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Undisturbed dust as a metric of long-term indoor insecticide exposure: Residential DDT contamination from indoor residual spraying and its association with serum levels in the VHEMBE cohort

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Abstract

Although approximately 123 million people may be exposed to high levels of insecticides through the use of indoor residual spraying (IRS) for malaria control, few studies exist on indoor insecticide contamination due to IRS and its relationship with human exposure. In the present study, we developed a sampling method to collect undisturbed dust from 50 homes in Limpopo, South Africa, a region where dichlorodiphenyltrichloroethane (DDT) has been used in IRS programs to prevent malaria for ~70 years. We quantified DDT and its degradation products, dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD) in dust samples to determine dust loading levels and compared these levels to paired serum concentrations of *p,p'*-DDT and *p,p'*-DDE in women residents. *p,p'*-DDT and *p,p'*-DDE had the highest detection frequencies in both dust (58% and 34% detection, respectively) and serum samples (98% and 100% detection, respectively). Significantly higher detection frequencies for *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD were observed in dust samples collected in buildings that had been previously sprayed for malaria control. We also observed a significant, positive association between dust

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2015.09.014.

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loading and serum concentrations of *p,p'*-DDT and *p,p'*-DDE (Spearman's rho = 0.68 and 0.54, respectively). Despite the low detection frequency in dust, our results indicate that undisturbed dust may be a good metric to quantify longterm home exposure to DDT-related compounds and that contamination of the home environment may be an important determinant/source of DDT and DDE exposure.

Keywords

Malaria control; DDE; DDD; South africa; Developing country

1. Introduction

Each year, malaria infects approximately 198 million people worldwide and results in nearly 584,000 deaths, with most deaths occurring among African children (World Health Organization, 2014). Indoor residual spraying (IRS), the application of insecticides to interior walls, ceilings, and eaves, is a malaria vector control policy adopted by 88 countries (World Health Organization, 2014). Although banned in many developed countries, dichlorodiphenyltrichloroethane (DDT) is exempted from the Stockholm Convention on persistent organic pollutants for public health uses and at least 10 countries use DDT for IRS because DDT has a long residual efficacy (>6 months) (World Health Organization, 2014) and non-contact spatial repellent properties (Grieco et al., 2007).

Although the benefits of decreased malaria infection are clear (Mabaso et al., 2004; Kim et al., 2012; West et al., 2014), the use of IRS for malaria control has contributed to uniquely high insecticide exposure in sprayed communities (Ortiz-Pérez et al., 2005; Aneck-Hahn et al., 2007; Channa et al., 2012; Bouwman et al., 2006; Sereda et al., 2009; Van Dyk et al., 2010) and uptake via home contamination is hypothesized to be a significant source of insecticide exposure (Channa et al., 2012; Van Dyk et al., 2010). In developed countries, house dust is a common environmental medium collected to assess long-term indoor chemical exposures because insecticides and other pollutants persist indoors due to reduced degradation from sunlight, moisture, and microorganisms (Lioy et al., 2002; Butte and Heinzow, 2002; Roberts et al., 2009). Additionally, indoor insecticide dust concentrations can also be a metric of indoor air concentrations because semi-volatile chemicals partition between the dust and gaseous phase (Weschler and Nazaroff, 2010; Weschler et al., 2008).

To date, few studies have measured insecticide levels in floor dust samples from homes sprayed for malaria control, but results indicate elevated insecticide contamination (Van Dyk et al., 2010; Herrera-Portugal et al., 2005). For example, Van Dyk et al. found Σ DDT floor dust loading levels two months after IRS in sprayed homes (n = 12) to be orders of magnitude higher than unsprayed homes (n = 9) in Limpopo, South Africa (medians = 910 vs. 1.3 μg/m^2, respectively) (Van Dyk et al., 2010). In Mexico, homes in a community sprayed with DDT three years prior to dust collection (n = 10) had a mean dust Σ DDT concentration of 30.8 μg/g, which was ~42 times higher than DDT concentrations in homes in a community that had not been sprayed for more than two decades (n = 10; mean = 0.7 μg/g) (Herrera-Portugal et al., 2005).

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However, floor dust may not be the best metric for long-term exposure in rural African communities due to frequent sweeping, mopping, and resurfacing of hard surfaces. Researchers in developed countries have successfully used attic dust as a metric of historical exposures such as air pollution (Ilacqua et al., 2003; Davis and Gulson, 2005; Feng et al., 2011). Although homes in rural Sub-Saharan Africa typically do not have attics, exposed indoor rafters or wall ledges accumulate large amounts of undisturbed dust and may be an innovative way to estimate historical home exposure to IRS insecticides in these communities.

In the present study, we collected undisturbed dust samples from 50 homes of women participating in the Venda Health Examination of Mothers, Babies and their Environment (VHEMBE) cohort study and quantified the dust loading levels of DDT and DDT breakdown products. We evaluated potential predictors of home contamination and the relationship between home contamination and serum DDT levels.

2. Methods

2.1. Study population

A subset of mothers (n = 50) participating in the VHEMBE study, a birth cohort investigating exposures and health effects from the use of IRS for malaria control, were enrolled between September 2012 and January 2013 to participate in a study about home environmental exposures. Participants were recruited when they presented to give birth at Tshilidzini Hospital in Limpopo Province, South Africa. However, participants were only considered eligible to continue to be in the study if field technicians observed accessible rafters or building material ledges to collect the undisturbed dust during a scheduled home visit one week after enrollment. We aimed to enroll an equal number of participants living in sprayed and unsprayed villages. Institutional Review Boards at the University of California-Berkeley, McGill University, and the University of Pretoria approved all study activities.

2.2. Maternal and home characteristics

VHEMBE study staff administered a baseline questionnaire after delivery at Tshilidzini Hospital. We obtained information on sociodemographic characteristics, housing history, and village IRS history. Approximately one week after delivery, the study team visited the home of the mother and child. At this home visit, a second questionnaire was administered to gather additional data on demographics and home IRS history. In addition, a home inspection was conducted at this visit to gather information on building characteristics.

2.3. Dust collection and analysis

Indoor dust samples (n = 50) were collected at the home visit. Sampling methods used hard surface wipe collection procedures previously developed by the United States Environmental Protection Agency in a national exposure survey (Stout et al., 2009; Tulve et al., 2006). Pre-cleaned acrylic cloths were used for dust sample collection. Using a portable ladder, approximately 0.018 m^2 (180 cm^2) of exposed rafters or building ledges were thoroughly wiped with a cloth wetted with 6 mL of insecticide-grade 2-propanol. The procedure was repeated in the same sampling area and both cloths were combined in a single high-density

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polyethylene (HDPE) jar. All samples were stored at − 20 °C in the HDPE jars until analyzed by the South African Agricultural Research Council's Plant Protection Research Institute. Samples were extracted then analyzed by gas chromatography-mass spectrometry (Agilent Technologies 5975) for DDT, dichlorodiphenyldichloroethylene (DDE) and dichlorodiphenyldichloroethane (DDD).

In preliminary analyses, matrix interferences occasionally co-eluted with the analytes of interest. To more accurately characterize the target analytes, matrix-matched standards, using a stable isotope labeled analogue of the analyte, were performed by spiking blank sample cloths with different concentrations of the analytical standards, representing each calibration level and analyzed following the procedure of the dust sample cloths. This method was performed to account for any enhancement or suppression effects as well as losses on extraction. The limit of quantification (LOQ) was 15 pg per sample for all isomers or, if converted to loading units using the average area of collection (0.018 m^2), 833.3 pg/m^2. Quality control (QC) procedures included five field and laboratory blanks, duplicates, and spiked dust samples. Briefly, across the five levels of analytical standards measured in triplicate, recovery ranged from 61.5 to 113.4%. The percent relative standard deviation (%RSD) of seven replicated analytical standard injections was 8.3 to 23.4%. Isomers in field blanks were typically below the LOQ and duplicate samples were typically either both below the LOQ or both above. Details of the quantification of DDT, DDE, and DDD in the dust samples and QC information are presented in the Supplemental Material.

2.4. Serum collection and analysis

Maternal blood (10 mL) was collected either before (n = 42) or after delivery (n = 8) by trained nurses into red-top Vacutainer® serum separator tubes. Blood was allowed to clot and the serum was separated via centrifuge and frozen at −80 °C within 2 hours. Serum aliquots (2 mL) were shipped on dry ice to Emory University's Rollins School of Public Health for analysis. One mL of serum was spiked with isotopically labeled internal standards, mixed, and extracted with hexane:dichloromethane. Residual fat was removed by passing extract through an acidified silica cartridge then concentrated. The *p,p′* and *o,p′* isomers of DDT/DDE were measured using gas chromatography–tandem mass spectrometry with isotope dilution quantification (Barr et al., 2003). The LOQs for DDT/DDE isomers ranged between 0.05 and 0.1 ng/mL. In each analytic run, 2 blank samples, 2 spiked quality control samples (0.75 and 3 ng/mL), and a full 8-point calibration set were analyzed concurrently with study samples.

2.5. Statistical analysis

Dust loading levels were calculated by dividing the mass ot each isomer by the total sampling area. For duplicate samples, if at least one of the two samples was above the LOQ, the sample was considered > LOQ and the average of the duplicated samples was used for that home. We tested for differences in dust detection frequencies between buildings that were and were not sprayed using Fisher's exact tests. Mann-Whitney U tests were used to determine if serum DDT/DDE concentrations were statistically different between women living in households with DDT/DDE dust levels either above or below the LOQ. We used Spearman's rank correlation tests to evaluate the association between dust loading levels and

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serum concentrations. Within the Spearman tests, we used instrument values for loading and serum levels below the LOQ. For instrument values below zero (n = 2, due to variability in the calibration curve), we assigned a value of LOQ / $\sqrt{2}$ (Hornung and Reed, 1990). We evaluated for the effect of time (years) lived in the current home on the association between DDT/DDE dust loading and serum concentrations with interaction terms within linear regression models. We considered *p*-values below 0.05 to be statistically significant.

3. Results

All VHEMBE participants were black, Africans. Most mothers were less than 24 years old (54%) and lived below the South African food poverty line (68%) (Table 1). Forty-eight dust samples were collected from western-style buildings with concrete walls and corrugated iron roofs and two samples were collected from rondavels (traditional round homes) with earth walls and thatched roofs. The majority of dust samples were collected from a ceiling rafter (86%) located in the mother's bedroom (92%). Thirteen homes were built before the year 2000, 25 homes were built after 2000, and 12 participants did not know the year of home construction. Eleven participants (22%) reported that the buildings where the dust sample was collected had been previously sprayed for malaria control and fifteen (30%) of the villages had been previously sprayed for malaria control. The median (interquartile range, IQR) of time mother had lived in the current home was 9.0 (1.6–20.3) years.

Analyte concentrations were above the LOQ in approximately 14% to 58% of the dust samples (Table 2). The most commonly detected analytes were *p,p'*-DDT and *p,p'*-DDE (34% and 58%, respectively) and the least commonly detected analytes were *o,p'*-DDE and o,p'-DDD (14% and 16%, respectively). The median loading level of *p,p'*-DDT was 4.5 ng/m^2. Some extreme values were observed in the loading levels. For example, the maximum loading level of *p,p'*-DDT was 451,000 ng/m^2 or approximately five orders of magnitude higher than the median. When stratified by whether the building in which the dust samples were collected had ever been sprayed for malaria control, we found that the detection frequencies for *o,p'*-DDT, *p,p'*-DDE, and *p,p'*-DDD were significantly higher in sprayed buildings than in non-sprayed buildings (p-values < 0.05) (Table 2). For all DDT-related compounds measured, detection frequencies were higher in buildings that had ever been sprayed (n = 11) than in buildings that had never been sprayed for malaria control (n = 37). The loading distributions were also higher in buildings that had ever been sprayed.

In the subset of VHEMBE mothers who provided blood as well as dust samples, serum concentrations were typically above the LOQ for *p,p'*-DDT (98%), *o,p'*-DDT (80%), and *p,p'*-DDE (100%), whereas *o,p'*-DDE serum concentrations were above the LOQ in 38% of the blood samples (Table 3). Median (IQR) *p,p'*-DDT and *p,p'*-DDE concentrations were 235 (31–964) and 112 (44.5–740) ng/g-lipid, respectively. Women living in a home with dust levels of *p,p'*-DDT, *o,p'*-DDT, *p, p'*-DDE, and *o,p'*-DDE above the LOQ had significantly higher serum concentrations of those respective analytes (p-values < 0.01) (Table 4). For example, median serum concentrations of *p,p'*-DDT and *p,p'*-DDE were 566 and 739 ng/g lipid, respectively, in mothers living in a home with dust levels above the LOQ for those analytes, which were an order of magnitude higher than median serum concentrations of mothers living in a home with dust levels below the LOQ (*p,p'*- DDT =

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56; *p,p'*-DDE = 57.8 ng/g-lipid). We found that *p,p'*-DDT and *p,p'*-DDE loading levels were significantly, positively associated with serum concentrations (Spearman's rho = 0.68 and 0.54, respectively; p-values < 0.01). This positive association is clearly evident when all observations were plotted using instrument values (Figure 1). We did not find that the number of years a mother had lived in the home modified the association between *p,p'*-DDT/DDE dust loading levels and serum concentrations.

4. Discussion

In this study, we collected undisturbed settled dust in 50 homes of mothers participating in the VHEMBE cohort study to quantify longterm exposure to DDT, DDE, and DDD from the use of IRS for malaria control. We found that dust loading levels were associated with whether the building had ever been sprayed for malaria control and with the serum levels of the mothers living in the homes.

Undisturbed dust loading levels were appreciably lower than floor dust levels as previously reported by Van Dyk et al., despite the samples being collected from the same province of South Africa and analyzed by the same laboratory (Van Dyk et al., 2010). For example, even in homes from *unsprayed* villages, Van Dyk et al. reported a median *p,p'*-DDT loading level of 1000 ng/m^2, which is ~20 times higher than our median level in buildings *ever sprayed* (51.2 ng/m^2). The samples collected by Van Dyk et al. were collected approximately four years prior (2008) to our sample collection and the reduction in loading levels may be due to the decreased use of DDT as an IRS insecticide for malaria control in Limpopo, South Africa (P. Kruger, Limpopo Malaria Control Programme, pers. comm.). In fact, this area of Limpopo had not been sprayed with DDT in the concurrent malaria season when our dust samples were collected. Although DDT is a persistent compound, we expect reduced persistence of DDT in a tropical area like Limpopo due to higher evaporation and microorganism degradation rates (ATSDR, 2002). Further, the difference in DDT dust loading levels may indicate DDT removal via mechanisms beyond evaporation and degradation (e.g., cleaning).

In IRS communities, few studies have formally evaluated the relationship between environmental contamination (e.g., indoor dust) and exposure biomarkers (e.g., serum). In a study in a malaria endemic area of Mexico where DDT was used, Herrera-Portugal etal. did not find an association between DDT/DDE floor dust concentrations and children's blood levels (n = 20) (Herrera-Portugal et al., 2005). Further, Ortiz-Perez et al. found that time since IRS with the pyrethroid delta-methrin corresponded to decreasing levels of two deltamethrin metabolites in children's urine, but that longitudinal soil samples in the homes were not associated with these biomarkers (Ortiz-Pérez et al., 2005). In non-IRS communities, there is suggestive evidence that indoor dust levels of DDT contribute to serum levels (Davies et al., 1975), but other studies have not found an association with DDT floor dust levels and serum concentrations (Roosens et al., 2010; Ali et al., 2014; Buckley et al., 1997). However, previous studies in non-IRS communities have found dust concentrations of other insecticides to be associated with biomonitoring levels (Buckley et al., 1997; Bradman et al., 2007; Ostrea et al., 2011; Gunier et al., 2013; Shealy et al., 1997).

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Our study has several strengths. For example, it is the largest indoor dust study in an IRS sprayed community conducted to-date and also the largest to investigate the relation of IRS insecticide home contamination to serum concentrations. In addition, we believe our dust collection method may be a better predictor of long-term residential contamination in our study population. Although residents may not be directly in contact with the undisturbed dust collected, the dust should be representative of general ambient contamination and potential exposures via non-dietary ingestion, inhalation, dermal absorption of adhered dust, and dermal absorption of gaseous compounds (Weschler and Nazaroff, 2012), as semi-volatile chemicals partition between the dust and gaseous phase (Weschler and Nazaroff, 2010; Weschler et al., 2008).

Limitations of this study include the low detection frequency in dust despite being in an area with historic use of DDT for IRS. The low detection frequencies may be due to matrix interferences from co-eluting compounds and not necessarily due to the low levels of DDT, DDE, and DDD within the home. In addition, due to the lack of records on IRS in this community, we relied on maternal self-report of malaria control spray history in the home and village, which may be subject to exposure misclassification. In addition, our study could not evaluate whether undisturbed dust is better than floor dust as a marker of home exposures because floor samples were not collected at the same homes for comparison. Future research would benefit from comparing detection frequencies, concentrations, and their correlation with biological markers of exposure between co-sampled undisturbed and floor dust.

5. Conclusion

Although we observed lower detection frequencies in dust than previous studies in IRS areas, the strong correlation between dust loading levels and serum concentrations of women living in the homes suggests that undisturbed dust samples may be an innovative way to estimate long-term home environmental exposures in IRS communities and that contamination of the home environment may be an important determinant/source of DDT and DDE exposure.

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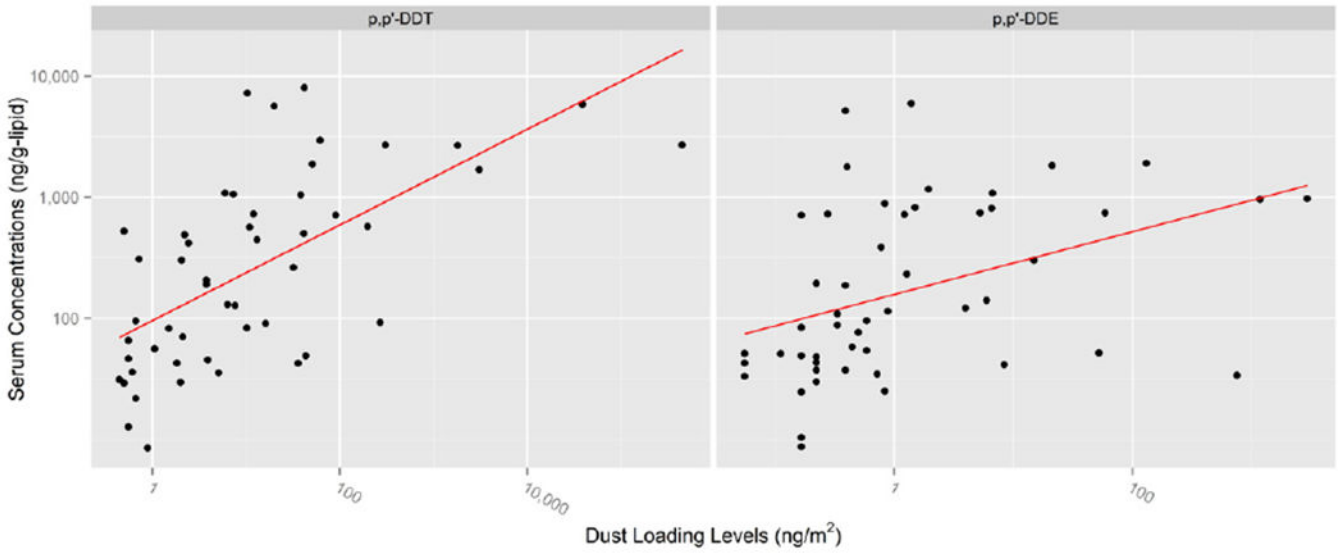

Fig. 1.

Comparison between *p,p'*-DDT and *p,p'*-DDE dust loading levels (x-axis) and serum concentrations (y-axis) of VHEMBE mothers. Red line is the linear regression line between loading and serum levels.

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Table 1

Demographics of participants and housing characteristics, n = 50.

Characteristic	n	(%)
Maternal age (years)		
18–24	27	(54)
25–30	8	(16)
30–35	6	(12)
>35	9	(18)
Poverty[a]		
Above food poverty line	16	(32)
Below food poverty line	34	(68)
Building type		
Rondavel	2	(4)
Western structure	48	(96)
Room of dust sample		
Mother's bedroom	46	(92)
Kitchen	4	(8)
Dust sample surface		
Rafter	43	(86)
Rondavel ledge	2	(4)
Other	5	(10)
Building construction date		
<2000	13	(26)
≥2000	25	(50)
Don't know	12	(24)
Building ever sprayed for malaria control		
No	37	(74)
Yes	11	(22)
Don't know	2	(4)
Village spray frequency		
Never	35	(70)
Some years	9	(18)
Most years	1	(2)
Every year	5	(10)

[a]Food poverty line developed by Statistics South Africa (370 Rands or $40.20 monthly income per household member) (Statistics South Africa, 2014; Ruch, n.d.).

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Table 2

DDT, DDE, and DDD isomer *dust* detection frequencies and loading distributions (ng/m^2) for all participants (n = 50) and stratified by whether building was ever sprayed for malaria control.

		***p,p'*-DDT**	***o,p'*-DDT**	***p,p'*-DDE**	***o,p'*-DDE**	***p,p'*-DDD**	***o,p'*-DDD**
All (n = 50)	Detect.freq.[b] (%)	58.0	26.0	34.0	14.0	26.0	16.0
	25th%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Median	4.5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	75th%	40.7	1.5	3.49	<LOQ	1.75	<LOQ
	90th%	272	49.2	53	3.05	34.3	3.17
	Max	451,000	21,700	2950	69.3	30,000	311
Building never sprayed (n = 37)[a]	Detect.freq.[b] (%)	48.6	13.5	24.3	10.8	13.5	10.8
	25th%	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Median	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	75th%	11	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	90th%	105	21.8	13.5	0.778	13.2	0.956
	Max	1810	340	131	11.4	273	24.4
Building ever sprayed (n = 11)[a]	Detect.freq.[b] (%)	81.8	54.5	63.6	27.3	63.6	27.3
	25th%	6.39	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
	Median	51.2	6.22	5.28	<LOQ	3	<LOQ
	75th%	1580	334	382	11.5	247	30.5
	90th%	39,000	8220	1180	55.9	4370	122
	Max	451,000	21,700	2950	69.3	30,000	311

[a] Homes where the mother did not know if the building had been ever sprayed for malaria control were excluded (n = 2).

[b] Detect. Freq. = detection frequencies, Limits of quantification (LOQ) for DDT/DDE isomers = 15 pg/sample.

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Table 3

DDT and DDE isomer *serum* detection frequencies and concentration distributions (ng/g-lipid, n = 50).

Analyte	Detect. freq.[a] (%)	Min	10th%	25th%	Median	75th%	90th%	Max
p,p'-DDT	98	<LOQ	31	50.7	235	964	2730	8000
o,p'-DDT	80	<LOQ	<LOQ	8.92	25.4	67.7	310	878
p,p'-DDE	100	8.69	32.8	44.5	112	740	1220	5920
o,p'-DDE	38	<LOQ	<LOQ	<LOQ	<LOQ	15.8	34.6	83.6

[a]Detect. Freq. = detection frequencies. Limits of quantification (LOQ) for DDT/E isomers were 0.05 ng/mL for 44 serum samples and 0.1 ng/mL for 6 samples.

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Table 4

Serum detection frequencies and concentrations (ng/g-lipid) stratified by whether dust sample was above the limit of quantification (LOQ).

	***p,p'*-DDT**		***o,p'*-DDT**		***p,p'*-DDE**		***o,p'*-DDE**	
Dust sample above LOQ?	No	Yes	No	Yes	No	Yes	No	Yes
	(n = 21)	(n = 29)	(n = 37)	(n = 13)	(n = 33)	(n = 17)	(n = 43)	(n = 7)
Detect. freq.[a] (%)	95.2	100.0	73.0	100.0	100.0	100.0	30.2	85.7
Min	<LOQ	35.4	<LOQ	6.99	8.69	33.9	<LOQ	<LOQ
10th%	21.7	66.1	<LOQ	21.9	25.9	47.6	<LOQ	5.46
25th%	31.2	127	<LOQ	45.2	37.3	121	<LOQ	14
Median	56	566	13.6	69.4	57.8	739	<LOQ	36.3
75th%	308	1870	50.7	249	231	971	10.3	73.6
90th%	524	3520	127	341	869	1430	19.1	82.8
Max	5630	8000	878	692	5920	1900	37.9	83.6

[a]Detect. Freq. = detection frequencies. Limits of quantification (LOQ) for DDT/E isomers were 0.05 ng/mL for 44 serum samples and 0.1 ng/mL for 6 samples.