohemia, NY, USA) and allowed to incubate at
136 room temperature for 20 min. An aliquot (250 µL) of permethrin-horseradish peroxidase enzyme conjugate was
137 added to each tube; mixed thoroughly by vortexing; and incubated at room temperature for 30 min. The test
138 tube rack was then affixed to the magnetic base. The samples were allowed to stand for 2 min for the magnetic
139 particles to separate and adhere to the wall of the tube. The rack assembly was inverted over a waste container
140 to decant unbound reagents. The rims of the test tubes were gently blotted on several layers of clean paper
141 towels. An aliquot (1 mL) of a buffered washing solution was added down the inside wall of each test tube. The
142 solution was allowed to stand for 2 min at room temperature before decanting. This washing step was repeated
143 one more time. The magnetic separation rack was then removed from the magnetic base. An aliquot (500 µL) of
144 the color reagent was added down the inside wall of each tube and mixed by vortexing. The solution was
145 allowed to incubate for 30 min at room temperature. At the end of the incubation period, an aliquot (500 µL) of
146 an acidic stopping solution was added down the wall of each tube without mixing. Each test tube was analyzed
147 on a RPA I RaPID photometric analyzer (SDI, Newark, DE, USA) at 450 nm within 15 minutes of the addition
148 of stopping solution.
149
150 GC/MS analyses were performed on a Hewlett-Packard 6890 capillary gas chromatograph equipped with a
151 5973 mass selective detector (Agilent Technologies, Palo Alto, CA, USA). The gas chromatograph was fitted
152 with a RTX 5 MS fused silica capillary column (60-m x 0.25-mm ID, 0.25 µm film thickness). Sample extracts
153 and standard solutions were analyzed at 70 eV electron impact in the selected ion monitoring mode.[13] Peaks
154 monitored were either the molecular ion peaks (if sufficient intensity was present) or the characteristic fragment

ion peaks (183, 165, 163 for cis/trans permethrin; 189, 190, 163 for $^{13}C_6$-labeled permethrin, and 314, 315 for diazinon-d_{10}).

Data Analysis

The non-detectable values were replaced with one-half the detection limit. The ELISA and instrumental analyses were performed on separate aliquots of each soil and sediment sample. Samples were mixed prior to aliquoting, but no measure of heterogeneity was performed. The ELISA-derived permethrin concentrations and the sums of the GC/MS cis- and trans-permethrin concentrations were used in the data analysis. Descriptive statistics were calculated to characterize the distribution of results for each method. Sample size, arithmetic mean, standard deviation, geometric mean, range and percentiles were calculated. The Pearson correlation coefficient measuring the extent of linear agreement between the ELISA and GC/MS data was also calculated.

RESULTS AND DISSCUSSION

Evaluation of Extraction Methods

Recovery data for the matrix spiked soil and dust samples provided the overall method accuracy including sample extraction, cleanup (when necessary) and final detection for the target analyte. Recovery data for the post-spiked sample extracts were used to determine the accuracy of the detection technique. Table 1 summarizes the recovery data for the matrix spiked samples. Quantitative recoveries (>90%) were obtained when the spiked soil samples were extracted by sonication using DCM, MTBE or 10% EE in n-hexane for both ELISA and GC/MS methods. Recoveries were less than 50% for the spiked soil samples when the shaking method was employed (shaking with methanol for 1 hour). A longer shaking time (16 hours, overnight) was

179 also evaluated using methanol, resulting in recoveries above 200% by ELISA. A similar finding was observed
180 when shaking was used to extract Aroclors from soil and sediment matrices.[25] This is mainly because
181 interferences were co-extracted during the longer shaking times. The GC/MS analysis indicated that the
182 interfering components remained in these extracts even after the SPE cleanup. Thus, good recovery data were
183 also not obtained by GC/MS because of the poor quality of the chromatogram showing a rising background.
184
185 Based on GC/MS results, the extraction procedure of sonication with DCM was optimal in quantitatively
186 removing permethrin from the dust matrix. Post-spiked dust sample extracts were analyzed to determine ELISA
187 matrix effects. Satisfactory recoveries were obtained from the post-spiked dust sample extracts with an average
188 of 94±17% (18%). The results suggest that for ELISA detection, a simple dilution is sufficient to remove any
189 of the potential matrix interferences in these real world dust samples. Note that levels of cis- and trans-
190 permethrin and other pollutants were generally higher in house dust samples as compared to yard soil samples
191 in these residential settings.[13, 23] Therefore, we used a higher dilution factor for the dust sample extracts
192 ranging from 0.01 to 0.05g of dust as opposed to the soil extracts representing 1g of soil. The higher dilution
193 factor used for the dust samples also helped to reduce the matrix interference of dust samples. The simple
194 dilution method minimized the dust sample matrix effect and yielded satisfactory recovery results.
195
196 For GC/MS analysis, quantitative recoveries of the spiked $^{13}C_6$-labeled cis/trans-permethrin were achieved in
197 all fortified soil and dust samples. Average recoveries were 89±10% and 98±7% of the spiked $^{13}C_6$-labeled
198 cis/trans-permethrin in dust and 99±10% and 95±13% in soil, respectively. Note that ^{13}C has a natural
199 abundance of only 1.1%. Thus, $^{13}C_6$-permethrin is not present to a significant extent in the non-spiked samples,
200 and recoveries of the $^{13}C_6$ spike would not be affected. The results indicated that permethrin can be
201 quantitatively removed from soil and dust sample matrices by sonication with DCM. As this extraction method
202 provided quantitative recoveries of the spiked samples, it was used to prepare samples for the subsequent

ELISA and GC/MS methods comparison. DCM was easily removed by evaporation and did not cause any loss of permethrin during the methanol exchange step necessary for ELISA analysis.

ELISA Method Performance

For ELISA analysis, the permethrin concentration in each of the 50 soil and dust samples collected from Pesticide Exposure of Preschool Children Over Time (PEPCOT) study was determined using a calibration curve generated from duplicate analyses of standard solutions at five concentration values (0, 0.75, 2.5, 5, and 15 ng mL^{-1}). Duplicate analyses were performed for all samples, and the means of the duplicate values were used to calculate the final concentrations of total permethrin. The acceptance criteria established for ELISA were: (1) the percent relative standard deviation (%RSD) of the absorbance values of each standard concentration should be less than 10%, where the %RSD is based on the ratio of the relative standard deviation and the average of the absorbance from the duplicate assays; and (2) a correlation coefficient (r) greater than 0.998 for the calibration curve. If the results were outside the calibration range, the sample extract was diluted and reanalyzed. All of the reported permethrin ELISA results met the acceptance criteria.

The percent difference (%D) of the derived concentration of each standard solution from duplicate analyses was within ±20%. The %D of the measured assay concentrations of sample extracts from duplicate analyses was within ±30% for soil samples and ±35% for dust samples. Note that the magnetic-particle ELISA had a small dynamic optical density (OD) range and small changes in OD correlate to large changes in derived concentrations. The differences between absorbance values from duplicate analyses of the sample extracts were within the acceptance requirement (%RSD <10%). The %D of the derived concentrations of all but three dust samples from duplicate analyses was less than 30%. The three dust samples having a %D ranging from 31-34% of the measured concentrations may be due to a small volume of sample retained in the pipette tip during the transfer step. A trace amount of aliquot not delivered to the test tube could result in a large variation in the data

from duplicate assays. A positive control solution was also analyzed in each assay set for quality assurance. The average value of the control solution from all ten assay sets was 3.18 ±0.47 ng mL^{-1}. The measured values agreed well with the expected value (3 ng mL^{-1}). The %RSD was 15% for the control solution among the ten assays from different days. Method blanks were analyzed with each sample set yielding all non-detectable values.

A cis/trans-permethrin ratio of 1:1.4 (41% cis- and 59% trans-permethrin) was used for generating the calibration curve as recommended in the method protocol. A single standard of cis- and a single standard of trans-permethrin were run against the assay calibration curve to determine the individual responses of the cis- and trans-isomers in the ELISA. Recoveries ranged from 99 to 123% for cis-permethrin, and from 182 to 196% for trans-permethrin. These findings indicated that trans-permethrin generated a higher ELISA response than the cis-isomer that could influence the overall ELISA response for total permethrin. Different ratios of the cis/trans-isomers have been reported in real-world samples, due in part to the variation of ratios in commercial formulations of permethrin as well as differential degradation rates of the isomers in the environment.[13, 26-28] Ratios of cis/trans-permethrin measured by GC/MS in this study ranged from 0.8 to 1.1 in soil and from 0.5 to 5.6 in dust. The vendor ELISA cross reactivity specifications were ~5% to cypermethrin and cyhalothrin and very low cross-reactivity (<0.2%) to resmethrin, cyfluthrin, and 3-PBA. Since sumithrin has a chemical structure very similar to permethrin (the two chlorine atoms on the C=C double in permethrin are replaced with hydrogen atoms), we examined the ELISA response for sumithrin. The results showed approximately 300% of cross-reactivity. As there is minimal cross-reactivity to other pyrethroids and 3-PBA, the interpretation of the ELISA-derived permethrin results should take into consideration the differential ELISA response toward cis/trans-permethrin as well as the high cross reactivity to sumithrin. Thus, the ELISA method is a qualitative screen but not a definitive quantitative measure of cis/trans-permethrin. The ELISA method can be used as a

low cost screening tool for pyrethroids in soil and dust for prioritizing important samples or eliminating samples not of interest for quantitative analysis.

GC/MS Method Performance

For GC/MS analysis, the acceptance criteria were: (1) a <15% value for the %RSD of the average response factors of cis- and trans-permethrin to the internal standard, diazinon-d_{10}; (2) a <20% value for %D of the measured and expected values of the standard solutions; and (3) a 80-120% recovery for the matrix spiked samples. The GC/MS data met all of these QA requirements. Quantitative recoveries were obtained in the spiked soil and dust samples (94±10% for cis-permethrin and 96±10% for trans-permethrin). The overall method precision of the GC/MS method was within ±15%, with method accuracy greater than 90%.

Comparison of ELISA and GC/MS Data

For ELISA results, cis/trans-permethrin was detected in four of the 14 soil samples and in all 36 of the dust samples from the observation field study. The estimated detection limits for soil and dust were 2 and 10 ng g^{-1}, respectively. ELISA-derived permethrin concentrations ranged from non-detect to 125 ng g^{-1} for soil samples and from 25 to 106,000 ng g^{-1} for dust samples. ELISA-derived permethrin concentrations were generally higher than the sum of the GC/MS-derived cis/trans-permethrin for each sample. Higher concentrations were observed in floor dust as opposed to yard soil for both ELISA and GC/MS; a similar trend was also reported in other studies.[13,23] This suggests that permethrin is more persistent and stable in indoor as compared to outdoor environments (weathering effects).

Cis/trans-permethrin was detected in five out of 14 soil samples and in all 36 dust samples by GC/MS. The estimated detection limits of the GC/MS method for soil and dust were 0.5 and 4 ng g^{-1}, respectively. Note that a low level (2.6 ng g^{-1}) of cis/trans-permethrin was detected by GC/MS in one soil sample but was not detected by the ELISA as the level was below the detection limit of the ELISA method (4 ng g^{-1}). Sums of the measured concentrations of cis/trans-permethrin ranged from non-detect to 80.5 ng g^{-1} for the soil samples and from 12 to 32,800 ng g^{-1} for the dust samples.

The discrepancies between the overall ELISA and the GC/MS methods are mostly due to the detection techniques (immunochemical or instrumental). In the comparison of ELISA and GC/MS data, the ELISA-derived data were calibrated against the calibration solutions with a constant cis/trans-isomer ratio (1:1.4) and the GC/MS data were the sums of individually measured cis/trans-permethrin. For any non-detects, half of the detection limit was used for descriptive statistics and correlation analysis. Descriptive statistics for the ELISA and GC/MS results are shown in Table 2. The ratio of the ELISA permethrin geometric mean to the GC/MS permethrin (sum of cis/trans-isomer) geometric mean was 2.5 for soil, 3.9 for dust, and 3.4 for combined soil and dust samples. The higher ELISA-derived permethrin concentrations were partly due to the various ratios of cis/trans-permethrin in the samples and also to the cross-reactivity of other pyrethroids (e.g., sumithrin) present in the samples as determined by GC/MS. Figure 1 displays the relationship of the ELISA and GC/MS combined soil and dust data and the linear regression line for these data. The linear regression equation was: ELISA = 3.1698 x GC/MS – 185.57 for the combined data. Different regression lines were obtained (ELISA = 3.1734 x GC/MS – 257.38, and ELISA = 1.2853 x GC/MS + 2.0482) for dust and soil samples, respectively. The sample preparation procedure for GC/MS required an SPE cleanup which may also have contributed to the variability between the methods. Generally, there was a strong and positive relationship between the ELISA and GC/MS data in soil, and dust, as well as for all dust and soil samples.

The ELISA and GC/MS data were highly correlated with a correlation coefficient (r) of 0.9470 for soil samples, 0.9852 for dust samples, and 0.9860 for combined soil and dust samples. The residential soil samples were fairly clean and only a few samples (29%) had detectable permethrin. The slope observed for soil samples was 1.2853 but increased to 3.1734 for dust samples. This is because the dust sample matrices were generally more complex and contained additional pollutants than the soil samples. The GC/MS results from the PEPCOT study showed that sumithrin which has a high cross-reactivity to the Py-1 antibodies was present in some of the dust samples. Other pyrethroids with a lower cross-reactivity (e.g., resmethrin, bifenthrin, cyhalothrin, cyfluthrin, esfenvalerate, and fenvalerate) were also detected in the dust samples and contributed to the overall ELISA response.

CONCLUSION

A low-cost, high throughput bioanalytical method (sonication/ELISA) for screening cis/trans-permethrin in soil and dust matrices was developed and applied to exposure samples. Additional dilutions were generally required for the dust sample extracts when analyzed by ELISA due to the high concentrations of cis/trans-permethrin. There was a positive and strong relationship between the ELISA and GC/MS data, although the ELISA-derived permethrin data were higher than the GC/MS data. The ELISA-derived permethrin concentrations should not be treated as quantitative measurements but rather as a screen to indicate the presence of permethrin and/or other pyrethroids, providing a broad indicator of exposure. The ELISA method offers lower overall analytical costs, as no SPE cleanup or expensive instrumentation is required, and provides a higher sample throughput as compared to the GC/MS method. Screening data obtained from house dust (a known sink and repository for indoor pyrethroid deposition) can be used as a measure of indoor contamination and provides useful information for the assessment of human indoor exposures. This streamlined, low cost bioanalytical method can be applied to large scale exposure field studies such as the National Children's Study[29] which will examine the

effects of environmental influences on children's health. Additionally, this screening method could be used to prioritize and rank large numbers of samples at selected threshold concentration levels for quantitative instrumental analysis minimizing analytical costs. The sonication/ELISA approach could easily be modified to monitor cis/trans-permethrin in food matrices as dietary ingestion is another important route of exposure.

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FIGURE CAPTION

Figure 1. Correlation of the ELISA and GC/MS data for soil and dust samples.

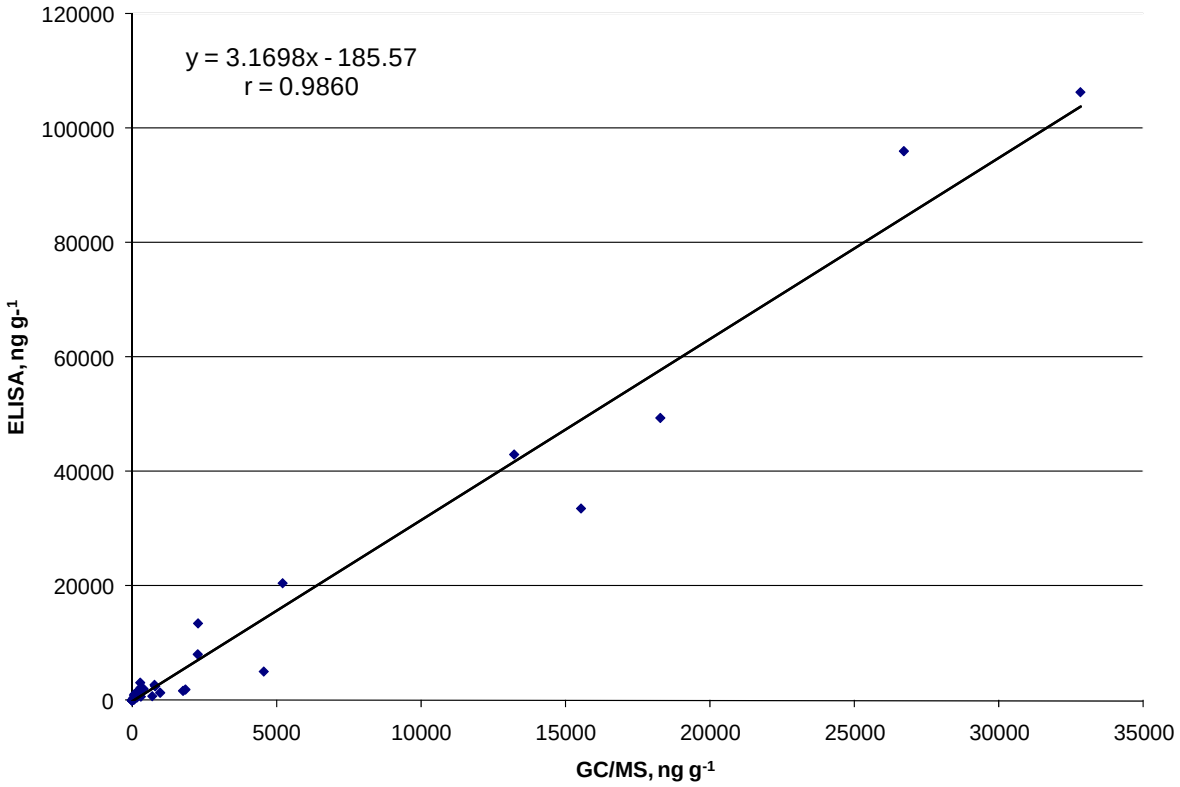
y = 3.1698x - 185.57
r = 0.9860
ELISA, ng g-1
GC/MS, ng g-1
120000
100000
80000
60000
40000
20000
0
0
5000
10000
15000
20000
25000
30000
35000

Fig. 1

Table 1. Recovery data of permethrin in soil and dust samples

Sample	Extraction Method	Analytical Method	Recovery, %[a]
Soil[b]	Sonication-MTBE	ELISA	92±11 (12%)
	Sonication-10% EE in n-hexane	ELISA	116±12 (10%)
	Sonication-DCM	ELISA	99±7 (6.8%)
	Shaking-methanol (1-hr)	ELISA	40±11 (28%)
	Sonication-DCM	GC/MS	99±10 (10%)[d] 95±13 (14%)[e]
Dust[c]	Sonication-DCM	ELISA	116%
	Sonication-DCM	GC/MS	89±10 (11%)[d] 98±7 (7.1%)[e]

[a] Recovery, % = (measured permethrin in the spiked sample - measured permethrin in the nonspiked sample)/spike level*100

[b] Two levels were used for the spiked soil samples (N=6): (1) 5 ng g^{-1} each of cis/trans-permethrin, and (2) 10 ng g^{-1} each of cis/trans-permethrin. Reported recovery data were the average ± standard deviation of all the spiked soil samples.

[c] Spike level was 400 ng g^{-1} for the dust sample (N=6).

[d] Recovery data for cis-permethrin.

[e] Recovery data for trans-permethrin.

Table 2. Summary statistics for permethrin concentrations by ELISA and GC/MS

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Summary Statistics	GC/MS	ELISA	GC/MS	ELISA	GC/MS	ELISA
Sample Type	Soil (ng g^{-1})		Dust (ng g^{-1})		Soil and Dust (ng g^{-1})	
Sample Size	14	14	36	36	50	50
Mean	15.0	20.3	3660	11400	2640	8180
Geometric Mean	1.30	3.26	552	2140	102	348
Minimum	0.25[a]	2.0[b]	11.6	25.0	0.25[a]	2.0[b]
25th Percentile	0.25[a]	2.0[b]	137	816	43.3	54.8
50th Percentile	0.25[a]	2.0[b]	298	1460	171	909
75th Percentile	14.9	27.3	1960	3570	799	2150
95th Percentile	70.1	87.5	20400	61100	17000	16500
Maximum	80.5	125	32800	106000	32800	106000

437 [a] The estimated detection limit was 0.5 ng g^{-1}; one half of the estimated detection limit was used for non-detects.

438 [b] The estimated detection limit was 4 ng g^{-1}; one half of the estimated detection limit was used for non-detects.

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