Trends in long term exposure to propoxur and pyrethroids in young children in the Philippines

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A B S T R A C T

Background/aim: Pesticides are neurotoxic and can adversely affect children’s neurobehavioral outcome. Ongoing pesticide exposure has to be monitored in the study of long term outcome of pesticide adverse effects since changes in the type and amount of exposure can influence outcome. The aim of this paper is to describe the trend in long term pesticide exposure in children through the analysis of pesticides in their hair.

Patients and methods: As part of an NIH study on the long term effects of pesticide exposure in young children, ongoing exposure to pesticides was determined by the analysis of children’s hair for propoxur and pyrethroids by gas chromatography/mass spectrometry at 2, 4 and 6 years of age.

Results: There were significant changes in the prevalence and concentration of propoxur and pyrethroids in children’s hair at 2, 4 and 6 years of age. At ages 2 and 4 years, the prevalence of propoxur exposure increased from 12.4% to 24.1% (p < 0.001) but dramatically decreased to 1.7% at 6 years (p < 0.001). For bioallethrin, the prevalence of exposure steadily increased from 2 years (0.7%, p < 0.001) to 4 years (12.4%, p < 0.001) and to 6 years (18.4% p < 0.001). Exposure to transfluthrin significantly increased from 4 years (10.0%) to 6 years (9.2%, p < 0.001).

There were also significantly higher median concentrations of bioallethrin at 2 compared to 4 years and for propoxur at 2 compared to 6 years. Between 4 and 6 years, there was a higher median concentration of propoxur at 4 compared to 6 years and for transfluthrin and bioallethrin, at 6 compared to 4 years.

Conclusion: Changes in the prevalence and concentration of exposure to propoxur and pyrethroids in children at 2, 4 and 6 years of age are related to the progress in ambulation of young children and to changes in the formulation of home spray pesticides. Thus, periodic monitoring of pesticide exposure is necessary when studying the long term effects of pesticide exposure in the neurodevelopment of young children.

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1. 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 (US Environmental Protection Agency, 1999, 2010; Sekiyama et al., 2007). Human exposure to pesticides is therefore inevitable and bioaccumulation of pesticide residues in human tissues has been reported (Waliszewski et al., 2002). Pesticides are neurotoxic and have adverse effects on the children’s neurobehavioral development. The child’s brain in utero, at infancy and at early childhood are in a state of rapid growth and development and are therefore highly vulnerable to the toxic effects of pesticides (Eriksson, 1997; Barone et al., 2000; Bruckner 2000). House dust is a major source of pesticide exposure in young children (Clayton et al., 2003; Bradman and Whyatt, 2005). Studies have shown that more pesticides, and higher pesticide concentrations are found in household dust as compared to air, soil, and food (Lewis et al., 1999; Simcox et al., 1995). Pesticides are commonly present in house dust as a consequence of indoor spraying of pesticides (Ostrea et al., 2009; Trunnelle et al., 2013; Quirós-Alcalá et al., 2011) and proximity to agricultural areas where pesticides are used and brought indoor by drift, worker’s work clothes and shoes and vehicles (Quirós-Alcalá et al., 2011; Trunnelle et al., 2013; Fenske et al., 2013; Coronado et al., 2011; Lu et al., 2000).

We are currently conducting a long term study of the pre- and postnatal effects of exposure to environmental pesticides on the neurobehavioral development of young children. At birth, we
found a high prenatal exposure rate in the newborn infants to propoxur, a carbamate and to the pyrethroids (Ostrea et al., 2009). The aim of this study is to determine the trend in long term pesticide exposure in children at 2, 4 and 6 years of age by the analysis of their hair for pesticides.

2. Materials and methods

The children are part of a cohort that was initially enrolled at birth and were born to mothers who delivered at the Bulacan Provincial Hospital in Malolos, Bulacan, an agroindustrial province of the Philippines (Ostrea et al., 2009). Their antenatal exposure to pesticides was predominantly to propoxur (a carbamate) and to the pyrethroids (transfluthrin, bioallethrin, cypermethrin and cyfluthrin) as determined principally by meconium analysis (Ostrea et al., 2009). The children were followed up at 2, 4 and 6 years of age, from 2002 to 2012 and the trend in pesticide exposure was determined by the analysis of the children’s hair for these pesticides. This study was approved by the Human Investigation Committees at both Wayne State University and the University of the Philippines. An informed consent was obtained from the mothers or caregivers for their participation and their children 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 (Wennig, 2000; Bouniba et al., 2006) and then wrapped in aluminum foil. All hair samples were packed in secondary, self-sealing polyethylene bags, labeled and kept frozen at ~20 °C until the time of analysis.

3. Pesticide analysis of hair samples

The hair samples were analyzed for pesticides that were previously found at birth which included propoxur, cyfluthrin, cypermethrin, bioallethrin and transfluthrin (Ostrea et al., 2009). Unwashed hair samples were analyzed for the pesticides by gas chromatography/mass spectrometry according to previously published procedure (Bielawski et al., 2005; Posecion et al., 2006; Ostrea et al., 2006). Using spiked samples, calibration curves were linear for all pesticides with coefficients of linearity > 0.950. Optimum recovery rates ranged from 87% to 112% at a spiked concentration of 31.25 μg/g. The inter-assay and intra-assay coefficients of variation for the parent pesticides were below 11%. Limits of detection (LOD) by empirical approach (Armbruster et al., 1994) ranged from 3.60 ng/g to 488.00 ng/g for the various pesticides.

4. Statistical analysis

The prevalence (%) and in positive samples, the median concentrations (ng/g) of pesticides in hair at 2, 4 and 6 years of age were determined and compared by the McNemar and Wilcoxon tests, respectively. Correlation between concentrations was assessed by the Kendall’s Tau test.

5. Results

There were significant changes in the prevalence and concentration of propoxur and pyrethroids in children’s hair at 2, 4 and 6 years of age (Table 1). At ages 2 and 4 years, the prevalence of propoxur exposure increased from 12.4% to 24.1% (p < 0.001) but dramatically decreased to 1.7% at 6 years (p < 0.001). For bioallethrin, the prevalence of exposure steadily increased from 2 years (0.6%, p < 0.001) to 4 years (12.4%, p < 0.001) and to 6 years (18.4% (p < 0.001). Exposure to transfluthrin significantly increased from 4 years (1.0%) to 6 years (9.2%, p < 0.001). Significant increases in exposure to cyfluthrin and cypermethrin was only seen between 2 to 4 years of age and the rate of increase was small (0% to 1.1–1.7%).

There were also significantly higher median concentrations of bioallethrin at 2 compared to 4 years and for propoxur at 2 years compared to 6 years (Table 1). Between 4 and 6 years, there was a higher median concentration of propoxur at 4 compared to 6 years and for transfluthrin and bioallethrin, at 6 compared to 4 years.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Prevalence and concentration</th>
<th>Comparing prevalence</th>
<th>Comparing concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two years</td>
<td>Four years</td>
<td>Six years</td>
</tr>
<tr>
<td>Propoxur</td>
<td>12.4 (125.8)</td>
<td>24.1 (29.5)</td>
<td>1.7 (28.6)</td>
</tr>
<tr>
<td>Transfluthrin</td>
<td>0.7 (252.3)</td>
<td>1.0 (107.9)</td>
<td>9.2 (102.4)</td>
</tr>
<tr>
<td>Bioallethrin</td>
<td>0.6 (2073.7)</td>
<td>12.4 (271.4)</td>
<td>18.4 (347.2)</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>0.0</td>
<td>1.1 (361.3)</td>
<td>1.0 (376.7)</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>0.0</td>
<td>1.3 (537.4)</td>
<td>1.7 (614.7)</td>
</tr>
</tbody>
</table>

a Values given are prevalence and for positive samples, their median concentrations.
b Exact test.

Table 2

Agreement of pesticide prevalence and concentration (ng/g) in children’s hair at 2, 4, and 6 years.

<table>
<thead>
<tr>
<th>Pesticide</th>
<th>Agreement of prevalence</th>
<th>Agreement of concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Two and four</td>
<td>Two and six</td>
</tr>
<tr>
<td>Propoxur</td>
<td>0.16 (&lt; 0.001)</td>
<td>0.01 (0.857)</td>
</tr>
<tr>
<td>Transfluthrin</td>
<td>−0.01 (0.815)</td>
<td>0.04 (0.380)</td>
</tr>
<tr>
<td>Bioallethrin</td>
<td>0.03 (0.027)</td>
<td>0.18 (0.002)</td>
</tr>
<tr>
<td>Cyfluthrin</td>
<td>−0.01 (0.837)</td>
<td>0.35 (&lt; 0.001)</td>
</tr>
<tr>
<td>Cypermethrin</td>
<td>−0.01 (0.837)</td>
<td>0.35 (&lt; 0.001)</td>
</tr>
</tbody>
</table>

When no measure is presented this is because at least one of the pesticides has no exposure for the pairs.

a Values given are Kappa for prevalence and Kendall’s Tau for concentration (p value).
Correlations between pesticide prevalence and concentration at 2, 4 and 6 years of age are shown in Table 2. There was significant correlation in the prevalence of propoxur \( (r=0.16, p < 0.001) \) and bioallethrin \( (r=0.03, p < 0.027) \) at 2 and 4 years and with bioallethrin \( (r=0.18, p < 0.002) \) and cypermethrin \( (r=0.35, p < 0.001) \) at 4 and 6 years. There was also significant correlation in the concentrations of propoxur \( (r=0.15, p < 0.001) \) and bioallethrin \( (r=0.08, p < 0.032) \) at 2 and 4 years and with the concentrations of bioallethrin \( (r=0.17, p < 0.001) \) and cypermethrin \( (r=0.34, p < 0.001) \) at 4 and 6 years.

6. Discussion

Monitoring the ongoing exposure to pesticides in very young children is essential when determining the long term adverse effects of pesticides on the neurodevelopment of children. This study has shown that the trend of exposure to the same types of pesticides changes and therefore must be taken into account when testing outcome effects of these toxicants. Our results show significant increases in the prevalence of exposure to children to propoxur and bioallethrin at 4 years compared to 2 years (Table 1), although the median concentrations of propoxur and bioallethrin were significantly higher at two years of age. The difference in exposure rate and concentration of pesticide is probably related to increase in ambulation of the children. At 2 years of age, the children are not as ambulant as compared to the 4 year old children and are likely to still be seated on the floor most of the time and in contact with large amounts of pesticides on floor dust. We have shown that house dust contains a significant amount of pesticides in the homes of these children (Ostrea et al., 2012; Birn et al., 2012) which explains the high concentration of pesticides in the children's hair. At 4 years, ambulatory activity is further increased and the children come in contact with more pesticides in the environment, but probably not at the same concentration as pesticides in floor dust, resulting in higher prevalence but lower concentrations of pesticides found.

House dust is a major source of pesticide exposure in young children (Clayton et al., 2003; Bradman and Whyatt, 2005). We have found significant correlation between the concentrations of bioallethrin and cypermethrin in house dust and children's hair (Ostrea et al., 2012). Studies have shown that more pesticides, and higher pesticide concentrations are found in household dust as compared to air, soil, and food (Lewis et al., 1999; Simcox et al., 1995). The exposure to pesticides in house dust can occur in three ways, i.e., via inhalation, oral ingestion, and dermal uptake. For small children, the oral and dermal routes are the most common (Berger-Preib et al., 2002). Some characteristics of children increase their exposure to pesticides in house dust (Landrigan et al., 2001): (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.

Non-persistent pesticides, such as carbamates (propoxur) 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 (Bradman and Whyatt, 2005; Hong et al., 2001). Carbamates and pyrethroids are semi- or less volatile pesticides that tend to settle in house dust (Lewis et al., 1999). We expect these pesticides to accumulate in house dust because spray pesticides and slow-burning mosquito coils which contain these pesticides are regularly used in the homes of the subjects (Ostrea et al., 2009). The dramatic fall in the prevalence of propoxur exposure in children at 6 compared to 4 years (from 24.1% at 4 years and 1.7% at 6 years) and the significant increase in exposure to transfluthrin (1% at 4 years to 9.2% at 6 years) also coincided with the change in formulation of a widely used home pesticide (Baygon) in the Philippines. This change occurred by virtue of a recommendation from the United States Environmental Protection Agency to ban the domestic use of propoxur in the United States in 2007 (NRDC, 2011). The manufacturers of Baygon in the Philippines eliminated propoxur in their Baygon home spray pesticides and substituted transfluthrin for propoxur. On the other hand, there was still continuous use of the slow burning mosquito coil which contained bioallethrin accounting for still persistently high exposure rate of children to this pesticide.

Hair constitutes an important matrix to determine pesticide exposure. Hair is suitable because of the ability of the hair to incorporate pesticides in the growing hair shaft either when ingested or secondary to passive exposure. In pregnant women, we have the maternal hair to be more sensitive than blood for detecting pesticide exposure during pregnancy (Ostrea et al., 2006). In paired analysis of maternal hair and blood samples obtained at mid-gestation and at delivery, there was a 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, we have found that postnatal exposure to pesticides was better detected in the children's hair compared to blood (unpublished data). In paired hair and blood samples, 9.5% of the infant's hair was positive for propoxur (median conc.=0.241 mcg/g) compared to 0.8% in blood (median conc.=0.0265 mcg/mL, \( p < 0.002 \)). The pesticides, diazinon was only found 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 (Tsatsakis et al., 1998; Tutudaki et al., 2003; Tutudaki and Tsatsakis, 2005). In humans, hair analysis of dialkyl phosphates has been used to confirm exposure to organophosphates (Tsatsakis et al., 2010). Similarly, hair analysis has been used to detect occupational exposures to DDT in adults (Covaci et al., 2002) and in children and to detect exposure to DDT and lindane from indoor pollution (Neuber et al., 1999). (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 (Barr and Needham, 2002). (3) Hair sampling is less invasive than blood sampling and is therefore a more desirable sampling method particularly in children. Hair collection is also less labor intensive compared to urine since the latter may require multiple spot samplings, early morning voids or 24 h collection (Bradman et al., 2013). In this study, we purposely did not wash the hair prior to analysis because we were interested in overall exposure of the children to pesticides, whether active or passive. However, we have conducted a study on the effect of hair washing on the concentrations of propoxur and bioallethrin in hair. There was no difference in the concentration of propoxur in the paired hair samples before and after washing \( (p=0.175, \text{Wilcoxon signed ranks test}) \), but for bioallethrin, the concentration of the pesticide was significantly higher in the pre-washed compared to the post-washed hair samples \( (p=0.001, \text{Wilcoxon signed ranks test}) \). However, the post wash concentration was undetectable in only one sample whereas all other samples were still positive after washing.
7. Conclusion

Changes in the prevalence and concentration of exposure to propoxur and pyrethroids in children at 2, 4 and 6 years of age are related to the progress in ambulation of young children and to changes in the formulation of home spray pesticides. Thus, periodic monitoring of pesticide exposure is necessary when studying the long term effects of pesticide exposure in the neurodevelopment of young children.

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