The Effects of Organophosphate Pesticide Exposure on Hispanic Children's Cognitive and Behavioral Functioning

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Objective This study investigates the effects of Organophosphate (OP) pesticides exposure on the cognitive and behavioral functioning of Hispanic children living in an agricultural community. **Methods** Forty-eight children were administered a battery of cognitive measures, and their parents and teachers completed behavior rating scales. Children provided a urine sample for analysis of OP pesticides metabolites. **Results** All children had a detectable level of at least one OP pesticide metabolite. Higher OP pesticide metabolite concentration levels were significantly correlated with poorer performance on some subtests of the Wisconsin Card Sorting Test. However, the significance of this association was dependent upon the inclusion of two samples with noticeable higher OP pesticide metabolite concentration levels. **Conclusions** Short-term OP pesticide exposure seems to have deleterious effects on children's speed of attention, sequencing, mental flexibility, visual search, concept formation, and conceptual flexibility. This study is among a relatively small number of studies investigating an extremely complex problem. Limitations and suggestions for future studies are discussed.

Key words children's cognitive and behavioral functioning; organophosphates; pesticides exposure.

Organophosphate (OP) pesticides are one group of insecticides commonly used for agricultural purposes. They are also used inside the homes and in yards in smaller quantities to control pests and are currently the most commonly used household insecticides (Kamrin, 1997). These pesticides are also regularly used in others settings such as hospitals and schools with the purpose of controlling pests [United States General Accounting Office (GAO), 1999]. The US Environmental Protection Agency (EPA, 1998) has reported that as much as 75% of all household pesticide use occurs inside the home, and 22% occurs in yards and gardens surrounding the home. OP pesticides are known to be highly toxic, but they have a short biologic half-life when compared to pesticides such as DDT (Wigle, 2003).

Professionals working with children such as pediatricians and child psychologists would benefit from understanding the negative effects of OP pesticide exposure on children's health. Some of the documented health effects in adults are cancer, respiratory illnesses, and liver and renal injuries (EPA, 1998). However, pesticides can be more harmful to children than to adults because children breathe more air and consume more food and beverage per pound of body weight than do adults (Hubal et al., 2000). In addition, since the nervous system undergoes rapid growth and development in the first years of life, children are more likely to have neurological problems based on OP pesticides exposure (Landrigan et al., 1999). If chemicals destroy cells in the developing brain, there is a risk that a resulting dysfunction might appear, which would be irreversible (e.g., lead exposure and decreased intelligence) (National Research Council, 1993). Furthermore, results of recent experimental studies investigating the effects of OP pesticide exposure have indicated that prenatal and postnatal exposures have significant neurodevelopmental consequences (Colborn, 2006; Dietrich et al., 2005; Qiao, Seidler, Tate, Cousins, & Slotkin, 2003; Slotkin,

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Levin, & Seidler, 2006); thus, understanding the potentially negative effects of OP pesticide exposure on children's neurodevelopment is relevant for those working with or treating children.

Despite the fact that OP pesticides are widely used, only limited knowledge exists about their effects on human health. Most research has focused on the carcinogenic effects of these chemicals on humans (EPA, 1998), particularly on adults exposed to pesticides in occupational settings. To date, however, little is known about the effects of OP pesticide exposure on children living in agricultural areas where these types of pesticides are frequently used. According to the US Census Bureau (2000), ~25% of the US population is under 18 years of age and the number of children living in rural areas grew by 3% between 1990 and 2000. Since 1990, demographically, the number of White children living in rural areas has declined ~4% while the number of Hispanic children has grown 8% (Kandel & Cromartie, 2004). These trends suggest that Hispanic children may be, presently and in the future, disproportionately exposed to pesticides used in agricultural regions. This study is an attempt to investigate the effects of OP pesticides on the cognitive and behavioral functioning of Hispanic children living in agricultural communities where pesticides are widely used. We begin by reviewing the limited literature on OP pesticide exposure and its effects on the cognitive and behavioral functioning of exposed humans, especially adult populations. We then indicate the necessity for further investigation with respect to children's exposure.

Neurobehavioral Effects of OP Pesticide Exposure

The belief that OP pesticides exposure might affect children's neuropsychological functioning stems from the fact that OP pesticides are designed to poison insects' nervous system by inhibiting the acetylcholinesterase enzyme (AchE) at the nerve endings. The enzyme is critical for the normal control of nerve impulse transmission from nerve fibers to muscle and gland cells and also to other nerve cells in the autonomic ganglia and in the brain. A fair amount of the tissue enzyme mass must be inactivated before symptoms and signs of poisoning are manifested. When there is a sufficient dosage, the loss of AchE enzyme function allows accumulation of the acetylcholine neurotransmitter at neuroefector junctions (muscarinic effects), at skeletal nerve-muscle junctions and autonomic ganglia (nicotinic effects), and in the brain (Morgan, 1989; O'Malley, 1997; Steenland et al., 1994). When a person is *poisoned* by OP pesticides, the major symptoms or signs appear within 12 hr of exposure. They include dizziness, anxiety, restlessness, muscle twitching, weakness, tremor, incoordination, hypersecretion, miosis, and pulmonary edema. Toxic psychosis can also occur (Morgan, 1989). Repeated exposure to OP pesticides can also cause anorexia, weakness, and malaise. Depression of respiration and pulmonary edema are the usual causes of death from OP pesticides poisoning (O'Malley, 1997).

With regard to studies exploring the neurobehavioral effects of OP pesticide exposure, most of the research has focused on acutely exposed people whose work involves the handling of OP pesticides, such as pest control applicators and farm workers. In these studies, participants who had been exposed to OP pesticides were assessed with different batteries of cognitive and behavior measures. Results from these studies have yielded mostly consistent findings with regard to the effects that OP pesticides have. That is, participants that had been exposed to OP pesticides performed worse on measures of visual-motor processing speed and pattern memory accuracy (Maizlish, Schenker, Weisskopf, Seiber, & Samuels, 1987); coordination, dexterity, memory, abstraction, and mood (Rosenstock, Keifer, Daniell, McConnell, & Claypoole, 1991); sustained visual attention, tension, and confusion (Steenland et al., 1994); and simple reaction time, symbol-digit substitution, and syntactic reasoning (Stephens et al., 1995). When study participants had been exposed to significantly higher levels of OP pesticides to be categorized as ''poisoned,'' their performance was poorer on tests of varying abilities such as intellectual functioning (WAIS Full Scale IQ), academic skills, abstraction and flexibility of thinking, and visual-motor coordination speed (Savage et al., 1988). However, in one study, exposure to OP pesticide not only did not have a negative association with performance, but it actually enhanced the exposed participants' performance on a measure of processing speed (Ames, Steenland, Jenkins, Chrislip, & Russo, 1995). More recently, epidemiological and experimental studies have also found that the cognitive areas affected by toxic exposure to OP pesticides are ''selective attention latency, symbol-digit latency, preferred-hand-finger tapping, alternating-hand finger tapping, and continuous performance hit latency'' (Rothlein et al., 2006, p. 694); motor speed and coordination, sustained attention, information processing speed, visual motor speed, verbal abstraction, attention, and memory (Dietrich et al., 2005; Qiao et al., 2003; Slotkin et al., 2006).

Although the effects of OP pesticides poisonings are well known and established in the literature, the chronic effects of low-level exposure have not been well researched and, once again, the available data are mainly from studies carried out with adult populations (Ames et al., 1995; Rosenstock, Daniell, Barnhart, Schwartz, & Demers, 1990). Pilkington et al. (2001) have reported that research with adults exposed to low levels of OP pesticides has also shown effects on neurological and neuropsychological functioning, but these effects are not as consistent as those found after acute poisonings. In their study, they found that there was a weak positive association between cumulative exposure to OP pesticides and neurological symptoms. The significance of this association, however, was dependent on the inclusion of a few individual workers with extremely high exposure. Thus, the neuropsychological effects of poisoning by OP pesticides have been documented in the literature of occupational exposure and experimental studies. However, what remains open to investigation is the effect of long-term, low-level exposures to OP pesticides, specifically of children who live in agricultural areas where pesticides are continuously applied and thus their likelihood of exposure increased.

Effects of OP Pesticides Exposure on Children's Neurological Development

Concerns about the effects of OP pesticides exposure on children's neurological development have existed for at least the last two decades (e.g., Eskenazi, Bradman, & Castorina, 1999; O'Brien, 1990; Stein, Schettler, Wallinga, & Valenti, 2002). In 1989, Whyatt noted that there were few studies conducted to determine the effects on neurological development of low-level exposure to OP pesticides during infancy. She indicated, ''In fact, federal regulations currently do not require that *any* pesticide be evaluated for the effects of low-level of exposure on behavior, including such processes as learning ability, activity level and memory, or on emotion, sight, and hearing'' (Whyatt, 1989, p. 9). Consistent with Whyatt's comments, there are few studies concerning chronic toxicity in children (National Research Council, 1993) and no studies published yet on the neurotoxic effects of low levels of children's exposure to OP pesticides (Aprea, Strambi, Novelli, Lunghini, & Bozzi, 2000). To date, there is only one study that has assessed the developmental differences in children exposed to OP pesticides (Guillette, Meza, Aquilar, Soto, & Garcia, 1998). In their study, Guillette et al. (1998) found that children that had been exposed to pesticides had significantly lower physical endurance, decreased ability to catch a ball, decreased fine eye-hand motor coordination, and decreased long-term memory than non-exposed children. The most striking difference was observed in the drawing of a person in that exposed children drew an average of 1.6 body parts versus 4.4 body parts drawn by non-exposed children.

Weiss (1997) and Weiss & Landrigan (2000) suggest that some neurodevelopmental disabilities might be related to exposure to various chemicals (e.g., lead, PCBs, organic mercury compounds, and certain pesticides), given that fewer than 25% of neurodevelopmental disabilities that affect children have a known cause. More recently Koger, Schettler, & Weiss (2005) have pointed out the need for interdisciplinary research in the area of environmental toxicants and developmental disabilities. They stated that a large number of environmental chemicals interfere with brain development during critical periods, thus impacting cognitive, motor, and sensory function. OP pesticides can be considered as one kind of such environmental toxicants.

Following the rationale that OP pesticides are one type of environmental pollutant that have the potential to adversely impact children's health and neurodevelopment, the purpose of the present study was to assess the cognitive and behavioral functioning of Hispanic children living in an agricultural area that has been continuously treated with OP pesticides. We hypothesized that the performance of children that had been exposed to OP pesticides would be poorer in different cognitive and behavioral measures than the performance of children who had not been exposed to OP pesticides. In addition, we expected that higher levels of OP pesticide would be associated with poorer performance on the cognitive measures.

Method

Participants

Forty-eight children from the Children Pesticide Survey (CPS) of southern Arizona (O'Rourke et al., 2000) were selected to participate. The CPS was a study that built upon two previous epidemiological studies conducted in Yuma County, Arizona: the National Human Exposure Assessment Survey (NHEXAS) and the Arizona Border Survey (ABS). The CPS was conducted from 1998 to 2000 and involved five stages: enrollment, screening, intensive sampling, subject education, and community education. For detailed information about the stages the reader is referred to O'Rourke et al. (2000).

Children were eligible to participate in this study if they had provided a urine sample for the screening stage in the CPS. The present study was designed entirely as the doctoral research project of the first author (Sánchez Lizardi, 2003); therefore, approval from the Human Subjects Protection Program was obtained prior to the enrollment of participants and consent for participation (either English or Spanish) was obtained separately from the CPS. Data for this study was collected in the Spring of 2002.

Children were originally selected based on the absence/presence of OP pesticide metabolites in urine samples collected during their participation in the CPS. Our original design had two groups of children with nearly identical demographic characteristics (Table I) that could be differentiated only by the presence/absence of OP pesticide metabolites in a urine sample and we identified them as Exposed and Nonexposed, respectively (Fig. 1). Twenty-five children that had a detectable level of OP pesticide metabolite in a urine sample during the CPS formed the Exposed group, and 23 children who had not had a detectable level of OP pesticide metabolite in a urine sample formed the Nonexposed group. However, as the reader will see in the results section, all children had detectable levels of OP pesticide metabolite in the urine sample collected *specifically for this study*, which meant that we had only an Exposed group ($n = 48$), according to the criteria just mentioned. Thus, we had to adjust the design to have only a single New-exposed group and explore the associations between OP pesticide exposure level and cognitive functioning.

Table I. Demographic Characteristics of the Originally Exposed and Nonexposed Groups

Characteristics	Exposed ($n = 25$)	Nonexposed ($n = 23$)
Mean age in years	7	7
Gender (Female)	14 (56%)	12 (52%)
Grade		
K-1	8 (32%)	4 (17%)
2	9 (36%)	9 (39%)
3	8 (32%)	10 (44%)
Mean GPA (Range: 1–4)	3.0	3.0
Preferred Language		
Spanish	18 (72%)	16 (70%)
English	2 (8%)	1 (4%)
Both Spanish and English	5 (20%)	6 (26%)
Two-parent family	19 (76%)	21 (91%)
Mean number of family members at home	5	5
Annual income category (Mode)	$10,000–$19,999	$20,000–$29,999
Mother's years of education (Mean)	10	12
Father's years of education (Mean)	9*	12*
Number of household members in contact with pesticides (Mean)	1	1

*$p < .05$.

Measures

The selection of tests for this study was based on their validity as it relates to what is known in the area of neuropsychological effects of OP pesticide exposure in adult populations. In addition, these tests are valid and reliable measures for evaluating the cognitive and behavior processes of interest (e.g., motor speed and coordination, sustained attention, information processing speed, visual motor speed, verbal abstraction, attention, and memory) and their standardization include Hispanic children. Since children participating in the present study had a strong Spanish-language environment, Spanish translations were used when necessary. To control for translation variability, the same instructions and directions were used by the first author (who is a native Spanish speaker), each time Spanish was the child's preferred language. The cognitive measures were: A short form of the Wechsler Intelligence Scale for

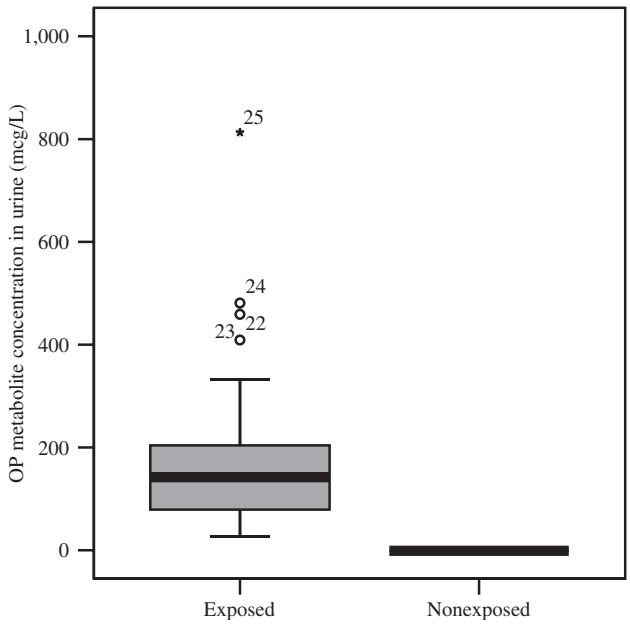

Figure 1. OP metabolite concentration levels found in the urine sample of children that were originally selected to form the Exposed and Nonexposed groups based on results of the CPS (1998–2000). Exposed children had $M = 195$ mcg/l ($SD = 182$) of OP metabolites. Nonexposed children had no detectable levels of OP metabolites. One child in the Exposed group had a noticeable higher OP metabolite concentration level.

Children—Third Edition (WISC–III; Wechsler, 1991); Children's Memory Scale (CMS; Cohen, 1997); Wisconsin Card Sorting Test (WCST; Heaton, Chelune, Talley, Kay, & Curtis, 1993); and Trail Making Test A & B (TMTA & TMTB; Lezak, 1995; Spreen & Strauss, 1998). The behavioral measures were: The Child Behavior Checklist/4-18 (CBCL/4-18) and The Teacher Report Form (TRF) (Achenbach & Rescorla, 2001).

Parent Interview

A structured interview about the child and the demographic characteristics of the family was conducted with all parents of participating children.

Urine Sample

A first-void urine sample was collected from each child the day of the cognitive assessment to analyze for OP metabolites (dialkylphosphates): dimethylphosphate (DMP), dimethylphosphorothioate (DMTP), dimethylphosphorodithioate (DMDTP), diethylphosphate (DEP), diethylphosphorothioate (DETP), and diethylphosphorodithioate (DEDTP). The urinalysis was conducted by Pacific Toxicology Laboratories (PTL) using gas chromatography with flame photometric detection (GC-FPD) with a detection limit of 25 μg/l. Calibration and control standards have been described in detail elsewhere (O'Rourke et al., 2000). In this study, absence of OP metabolite means a concentration level below the detection limit (25 μg/l) and presence means the actual concentration level detected in μg/l. The detection of dialkylphosphates in urine has been used as an indication of exposure (PTL, 1998). This, however, can only be related to events or exposures that take place within 24–48 hr prior to the collection of the urine sample (Krieger, 1999; Walker & Nidiry, 2002). The sum of the six OP metabolite concentrations was used for all data analyses. For comparison with national values, only DMP concentration levels were used.

Procedure

All children's parents were sent a letter of invitation to participate in this study and a follow-up phone call was conducted to ensure receipt of the letter and to enroll participants if they expressed interest. If parents agreed to participate, an appointment for a home visit was scheduled.

Home Visit

During the home visit, written consent was obtained (English or Spanish), the interview was conducted, and the CBCL was completed. In addition, the specimen cup for a first-void urine sample and directions for its collection were delivered. The child was informed of the first author's visit to his or her school the following day and the child's assent was obtained according to the guidelines for protecting study volunteers in research (Dunn & Chadwick, 1999). First-void urine samples were collected the day of the cognitive assessment as agreed to with the parent.

School Visit

Children were visited at their school for cognitive assessment with parental and school permission. If children had not provided assent during the home visit, assent was obtained at this time. When a school visit was not possible due to scheduling conflicts, specific parental request, or school refusal to participate, a second home visit was conducted to complete the cognitive assessment. Written consent was obtained from teachers of participating children and they were given the TRF to complete.

Results

Urinary OP Pesticide Metabolites

Because children selected for this study had participated in the CPS, it was known that they had been exposed (Exposed group) or not exposed (Nonexposed group) to OP pesticides according to the urinalysis results. In addition, children continued living at the same address, which we assumed controlled for sources of exposure. Given these facts, the authors expected children to remain in their original (Exposed/Nonexposed) group when the results of the urinalysis for this study were obtained. However, contrary to our expectations, urinalysis' results indicated that all 48 children had a detectable level of the OP pesticide metabolite DMP ($M = 65.5$ mcg/l, $SD = 78$; 95% Confidence Interval [CI] = 43–88) in their urine sample the day of cognitive assessment. Comparatively, the mean concentration level for the CPS was $M = 110.8$ mcg/l, $SD = 163$; 95% CI = 56–164; and the mean national value reported by the National Health and Nutrition Examination Survey [NHANES, Department of Health and Human Services (DHHS), 2003] was $M = 21.7$ mcg/l; 95% CI = 10–41. The NHANES value for Mexican-American population, ages 6–59, was $M = 15$ mcg/l; 95% CI = 10–23.

Because in our initial design we had divided children into Exposed and Nonexposed groups based on the presence/absence of OP pesticide metabolite in a urine sample during the CPS, we compared the sum of OP pesticide metabolite concentration levels of the day of cognitive assessment between these originally Exposed and Nonexposed groups. We found that (a) two samples

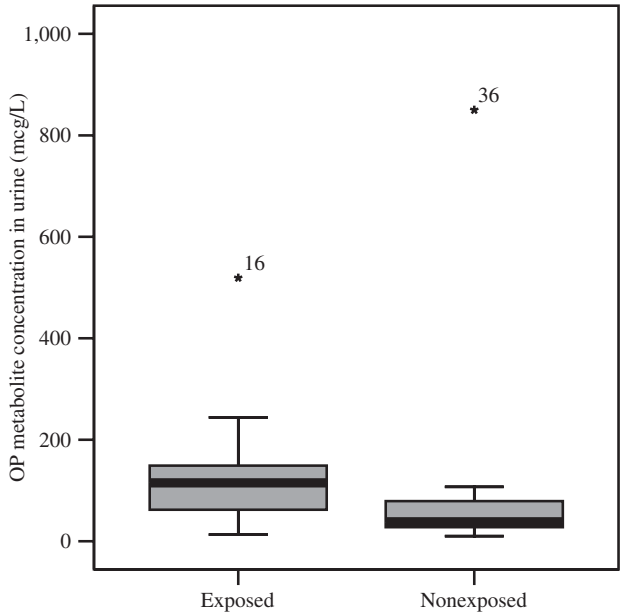

Figure 2. OP metabolite concentration levels found in the urine sample of children the day the cognitive assessment was conducted. All children, regardless of their original membership to either Exposed or Nonexposed groups, had a detectable level of OP metabolite. Note that one of the two samples with the highest OP metabolite level was part of the Exposed group and the other one was in the Nonexposed group.

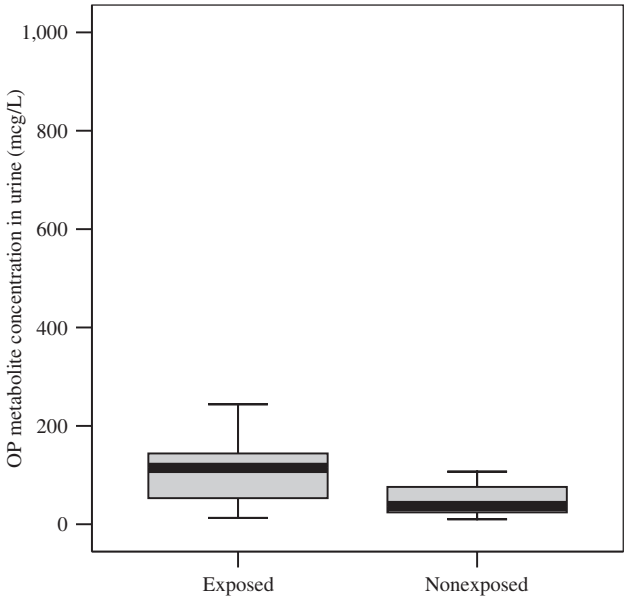

Figure 3. Comparison of the Exposed and Nonexposed groups after the two samples with the highest OP metabolite levels of Fig. 2 were removed. The Exposed group had significantly ($p < .05$) higher OP metabolite concentration levels ($M = 110$ mcg/l) than the Nonexposed group ($M = 49$ mcg/l).

had significantly higher levels than the rest of the samples; (b) one of those samples belonged to a child that was originally part of the Exposed group, and the other sample belonged to a child that was originally part of the Nonexposed group (Fig. 2); (c) if these two outliers were removed for group comparison, then there was a significant difference on the OP pesticide metabolite concentration levels between the originally Exposed ($M = 110$ mcg/l; 95% CI = 83–139) and Nonexposed ($M = 49$ mcg/l; 95% CI = 36–63) groups, $F(1,44) = 15.83$, $p < .01$, $d = 1.19$ (Fig. 3). That is, children that were part of the Exposed group according to the CPS continued to be exposed and to statistically significant higher levels than the Nonexposed children.

Demographic Characteristics of the Sample

The demographic characteristics based on the original Exposed/Nonexposed design indicate that children had nearly identical characteristics (Table I). Father's years of education was the only characteristic that was significantly different ($p < .05$) between the original Exposed and Nonexposed groups. That is, fathers in the Exposed group had significantly less number of years of education ($M = 9$; 95% CI = 8–10) than the fathers in the Nonexposed group ($M = 12$; 95% CI = 11–14), $F(1, 42) = 11.45$, $p = .002$; $d = -1.03$.

Given that all children had detectable OP pesticide metabolite in their urine the day of cognitive assessment and that their demographic characteristics were essentially the same, both Exposed and Nonexposed groups, from the original design, were treated as a single group, New-exposed, to explore the association between OP pesticide levels and performance in the cognitive and behavioral measures.

Cognitive and Behavioral Functioning

Before presenting the results of the correlations, the original Exposed and Nonexposed groups, after removing outliers, were compared to see if more exposure had an effect on the cognitive and behavioral functioning of children. Results showed that there were significant effects for Exposure on the TMTB, $F(1,40) = 6.01$, $p = .01$, indicating that children in the Exposed group took more time ($M = 283$ s; 95% CI = 224–341) to complete this measure than the Nonexposed group ($M = 204$ s; 95% CI = 172–236), $d = .76$. Results also showed that there were no significant effects of Exposure on the other cognitive and behavioral measures.

Cognitive Functioning and OP Pesticide Metabolites the Day of Assessment

As indicated earlier, all children were treated as a single Newexposed group to explore the association between OP pesticide levels and performance in the cognitive measures. Because behavioral measures refer to a 6-month period, they were not considered in this analysis.

Results indicate that there were significant correlations between the concentration levels of OP metabolites the day of the cognitive assessment and the performance of children on some of the measures of the WCST. Specifically, there were significant positive correlations between the OP metabolite concentration levels and the total *Number of Errors* made ($r = .31$, $p = .03$); the *Number of Perseverative Responses* ($r = .34$, $p = .01$); the *Number of Perseverative Errors* ($r = .35$, $p = .01$); the *Conceptual Level Responses* provided ($r = .38$, $p = .01$); and, the *Failure to Maintain Set* ($r = .38$, $p = .02$). There were no significant correlations ($p < .05$) between the concentration levels of OP metabolites found in the urine of children the day of the cognitive assessment and their respective performance on the WISC-III SF, the CMS, the TMTA, and the TMTB.

Since Pilkington et al. (2001) reported that the associations they found between OP pesticide concentration levels and neurologic impairment in OP pesticide applicators were dependent upon the inclusion of a few samples that had significant higher OP pesticide levels, we decided to reanalyze our data to determine if we would find the same trend. Thus, the two samples with the highest concentration levels of OP metabolite were removed and the data reanalyzed. When these concentration values were removed, there were no significant correlations between the concentration levels of OP pesticide metabolite on the day of cognitive assessment and the measures of the WCST that were previously reported. This suggests that the two removed samples that had the highest concentration levels of OP pesticide metabolite (519 mcg/l and 850 mcg/l) might have accounted for the initial association, which is consistent with the findings of Pilkington et al.

Discussion

This study assessed the cognitive and behavioral functioning of Hispanic children living in an agricultural community who had documented levels of exposure to OP pesticides. The findings of this study add relevant information to the relatively limited body of knowledge about the effects of OP pesticides on children's neurodevelopment. To date, research had focused primarily on documenting the effects of OP pesticide exposure on the neurobehavioral functioning of farm workers or pesticide applicators in occupational settings. Our results are consistent with the findings of these studies and with those of experimental ones (e.g., Pilkington et al., 2001; Qiao et al., 2003; Rothlein et al., 2006; Steenland et al., 1994; Stephens et al., 1995). Our study design initially involved Exposed and Nonexposed groups; however, contrary to our expectation, we found that all children had a detectable level of at least one OP metabolite, DMP, in the urine sample they provided the day of cognitive assessment. This finding alone deserves attention for further investigation. It also meant that we had to adjust our study design that initially included Exposed and Nonexposed children and treat them as a single New-exposed group. Even though all children had a detectable level of OP pesticide metabolite, it was found that the original Exposed group had significantly higher levels than the Nonexposed group.

Results from the comparison between the original Exposed and Nonexposed groups and those of the correlations between OP pesticides levels and cognitive performance of the New-exposed group suggest that OP pesticide exposure might have detrimental effects on children's cognitive skills, as measured by the TMTB and WCST. The specific cognitive skills were: speed of attention, sequencing, mental flexibility, visual search, motor functioning, concept formation, and conceptual flexibility. These results are consistent with the findings reported in other studies (e.g., Pilkington et al., 2001; Qiao et al., 2003; Rothlein et al., 2006). However, the dependent measure of the WCST associated with insight into the correct sorting principle showed a significant correlation in the opposite direction from that expected, suggesting that the higher the levels of OP pesticide concentration the better the performance in this area. This latter finding is consistent with the study of Ames et al. (1995), who found enhanced performance of their exposed subjects on a measure of processing speed. It would seem, therefore, that the findings associated with the WCST deserve further study, especially given the fact that when the correlations were recalculated using a distribution without the two highest concentrations of OP pesticides, none of the aforementioned correlations were found to be significant. Furthermore, this also suggests that a ''threshold'' of OP pesticide exposure exists in order to detect an association between exposure and

these cognitive functions (the two cases that were removed had OP pesticide concentration levels >500 mcg/l). This finding is consistent with the results reported by Pilkington et al. (2001).

In summary, findings from this study are consistent with what is known about the effects of OP pesticide exposure in adult populations. Short-term OP pesticide exposure appears to have a deleterious effect on the cognitive functioning of children living in an agricultural community where OP pesticides are continuously used. However, the association between levels of OP pesticide exposure and poor performance on the cognitive measures was dependent upon the inclusion of cases that had significantly higher OP pesticide concentration levels. Considering the limited research in this area, the findings of this study support the notion that chemicals in the environment have the potential to negatively impact children's neurodevelopment with possible implications for learning ability and behavior.

Limitations of the Study and Suggestions for Future Research

The fact that children in the original Exposed group had significantly higher OP metabolite concentration levels than the original Nonexposed group raises some questions that need further investigation: What in their environment makes these children have higher levels of OP metabolite? (e.g., proximity to agricultural fields? Drift direction?). In addition, when compared to national levels (NHANES), children in this agricultural area had higher levels of OP metabolite concentration than children in the rest of the country. Furthermore, children in the CPS had noticeable higher levels than the levels found in this study. The authors did not investigate the reasons for these differences any further, but acknowledge that there might be some factors influencing this outcome that deserve attention. For example, are these differences the result of a pesticide seasonal application trend/variability and the time when the urine sample collection took place? Also important, why did children that were not exposed in the CPS have a detectable level of OP pesticide at this time? Is this related to the pesticide seasonal application trend/variability?

In addition to these suggestions, there are some potential limitations to the present study that should be considered in future research. The first one is related to the limited number of participating children. However, the only other available study (Guillette et al., 1998) evaluating developmental differences of exposed and nonexposed children also included a small number of participants. This indicates that research in this area is just beginning and that the data generated by the present study can help in the generation of new hypotheses and better methodologies.

A second limitation relates to the specific cognitive and behavioral measures used in this study. The measures were selected based on the frequency with which they had been used in studies involving adult populations. However, they may not be the most sensitive (or appropriate) ones in detecting differences in the cognitive and behavioral functioning of Hispanic children living in an agricultural community, whose primary language is Spanish. Nonverbal tests such as the WCST and the TMTB seem to be the most appropriate measures, and should be included in future studies.

Finally, another limitation involves the difficulty in identifying a ''true'' Nonexposed group within the same rural agricultural community where OP pesticides are continuously used. To date, assessment of OP pesticide exposure using urinary biomarkers seems to be the best method available. However, this method has the limitation of only providing information about an exposure occurring 24–48 hr *prior* to the collection of the urine sample. Given that the community in which this study took place has been mainly agricultural for the past 30 years and is heavily farmed year round, cumulative exposures cannot be accurately assessed by this urinary biomarker method. The methods necessary to assess long-term exposures are being developed and issues regarding accurate characterization of children's exposure to pesticides are being addressed by research in the area of children's exposure assessment (e.g., Fenske et al., 2000; Freeman et al., 2005; Hubal et al., 2000; Landrigan et al., 1999; Needham & Sexton, 2000). Multidisciplinary research in this area is not only advisable, but also necessary. Future research should include a group of Hispanic children living in a nonagricultural community where OP pesticides are not used and thus exposure is less likely.

In conclusion, even though the results of this study have the mentioned limitations, they also add important information to the limited body of knowledge about the effects of OP pesticide exposure on children's cognitive functioning. Recently, a number of experimental studies (Qiao et al., 2003; Slotkin et al., 2006) as well as large epidemiological ones (Dietrich et al., 2005; Eskenazi et al., 1999; Rothlein et al., 2006; Whyatt et al., 2004) have begun to document the deleterious effects of OP pesticides in neurodevelopment. Thus, it is relevant for all of us working with pediatric populations to look at

our children who are extremely vulnerable and do not have the potential of self-protection. We need to be aware of the potential harm of the chemicals present in the environment in which we live. We need to educate the children and families that we serve and participate and encourage participation in movements advocating for the restriction of the use of pesticides and other chemicals in our foods. We have this responsibility to children so that they can have a healthy development and future.

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