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Analysis of House Dust and Children's Hair for Pesticides: A Comparison of Markers of Ongoing Pesticide Exposure in Children

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Abstract

Background/Aim: The long term study of the adverse effects of pesticides on child neuro development requires monitoring not only of initial, but ongoing pesticide exposure. Our aim was to compare house dust and children's hair as environmental and biological markers of ongoing pesticide exposure in children.

Design/Methods: In a continuing NIH study on the adverse effects of prenatal pesticide exposure on child neurodevelopment, ongoing pesticide exposure after birth was measured in swept house dust and hair in the children at 4 years of age for propoxur and pyrethroids (transfluthrin, bioallethrin, cyfluthrin and cypermethrin) by gas chromatography/mass spectrometry. The prevalence and concentration of pesticides in the two matrices were compared.

Results: Prevalence of propoxur was higher in hair compared to house dust ($p<0.001$) whereas prevalence of the pyrethroids was higher ($p<0.001$) in house dust. The overall concentrations of the pyrethroids were also higher ($p<0.007$) in house dust compared to hair. There was a significant ($p<0.001$) correlation between dust and hair for bioallethrin and cypermethrin.

Conclusions: Ongoing exposure of children to environmental pesticides is sensitively detected by analysis of children's hair and house dust. However, prevalence of propoxur was higher in hair compared to swept house dust, but the opposite was found for the pyrethroids. Thus, both matrices should be analyzed. There was a significant ($p<0.001$) correlation between house dust and hair for bioallethrin and cypermethrin.

Keywords: House dust; Hair; Pesticides; Environmental exposure; Biomarkers

Introduction

There is widespread use of pesticides and vast quantities are dispersed in the environment and are subsequently found in the air, water, soil and food sources [32,38]. Human exposure to pesticides is therefore inevitable and bioaccumulation of pesticide residues in human tissues has been reported [39]. Pesticide exposure in women while pregnant is a major concern, since most pesticides are neurotoxicants and the brain of the fetus and newborn infants are highly vulnerable to these toxicants due to the rapid growth and development of their brain [3,10,16] higher dose of pesticides per body weight [40] and lower activity and levels of enzymes that detoxify the pesticides [21]. Although the recognizable effects of maternal exposure to low doses of environmental pesticides are minimal, serious concerns have been raised about their adverse effects on the fetus, particularly on subsequent neurodevelopment, learning and behavioral difficulties in the children. A number of studies on prenatal exposure to organophosphate pesticides have been found to be associated with increased number of abnormal reflexes in newborn infants as assessed by the Brazelton Neonatal Behavioral Assessment Scale [15,42]. In young children, prenatal as well as ongoing, postnatal exposure to organophosphates have been associated with decreased scores on the Stanford-Binet copying test, mean reaction time, poorer short term memory, executive function and lower MDI and PDI scores on the Bayley Scales of Infant Development term memory [17-20,30,31].

Among children exposed to pesticide, ongoing exposure has to be monitored to minimize further exposure and to assess the effectiveness of preventive intervention, if such measures are undertaken. Furthermore, for any study dealing with the longitudinal effect of pesticide exposure on child development, ongoing pesticide exposure has to be monitored. We report on the analysis of children's hair and house dust as surrogates of environmental and biological markers of ongoing exposure of children to pesticides.

Materials and Methods

The children are part of a cohort that was initially enrolled at birth and were born to pregnant women who delivered at the Bulacan Provincial Hospital in Malolos, Bulacan, and an agro industrial province of the Philippines [28]. The antenatal exposure of the women to pesticides was predominantly to propoxur (a carbamate) and the pyrethroids (transfluthrin, bioallethrin, cyfluthrin and cypermethrin) as determined by meconium analysis [28]. The children were followed up at 4 years of age for neurobehavioral testing and ongoing exposure to pesticides was determined by the analysis of house dust and children's hair. This study was approved by the Human Investigation Committees

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at both Wayne State University and the University of the Philippines. An informed consent was obtained from the mothers or caregivers for their and their children's participation in the study.

Hair specimens from the children, about the size of a pencil eraser in diameter, were obtained from the occipital region of the head close to the scalp [8,41] and then wrapped in aluminum foil. All hair samples were packed in a secondary, self-sealing polyethylene bag labeled and kept frozen at -20°C until the time of analysis. House dust was collected by sweeping with a household broom. The dust samples were placed in a plastic bag, sealed, labeled, and stored at -20°C until the time of analysis.

Analysis of hair and dust samples

The hair and dust samples were analyzed for the predominant pesticides that were previously found at birth [28] which included propoxur, cyfluthrin, cypermethrin, bioallethrin and transfluthrin.

Hair analysis: Unwashed hair samples were analyzed for the pesticides by gas chromatography/ mass spectrometry according to previously published procedure [26,30]. Briefly, hair was powdered for 10 minutes in a ball mill (Retsch, Germany) and about 50-100 mgs of the powdered hair was weighed and placed into a test tube. Hair that was negative for pesticide was used for negative and positive control. For the latter, hair was spiked with 1560 ng/mL of pestmix 11 (Cerilliant, Austin, TX). Two milliliter of hexane was added to each test tube and placed on a Vibrax machine for 6 hours for pesticide extraction. The test tubes were centrifuged at 4000 rpm for 15 minutes. About 1.6 mL of supernatant was pipetted out and transferred into a high recovery vial and dried to completion under nitrogen. The dried sample was reconstituted in 100 µl hexane, and 4 µL of 615.4 µg/mL 1,4 dichlorobenzene (internal standard) was added and vortexed. Exactly 1 µL sample was drawn and injected into the GC/MS using previously published settings [30].

Dust analysis: All dust samples were sieved using a stainless steel sieve with particle collection size of 150 µm (Fisher Scientific) [20]. The samples were sieved in a Retsch AS 200 sieve shaker (Haan, Germany) with the amplitude set at 30 Hz for 4 minutes. Each sample was then weighed and placed into a test tube. The sample weights ranged between 50-100 mg. Since many of these samples included dirt or sand, sand was used as the matrix for the negative and positive controls (Michael Ruby, personal communication). Pesticide extraction and clean-up protocols were a variation of the procedure established by Colt et al. [12]. Solvent volumes used by Colt et al. [12] were 2 to 3 times more volume than that utilized in our protocol (personal communication). Three positive controls and one negative control were prepared using sand as the matrix. The three positive controls were spiked with 1,560 ng/mL of Pestmix 11. For this study, the pesticides were extracted using 6 mL of a 1:1 hexane: acetone solution. The samples were sonicated for 10 minutes and then centrifuged at 4,000 rpm for 15 minutes. Five mL of the supernate was transferred to a clean test tube, dried to completion under nitrogen and reconstituted in 1 mL of hexane. The SPE columns were conditioned as follows: 6 mL 20% acetone in ethyl acetate, 6 mL methylene chloride, 6 mL 15% diethyl ether in hexane, and 6 mL hexane. The samples were then added to the conditioned SPE columns. The pesticides were eluted sequentially with 1.5 mL hexane, 6 mL 15% diethyl ether in hexane, 3 mL methylene chloride, and 6 mL 20% acetone in ethyl acetate. The combined eluates were dried to approximately 1 mL under nitrogen, transferred to high recovery vials and then dried down to completion under nitrogen. The sample was finally reconstituted in 100 µl of hexane and 4 µl of the diluted internal standard (1,4-DCB, 16,000 ng/mL) was added for a final concentration of 615.4 ng/mL.

Calibration curves for propoxur, transfluthrin, bioallethrin, cyfluthrin, and cypermethrin were constructed by spiking sand with varying concentrations of the parent pesticide standard mix (Pestmix 11). These concentrations ranged from 3 ng/mL to 6,250 ng/mL. Empirical limits of detection were determined [1]. Pesticide analysis was done using an HP 6890 GC and a HP 5973 MS. An Agilent Technologies 7683 series auto sampler was used for sample injection. The GC column was a 30m J&W DB-5MS capillary column ([5%-phenyl]-methylpolysiloxane, 0.25 mm I.D., 1 µm film thickness) obtained from Agilent Technologies. An HP Chemstation version B.01.00 was used to generate analytical data. The GC/MS conditions are described in a previous publication [7]. One µL of the sample was injected into the GC front inlet (250°C) using the auto sampler in splitless mode. The initial oven temperature was set at 70°C, and increased at a rate of 10°C min^{-1} until the final temperature of 300°C was reached and maintained for 10 minutes. The Data Analysis computer program (HP Chemstation version B.01.00) was used for quantifying the acquired data. The concentration of pesticides present in each sample was determined using the calibration curves. The identity of a pesticide in the sample was established if the following criteria were satisfied: 1) the peak was +/- 0.03 min from the retention time as determined from positive control standards and 2) the target and qualifier ion(s) were within the established ratio (+/- 30%). Once positivity and identity of the pesticides were determined, corroboration by another investigator was also required.

Statistical analysis

The prevalence (%) and concentrations (ng/g) of pesticides in paired hair and swept house dust were calculated. Comparisons of prevalence and concentration were done by the McNemar and Wilcoxon Signed Ranks tests, respectively. Correlation between concentrations was assessed by the Kendall's Tau test.

Results

For the hair analysis of pesticides , the target and qualifier ions, retention time for each parent pesticide, validation method and empirical limits of detection have been previously reported [30,26]. For each batch of hair samples analyzed for pesticides in this report, a validation test was conducted and a representative example is shown in Table 1.

For the dust analysis of pesticides, the target and qualifier ion(s) along with the retention time (t_R) for each parent pesticide are listed in Table 2. Results of the validation test of 32 initial dust samples analyzed for pesticides are shown in Table 3. The mean (SD) recoveries ranged from 96.32% (±14.77) for cyfluthrin to 107.92% (±12.66) for propoxur. The mean (SD) for the CVs ranged from 6.70% (±4.44) for cyfluthrin to 7.28% (± 4.41) for cypermethrin. The empirical LODs were 3.75 $ng/g^{-1}$for propoxur, transfluthrin, and bioallethrin and 60.00 ng/g^{-1} for cyfluthrin and cypermethrin. All recoveries and coefficients of variation are based on spiked sand samples with a concentration of 1,950 ng/g^{-1}.

The prevalence and median concentration of pesticides in paired hair and dust samples are shown in Table 4. Due to the skew of the concentrations, overall median concentration values for all pesticides are 0. To provide a meaningful value for concentrations, the median concentration is presented using only cases with positive exposure.

	Mean (ng/mL)	SD	CV (%)	Recovery (%)	LOD (ng/mL)
Propoxur	1530.71	4.25	0.28	98.12	6
Transfluthrin	1529.10	21.27	1.39	98.02	6
Bioallethrin	1533.72	37.14	2.42	98.32	12
Cyfluthrin	1589.24	2.65	0.17	101.87	190
Cypermethrin	1572.34	11.38	0.72	100.79	390

Table 1: Representative validation test [coefficient of variability (%), recovery (%) and limit of detection (LOD)] in hair analysis.

Compound	Target Ion *m/z*	Target Ion *m/z*	t_r(min)	r^2
1,4-dichlorobenzene (IS)	152	150, 115	8.89	N/A
Propoxur	110	152	17.49	0.987
Transfluthrin	163	91, 335	20.71	0.988
Bioallethrin	123	79, 136	22.53	0.994
Cyfluthrin	206	226	31.84	0.999
Cypermethrin	181	209	32.99	1.000

Table 2: Target and qualifier ion(s), retention times (t_R), and coefficients of determination (r^2) for parent pesticides in dust.

Pesticide	Recovery (SD)	%CV(SD)	LOD (ng/g)
Propoxur	107.92(12.66)	7.08(4.84)	3.75
Transfluthrin	106.93 (14.05)	7.24 (5.13)	3.75
Bioallethrin	106.64 (13.24)	7.48 (4.48)	3.75
Cyfluthrin	96.32 (14.70)	6.70 (4.44)	60.0
Cypermethrin	97.54 (12.01)	7.28 (4.41)	60.0

Table 3: Mean (SD) of pesticide recovery (%) and coefficients of variation (%CV), for the analysis of parent pesticides in swept dust (N=32) spiked with 1,950 ng/g^{-1} pesticides and the lowest empirical limits of detection (LODs) based on calibration curves.

	Prevalence and Concentration				
Pesticide	Hair [1]	Swept Dust [1]	Comparing Prevalence [2]	Comparing Concentration [3]	Correlation of Hair and Swept House Dust Concentration [4]
Propoxur	20.1 (29.5)	11.6 (67.1)	<0.001	0.51	0.047 (p=0.23)
Transfluthrin	0.007 (79.3)	4.1 (39.6)	0.001	0.007	-0.017 (p=0.68)
Bioallethrin	13.1 (294.2)	22.9 (204.6)	<0.001	0.001	0.203 (p<0.001)
Cyfluthrin	0.009 (407.7)	4.1 (724.0)	0.001	0.002	0.076 (p=0.07)
Cypermethrin	1.3 (528.8)	6.3 (538.6)	<0.001	<0.001	0.171 (p<0.001)

[1]Values given are prevalence (%) and median concentration (ng/g) for positive samples
[2]Significance of McNemar test
[3]Significance of Wilcoxon Signed Ranks test
[4]Estimate and (significance) of Kendall's Tau

Table 4: Prevalence (%) and median concentrations (ng/g) for positive samples of pesticides in paired children's hair versus swept house dust (N=557).

For the comparison of the concentrations between the two markers, all cases with and without measurable exposure were used. The prevalence of propoxur was higher in hair compared to swept dust (20.1% versus 11.6%, p<0.001) whereas the prevalence of each of the pyrethroids was higher in swept dust compared to hair (p<0.001). The concentrations of the pyrethroids were also higher in house dust compared to hair for transfluthrin (p<0.007), bioallethrin (p=0.001), cyfluthrin (p=0.001), and cypermethrin. There was a significant correlation between dust and hair for bioallethrin (r=0.203, p<0.001) and cypermethrin (r=0.171, p<0.001).

Discussion

The monitoring of ongoing exposure to pesticides in very young children is essential in a study of the long term adverse effects of pesticides on child neurodevelopment. Likewise, in children at risk, ongoing exposure to pesticides should be monitored to reduce further pesticide exposure and to determine the effectiveness of preventive interventions if such measures are undertaken. In a cohort of 4 year old children who have been participants since birth in a longitudinal study of the adverse effects of prenatal pesticide exposure, this study has shown that ongoing exposure of children to pesticides occurs and that the analysis of an environmental (house dust) and biological marker (child's hair) are sensitive surrogates to detect such exposure.

Children's exposure to pesticides from house dust occurs in three ways, i.e., via inhalation, oral ingestion, and dermal uptake. For small children, the oral and dermal routes are the most common [5]. Some characteristics of children increase their exposure to pesticides in house dust [23]: (1) Their hand to mouth behavior increases their ingestion of any toxic chemicals in dust or soil, and (2) the likelihood of playing close to the ground increases their exposure to toxins in the dust, soil and carpets as well as to any toxicants that form low-lying layers in the air, such as certain pesticide vapors. Thus, exposure to house dust is a significant pathway for the children's exposure to pesticides [9,11]. Studies also indicate that more pesticides and higher pesticide concentrations are found in household dust as compared to air, soil, and food [24,35].

Non-persistent pesticides, such as carbamates and pyrethroids, biodegrade in the environment easily but their persistence in the indoor dust environment appears to be more stable and they degrade

more slowly than outdoors because they are protected from sunlight, moisture, temperature extremes, wind and rain dispersal, and microbial activity [9,22]. Carbamates and pyrethroids are semi- or less volatile pesticides that tend to settle in house dust [24]. We expect that these are the pesticides that will accumulate in the dust samples because spray pesticides and slow-burning mosquito coils which contain these pesticides are regularly used in the homes of the subjects [28].

Hair is a suitable matrix for pesticides analysis because hair can incorporate pesticides in the growing hair shaft either by ingestion or passive exposure. We have previously reported on the higher sensitivity of hair compared to blood analysis in detecting pesticide exposure in pregnant women [26]. Analysis of paired maternal hair and blood samples obtained at mid-gestation and at delivery for several pesticides showed significantly higher prevalence and concentration of the pesticides, particularly propoxur and bioallethrin, in maternal hair compared to blood.

Similarly, in a subset of 1 year old children (N=115) in our study cohort, we have found that postnatal exposure to pesticides was better detected in hair compared to blood (unpublished data). In paired hair and blood samples, propoxur and diazinon were detected at a significantly higher rate and concentration in infant hair compared to blood: (1) 9.5% positive for propoxur (median concentration = 0.241 ug/g) in infant hair compared to 0.8% in blood (median concentration = 0.0265), $p<0.002$, (2) Diazinon was also found only in infant hair (0.8%).

There are several advantages in using hair to test for ongoing exposure to pesticides in children: (1) Hair analysis has a wide window to detect exposure due to the ability of hair to incorporate pesticides in the growing hair shaft. The incorporation of methomyl, a carbamate pesticide, and diazinon, have been studied in the hair of laboratory animals and showed a dose dependent response [37-39]. In humans, hair analysis of dialkyl phosphates has been used to confirm exposure to organophosphates [36]. Similarly, hair analysis has been used to detect occupational exposures to DDT in adults [14] and in children, to detect exposure to DDT and lindane from indoor pollution [25]. (2) There is no active metabolism and excretion of pesticides in hair. Thus, pesticides remain unchanged as the parent compounds. On the other hand, pesticides in blood and urine are subject to metabolism and excretion in the body; thus they are more difficult to detect in these matrices and if detected, their presence is frequently indicative of short term or recent exposure [4]. (3) Hair sampling is less invasive than blood sampling and is a more desirable method particularly if children are the subjects of sampling. Hair collection is also less labor intensive compared to urine collection which requires multiple spot samplings, early morning voids or 24 hour collection. Unwashed hair was analyzed in this study because we were interested in overall exposure, both active and/or passive exposure of the children to pesticides. Besides, our previous study demonstrated that preliminary hair washing with 1:10 solution of commercial shampoo and deionized water for 5 minutes using an orbital shaker did not show a significant difference in the concentrations of pesticides (propoxur and bioallethrin) in hair [30].

There was a difference in the rate and concentration of pesticides found in dust and hair. Propoxur was found more frequently in children's hair compared to house dust, but the opposite was true for the pyrethroids.The propensity of propoxur deposition in hair has also been seen in our previous study wherein maternal hair was found to show a higher prevalence and concentration of pesticides compared to maternal blood [28]. We found a significant correlation between the hair and dust for bioallethrin and cypermethrin. It is not surprising to see this relationship particularly for bioallethrin, because bioallethrin, the principal pesticide in mosquito coil, burns slowly and remains in air for a more prolonged period, thereby allowing the pesticide to permeate both hair and house dust more effectively.

Conclusion

We conclude that ongoing exposure of children to environmental pesticides can be sensitively detected by the analysis of children's hair and house dust. The prevalence of propoxur was higher in hair compared to house dust, but the opposite was true for the pyrethroids. Thus both matrices should be analyzed. A significant ($p<0.001$) correlation was also observed between house dust and hair for bioallethrin and cypermethrin.

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