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Children's residential exposures to flame retardants, pesticides and pesticide degradation products, and the relationship of pesticides with autonomic nervous system functioning

By

Lesliam Quiros Alcala

A dissertation submitted in partial satisfaction of the

requirements for the degree of

Doctor of Philosophy

in

Environmental Health Sciences

in the

Graduate Division

of the

University of California, Berkeley

Committee in charge:

Professor Brenda Eskenazi, Co-Chair
Professor Mark Nicas, Co-Chair
Professor S. Katharine Hammond
Professor Alan Hubbard

Spring 2010

Children's residential exposures to flame retardants, pesticides and pesticide degradation products, and the relationship of pesticides with autonomic nervous system functioning

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by

Lesliam Quiros Alcala

Abstract

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by

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Doctor of Philosophy in Environmental Health Sciences

University of California, Berkeley

Professor Brenda Eskenazi, Co-Chair

Professor Mark Nicas, Co-Chair

Protecting children's environmental health is a significant public health challenge given children's unique exposure pathways and special vulnerabilities to environmental contaminants compared to adults. This dissertation focused on topics surrounding children's environmental health research with an emphasis on exposure assessment and application in an epidemiologic investigation. The environmental contaminants that this work focused on included pesticides and polybrominated diphenyl ether (PBDE) flame retardants.

Chapter 1 provides a general introduction to children's environmental health and highlights the background and significance and specific aims for each study/chapter.

Chapter 2 focuses on children's residential exposures via house dust to pesticides and PBDEs in low-income homes. House dust was used to assess indoor residential exposures to these environmental contaminants given that for young children this medium serves as a reservoir for contaminants tracked-in or used indoors and a source of non-dietary ingestion. Additionally, the contaminants of interest have been routinely measured in this medium. In this study, concentrations for 24 pesticides, one pesticide synergist, and three PBDE congeners (major constituents of the pentaBDE flame retardant commercial mixture commonly used on furniture) were measured in house dust samples from farmworker and urban homes in California. Pesticides frequently detected in most homes included: organophosphates (chlorpyrifos and diazinon) which were voluntarily phased-out for residential uses prior to this study by the urging of the United States Environmental Protection Agency (EPA); pyrethroids such as permethrins, allethrins, cypermethrins; and the synergist piperonyl butoxide. Interestingly, chlorthal-dimethyl was detected solely in farmworker homes, suggesting contamination due to regional agricultural use. All three PBDE congeners were detected in all homes and maximum concentrations for each of these congeners are the highest reported to date in house dust (BDE-47: 125 632 ng/g, BDE-99: 218 768 ng/g, BDE-100: 41 149 ng/g). Possible explanations for the high PBDE concentrations observed include California's stringent flammability standards and the presence of poorly constructed or deteriorating furniture treated with PBDEs as previously hypothesized by other researchers.

Additionally, diazinon and chlorpyrifos concentrations in Salinas farmworker homes were lower than concentrations reported in samples from Salinas farmworker homes studied between 2000-2002, suggesting a temporal reduction after the U.S. Environmental Protection Agency's voluntary residential phase-out. Lastly, for some resident children, estimated non-dietary PBDE but not pesticide intake exceeded U.S. EPA recommended chronic reference doses (RfDs).

In chapter 3 the presence in the environment of dialkylphosphates (DAPs), non-specific urinary OP pesticide metabolites, and their relation to children's urinary DAP metabolites was investigated. Although DAPs were found to be present in the environment, as assessed in house dust, this medium may not play a significant contribution to the DAPs observed in children's urine. The non-dietary ingestion exposure route for environmental DAPs was estimated to be $\leq$5% of the dose calculated from DAP levels in children's urine. The distribution of concentrations of diethyl and dimethyl DAPs in dust differed from those observed in children's urine, a finding suggesting that DAPs behave differently in the environment and in the body. However, if humans excrete DAPs unchanged then it is possible for urinary DAPs to reflect exposure to both OP pesticides and DAPs present in one's environment and/or food. Results from this study indicate other sources and pathways, such as DAPs in food, may impact urinary DAP levels more significantly than DAPs in dust. More research is needed on the pharmacokinetics and toxicodynamics of preformed DAPs and other specific OP metabolites to determine the extent of their contribution to urinary biomarkers in humans.

In chapter 4 the effects of early life exposures to OP pesticides, as assessed by urinary DAP metabolites, on children's autonomic dysregulation (concomitant sympathetic activation and parasympathetic withdrawal) were assessed at several time points (i.e., when children were 6 months and 1, 3 ½ and 5 years of age). This is the first study to use ANS response measures as outcomes to investigate the association between OP pesticide exposures in children and ANS regulation. The study population was part of the Center for Children's Environmental Health Research longitudinal birth cohort study (CHAMACOS). Children in this cohort live in the Salinas Valley, an agricultural region in California with intense OP pesticide use and were predominantly from Mexico or Mexican-American. Children's autonomic nervous system (ANS) function was assessed using resting and reactivity measures of respiratory sinus arrhythmia (RSA), pre-ejection period (PEP), and heart rate (HR), while OP pesticide exposures were assessed *in utero* and postnatally by using urinary DAPs. Although the results suggest that OP pesticides at the exposure levels observed are not associated with children's ANS dysregulation, the study focused on a relatively demographically and ethnically homogeneous study population; thus, the results may not be generalizable to other populations. Future investigations in this population will involve evaluating what factors predict ANS regulation and whether ANS resting and reactivity measures of HR, RSA, and PEP are related to later physical and mental problems as observed in prior studies.

Finally, chapter 5 highlights the major findings, public health implications and future directions for each chapter/study.

Dedication

I dedicate this work to the light of my life: my parents, Amalia and Leslie, my brother, Leslie, and my dear husband, Luis. Thank you for your unconditional love and support throughout my journey, for pushing me, for believing in me, and for being there when I have needed you the most. I also dedicate this work to our baby boy on the way.

You are all a blessing in my life!

¡Los amo infinitamente!

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List of Abbreviations (in alphabetical order):

AChE: Acetylcholinesterase
ACN: Acetonitrile
ANOVA: Analysis of Variance
ANS: Autonomic Nervous System
AUC: Area-under-the-concentration–time-curve
CDC: Centers for Disease Control and Prevention
CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas
CI: Confidence interval
cPAD: Chronic Adjusted Population Dose
DAP(s): Dialkylphosphate(s)
DE: Diethyl alkylphosphates
DE: Diethyl alkylphosphates
DEDTP: Diethyldithiophosphate
DEP: Diethylphosphate
DETP: Diethylthiophosphate
DF: Detection frequency
DM: Dimethyl alkylphosphates
DM: Dimethyl alkylphosphates
DMDTP: Dimethyldithiophosphate
DMP: Dimethylphosphate
DMTP: Dimethylthiophosphate
ECG: Electrocardiogram
FA: Formic Acid
GC-MS/MS: Gas chromatography-tandem mass spectrometry
GC/MS: Gas chromatography/mass spectrometry
GEE: Generalized Estimating Equation
GM: Geometric mean
HQ: Hazard Quotient
HR: Hear rate
HVS3: High Volume Small Surface Sampler
ICC: Intraclass Correlation Coefficient
ISTD: Isotopically labeled internal standard
Kow: Octanol:Water Partition Coefficient
LOD: Limit of Detection
LOD: Limit of detection
MeOH: Methanol
OP: Organophosphorous or organophosphate
PBDE: Polybrominated diphenyl ether
PBO: Piperonyl butoxide
PDI: Potential daily intake

PEP: Pre-ejection period
PON1: Paraoxonase 1
PUR: Pesticide Use Reporting Database
QA/QC: Quality Assurance/Quality Control
RfD: Reference Dose
RSA: Respiratory sinus arrhythmia
RSD: Relative standard deviation
RV: Recreational vehicle
SD: standard deviation
SI: Supporting Information
SPE: Solid phase extraction
SRS: Surrogate Recovery Standard
TEA: Triethylamine
U.S. EPA: United States Environmental Protection Agency

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Chapter 1: Introduction

INTRODUCTION

Protecting children's health is an important public health challenge. It is well recognized, largely in part as a result of the 1993 National Research Council's report on "Pesticides in the Diets of Infants and Children" (NAS 1993), that children have unique exposures and special vulnerabilities to environmental contaminants compared to adults. Children are more vulnerable to environmental contaminants because they eat, breathe, and drink more relative to their body weight, have unique behaviors (e.g., crawling and mouthing behaviors) that predispose them to higher contaminant exposures, and spend a large amount of their time indoors (Bearer 1995; Roberts, Wallace et al. 2009). Low doses of environmental contaminants may affect children's developing neurological, immune, digestive, and other bodily systems as these systems are less efficient at metabolizing, detoxifying, and excreting contaminants (Grandjean and Landrigan 2006). For example, *in utero* and/or postnatal exposures to organophosphate (OP) pesticides have been associated with altered neurodevelopment in children (e.g., abnormal neonatal behavior and reflexes, altered mental development, and pervasive developmental problems) (Young, Eskenazi et al. 2005; Grandjean, Harari et al. 2006; Engel, Berkowitz et al. 2007; Eskenazi, Marks et al. 2007). Critical neurologic developments that define the normal functioning of the autonomic nervous system occur in the first years of life (e.g., maturation of the central regulatory nuclei of cardiovascular activity and myelination of the vagus nerve) (Pereyra, Zhang et al. 1992), thus OP pesticide exposures in children could also lead to autonomic dysregulation, although this has not been investigated previously. Animal and limited human studies also suggest that contaminants like polybrominated diphenyl ether (PBDE) flame retardants are endocrine disruptors which may affect thyroid hormone homeostasis, exert anti-androgenic effects (Talsness 2008), and affect children's neurodevelopment (Herbstman J, Sjödin A et al. 2010).

Factors which may predispose children to pesticide and PBDE exposures include: conditions in low-income homes and/or location of residence. Conditions in low-income homes, like overcrowding and housing disrepair, are associated with pest infestations and increased home pesticide use in both urban and agricultural communities (Whyatt, Camann et al. 2002; Bradman, Chevrier et al. 2005). Additionally, the presence of farmworkers in the home and/or proximity of homes to nearby fields in agricultural communities has been associated with higher residential pesticide concentrations (Lu, Fenske et al. 2000; Harnly M., Bradman A. et al. 2009). Lastly, although PBDEs have been found in residences of different socioeconomic status, a study by Imm et al. (Imm, Knobeloch et al. 2009) found that upper end exposures of PBDEs in serum from a Wisconsin cohort were associated with lower household income. Researchers have hypothesized that older, deteriorating or poorly manufactured furniture treated with PBDEs present in low-income homes may be responsible (Zota A.R., Rudel R. et al. 2008). Thus, intrinsic and extrinsic factors play major roles on environmental exposures of children.

To assess children's residential exposures to pesticides and/or PBDEs scientists have relied on environmental monitoring and/or biological sampling. For example, pesticides have been measured in soil, air, toy wipes, surface wipes, and house dust (Fenske, Bradman et al. 2005). House dust serves as a reservoir of contaminants tracked in and/or used indoors. Research indicates that house dust is a major source of exposure to pesticides and PBDEs and it is considered a potential source of non-dietary ingestion for children (Butte and Heinzow

2002; Roberts, Wallace et al. 2009). According to the U.S. Environmental Protection Agency (U.S. EPA), young children ingest twice as much dust as adults (100 mg vs. 50 mg/day) and are up to ten times more vulnerable to environmental contaminant exposures via house dust compared to adults (U.S. EPA 2002; U.S. EPA 2003). In addition to household factors such as the presence of carpets in the home, the physical and chemical properties of various pesticides and PBDEs may be determined by the persistence of the individual contaminants indoors.

Biological media commonly used to assess children's exposures to these contaminants include blood (PBDEs and pesticides) and urine (pesticides). Both of these biological media offer an integrated measure of exposure. Selection of biological media depends on many factors including kinetics, ease of sample collection, and the possibility of sample contamination. Collection of urine samples is often preferred to assess pesticide exposures because collection is simple and noninvasive, particularly in studies involving children (Barr and Angerer 2006). For example, dialkylphosphates (DAPs) which are non-specific urinary pesticide metabolites have been routinely measured in multiple studies to assess OP pesticide exposures (Loewenherz, Fenske et al. 1997; Fenske, Kissel et al. 2000; Castorina, Bradman et al. 2003; Duggan, Charnley et al. 2003; Barr, Bravo et al. 2004). However, these biomarkers of exposure are not without their own limitations. Although researchers have used DAPs to derive biologically-based OP pesticide dose estimates, a recent study indicates that DAPs in urine may also reflect exposure to preformed DAPs in food (Lu, Bravo et al. 2005); and though not previously confirmed, preformed DAPs could also be present in the environment.

This dissertation focuses on children's residential exposures and potential non-dietary exposures to pesticides and PBDEs in house dust (Chapter 2), the presence of DAPs in environmental media and its association with urinary DAP levels in children (Chapter 3), and the association between children's *in utero* and postnatal OP pesticide exposures as measured by maternal and child urinary DAPs and autonomic dysregulation at ages 6 months and 1, 3 ½ and 5 years (Chapter 4).

Chapter 2:

Background & Significance. To date, no studies have examined pesticide exposures to children residing in low-income urban versus agricultural environments, where general home conditions may be similar (e.g. high pest infestations and pesticide use) but where additional agricultural sources and exposure pathways are present in the rural community. Additionally, it is unclear to what extent the residential phase-out on certain OP pesticides has resulted in lower indoor levels in agricultural regions where these pesticides are still widely used in agriculture. Data on indoor exposures to other contemporary-use pesticides like pyrethroids are also scarce. Other environmental contaminants of concern in low-income homes include PBDE flame retardants. The highest PBDE concentrations in dust and serum in the U.S. have been reported in California, and recent findings published by Zota et al. (Zota A.R., Rudel R. et al. 2008) suggest that low-income residents may experience higher PBDE exposures.

Specific Aims. In this chapter, exposures in low-income homes were assessed via house dust. House dust serves as a non-dietary exposure source for children and environmental contaminants are commonly found in this media. House dust levels of select agricultural and home-use pesticides and PBDEs from low-income homes in an urban area (Oakland, CA) were compared to those observed in an agricultural area with intense pesticide use (Salinas, CA). Additionally, the variability of analyte levels within homes over short time frames was evaluated. Children's potential non-dietary ingestion exposures via house dust were estimated to determine if these exceeded health-based reference doses. This information will be valuable to policy makers considering whether population pesticide exposures are higher in agricultural versus non-agricultural homes and to future researchers considering the stability of pollutant measurements in dust over time.

Chapter 3:

Background & Significance. OP pesticides account for approximately half of all insecticides sold in the United States (Wessels, Barr et al. 2003) and are the largest group of insecticides sold worldwide (Costa 2006). OP pesticides are popular due to their broad spectrum of applications, toxicity to insects, and diminished persistence in the environment compared to other pesticides (e.g. organochlorines). They are widely used on many food crops, as well as in residential and commercial buildings, and for ornamental plants and lawn care, potentially resulting in human exposure. They are known neurotoxicants that act via inhibition of acetylcholinesterase (Wessels, Barr et al. 2003). OPs are of particular public health concern because recent evidence suggests that low-level chronic exposure to OP pesticides *in utero* is associated with poor neurodevelopment in children (Young, Eskenazi et al. 2005; Engel, Berkowitz et al. 2007; Eskenazi, Marks et al. 2007). Due to their widespread use, common mode of toxicity, and health effects, they were placed in the first priority group of compounds to be reviewed under the Food and Quality Protection Act of 1996. This Act mandates that the EPA reassess all pesticide residue tolerances on food while giving special consideration to potential cumulative and aggregate exposures to susceptible populations including children (FQPA 1996).

The biomarkers of exposure most frequently used to assess OP pesticide exposure are urinary dialkylphosphates or DAPs. Researchers have used urinary DAP levels to derive biologically-based OP pesticide dose estimates, given that 70% of the OP pesticides registered for use in the U.S. devolve to DAPs (Wessels, Barr et al. 2003). A factor that complicates the interpretation of OP pesticide breakdown products as biomarkers of exposure is the observation that certain OP pesticides undergo hydrolysis reactions in the environment resulting in the same breakdown products in the environment as in the body (Racke 1992). A recent study by Lu et al. (Lu, Bravo et al. 2005) also reported finding DAPs in food. Prior studies have assumed that the potential doses estimated from urinary DAP levels result from exposure to parent OP compounds and not a combination of both parent OP pesticides and preformed DAPs in the environment and/or food (Fenske, Kissel et al. 2000; Castorina, Bradman et al. 2003). If people are exposed to DAPs and excrete them unchanged, the use of urinary DAPS as a biomarker of OP pesticide exposure would lead to an overestimation of

exposure and would likely result in exposure misclassification in epidemiology studies and less accurate exposure characterizations in risk assessments. Additionally, if environmental levels of DAPs are the largest contributor to the levels observed in urine, then DAPs may not be a reliable biomarker for OP pesticides, particularly in non acute exposure settings. However, if DAPS in the environment contributed to urinary DAP measurements then it is likely to result in non differential misclassification of exposure, i.e, exposure to DAPS in the environment would be random and unrelated to an individual's exposure to parent compounds in the environment.

Specific Aims. To date, no studies have documented the existence of DAPs in the environment nor their impact on human urinary DAP levels. A laboratory method to analyze DAPs in dust (Weerasekera, Smith et al. 2009) was previously developed in collaboration with chemists at the Centers for Disease Control and Prevention. In this study, this laboratory method was applied to: (1) determine whether DAPs are present in the environment using house dust as a proxy and evaluate differences in concentrations between homes located in urban and agricultural communities; (2) evaluate the association between OP pesticide and DAP residues in dust; and (3) determine the relationship between DAPs present in house dust and urinary DAP metabolite levels in young children. Establishing the presence of DAPs in house dust and its relation to urinary DAPs will help determine if preformed DAPs in dust significantly contribute to urinary DAP levels observed in children.

Chapter 4:

Background & Significance. The autonomic nervous system (ANS) consists of two branches, the parasympathetic and sympathetic nervous systems, which act in complementary ways to respond to and recover from environmental stressors. OP pesticides exert their pharmacologic effects through the inhibition of acetylcholinesterase (AChE) which leads to the accumulation of the neurotransmitter acetylcholine. Because acetylcholine is the preganglionic neurotransmitter for both ANS branches, as well as the postganglionic neurotransmitter of parasympathetic neurons it is essential for the normal functioning of the ANS. Accumulation of acetylcholine could lead to systemic effects in the ANS (e.g., by disrupting cholinergic functions essential to skeletal-muscle motor neurons and peripheral parasympathetic and sympathetic neurons) and other central nervous system components (Eskenazi, Bradman et al. 1999; Rosas and Eskenazi 2008). Vital neurologic developments that define the normal functioning of the ANS occur in the first years of life (e.g., maturation of the central regulatory nuclei of cardiovascular activity and myelination of the vagus nerve) (Pereyra, Zhang et al. 1992). Thus, inhibition of AChE during this period may impact the cholinergic receptors of the ANS possibly leading to short- and long-term effects on the regulation of ANS-controlled functions. Autonomic dysregulation may be one the earliest and most sensitive measures of long-term systemic physiologic effects of OP pesticide exposure in infants and young children (Eskenazi, Bradman et al. 1999) given AChE inhibition is central to the toxic effects of these pesticides.

To date, no studies have assessed the effects of prenatal and postnatal OP pesticide exposures on children's ANS functioning. ANS regulation in children has been assessed by relying on measures of the parasympathetic nervous system and the sympathetic nervous system (Alkon, Goldstein et al. 2003). Respiratory sinus arrhythmia (RSA) is a measure of the parasympathetic nervous system which measures the periodic oscillation in sinus rhythm occurring in synchrony with respiration. Another measure of ANS function includes pre-ejection period (PEP) which is a noninvasive and indirect measure of the sympathetic nervous system's influence on the cardiac cycle and measures the duration of isovolumetric ventricular contraction. Heart rate (HR) is also used as a measure of ANS regulation; it is an integrated measure of the parasympathetic and sympathetic nervous systems (Berntson, Cacioppo et al. 1993; Sherwood 1993; Beauchaine 2001). Measures of RSA, PEP, and HR reactivity are important because they have been related to physical and mental health problems (Porges, Doussard-Roosevelt et al. 1996; Doussard-Roosevelt, Porges et al. 1997) and may serve as markers of developmental outcomes in children (Doussard-Roosevelt, Porges et al. 1997). As such, measures of ANS dysregulation (concomitant sympathetic activation and parasympathetic withdrawal) may offer insights into the neurobehavioral effects of OP pesticide exposure.

Specific Aims. A study was conducted to assess the effects of OP pesticide exposure in children on ANS functioning using measures of RSA, PEP and HR by having children living in an agricultural region complete a series of standardized tasks designed to elicit ANS responses. The main aims of this study were to: (1) examine the cross-sectional effects of prenatal and postnatal OP pesticide exposure on children's ANS at four time points: 6 months, 1 year, 3 ½ and 5 years of age; (2) determine whether cumulative measures of prenatal and postnatal pesticide exposure were associated with ANS function at 5 years; and (3) determine whether children with consistently high versus consistently low OP pesticide exposures had significantly different mean ANS responses at 5 years. Findings from this study will help elucidate if early life exposures to OP pesticides are associated with ANS dysregulation in young children.

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Chapter 2:
Pesticides and polybrominated diphenyl ethers in dust from low-income urban and farmworker households in California and potential non-dietary exposures to resident children

CHAPTER SUMMARY

California has intense pesticide use and strict flammability standards. Exposure data on pesticides and polybrominated diphenyl ether (PBDE) flame retardants in low-income homes are limited. In 2006, we collected two house dust samples 5-8 days apart from each of 13 urban homes in Oakland, CA and 15 farmworker homes in Salinas, CA, an agricultural community (54 samples from low-income homes). We measured 24 pesticides, one pesticide synergist, and three PBDE congeners which are major constituents of the pentaBDE flame retardant commercial mixture. Pesticides frequently detected in both locations included chlorpyrifos, diazinon, permethrins, allethrin, cypermethrin, and the synergist piperonyl butoxide. Chlorthal-dimethyl was detected solely in farmworker homes, suggesting contamination due to regional agricultural use. We detected BDE-47, BDE-99, and BDE-100 in every home; maximum concentrations for each of these congeners are the highest reported to date (BDE-47: 125 632 ng/g, BDE-99:218 768 ng/g, BDE-100: 41 149 ng/g). Concentrations for frequently detected analytes were generally strongly correlated within homes (Spearman ρ=0.70-1.00, $p<0.01$). Additionally, diazinon and chlorpyrifos concentrations in Salinas farmworker homes were 40-80% lower than concentrations reported in samples from Salinas farmworker homes studied between 2000-2002, suggesting a temporal reduction after the U.S. Environmental Protection Agency's (U.S. EPA) voluntary residential phase-out. Finally, for some resident children, estimated non-dietary PBDE, but not pesticide, intake exceeded U.S. EPA recommended chronic reference doses.

INTRODUCTION

Exposures to pesticides and polybrominated diphenyl ether (PBDE) flame retardants may be associated with adverse health effects, particularly among vulnerable populations. For example, *in utero* and/or postnatal chronic exposures to pesticides, such as organophosphorous (OP) pesticides, have been associated with poorer neurodevelopment in children (Engel et al., 2007; Eskenazi et al., 2007; Rauh et al., 2006), and altered fetal growth (Whyatt et al., 2004) and gestational duration (Eskenazi et al., 2004). Animal studies indicate that pre- and postnatal PBDE exposure is associated with developmental neurotoxicity (e.g., hyperactivity and learning and memory impairments) (Costa and Giordano, 2007) and a recent study by Herbstman et al. (Herbstman J et al., 2010) found that children with higher prenatal exposure to BDE-47,-99, or -100 scored lower on tests of mental and physical development at 1-4 and 6 years. Additionally, animal and limited human studies suggest that PBDEs are endocrine disruptors which may affect thyroid hormone homeostasis and exert anti-androgenic effects (Talsness, 2008).

Pesticides and PBDEs have been measured in residential environments, most notably in indoor dust (Bradman et al., 2006; Harrad et al., 2008; Roberts et al., 2009; Schecter et al., 2005; Zota A.R. et al., 2008). Residential persistence of pesticides indoors is likely due to the lack environmental factors that aid in degradation such as sunlight, rain, temperature extremes, and microbial action (Lorber, 2008; Roberts et al., 2009). Semi- and non-volatile pesticides (e.g., OPs and pyrethroids) and PBDEs have chemical properties that increase binding affinity for particles and the tendency to adsorb onto household surfaces such as carpet or dust, also prolonging their persistence indoors (Butte and Heinzow, 2002; Hwang et al., 2008). Young children are particularly vulnerable to ingesting pesticide- or PBDE-contaminated dust due to their frequent hand-to-mouth activity and contact with indoor surfaces (Roberts et al., 2009). Several studies suggest that house dust is a significant source of pesticide exposure for children and the primary source of exposure to PBDEs (Lorber, 2008; Roberts et al., 2009). For example, according to a recent study by Lorber et al. (Lorber, 2008), children's exposures to PBDE-contaminated dust via the non-dietary and dermal pathways could account for more than 80% of aggregate PBDE intake.

Other factors that may affect indoor exposures to pesticides and PBDEs include housing characteristics of low-income homes. Conditions in low-income homes, such as overcrowding and housing disrepair, are associated with pest infestations and increased home pesticide use in both urban and agricultural communities (Bradman et al., 2005; Whyatt et al., 2002). Additionally, the presence of farmworkers in the home and/or proximity of homes to nearby fields in agricultural communities has been associated with higher pesticide concentrations indoors (Harnly M. et al., 2009; Lu et al., 2000). Finally, although PBDEs have been found in residences of different socioeconomic status, a study by Imm et al. (Imm et al., 2009) found that upper end exposures of PBDEs in serum from a Wisconsin cohort were associated with lower household income. Zota et al. also observed a significant association between lower income status and PBDE concentrations in blood using data from a nationwide U.S. sample. Although, no studies are available to confirm why low income status is associated with PBDE concentrations indoors, researchers have hypothesized that older, deteriorating or poorly manufactured furniture present in low-income homes may be responsible (Zota A.R. et al., 2008).

California has intense agricultural production and leads the nation in agricultural pesticide use (Gunier et al., 2001). The highest PBDE concentrations in dust and serum in the U.S. have also been reported in California, likely due to the state's stringent flammability standards (Zota A.R. et al.). Exposure data on these indoor contaminants in low-income households is limited. Additionally, residential uses of the OP pesticides chlorpyrifos and diazinon were voluntarily phased out by the U.S. Environmental Protection Agency (U.S. EPA) in 2001-04 (U.S. EPA, 2000; U.S. EPA, 2001), resulting in decreased air concentrations among low-income households in New York (Whyatt et al., 2007). However, trends in residential contamination of these compounds have not been studied in agricultural areas, where pesticide drift and transport from fields on work clothing may impact indoor pesticide dust concentrations (Lu et al., 2000). In this study, we characterized and compared house dust levels of pesticides and PBDEs from low-income homes in an urban community (Oakland, CA) and an agricultural community (Salinas, CA). We then evaluated the intra-home variability of analyte concentrations in samples collected several days apart. To determine whether chlorpyrifos and diazinon indoor concentrations have declined since the U.S. EPA's voluntary residential phase-out, we compared concentrations in Salinas dust samples collected for this study in 2006 to concentrations in samples collected from similar homes studied between 2000-2002 (Bradman et al., 2006; Harnly M. et al., 2009). Finally, to determine if pesticide and PBDE concentrations in these homes led to exposures that exceed health-based regulatory guidelines, we estimated resident children's potential non-dietary ingestion exposures to these indoor contaminants.

METHODS

Study Population

The study population consisted of children participating in a biomonitoring exposure study conducted from July through September 2006 whose primary aim was to assess the effects of diet on children's urinary pesticide metabolite concentrations. Through community health clinics and organizations serving low-income populations, we recruited a convenience sample of 20 families living in Oakland, CA, (an urban community in Alameda county) and 20 families living in Salinas, CA (an agricultural community in Monterey county). Participants included families with children 3-6 years old who were toilet-trained. Salinas households included at least one resident who worked in agriculture. We restricted ethnicity to Mexican immigrants and Mexican-Americans to minimize cultural disparities between locations. The University of California, Berkeley Committee for the Protection of Human Subjects approved all study procedures and we obtained informed written consent from parents upon enrollment.

Data collection

Bilingual staff administered a validated questionnaire (Bradman et al., 2006) to ascertain demographic information on children and household members as well as information on factors potentially related to exposures such as: pesticide storage and use (i.e.,

usage in the previous "0-6 days", "7-30 days", "31-90 days" and " >90 days"), occurrence of pest infestations, and timing of pesticide applications. We also conducted a home inspection to obtain information on housing quality and residential proximity to the nearest agricultural field or orchard.

Dust Sample Collection

Most dust samples were collected from carpets where parents indicated children spent time playing. In two homes with no carpets or rugs, we collected samples from upholstered furniture. Samples were collected with a High Volume Small Surface Sampler (HVS3), which collects particles >5 μm, from over an area 1 to 2 m^2 using standard protocols (ASTM, 1994). To assess within-home temporal variability, we collected two dust samples from the same general location in each home, 5-8 days apart. We sieved samples to obtain the fine fraction (<150 μm), which is more likely to adhere to human skin (Roberts et al., 2009). This fraction was stored at -80 °C until shipment to Battelle Memorial Institute in Columbus, Ohio for laboratory analysis.

Laboratory Analysis

Of the 40 homes sampled, 15 Salinas farmworker and 13 Oakland urban homes had sufficient sample mass ($\geq$ 0.5 g) for analysis after measurement of other analytes (to be presented elsewhere). We analyzed two dust samples per home except for one home in each location from which one sample was analyzed yielding a total of 54 dust samples. We measured 28 analytes including broad spectrum insecticides (OPs, pyrethroids), a pyrethroid synergist (piperonyl butoxide--PBO), an herbicide (chlorthal-dimethyl), a fungicide (iprodione), and three PBDE congeners (BDE-47, BDE-99, BDE-100). Pesticides were selected based on regional agricultural use (California Department of Pesticide Regulation; California Department of Pesticide Regulation, 2006), active ingredients in pesticides used or stored indoors, detection in our prior studies (Bradman et al., 2006; Harnly M. et al., 2009), and laboratory feasibility. The three PBDE congeners were selected because (1) they are the principal penta-PBDE formulation constituents mostly used in furniture for sale in California to comply with the state's flammability standards (California State Senate AB513), (2) they are some of the most abundant congeners found in human tissue (Hites, 2004), (3) they have been frequently detected in prior studies (Rudel et al., 2003; Schecter et al., 2005; Sharp and and Lunden, 2004; Zota A.R. et al., 2008), and (4) because of laboratory feasibility to simultaneously measure these congeners along with the selected pesticides. Analytes and limits of detection (LODs) are presented in Table 1. Select physico-chemical properties of the target analytes and information on county-level agricultural and non-agricultural pesticide use in both study locations are provided in the Supporting Information (SI) (Table S1).

A 0.5 g dust aliquot from each sample was fortified with 250 ng of two surrogate recovery standards (SRSs)--fenchlorphos and $^{13}C_{12}$-trans-permethrin--and extracted using ultrasonication in 1:1 hexane:acetone. The extract was cleaned-up using an aminopropyl solid phase extraction cartridge, concentrated to 1 mL and fortified with the internal standard, dibromobiphenyl. We analyzed extracts with an electron impact GC/MS in the multiple ion detection mode, using a Phenomenex ZB-35 column (30 m x 0.25 mm ID, 0.25 μm film) with

temperatures programmed from 130-340 °C @ 6 °C/min. We analyzed samples with seven calibration curve solutions spanning the range of 2-750 ng/mL (five times higher for deltamethrin and acephate). To facilitate analysis of the PBDEs in the same analytical run as the pesticides, we used a slightly elevated GC injector temperature (300°C). We prepared calibration curves using a linear least squares regression and the internal standard method of quantification. For QA/QC purposes, we included method solvent blanks, matrix spike samples (spike=250 ng), and duplicate study samples in each analytical run. To verify that the analytical precision was within acceptable limits, the relative percent difference of the duplicate samples was also determined for each analyte measured.

No analytes were detected in the four solvent method blanks indicating no laboratory contamination. Analyte recoveries in four randomly-selected matrix spike samples averaged 117 ± 19% for OP analytes, 115 ± 16% for pyrethroid analytes, 82 ± 5% for chlorthal-dimethyl, 112 ± 14% for iprodione, and 98 ± 10% for PBDEs; average SRS recoveries were 113 ± 6% and 128 ± 5% for fenchlorphos and $^{13}C_{12}$-trans-permethrin, respectively. The average relative percent difference in concentration for the 17 analytes detected in duplicate samples was 12 ± 16% (n=55 difference values spread across 17 analytes) indicating good analytical precision.

Data Analysis

We first summarized demographic characteristics and computed descriptive statistics for all analytes by location. For subsequent statistical analyses, we focused on analytes frequently detected (i.e., detection frequencies $\geq$50%). Concentrations <LOD were assigned a value of LOD/$\sqrt{2}$ (Hornung and Reed, 1990) and results were considered significant at $p<0.05$. We computed Spearman rank order correlations between analyte concentrations in samples collected during the first and second visits. Random effects models were used to compute between- and within-home variance and intraclass correlation coefficients (ICCs) (Rosner, 2006).

We used Fisher's Exact tests to determine if analyte detection frequencies differed between locations. To assess differences in analyte concentrations between study locations, we used linear regression models with a generalized estimating equations (GEE) approach (Hedeker D. and Gibbons R.D., 2006) which accounts for the non-independence of multiple samples collected within households. Given the limited number of homes sampled, we excluded demographic characteristics as covariates in GEE models. We also examined location differences using analyte loadings ng/m^2) (Egeghy P. et al.). We calculated loadings by multiplying analyte concentrations by the sieved fine mass and dividing by the area sampled.

To examine temporal trends, we used Wilcoxon-Mann-Whitney tests to compare chlorpyrifos and diazinon concentrations in the Salinas farmworker homes sampled for this study and concentrations in Salinas farmworker homes sampled during 2000-2002 in the same zip codes (Bradman et al., 2006; Harnly M. et al., 2009). Using the California Department of Pesticide Regulation's Pesticide Use Reporting (PUR) database (California Department of Pesticide Regulation), we also computed county-level agricultural and non-agricultural usage of these OPs during 1999-2007 to determine whether temporal changes in dust concentrations were concurrent with regional use patterns. Non-agricultural uses included applications for

landscape maintenance, public health, commodity fumigation, rights-of-way, and structural pest control applications by licensed applicators which are reported to the state. Information on the PUR system is available in the SI.

Finally, to prioritize analytes for future research and determine if exposures via the non-dietary ingestion pathway exceed U.S. EPA guidelines, we calculated hazard quotients (HQ) for non-dietary ingestion of all detected analytes. We focused on the resident children given their unique vulnerabilities to environmental toxicants (Bearer, 1995). We calculated the HQ using the following equation:

$$HQ = \frac{PDI}{RfD},$$

where PDI is the child's potential daily toxicant intake at home via non-dietary dust ingestion (mg/kg/day) and RfD is the respective toxicant chronic reference dose (mg/kg/day) (U.S. EPA, 1993). The PDI was calculated as follows:

$$PDI(mg/kg/day) = \frac{(C_{dust} \times IR)}{BW_{child}},$$

where C_{dust} is the toxicant dust concentration in the child's home (mg/g), IR is the dust intake rate--assumed to be 60 mg/day (U.S. EPA, 2008), and BW_{child} is the child's body weight (kg) obtained at the initial visit. The RfD is considered by the U.S. EPA to be an estimate of daily oral exposure likely to be without appreciable risk of adverse health effects (U.S. EPA, 2009). We used chronic RfDs because children ingest small amounts of dust every day . Chronic oral RfDs were available for 15 of the detected pesticides and two of the BDE congeners (BDE-47 and -99) (U.S. EPA, 2009). For those pesticides that have been re-registered in response to the Food and Quality Protection Act, chronic population adjusted doses (cPAD) were used as the reference dose. An HQ >1.0 would suggest that the child's exposure via non-dietary ingestion, independent of other exposure routes, may exceed the U.S. EPA's RfD.

All statistical analyses were done using Stata 10 for Windows (StataCorp, College Station, TX).

RESULTS

Household demographics and pesticide use

Demographic characteristics, unrelated to farmworker status, were similar in both study locations (SI, Table S2). All households were within 200% of the poverty line and ~50% or more of the homes had six or more household members. Most participants reported using pesticides indoors in the three months preceding the study (67% and 85% of farmworker and urban homes, respectively). Hand-held pyrethroid sprays were the most common application method in both locations and applications were mostly targeted at ants and cockroaches. Fifty-four percent versus 20% of participants living in urban and farmworker homes, respectively, reported storing pyrethroids indoors and a similar trend (46% and 27%) was reported in applications in the three months preceding the home visit. Participants from three homes (one Salinas farmworker home and two Oakland urban homes)

reported applying pyrethroid insecticides between the two sampling dates. No products with OPs pesticides were stored or reported applied in the homes, at the workplace or on pets. Most participants from Salinas households (80%) reported that farmworkers residing in the home wore their work clothing indoors and about half of them also wore their work shoes indoors. Lastly, approximately 27% of Salinas farmworker homes were located <1/4 mile from the nearest agricultural field.

Dust Levels: Trends and location differences

We detected 24 of the 28 analytes measured (Table 1). Permethrins and all three PBDE congeners were detected in every home. Of the 12 OP pesticides measured, diazinon and chlorpyrifos were most frequently detected in both locations. Other commonly detected analytes included: allethrin, cypermethrin, and PBO. Detection frequencies were only significantly different between locations for chorthal-dimethyl, which was detected solely in Salinas farmworker homes.

Median concentrations of diazinon, chlorpyrifos, permethrins, allethrin, and chlorthal-dimethyl in Salinas farmworker homes were higher than in Oakland urban homes; however, only chlorthal-dimethyl concentrations were significantly different between locations. Analyses of location differences using pesticide loadings (ng/m^2) did not change our findings (see SI, Table S3 for more information). Some of the less frequently detected analytes (e.g., tetrachlorvinphos, sumithrin) were detected with greater frequency in Salinas farmworker homes and at higher maximum concentrations than in Oakland urban homes. Conversely, the 95^{th} percentile and maximum concentrations for malathion and deltamethrin were higher among Oakland urban homes (Table 1).

For eight of eleven homes reporting home pesticide use up to < 90 days prior to the study and for which we were able to obtain active ingredient information on the formulations used and measure these ingredients in dust, we observed higher dust concentrations compared to median concentrations among other homes. For example, in one home where bifenthrin had been applied less than a week before the study, concentrations were up to 200 times higher than the median concentration observed in other homes.

We did not observe statistically significant differences in PBDE concentrations (or loadings) between the urban and farmworker homes. Concentration ranges in Salinas farmworker homes were: 185-29 158 ng/g for BDE-47, 367-43 247 ng/g for BDE-99, and 84-7493 ng/g for BDE-100. For Oakland urban homes, concentration ranges were: 301-125 632 ng/g for BDE-47, 613-218 768 ng/g for BDE-99 and 141-41 149 ng/g for BDE-100. The PBDE congener concentrations in the two homes where samples were collected directly from furniture were at the higher end of the distribution (95^{th} percentile), however maximum concentrations for two of the three congeners (BDE-99, -100) were observed in carpet dust.

Variability within and between homes

Concentrations in samples collected 5-8 days apart in the same home were significantly correlated for the most frequently detected pesticides (except allethrin) and all three PBDE congeners in homes from both locations. Spearman correlation coefficients ranged from 0.70 to 1.00 ($p<0.01$) for the frequently detected pesticides and from 0.90-0.94 ($p\leq0.0001$) for the PBDE congeners. The ICCs for the majority of the frequently detected pesticides ranged from 0.61-0.99 (except chorthal-dimethyl, ICC=0.33 in Salinas farmworker homes and allethrin, ICC=0.03 in Oakland urban homes) and from 0.92 to 0.95 for PBDE congeners, indicating that most of the variability in concentrations was due to between-household differences (see SI, Table S4 for more information).

Temporal trends of chlorpyrifos and diazinon among Salinas farmworker homes located in Monterey County

Residential use of chlorpyrifos and diazinon was discontinued between 2001 and 2004 (U.S. EPA, 2000; U.S. EPA, 2001), respectively. However, agricultural use of chlorpyrifos and diazinon in Monterey county generally increased from 1999-2007 (trendline in Figure 1), most notably for diazinon. Non-agricultural uses in Monterey County (i.e., applications for landscape maintenance, public health, commodity fumigation, rights-of-way, and structural pest control) for both of these OP pesticides was a small fraction (<6%, ≈ 20-1,500 kgs/yr) of agricultural use between 1999 and 2005, and declined further through 2006-2007 (SI, Table S5) (California Department of Pesticide Regulation). Median dust concentrations of chlorpyrifos and diazinon were 70-80% and 40-50% lower, respectively, in the present farmworker homes compared to samples we collected in 1999-2002 from farmworker homes in the same Salinas zip codes (Figure 1 and SI, Table S6). Wilcoxon Mann-Whitney tests between concentrations measured in the present study and those measured in previous years were statistically significant for all chlorpyrifos comparisons, but were only significant for diazinon for homes sampled before 2002 (Bradman et al., 2006; Harnly M. et al., 2009). These findings suggest that residential contamination by chlorpyrifos and diazinon declined due to the U.S. EPA's voluntary residential phase-out.

Hazard quotients (HQs)

For the 15 detected pesticides with RfDs, none of the hazard quotients for children's non-dietary dust ingestion exceeded 1.0 (i.e., estimated intake did not exceed the RfD) (SI, Table S7). However, the hazard quotient for non-dietary dust ingestion of BDE-47 for two children, and BDE-99 for three children, respectively, exceeded 1.0. Dust samples for these children were all collected from carpets. Notably, two children with estimated intakes exceeding the BDE-47 RfD also had intakes that exceeded the BDE-99 RfD. The estimated intake for BDE-47 ranged from 0.6 to 360 ng/kg/day (i.e. up to 3.6 times the RfD) and for BDE-99 from 1.2 to 640 ng/kg/day (i.e. up to 6.4 times the RfD). The RfD for both BDEs is 0.0001 mg/kg/day (100ng/kg/day) (U.S. EPA, 2009).

DISCUSSION

Pesticides

We consistently detected several pesticides in most homes, including OP pesticides, pyrethroids, and PBO. Biological exposure metrics for these pesticides are relatively transient and highly variable, typically reflecting recent exposures (Barr, 2008). The consistency of concentrations in dust samples collected 5-8 days apart suggests that, for some pesticides, measurements in house dust are relatively stable indicators of potential indoor exposure for this time frame.

Concentrations of OP pesticides in dust were generally lower than pyrethroids which reflect general residential use patterns. However, the absence of a difference in dust concentrations for chlorpyrifos and diazinon between homes in Salinas and in Oakland is notable given that >28 000 and 65 000 kgs of chlorpyrifos and diazinon, respectively, were applied for agricultural purposes in Monterey County in 2006 (SI, Table S1) and minimal applications (65 kgs and 3 kgs of chlorpyrifos and diazinon, respectively) occurred in Alameda County. Previously, we showed a significant correlation with local agricultural use and chlorpyrifos dust concentrations (but not diazinon) for homes throughout the Salinas Valley (Harnly M. et al., 2009). Mapping of dust concentrations and agricultural use suggests that chlorpyrifos dust concentrations are higher in the center of the Valley (south of the city of Salinas), where agricultural use is higher (Harnly M. et al., 2009). Farmworker homes in the present study were from the city of Salinas where the impact of drift from agricultural applications may have been lower. In addition, we observed significantly lower house dust concentrations of chlorpyrifos and diazinon in the present study compared to levels measured in house dust from the same zip codes between 2000-2002 (Bradman et al., 2006; Harnly M. et al., 2009), suggesting that indoor concentrations in the city of Salinas are decreasing despite continued agricultural use in the area. In New York City, air concentrations for these OP pesticides in low-income homes also significantly decreased between 2001-2004 (Whyatt et al., 2007). The temporal declines in indoor concentrations reported here and in the New York City study may reflect the decreasing usage of these OP pesticides for home or structural applications per the U.S. EPA's residential phase-out. Nonetheless, despite declining concentrations indoors, detection of these OP pesticides underscores their persistence indoors.

Similar to low-income homes in Boston, MA (Julien R et al., 2008), pyrethroids and PBO, were detected in higher concentrations and used more frequently in our study homes compared to other pesticides. Although over 19 000 kgs of permethrins were applied in Monterey County in 2006 for agricultural purposes (SI, Table S1), we did not observe significant differences in permethrin concentrations between locations. Allethrin and cypermethrin were also widely detected in most homes. We observed higher dust concentrations of these pesticides in Oakland urban homes than in the Salinas farmworker homes, which were also more than 2 times higher than concentrations reported in urban public housing units in Boston, MA (Julien R et al., 2008). Additionally, we generally observed higher dust concentrations (compared to the median concentrations) of pyrethroid pesticides in households which reported recent residential use. Our findings suggest that home use likely contributed to the presence of pyrethroid pesticides in house dust since pyrethroids were commonly used indoors and negligible to no agricultural applications took place at the county level (except for permethrin). It is also possible that structural pest control

applications influenced indoor detection of certain pyrethroids in some homes. For example, it is estimated that ~80% of the non-agricultural cypermethrin use reported in Alameda County in 2006 was for structural pest control (California Department of Pesticide Regulation, 2006). The presence of pyrethroids in house dust is also consistent with their physical and chemical properties, including high octanol:water partition coefficient values (log $K_{ow} > 4.0$) and low vapor pressures (SI, Table S1).

The detection of chlorthal-dimethyl in all Salinas farmworker homes and none of the Oakland urban homes is consistent with other Salinas Valley studies showing an association between agricultural use and house dust contamination (Harnly M. et al., 2009) and a positive correlation between outdoor and indoor air concentrations (Bradman et al., 2006). This herbicide had relatively high agricultural use (~ 33 970 kgs) in the Salinas Valley. Chlorthal-dimethyl also has a high K_{ow} value and low vapor pressure (SI, Table S1), and may be bound to particulate matter at room temperature.

Over 16 000 kgs of malathion and iprodione were used in 2006 for agricultural applications (Table S1); however, they were not commonly detected in farmworker homes from the city of Salinas. For some of these pesticides, e.g., iprodione, LODs were higher than for other analytes. Other factors including physico-chemical properties, e.g., high vapor pressure and low K_{ow} values (≤ 3), may have resulted in lower detection frequencies. These pesticides were also not frequently detected in dust samples from our previous Salinas study in the city of Salinas (Bradman et al., 2006).

PBDEs

Previous studies have reported 100 percent detection frequencies for the PBDE congeners we measured in dust (Allen et al., 2008; Harrad et al., 2008; Hwang et al., 2008; Rudel et al., 2003; Schecter et al., 2005; Sharp and and Lunden, 2004; Stapleton et al., 2005; Zota A.R. et al., 2008) and strong correlations between samples collected as much as eight months apart (Allen et al., 2008). We also found PBDEs in every home, high within-home correlations, and low intra-home variability indicating that PBDEs in dust are a stable and reliable indicator of indoor PBDE exposures provided sources of exposure do not vary over time (e.g, changes in furniture or other household products with more or less PBDEs). Median PBDE concentrations in dust for the three congeners measured in our study were up to 20 times higher than that found in homes elsewhere in the U.S. (Figure 2) (Allen et al., 2008; Harrad et al., 2008; Hwang et al., 2008; Rudel et al., 2003; Schecter et al., 2005; Sharp and and Lunden, 2004; Stapleton et al., 2005; Zota A.R. et al., 2008), substantially higher than in Europe and Asia (Harrad et al., 2008; Tan et al., 2007), and maximum concentrations for each of the three congeners (BDE-47, -99 and -100) are the highest reported to date. Summed concentrations for these three PBDE congeners ranged from 670-385 550 ng/g which is a higher range than that reported in a previous study by Stapleton et al. (290 to 23 500 ng/g) (Stapleton et al., 2005) and Schecter et al. (141-26 984 ng/g); samples collected in prior studies were obtained with commercial vacuums. Our study, however, is the first to collect samples with an HVS3 versus other standardized methods or vacuum cleaner bags, and a recent study suggests that collection method may affect measured concentrations (Allen et al., 2008). The best method to collect dust for estimating human PBDE exposure remains unknown. The high PBDE concentrations observed in California homes, including those

sampled in the present study (Figure 2), may be due to the state's stringent flammability standards (Zota A.R. et al., 2008). Although the state enacted a ban on the penta-BDE mixture that went into effect on January 2008 (California State Senate AB513), indoor exposures are likely to continue as long as the sources of exposure remain indoors.

Researchers have also hypothesized that PBDE concentrations may be higher in lower-income homes due to the presence of poorly manufactured or aging, deteriorated furniture(Zota A.R. et al., 2008); (Imm et al., 2009). Future studies that include both environmental and biological monitoring should be conducted to determine the importance of the dust exposure pathway, particularly in California where concentrations of PBDEs in dust are elevated, and to determine whether PBDE exposures vary by income.

Public Health Implications

Studies of contaminants in low-income homes, including our study, have been limited in sample size and, often, selection of participants has not been random. In addition, collection methods, analytical techniques, and timing of data collection differ. Nonetheless, the results from these studies indicate that low-income children are potentially exposed to a mixture of pesticides and PBDEs. Agricultural pesticide use may contribute to additional exposures to some pesticides in rural areas; historical or current residential use is also likely to contribute to ongoing exposures. The frequent pesticide use reported among participating households suggests there is a need to educate families on the impacts of pesticide use and effective integrated pest management strategies to control pests (Williams et al., 2006).

The fact that non-dietary ingestion exposure estimates of PBDEs for some children exceeded the U.S. EPA's RfD is of concern, particularly since we did not account for other sources or pathways of exposure (e.g., dermal absorption or dietary consumption). A recent study by Meeker et al. (Meeker et al., 2009) found that PBDE dust concentrations at substantially lower concentrations than those observed herein were associated with altered hormone levels in men. Results from recent studies and those presented herein suggest that PBDEs should be a high priority for future exposure and health research and that exposure mitigation may be warranted.

Although estimated intakes for select pesticides were below EPA RfDs (i.e., HQ < 1.0), it should not be concluded that intakes below RfDs are "acceptable" or free of any health risks. For example, recent studies have identified mechanisms of OP pesticide toxicity that were not considered in defining current U.S.EPA RfDs (Slotkin and Seidler, 2008). Moreover, RfDs do not account for differences in vulnerability to pesticides due to genetic factors, such as paraoxonase (PON1) polymorphisms (Holland et al., 2006). Additionally, our intake calculations for pesticides (and PBDEs) do not account for other exposure pathways (e.g., inhalation, dermal absorption or diet); nor did we consider that some children could have pica or other behaviors that could increase or decrease intake. RfDs were also not available for some of the compounds we measured (e.g., BDE-100). The lack of an RfD does not mean there are no potential health risks associated with exposure. Lastly, children in the homes sampled are clearly exposed to multiple indoor contaminants and our hazard evaluation does not account for exposure to complex mixtures.

Children are particularly vulnerable to contaminants in house dust due to their mouthing, crawling, and other exposure-prone behaviors in addition to their physiological

and developmental immaturity (Bearer, 1995; Roberts et al., 2009). Additional research is needed to quantify exposures and potential health effects from these compounds. Finally, such research should consider the complex mixture of chemicals found in indoor environments and include both environmental and biomonitoring measurements to assess cumulative exposures.

Supporting Information:

The Supplemental Information (SI) for this chapter provides additional information on select physico-chemical analyte properties, county-level agricultural/ non-agricultural uses on measured analytes, analyte concentration variability in homes, summary statistics on analyte loadings, background information on previous Salinas studies used to evaluate temporal dissipation of chlorpyrifos and diazinon, and statistics on hazard quotients. This information is available in the Appendix.

Chapter 2: Tables and Figures

Table 1. Limits of detection and summary statistics for dust concentrations (ng/g) in samples collected in 2006 from low-income farmworker and urban homes.[a]

		Salinas farmworker homes (n=29 samples collected from 15 homes)							Oakland urban homes (n=25 samples collected from 13 homes)						
Analyte	**LOD (ng/g)**	**DF**	**min**	**p25**	**p50**	**p75**	**p95**	**max**	**DF**	**min**	**p25**	**p50**	**p75**	**p95**	**max**
Organophosphates															
Diazinon	4	79	--	8	14	18	36	56	52	--	--	7	18	133	139
Chlorpyrifos	10	55	--	--	22	28	135	200	36	--	--	--	35	44	56
Malathion	10	7	--	--	--	--	52	71	12	--	--	--	--	877	1,158
Tetrachlorvinphos	50	10	--	--	--	--	252	271	4	--	--	--	--	--	16
Diazinon-oxon	4	ND	--	--	--	--	--	--	4	--	--	--	--	--	5
Methidathion	10	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
Methyl Parathion	10	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
Phorate	10	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
Pyrethroids															
cis-permethrin	4	100	46	85	568	908	5,925	6,297	100	12	84	291	946	21,620	26,741
trans-permethrin	4	100	88	144	952	1,380	9,175	9,688	100	18	166	504	1,622	36,431	46,776
Allethrin[b]	10	83	--	18	57	129	652	694	80	--	20	50	158	276	289
Cypermethrin[c]	20	55	--	--	230	918	4,542	13,452	64	--	--	587	1,051	5,988	13,057
Bifenthrin	10	14	--	--	--	--	24	24	44	--	--	--	45	2,049	2,117
Sumithrin	10	24	--	--	--	--	591	807	8	--	--	--	--	104	116
Deltamethrin	250	17	--	--	--	--	3,778	5,588	12	--	--	--	--	13,014	16,258
Imiprothrin	50	7	--	--	--	--	253	2,141	4	--	--	--	--	--	160
Prallethrin	2	ND	--	--	--	--	--	--	4	--	--	--	--	--	34
Esfenvalerate	50	3	--	--	--	--	--	66	ND	--	--	--	--	--	--
Other															
Piperonyl butoxide[d]	2	86	--	31	92	283	9,057	9,349	96	--	52	353	751	40,332	46,630
Chlorthal-dimethyl[e]	2	97	--	13	16	24	34	35	ND	--	--	--	--	--	--
Iprodione[f]	100	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
PBDEs															
BDE-47	10	100	185	1,493	3,103	8,262	28,461	29,158	100	301	1,334	2,779	6,910	97,052	125,632
BDE-99	10	100	367	3,128	5,482	17,117	24,261	43,247	100	613	2,241	4,454	12,062	202,403	218,768
BDE-100	10	100	84	658	1,059	3,335	4,508	7,493	100	141	464	1,049	2,432	36,550	41,149

a. Two samples were obtained from each home in both locations except for one home in each location due to inadequate sample volume. b. Reported as the sum of two isomers (cis/trans) isomers. c. Reported as the sum of four isomers. d. Insecticide synergist. e. Phthalate herbicide f. Dicarboximide fungicide. Abbreviations and notation: LOD= limit of detection; DF= detection frequency (based on the number of samples obtained); ND or `--' indicates that analyte was not detected or detected <LOD so a summary statistic is not reported.

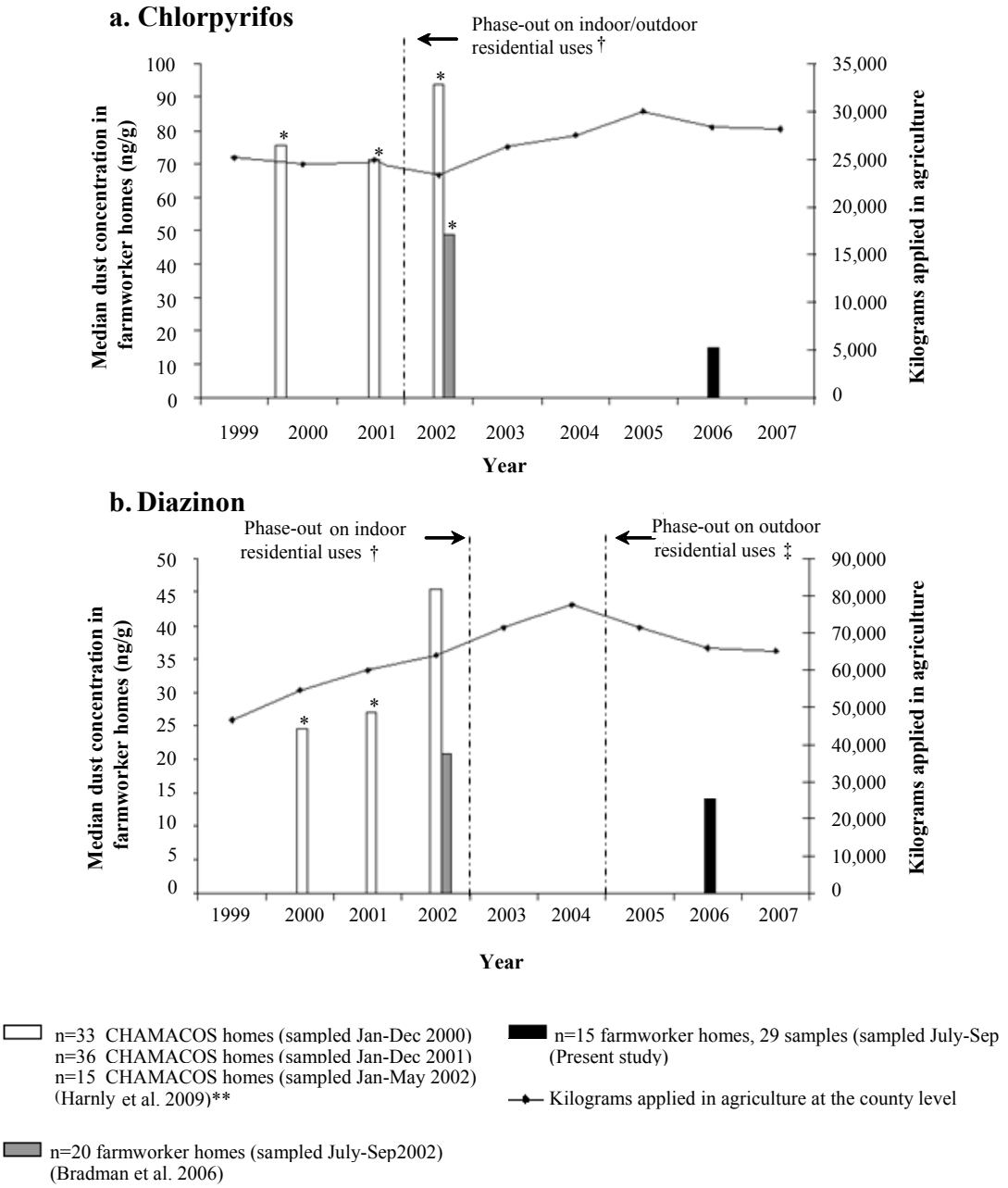


Figure 1. Median chlorpyrifos and diazinon dust concentrations in samples from farmworker homes in the city of Salinas, CA by year of collection and kilograms applied in at the county-level (Monterey County) for agricultural purposes from 1999-2007.

† In December 2001 and 2002, residential products containing chlorpyrifos and diazinon, respectively were canceled. ‡ Technical registrants were to buy back existing products from retailers by the end of December 2004. * Indicates that study had significantly higher dust concentrations compared to those observed in farmworker homes sampled in this study (Wilcoxon Mann-Whitney tests, $p \leq 0.05$). € CHAMACOS: Center for the Health Assessment of Mothers and Children of Salinas longitudinal birth cohort study evaluating the effects of environmental chemicals on maternal and child health in a farmworker population. (Harnly M., et al., 2009)

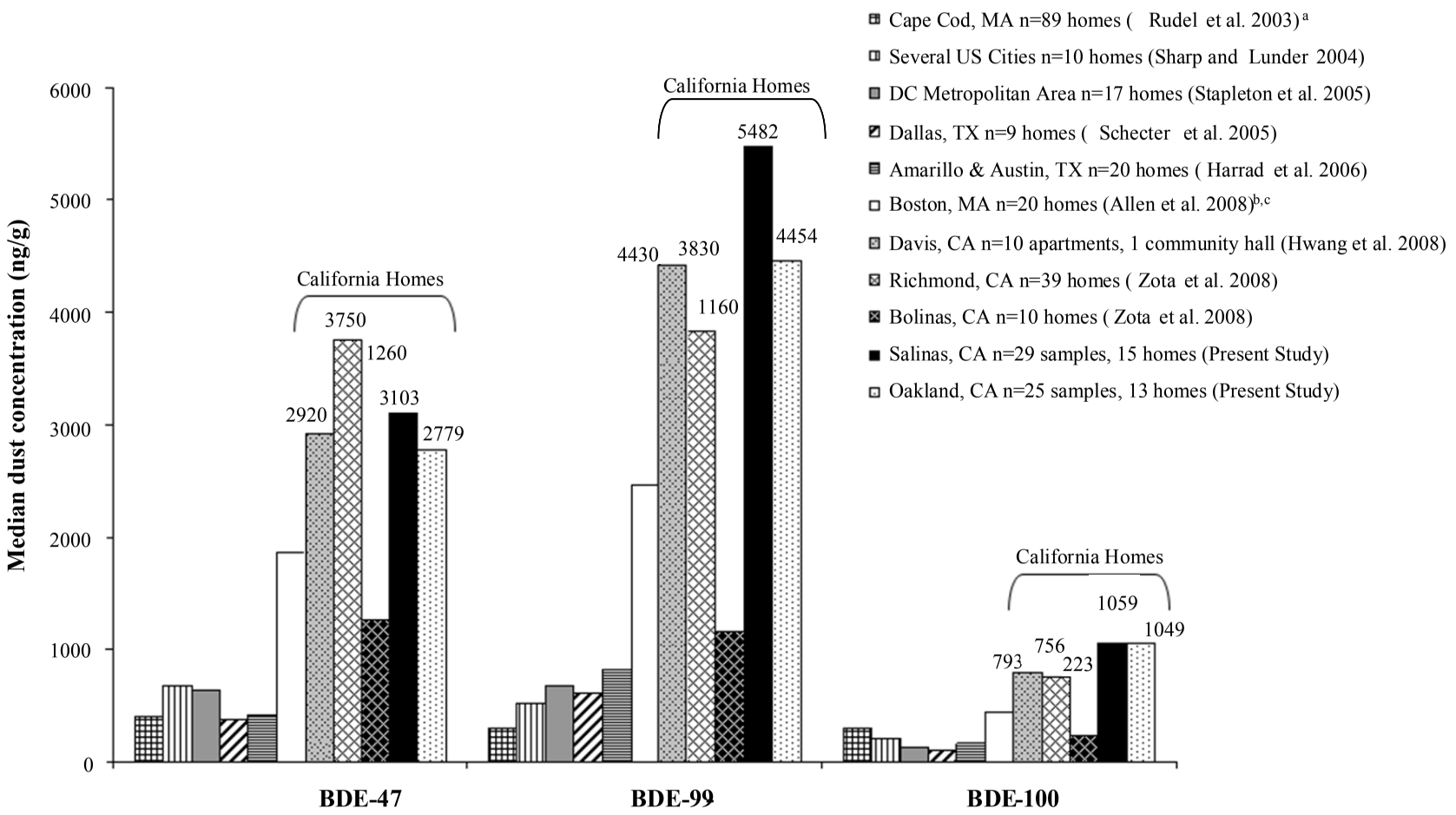


Figure 2. Median dust concentrations for PBDE congeners from select US studies. a. Median concentration was below LOD, LOD displayed as the median. b. Median concentration not provided; the geometric mean (GM) is displayed. c. Concentrations for several rooms were reported so the highest median concentration among the rooms sampled is reported.

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Chapter 3:
The presence of diakylphosphates in house dust and their association with organophosphate pesticides in house dust and urinary dialkylphosphate metabolite levels in children.

CHAPTER SUMMARY

Preformed dialkylphosphates (DAPs, urinary exposure biomarkers for organophosphate (OP) pesticides) have been found in food, but no studies have investigated their presence in indoor residential environments. We conducted a study to determine whether DAPs were present in house dust and whether they were associated with OP pesticides in house dust or DAPs in urine collected from resident children. With a single exception, we collected two dust samples from homes in farmworker and urban communities in California (20 homes per community, n=79 total samples); and up to two urine samples from resident children. We measured six DAPs in all samples (DEP, DETP, DEDTP, DMP, DMTP, and DMDTP) and eight DAP-devolving OPs in a subset of dust samples (n=54). DAPs were detected in dust with DEP being the most frequently detected in homes from both locations ($\geq$60% detection frequency at each collection); detection frequencies for the other DAPs ranged from zero to 50%. DEP dust concentrations did not significantly differ within or between communities. Dust concentrations of DEP were not correlated with dust concentrations of the two most frequently detected OP pesticides, chlorpyrifos and diazinon (Spearman ρ= -0.18 to 0.07). Further, we observed no agreement in detection between residues in dust (for DEP, chlorpyrifos, and diazinon) and respective metabolite(s) in urine (kappa coefficients= -0.33 to 0.16). Finally, estimated non-dietary ingestion intake from DEP in dust was found to be $\leq$ 5% of the dose calculated from DEP levels in urine, suggesting that dust through the non-dietary ingestion pathway is not a significant source of DAPs in urine.

INTRODUCTION

Organophosphate (OP) pesticides have been the focus of recent exposure and epidemiologic studies due to their potential adverse health effects, particularly in children (Eskenazi, Harley et al. 2004; Whyatt, Rauh et al. 2004; Bradman, Whitaker et al. 2006; Rauh, Garfinkel et al. 2006; Engel, Berkowitz et al. 2007; Eskenazi, Marks et al. 2007). Several of these studies have relied on urinary dialkylphosphate (DAP) metabolites as exposure biomarkers (Loewenherz, Fenske et al. 1997; Fenske, Kissel et al. 2000; Castorina, Bradman et al. 2003; Duggan, Charnley et al. 2003; Wessels, Barr et al. 2003; Barr, Bravo et al. 2004; Eskenazi, Harley et al. 2004; Bradman, Whitaker et al. 2006; Eskenazi, Marks et al. 2007).

DAP metabolites represent a class-specific exposure metric, which cannot be used to quantify exposure to specific OP pesticides except in acute exposure settings (Barr and Angerer 2006). Over 70% of the OP pesticides registered for use in the U.S. by the Environmental Protection Agency (EPA) will devolve to DAPs (U.S. EPA March 2010), which consist of six species: three diethyl (DE) phosphate species: diethylphosphate (DEP), diethylthiophosphate (DETP), and diethyldithiophosphate (DEDTP); and three dimethyl (DM) phosphate species: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), and dimethyldithiophosphate (DMDTP). Measurement of these metabolites in urine is often preferred over exposure biomarkers in other matrices such as in blood because sample collection is simple and noninvasive and laboratory methods are available to measure these metabolites at low detection levels (Weerasekera, Smith et al. 2009). Additionally, urinary DAPs may be detected at exposure levels below those affecting cholinesterase activity, the common mechanism of toxicity of OP pesticides (Wessels, Barr et al. 2003).

Several studies have used urinary DAP metabolites to derive biologically-based OP pesticide dose estimates by attributing metabolite levels in urine solely to OP pesticide exposure (Fenske, Kissel et al. 2000; Castorina, Bradman et al. 2003). However, OP pesticides can undergo environmental degradation or be metabolized by plants, leading to the presence of DAPs or pesticide-specific metabolites in environmental media (Racke 1992; Krieger, Dinoff et al. 2003). For example, recent studies have detected DAPs in fruit juices and produce (Lu, Bravo et al. 2005; Zhang C, Driver JH et al. 2008); and a study by Morgan et al. (Morgan, Sheldon et al. 2005) detected the specific metabolite of the OP pesticide chlorpyrifos, TCPY, in environmental samples (i.e., air, soil and dust) and food. Although DAPs have not been measured in environmental media, the presence of DAPs in food and TCPY in environmental samples indicates that urinary DAPs may represent exposure to both parent OP pesticides and the preformed degradation products present in food and environmental media. A recent study with rodents suggests that ingested DAPs may be excreted unchanged in urine (Timchalk, Busby et al. 2007). Thus, attributing urinary DAP metabolites solely to exposure to OP pesticides could lead to overestimation and misclassification of exposure for epidemiologic studies and risk assessments.

We previously developed a laboratory method to analyze DAPs in dust (Weerasekera, Smith et al. 2009). In the present study, we applied our laboratory method to: (1) determine whether DAPs are present in house dust and evaluate differences in concentrations between homes located in urban and agricultural communities; (2) evaluate the association between

OP pesticide and DAP residues in dust; and (3) determine the relationship between DAPs present in house dust and urinary DAP metabolite levels in young children.

METHODS

Study Population

The study population has been described elsewhere (Quirós-Alcalá, Bradman et al. Submitted,2010). Briefly, participants were selected from families of children participating in a 16-day biomonitoring study whose primary aim was to evaluate the effect of conventional and organic diets on pesticide urinary metabolite levels. Children followed a conventional diet for four days, then an organic diet for seven days, and returned to a conventional diet for the remaining five days. The study was conducted during July through September of 2006. We recruited a convenience sample of 20 families residing in a predominantly urban community (Oakland, CA; located in Alameda County) and 20 families residing in an agricultural community with intense agricultural OP pesticide use (Salinas, CA; located in Monterey County) from local community clinics and organizations serving low-income populations. Urban homes consisted of inner-city dwellings located more than 25 km from the nearest field where agricultural pesticide applications had been reported. A map indicating general county-level OP pesticide usage in both study locations is available in the Supporting Information (SI) section (Figure S1). Only families that did not habitually consume organic foods were selected for the study. Ethnicity was restricted to Mexican immigrants or Mexican-American families to minimize cultural disparities between populations from the two study locations. All Salinas households included at least one farmworker resident. Eligible children were toilet-trained and were between 3 and 6 years old. All study procedures were reviewed and approved by the University of California, Berkeley Committee for the Protection of Human Subjects and written informed consent was obtained from parents upon enrollment in the study.

Data Collection

Bilingual staff administered a baseline questionnaire and home inspection before sampling to collect demographic information on children and household members and information on factors potentially related to indoor pesticide contamination including occupational status of household residents, storage and residential use of OP pesticides, general housing quality, and proximity of the homes to the nearest agricultural field, orchard or golf course where pesticide applications may have taken place. Daily questionnaires were administered during the study to ensure that new exposure information was captured (e.g., use of pesticides at home or at work on the previous day).

Dust Sample Collection

We collected two dust samples in each home with a High Volume Small Surface Sampler (HVS3) (Envirometrics, Inc., Seattle, Washington). The first sample was collected during the first conventional diet phase (i.e., days 3 or 4 of the study) and the second sample was collected towards the end of the organic diet phase (5-8 days after the first sample collection). One participant was lost to follow-up prior to collection of their second sample yielding a total of 79 dust samples (40 samples from 20 farmworker homes and 39 samples from 20 urban homes). Dust samples were collected using standard collection procedures (ASTM 1994). The majority of samples were collected from carpets in areas where children spent time playing. For three agricultural homes with no carpeted areas, we collected samples from upholstered furniture using a furniture attachment on the HVS3. Samples were collected from the same general area during the second collection. The dust samples were sieved to obtain the fine fraction (<150 μm) which is more likely to adhere to human skin (Roberts, Wallace et al. 2009) and were stored at -80°C until shipped on dry ice for laboratory analysis. All 79 dust samples were analyzed individually for DAPs at the Centers for Disease Control and Prevention (CDC), National Center for Environmental Health in Atlanta, GA and those samples with $\geq$ 0.5 grams of dust remaining (n=54) were analyzed for OP pesticides at Battelle Memorial Institute in Columbus, Ohio.

Urine Sample Collection

Parents were instructed to collect children's first morning voids for 15 consecutive days (no specimens were collected on the first day of the study). If parents were not able to collect the child's first morning void then the next spot sample was collected. Children voided directly into a collection jar or into a clean, sterile Specipan™ (Baxter Scientific, McGaw Park, IL). If a Specipan™ was used, parents transferred the smple into a collection jar. For all specimens, parents, recorded the collection time, and stored the sample in a cooler with ice packs. Study staff collected urine samples from parents on each collection day and provided them with fresh ice packs and materials to collect the next day's specimen. In total, 148 first morning voids and nine random spot samples were collected for the analysis presented herein. Urine specimens were aliquoted at the field laboratory and stored at -80°C. Frozen field blanks and spikes, previously prepared by CDC, were defrosted and then re-packaged in the field according to collection procedures used for study samples. All samples were shipped on dry ice to CDC for laboratory analysis of DAPs.

Laboratory Analysis

DAPs in dust (n=79). Six DAPs (DEP, DETP, DEDTP, DMP, DMTP, and DMDTP) were measured in dust samples using a previously validated laboratory method (Weerasekera, Smith et al. 2009). Briefly, each analytical run consisted of seven calibration standards with concentrations ranging from 2 to 600 ng/g, 19-30 study samples, two blanks and two matrix QC samples (one QC low, 20 ng/g, and one QC high, 100 ng/g) (Weerasekera, Smith et al. 2009). Blanks consisted of pre-cleaned dust samples with DAP levels below the lowest calibration standard used. To analyze samples, a 1 g dust aliquot was added to the top portion

of a centrifuge filter (Whatman® Vecta Spin 20 Polypropylene Mesh centrifuge filter). All samples were then fortified with 62.5 µL of the isotopically labeled internal standard (ISTD) solution. The ISTD solution consisted of DMP (dimethyl-$^{2}H_{6}$), DEP (diethyl-$^{2}H_{10}$), DMTP (dimethyl-$^{2}H_{6}$), DMDTP (dimethyl-$^{2}H_{6}$), DETP (diethyl-$^{2}H_{10}$), and DEDTP (diethyl-$^{13}C_{4}$). A 3 mL phosphate buffer (pH 7.0) was then added to each sample. Samples were then shaken to homogenize the contents and centrifuged at 2500 × *g* for 5 min. A second phosphate buffer extraction was performed repeating this process. The pH of the dust buffer extract was then adjusted to 2.0 by adding formic acid (FA). For sample cleanup, solid phase extraction (SPE) cartridges were conditioned with 3 mL of 1% FA in methanol and then with 3 mL of 1% FA in water. All samples were then loaded into the SPE cartridges and a vacuum was applied for 30 sec. Cartridges were washed with 1% FA in methanol. After application of a vacuum for 5 min., cartridges were eluted twice with a 2 mL 20% triethylamine (TEA) in acetonitrile (ACN) solution into clean centrifuge tubes. These samples were then concentrated to dryness at 40 °C. The dried residue was reconstituted in 1 mL of ACN and vortex-mixed for 15 sec. K_2CO_3 (10 mg) and 50 µL of 1-chloro-3-iodopropane were added to each sample to form chloropropyl esters of each DAP, and then placed in a dry bath set at 60 °C for three hours. Samples were then centrifuged for 5 minutes, decanted into new 15 mL centrifuge tubes and concentrated to dryness at 40 °C. This dry residue was reconstituted in toluene (100 µL), vortex-mixed, and transferred to autosampler vials. Derivatized extracts were analyzed by isotope dilution gas chromatography-tandem mass spectrometry (GC-MS/MS) according to the method of Bravo et al. (Bravo, Caltabiano et al. 2004). No DAPs were present in any blank dust samples indicating that there was no contamination during laboratory sample processing. The relative standard deviation (RSD) for DE DAPs ranged from 5.9-14.4% for QC high samples and from 0.6-21.1% for QC low samples. For DM DAPs, RSDs ranged from 5.0-8.8% for QC high samples and from 9.3-17.1% for QC low samples. The limits of detection (LOD) for DE DAPs were 10.4 ng/g, 5.8 ng/g, and 5.2 ng/g for DEP, DETP and DEDTP, respectively, and for DM DAPs, 4.8 ng/g, 2.8 ng/g, and 9.9 ng/g for DMP, DMTP, and DMDTP, respectively.

OP pesticides in dust (n=54). Of the 40 households sampled, 15 agricultural homes and 13 urban homes had adequate dust sample volumes (≥ 0.5 grams) for analysis of OP pesticides after initial analysis of DAPs. For the majority of these homes, we were able to analyze OP pesticides in samples collected at each time point, except for one home in each location for which we only measured OP pesticides in one sample. There were no demographic or housing differences in those homes with adequate vs. inadequate volume of remaining dust. Target OP pesticides were selected based on active ingredients in household products stored or used indoors, laboratory feasibility, and county-level agricultural and non-agricultural pesticide use in both study locations as reported in the California Pesticide Use Reporting Database (California Department of Pesticide Regulation ; California Department of Pesticide Regulation 2006). Table 1 presents DAP-devolving parent OP pesticides and respective degradation products and/or metabolites along with general usage at the county-level where our study homes were located. We measured eight DAP-devolving OP pesticides. Target OP pesticides and respective LODs included four DM-devolving OPs: malathion (10 ng/g), methidathion (10 ng/g), methyl parathion (10 ng/g), and tetrachlorvinphos (10 ng/g) and four

DE-devolving OPs: chlorpyrifos (10 ng/g), diazinon (4 ng/g), diazinon-oxon (4 ng/g), and phorate (10 ng/g). Diazinon-oxon is not used as a pesticide, but is an intermediary breakdown product of its precursor compound diazinon; it is also a precursor of DEP and its respective specific degradates.

Samples were extracted and cleaned with an SPE cartridge, concentrated to 1 mL and fortified with the internal standard, dibromobiphenyl, and analyzed using electron impact GC/MS in the multiple ion detection mode, using a Phenomenex ZB-35 column (30 m x 0.25 mm ID, 0.25 μm film) with the temperature programmed from 130-340 °C @ 6 °C/min. Seven calibration curve solutions were used in each analytical run. No analytes were detected in the four solvent method blanks indicating that there was no contamination during laboratory sample processing. Analyte recoveries (spike=250 ng) in the four randomly-selected matrix spike samples averaged 117±19% for OP pesticides. The average relative percent difference in concentration for the two OP pesticides detected in duplicate samples, chlorpyrifos and diazinon, was 8±10% (n=6 analyte pairs in duplicate samples). For additional details on pesticide measurement methods and QA/QC, see Chapter 2.

DAPs in urine (n=157). As noted earlier, the six DAPs (DEP, DETP, DEDTP, DMP, DMTP, and DMDTP) were measured in children's urine samples using a previously validated method (Bravo, Caltabiano et al. 2004). Briefly, urine specimens were lyophilized to remove water and the remaining residue redissolved in acetonitrile:diethyl ether (1:1). DAPs were then derivatized to their chloropropyl phosphate esters and concentrated extracts were analyzed by isotope dilution GC-MS/MS. Analytical QC procedures included repeat analysis of three in-house urine pools enriched with known amounts of pesticide residues whose target values and confidence limits were previously determined. Westgard rules for quality control were used to validate each analytical run (Westgard 2003). LODs for DAP metabolites were as follows: 0.2 μg/L, 0.1μg/L, and 0.1μg/L for DEP, DETP, and DEDTP, respectively; and 0.6 μg/L, 0.2 μg/L, and 0.1 μg/L for DMP, DMTP, and DMDTP, respectively. Creatinine concentration (mg/dL) in each specimen was determined with a commercially available method (Vitros CREA slides, Ortho Clinical Diagnostics, Raritan, NJ, USA). In addition to the use of field QC samples (blank and spiked samples), we also analyzed duplicate urine samples to assess the precision of our analytical runs. No DAP metabolites were present in any blank samples indicating that no contamination occurred in the field, during sample processing, or during shipment to the laboratory.

Data Analysis

We used Fisher Exact tests to determine whether there were any differences in demographic characteristics between participants from urban and agricultural homes. We calculated detection frequencies (DFs) and descriptive statistics for each analyte in both dust and urine samples stratified by location and collection time point (i.e., conventional and organic diet phase). For subsequent statistical analyses, we focused on the most frequently detected DAPs and OP pesticides in dust (i.e., DF$\geq$50% in at least one location). Analyte concentrations below the LOD were assigned a value of LOD/$\sqrt{2}$ for statistical analyses. Results were considered statistically significant at $p<0.05$.

DAP dust concentrations within and between homes. We performed Wilcoxon signed-rank tests to determine if dust DAP concentrations differed significantly between collection time points. We computed Spearman rank-order correlations and intraclass correlation coefficients (ICCs) (Rosner 2006) to evaluate the correlation and within- and between-home variability of DAP concentrations in house dust (an ICC=1 would indicate that all of the variability in concentrations is due to between-household differences while an ICC=0 would indicate that all of the variability in concentrations is due to within-household differences and that more measurements may be needed to properly characterize exposure). To determine if dust DAP concentrations significantly differed between agricultural and urban homes at each collection we used Wilcoxon rank-sum tests.

Relationship between OP pesticide and DAP residues in dust. We used a Spearman rank-order correlation procedure to assess the relation between OP pesticide and DAP residues in dust at each collection. We also computed the molar ratio of DAPs:OP pesticides in each dust sample. The molar ratio was calculated as follows:

$$\frac{\sum DAPs(nmol/gdust)}{\sum OPs(nmol/gdust)},$$

where ∑DAPs represents either measured DM or DE phosphates and ∑OPs represents the molar sum of the corresponding DM- or DE-devolving parent OP pesticide residues in dust. Both Spearman correlations and molar ratios were calculated for the most frequently detected (DF>50%) DAPs and OP pesticides in dust.

Association between residues in dust (OPs and DAPs) and DAPs in children's urine. Because OP pesticides are rapidly metabolized and excreted (Barr 2008) and no published human data are available on the metabolism of preformed DAPs, statistical analyses focused on urine samples collected on the same day (n=79) and on the day after (n=78) dust sample collection. All analyses to determine the relationships between dust residues (OPs and DAPs) and DAPs in urine were performed twice: once using urine samples collected on the day of dust sample collection and again using urine samples collected on the day after dust sample collection. We examined the relationship between frequently detected OP pesticides (chlorpyrifos and diazinon) and DEP in dust (i.e., OP pesticides and DAPs with DF ≥50% at both collections in at least one location) with respective DAPs in urine three ways:

(1) For each collection time point, we used a Spearman-rank order correlation procedure to determine the relation between DEP in dust and DEP in urine. We restricted these correlations to children with detectable DEP concentrations in both media (13 children at the first collection and 17 children at the second collection). Because the number of children with concurrent detection of parent OP pesticides in dust and respective metabolites in urine was small (n<9) we did not calculate correlations between frequently detected OP pesticides in dust and respective urinary metabolites.

(2) We determined the level of agreement in DEP, chlorpyrifos and diazinon detection in dust and detection of respective metabolites in urine at each collection time point, by calculating Cohen's Kappa coefficients (where 1=perfect agreement, 0=no agreement above that expected by chance, and −1=perfect disagreement). For this analysis, measured concentrations in each media were categorized as ≥LOD or <LOD.

(3) We constructed longitudinal logistic regression models using a generalized estimated equations approach (GEE) (Rosner 2006) where the dependent variable was detection of metabolites in urine (DEP or DEP+DETP considered in separate models) (0/1) and the independent variable was considered in separate models as the detection of (i) DEP in dust (categorized as ≥LOD or <LOD), (ii) detection of each precursor OP pesticide (chlorpyrifos and diazinon considered in separate models and categorized as ≥LOD or <LOD), (iii) concurrent detection of both parent precursor OP pesticides (categorized as both ≥LOD or either one or both of them <LOD), (iii) detection of all frequently detected analytes in dust (chlorpyrifos + diazinon + DEP, categorized as all of the analytes ≥LOD or one or more of these analytes <LOD). Detection of DEP+DETP in urine was not considered in the model where detection of DEP in dust was the main predictor as metabolism of DEP is not likely to result in DEP in urine. In each model, we controlled for location and diet (conventional or organic) at the time of dust and urine sample collection. For all GEE models, we used an exchangeable working correlation structure with robust inference and used the xtgee command in Stata 10 for Windows (StataCorp LP, College Station, TX) to run the models. Further details on these GEE logistic regression models are provided in the SI section.

DEP dose estimation. We estimated the potential contribution of the most frequently detected DAP in dust, DEP, via the non-dietary ingestion pathway to the estimated DEP dose predicted from the individual DEP urine concentrations. This was done by calculating the ratio of non-dietary DEP intake from dust to the DEP dose calculated from urine concentrations. To estimate non-dietary intake, we used individual DEP dust concentrations (ng/g) observed in homes, assumed a dust ingestion rate of 0.100 g/day (U.S. EPA) and 100% absorption of the dose based on animal data (Timchalk, Busby et al. 2007). Children's body weights (kg) were obtained during the baseline questionnaire and were used in our intake calculations. We calculated intake by multiplying the DEP concentration in dust by the dust ingestion rate and then dividing by the child's body weight. Intake was calculated for those children with detectable levels of DEP in urine samples (n=32 children, 46 urine samples with detectable levels of DEP).

To estimate the children's total daily DEP dose (D_{Urine_DEP}, ng/kg/day), we used the following equation:

$$D_{Urine_DEP}(ng/kg/day) = C_{urine}(nmol/L) \times \frac{Cre_{24}(mg/day)}{Cre_i(mg/L)} \times MW(ng/nmol) \times \frac{1}{BW(kg)}$$

where, C_{urine} is the DEP concentration in urine in nmol/L and Cre_{24} is the estimated daily creatinine excretion in mg/day based on the child's sex and age. To estimate Cre_{24} we used 24-hour creatinine excretion data obtained from a study we conducted on low-income Mexican-American children between 3 and 6 years of age (personal communication). Based on that study, we assigned the mean daily age- and sex-specific creatinine excretion rate to the children in our study. Cre_i is the creatinine concentration in the child's urine sample in mg/L; creatinine was was originally reported by the lab in units of mg/dL; however, for each child, we multiplied their urine sample creatinine by a factor of (1L/10dL) to get creatinine concentration in units of mg/L. MW is the molecular weight for DEP in ng/nmol (154 ng/nmol), and BW is the child-specific body weight in kilograms. Our estimates were based on the following assumptions: (1) DEP metabolite concentrations in urine voids were representative of steady state conditions; and (2) 100% of the absorbed DEP dose from DE-devolving parent OP pesticides and DE-DAPs was expressed in urine as DEP. Available information on the elimination rate of DEP in urine suggests that the above equation provides an adequate, albeit uncertain, estimate of DEP intake in the previous 24 hours. In a recent rodent study by Timchalk et al. (Timchalk, Busby et al. 2007), approximately 86% of a DEP dose was eliminated in urine according to a first-order process with a half time of 13 hours. If one assumes (i) constant intake of DEP during 14 waking hours, (ii) no intake during 10 sleeping hours, (iii) a constant excretion rate of creatinine, and (iv) the elimination parameters observed in the rodent study, it can be shown that the equation provides an estimate of 24-hour DEP intake about 10% less than the actual intake.

We performed all statistical analyses using Stata 10 for Windows (StataCorp LP, College Station, TX).

RESULTS

Population and Household Characteristics

Table 2 summarizes select household characteristics for participants. The income for all households was $\leq$ 200% of the U.S. poverty level and the majority of the children's parents had completed less than 10 years of education. Most participants reported applying a pesticide in the home at least 3 months prior to sample collection (~60% of agricultural households and ~80% of urban households, not shown). One agricultural participant reported applying the insecticide tetrachlorvinphos (a DMP-devolving OP pesticide) for flea treatment on the house pet 7-30 days preceding sample collection. No other home OP pesticide use was reported. The majority of agricultural households had 1-3 farmworkers living in the home and lived more than ¼ mile from the nearest agricultural field or orchard. Other than occupation, demographic characteristics were similar in the two study locations.

Detection, concentrations, and variability of DAPs in dust for agricultural and urban homes

DEP and DMP were the most frequently detected DAPs in dust samples in both locations (Table 3). DEP was detected in ≥60% of the samples at each collection and in both locations, while DMP was detected in ≥45% of the samples at each collection in agricultural homes. In urban homes, we detected DMP in 40% and 26% of the samples collected at the first and second time point, respectively. Other DAPs were not detected or detected at much lower frequencies. For example, DETP and DEDTP were not detected in any urban homes while DMTP was not detected in any agricultural homes. For the participant in the agricultural home who reported applying tetrachlorvinphos on their pet, we only detected DMP in only one of the two samples collected; the DMP concentration (44 ng/g) was above the 95th percentile concentration observed among all samples in agricultural homes.

For DEP, the most frequently detected DE-DAP, the overall detection frequency in dust was 65% for agricultural homes and 67% for urban homes. Among all samples in each location, median DEP concentrations were slightly higher in the urban homes (47 ng/g) compared to agricultural homes (35 ng/g). However, the maximum DEP concentrations were higher in agricultural homes (859 ng/g) than in urban homes (316 ng/g). For DMP, the most frequently detected DM-DAP, the overall detection frequency was 48% and 33% among agricultural and urban homes, respectively. Median DMP concentrations were below the LOD in both locations, but maximum concentrations were higher in urban (1588 ng/g) than in agricultural (806 ng/g) homes.

Given DEP was the only DAP with a DF > 50% in both locations, we report subsequent dust analyses only for DEP. DEP dust concentrations observed during the first collection were not significantly different than concentrations observed during the second collection by location or overall (Wilcoxon-Mann Whitney, $p>0.05$). DEP concentrations within homes were moderately correlated in agricultural homes (Spearman $\rho=0.49$, $p=0.03$) with concentrations varying more between than within homes (ICC=0.61). However, when we removed one influential point, the correlation became weaker (Spearman rho=0.39, $p=0.09$) and the ICC decreased (ICC=0.41) indicating more variability within than between homes. A weak correlation was observed for DEP concentrations within urban homes (Spearman rho=0.28, $p=0.25$) where concentrations were observed to vary more within than between homes (ICC=0.36). We also found that DEP dust concentrations did not significantly differ between urban and agricultural homes at each collection (Wilcoxon rank-sum tests, not shown).

Association between OP pesticides and DAPs in dust

We detected several OP pesticides in dust samples from both locations, including two DE-devolving OP pesticides: diazinon and chlorpyrifos, one DE-devolving OP intermediary product, diazinon-oxon, and two DM-devolving OP pesticides: tetrachlorvinphos and malathion (Table 4). (For the participant who applied tetrachlorvinphos on their house pet, we were not able to analyze their dust sample for OP pesticides due to insufficient sample mass after analysis of DAPs.) Diazinon and chlorpyrifos were the only OP pesticides with detection frequencies ≥50% in at least one location. Environmental degradation of these DE-devolving OP pesticides could result in the presence of DEP and DETP. Given we did not

detect DETP in the majority of dust samples (i.e., DETP was only detected in two agricultural homes), we restricted subsequent analyses and comparisons between concentrations of diazinon and chlorpyrifos to DEP residues in dust.

Diazinon concentrations in dust were not significantly correlated with DEP dust concentrations among urban and agricultural homes or overall at each collection (Spearman ρ= -0.02 to 0.07; Table 5). Similarly, chlorpyrifos concentrations in dust from agricultural homes were not correlated with DEP concentrations in dust (Spearman ρ= -0.18 to -0.03; Table 5); correlations between DEP and chlorpyrifos in urban homes were not conducted because detection frequency of chlorpyrifos in these homes was <50% at each collection. We also observed wide variation in the mole ratio of DEP to the molar sum of chlorpyrifos and diazinon. Median and maximum DEP mole ratios were 2.2 and 24.1, respectively, for samples from agricultural homes and 1.6 and 70.0, respectively, for samples from urban homes. Sixty-six percent and 76% of the dust samples analyzed in agricultural and urban homes, respectively, had mole ratios >1 indicating that concentrations for DEP were generally greater than concentrations for diazinon and chlorpyrifos combined.

Association between OP pesticides and DAPs in dust and DAPs in children's urine

Because the inference of our findings did not differ based on whether we examined the association of analytes in dust and DAPs in urine samples collected on the same day or the day after dust collection, we present results related to urine samples collected on the same day as dust collection. Table 6 presents summary statistics for DAPs in urine by location and collection time point. Overall, the most frequently detected DEs in urine were DEP and DETP. DEP was detected more frequently in urine samples from urban children compared to samples from agricultural children ($\geq$ 65% vs. < 50% detection frequency, respectively) while the opposite was observed for DETP ($\leq$60% vs. $\leq$65%, respectively). Maximum urinary DEP concentrations were higher in agricultural children (401 nmol/L) compared to urban children (181 nmol/L); while maximum DETP concentrations were higher in urban children (169 nmol/L) compared to agricultural children (104 nmol/L). The DMs most frequently detected in urine included DMTP followed by DMP. DMP was detected more frequently in urine samples from urban children compared to agricultural children ($\geq$ 60% vs. < 50 % detection frequency, respectively) while we observed comparable DMTP detection frequencies in urine samples from children in both locations (DF >80%). Maximum urinary DMP concentrations were comparable for agricultural and urban children (227 and 223 nmol/L, respectively), while maximum DMTP concentrations were higher in agricultural children (993 nmol/L) compared to urban children (777 nmol/L). We also found that higher molecular weight DEs and DMs such as DETP and DMTP were frequently detected in urine, although they were not frequently detected in dust.

Detection of DEP in dust and same day urine samples was observed for 13 (five agricultural and 8 urban) and 17 (seven agricultural and 10 urban) children at each collection, respectively. However, these DEP residues in dust were not significantly correlated with urinary DEP metabolite concentrations at either collection time point (Spearman ρ=-0.09 and -0.15, respectively, $p>0.55$). Additionally, we did not observe agreement in detection for frequently detected analytes in dust (chlorpyrifos, diazinon, and DEP) and respective metabolites in urine (Table 7); Kappa coefficients ranged from -0.33 to 0.16 ($p\geq0.12$)

indicating no agreement in detection between media. Results from logistic regression models also confirmed that detection of analytes in dust did not increase the odds of detecting respective urinary metabolites (not shown).

Finally, the estimated potential non-dietary ingestion intake of DEP ranged between 0.02 to 3 ng/kg/day, accounting for 0% to 5 % of the overall dose estimated from urinary DEP concentrations. The distribution of concentrations for total DEs (molar sum of DEP, DETP and DEDTP) and total DMs (molar sum of DMP, DMTP and DMDTP) was also found to differ between media (Figure 1). For example, total DAPs (molar sum of total DEs + total DMs) in children's urine consisted mostly of total DMs; however, total DAPs in dust samples consisted mostly of total DEs. Results suggest that DAPs in dust via the non-dietary ingestion exposure pathway do not seem to represent a significant source of DAPs in urine.

DISCUSSION

Urinary DAP metabolites have been widely used to assess human OP pesticide exposure in epidemiologic and biomonitoring studies. However, the validity of their use as biomarkers of exposure has been questioned due to recent studies reporting detection of an OP urinary metabolite in environmental and food media (Morgan, Sheldon et al. 2005) and preformed DAPs in fruit juices and produce (Lu, Bravo et al. 2005; Zhang C, Driver JH et al. 2008). The present study is the first to report detection of DAPs in house dust from homes located in agricultural and urban communities. We also report no association between DAP residues in dust with parent OP pesticide residues in dust nor with detection of DAPs in urine from young children residing in those homes.

DEP was the most frequently detected DAP in dust samples from both the urban and agricultural homes and concentrations within homes did not differ significantly between collections. Other DAPs were not detected or detected at much lower detection frequencies (e.g., higher molecular weight DAPs like DETP, DEDTP, DMTP and DMDTP). It is not likely that degradation of these higher molecular weight DAPs occurred during sample storage, processing or laboratory analysis (Weerasekera, Smith et al. 2009). The low detection frequency of higher molecular weight DAPs in dust suggests that these compounds may have degraded to the lower molecular weight species (e.g., DEP) in the environment. Consistent with this finding, Zhang et al. reported an increase in DMP residues and a decrease in DMTP residues over time in strawberries treated with malathion (Zhang C, Driver JH et al. 2008). Information on the environmental fate of OP pesticide degradation products is scarce; research in this area could help inform future pesticide exposure studies.

Higher OP pesticide dust concentrations have been reported in agricultural homes compared to non-agricultural homes (Simcox, Fenske et al. 1995). Thus, we hypothesized that OP pesticide degradation in the environment would result in higher DAP concentrations in dust from agricultural homes compared to urban homes. However, we did not observe significant differences in detection frequencies or median concentrations for DEP in dust between locations despite intense agricultural OP pesticide use in the agricultural community. Comparable detection frequencies and median concentrations for DEP in dust in homes from both locations suggest that other sources may also be responsible for its presence indoors. One

possible source could be historical residential use of DE-devolving OP pesticides. General pesticide use was prevalent in homes from both locations and while no participants reported residential applications of DE-devolving OP pesticides in the three months preceding the study, we do not have information on prior use or on the persistence of DAPs in the environment after application of OP pesticides. Although chlorpyrifos and diazinon were voluntarily phased-out for residential uses by the U.S. EPA by the end of 2001 and 2004 (U.S. EPA 2000; U.S. EPA 2001), respectively, these DE-devolving OPs were the most frequently detected OPs in house dust from participants' homes. If DAPs persist in the environment then application of these DE-devolving OP pesticides prior to our study could explain detection of DEP indoors. It is also not known whether DEP or other preformed DAPs could arise from other household or industrial chemicals.

We found that detection of DEP or frequently detected parent OP pesticides in dust were not associated with detection of respective urinary DAP metabolites, and that DEP in dust does not contribute more than 5% of the DEP excreted in urine. These findings suggest that this non-dietary ingestion of DAPs in dust may not significantly impact urinary DAP metabolite levels. Additionally, as observed in previous studies, (CDC 2005; Fenske, Lu et al. 2005) we found that total DAPs in urine consisted mostly of total DMs; however, this was not the case in our dust samples where total DAPs consisted mostly of total DEs. Similarly, DMTP was frequently detected in urine samples in our study population, but not in our dust samples. These findings suggest that DAPs might break down differently in the body than in the environment and that other sources and routes of exposure may be more important contributors of DAPs in urine.

Currently, it is not known whether a person who is exposed to preformed DAPs will excrete them unchanged or if further metabolism occurs. A recent study by Timchalk et al. (Timchalk, Busby et al. 2007) reported that oral doses of DEP and DETP to rats were well absorbed and excreted unchanged in the urine. However, Imaizumi et al. (Imaizumi H, Nagamatsu K et al. 1993) found that dosing rats with high doses of DAP compounds resulted in inorganic phosphate in the urine, suggesting further metabolism. More research is clearly needed on the pharmacokinetics and toxicodynamics of preformed DAPs and other specific OP metabolites to determine the extent of their contribution to urinary biomarkers in humans.

This study has several limitations. The sample size was small, limiting statistical power. Additionally, the results may not be generalizable to other populations given we focused on low-income Mexican-American families which may have different patterns of residential pesticide usage and/or other genetic factors influencing metabolism and excretion of OP pesticides and preformed DAPs. Another limitation is that we were not able to measure every DAP-devolving OP pesticide in dust that could have led to detection of DAPs in dust and/or urine. For example, the DM-devolving OP pesticide, oxydemeton-methyl was heavily used (> 30,000 kgs applied for crops) for agricultural purposes in the county where our agricultural homes were located. However, we did measure most DE-devolving OP pesticides that could have led to DEs in dust, at least in urban homes; chlorpyrifos and diazinon alone made up approximately 97% and 100% of the DE-devolving OP pesticides used at the county-level where our agricultural and urban homes were located, respectively. Additionally, we did not measure parent OP pesticides and DAPs in food or other environmental media (e.g. soil and air) through which children may have been exposed (Morgan, Sheldon et al. 2005). Measurement of both parent OP pesticides and respective DAPs in food and

environmental samples is necessary to determine the relative contribution of OP pesticides and DAPs from all sources to DAPs in urine. We also did not consider exposure to DEP in dust resulting from dermal absorption as no hand wipes or surface wipes on objects which children may come into contact with (e.g., toys) were collected nor did we consider DEP intake as a result of resuspended particles as no air measurements were collected and information on the volatilization and deposition rate of DEP in dust is unknown. Lastly, our exposure estimates were based on several assumptions that may under- or overestimate true exposure to DAPs. For instance, if a child were to ingest more than 0.100 g/day of dust, our potential daily intakes would underestimate exposure through the non-dietary ingestion pathway. However, the lack of agreement in detection for residues in dust for frequently detected OP pesticides and DEP with respective metabolites in urine suggests that the non-dietary ingestion pathway does not significantly impact metabolite levels in urine.

In summary, we have documented the presence of preformed DAPs in house dust from agricultural and urban homes. Our findings suggest that preformed DAPs in dust (via ingestion) are not a significant source of DAPs in urine. However, it is still possible that other sources (e.g., diet) of preformed DAPs may contribute to urinary DAPs (Krieger, Dinoff et al. 2003; Lu, Bravo et al. 2005; Morgan, Sheldon et al. 2005). Future analysis in this study population will include more in depth assessment of the effects of diet on urinary pesticide metabolite concentrations.

Supporting Information (SI)

Supporting information is provided for this chapter on (i) Maps on general county-level OP pesticide use in both study locations (Figure S1), (ii) general statistics on the mole ratio of DEP to OP pesticides (Table S1), (iii) results for kappa statistics for all samples (Table S2), and (iv) results from GEE logistic regression models (Table S3) and explanation of models. This information is available in the Appendix.

Chapter 3: Tables and Figures

Table 1. DAP-devolving OP pesticide compounds, respective dialkylphosphate degradation products, and amount of OP pesticide applied at the county level in the two study locations during the year in which samples were collected.

OP Parent Compound	Potential environmental degradates and/or metabolites [a]	Amount applied at the county level in 2006 (kgs) in the locations sampled.[d]	
		Monterey County (predominantly agricultural region; location of Salinas homes) Ag use(non-ag use)[e]	Alameda County (predominantly urban region; location of Oakland homes) Ag use(non-ag use)[e]
Azinphos-methyl	DMP, DMTP, DMDTP (BTA, MSMB)	2(0)	N/A
Chlorethoxyphos	DEP, DETP	N/A	N/A
Chlorpyrifos	DEP, DETP (3,5,6-TCPY)	27,959(126)	62(6)
Chlorpyrifos-methyl	DMP, DMTP (3,5,6-TCPY)	N/A	N/A
Coumafos	DEP, DETP (CMHC)	N/A	N/A
Diazinon	DEP, DETP (IMPY)	65,268(24)	0(5)
Dichlorvos[b]	DMP	N/A	N/A
Dicrotophos[c]	DMP	N/A	N/A
Dimethoate	DMP, DMTP, DMDTP	16,024(8)	36(0)
Disulfoton	DEP, DETP, DEDTP	2,161(0)	0(<1)
Ethion[b]	DEP, DETP, DEDTP (p-Nitrophenol)	N/A	N/A
Fenitrothion	DMP, DMTP	N/A	N/A
Fenthion[b]	DMP, DMTP	N/A	N/A
Isazofos-methyl[b]	DMP, DMTP	N/A	N/A
Malathion	DMP, DMTP, DMDTP (Malathion dicarboxylic acid, Malathion mon-carboxylic acid)	16,686(120)	0(577)
Methidathion	DMP, DMTP, DMDTP	3,287(0)	N/A
Methyl Parathion	DMP, DMTP (p-Nitrophenol)	93(0)	0(11)
Naled	DMP	6,968(12)	N/A
Oxydemeton-methyl	DMP, DMTP	32,215(0)	N/A
Parathion	DEP, DETP	N/A	N/A
Phorate	DEP, DETP, DEDTP	274(0)	N/A
Phosmet	DMP, DMTP, DMDTP	32(0)	N/A
Pirimiphos-methyl	DMP, DMTP (DEAMPY)	N/A	N/A
Sulfotepp[b]	DEP, DETP	N/A	N/A
Temephos	DMP, DMTP	N/A	N/A
Terbufos	DEP, DETP, DEDTP	N/A	N/A
Tetrachlorvinphos	DMP	N/A	N/A
Trichlorfon[b]	DMP	N/A	N/A
Total amount of Diethyl-devolving OP pesticides applied		95,812	73
Total amount of Dimethyl-devolving OP pesticides applied		75,447	624
Total amount of OP pesticides applied		171,259	697

a. Specific metabolites/degradates to which parent OP pesticides may devolve into are included in parenthesis. b. Uses for this OP pesticide were canceled in the U.S. by the EPA. Source: http://www.epa.gov/pesticides/reregistration/status_op.htm. c. Registered for use in the U.S. but not for use in California. Source: http://www.epa.gov/pesticides/reregistration/status_op.htm. d. Source: California Department of Pesticide Regulation Pesticide Use Reporting Database. Available at: http://www.cdpr.ca.gov/docs/pur/-pur06rep/06_pur.htm e. Non-agricultural (non-ag) uses refers to applications for landscape maintenance, public health, commodity fumigation, rights-of-way, and structural pest control applications by licensed applicators reported to the state of California. Abbreviations: DEP=diethyphosphate, DETP= diethylthiophosphate, DEDTP= diethyldithiophosphate, DMP=dimethyphosphate, DMTP= dimethylthiophosphate, DMDTP= dimethyldithiophosphate, BTA=1,2,3-benzotriazin-4(3*H*)-one/ol; MSMB= methy-sulfonylmethylbenzazimide; TCPY=3,5,6-trichloro-2-pyridinol; CIT=5-chloro-1-isopropyl-(1*H*)-1,2,4-triazol-3-ol/one; CMHC= 3-chloro-7-hydroxy-4-methyl-2*H*-chromen-2-one/ol; DEAMPY= 2-(diethylamino)-6-methylpyrimidin-4-ol/one; IMPY= 2-isopropyl-4-methyl-6-hydroxypyrimidine.

Table 2. Select demographic characteristics for participants living in Salinas agricultural and Oakland urban homes sampled in 2006.

	Salinas, CA (Agricultural homes) (n=20)		Oakland, CA (Urban homes) (n=20)	
	n	(%)	n	(%)
Family income relative to federal poverty level [a]				
≤ Poverty level	13	(65.0)	13	(65.0)
>Poverty level but <200% poverty level	7	(35.0)	7	(35.0)
Maternal education (highest grade completed)				
completed 9th grade or lower	12	(60.0)	12	(60.0)
Grades 10-12 (no diploma)	3	(15.0)	3	(15.0)
High school diploma/GED or technical school	3	(15.0)	5	(25.0)
College graduate	2	(10.0)	0	(0.0)
Paternal education (highest grade completed)[b]				
completed 9th grade or lower	15	(79.0)	13	(72.2)
Grades 10-12 (no diploma)	1	(5.3)	1	(5.6)
High school diploma/GED or technical school	3	(15.8)	4	(22.2)
College graduate	0	(0.0)	0	(0.0)
Number of household members				
3-5	12	(60.0)	10	(50.0)
>6	8	(40.0)	10	(50.0)
Reported OP pesticide application in the 3 months preceding sample collection				
Yes	1	(5.0) [c]		---
No	19	(95.0)	20	(100.0)
Farmworkers living in the home (past 3 months)				
0	1	(5.0) [d]	18	(90.0)
1-3	15	(75.0)	2	(10.0) [e]
4-7	4	(20.0)		---
Farmworkers currently living in the home				
0	1	(5.0)[d]	20	(100.0)
1-3	15	(75.0)		---
4-7	4	(20.0)		---
Farmworkers wore work clothing indoors[f]				
Yes	17	(10.5)		---
No	2	(89.5)		---
Farmworkers wore work shoes indoors[f]				
Yes	10	(52.6)		---
No	9	(47.4)		---
Distance of home to nearest field/orchard				
50-20 feet	1	(5.0)		---
>200 feet-1/4 mile	3	(15.0)		---
>1/4 mile	16	(80.0)		---

a. Families' poverty levels were based on U.S. Department of Health and Human Services thresholds for 2006. b. Information was not available for one father living in an agricultural home and two fathers living in urban homes. c. One participant reported applying tetrachlorvinphos 7-30 days prior to the study for flea treatment on their pet dog. d. One participant in the agricultural group reported that the father was a farmworker during eligibility screening; however, the father was not living in the home during the sample collection period. e. Two participants reported having a parent or parent's sibling working in a field /golf course doing maintenance/landscaping work which may have involved pesticide use in the 3 months preceding the study; however, they were not doing this work at the time of the study. f. Information not available for one father living in an agricultural home.

Table 3. Limits of detection and summary statistics for dialkylphosphate compounds in house dust for 20 Salinas agricultural homes and 20 Oakland urban homes by collection time point (ng/g).[a,b]

Dialkylphosphate	LOD (ng/g)	Homes	Collection time point	%DF	Concentration in Dust, ng/g			
					p50	p75	p95	Max
DEP	10.4	Agricultural	1	60	30	57	246	386
			2	70	43	66	484	859
		Urban	1	70	50	79	246	316
			2	63	47	64	245	245
DETP	5.8	Agricultural	1	10	<LOD	<LOD	154	183
			2	0	--	--	--	--
		Urban	1	0	--	--	--	--
			2	0	--	--	--	--
DEDTP	5.2	Agricultural	1	5	<LOD	<LOD	<LOD	31
			2	5	<LOD	<LOD	<LOD	11
		Urban	1	0	--	--	--	--
			2	0	--	--	--	--
DMP	4.8	Agricultural	1	45	<LOD	8	28	29
			2	50	<LOD	20	425	806
		Urban	1	40	<LOD	9	822	1,588
			2	26	<LOD	8	79	79
DMTP	2.8	Agricultural	1	0	--	--	--	--
			2	0	--	--	--	--
		Urban	1	5	<LOD	<LOD	<LOD	17
			2	5	<LOD	<LOD	<LOD	20
DMDTP	9.9	Agricultural	1	20	<LOD	<LOD	70	98
			2	10	<LOD	<LOD	20	22
		Urban	1	5	<LOD	<LOD	<LOD	17
			2	11	<LOD	<LOD	18	18

a. One participant in the urban cohort (Oakland, CA) was lost to follow up prior to collection of the second dust sample; thus, statistics reported for the second collection time point are for 19 urban homes.
b. Samples 1 and 2 were collected 5 to 8 days apart from the same general location in the home.
Abbreviations and notation: LOD=Limit of detection; DF=Detection frequency; "--"statistic not reported when analyte was not detected.

Table 4. Limits of detection and summary statistics for detected organophosphate pesticides in house dust from 15 agricultural homes and 13 urban homes by collection time point (ng/g).[a,b]

Parent Organophosphate Compound	Dialkylphosphate breakdown product(s)	LOD (ng/g)	Homes	Collection time point	# of homes sampled	%DF	Concentration in dust, ng/g			
							p50	p75	p95	Max
Diazinon	DEP, DETP	4	Agricultural	1	14	86	15	19	56	56
				2	15	73	14	17	36	36
			Urban	1	13	54	9	16	139	139
				2	12	50	5	21	133	133
Chlorpyrifos	DEP, DETP	10	Agricultural	1	14	57	22	32	200	200
				2	15	53	20	26	135	135
			Urban	1	13	39	<LOD	35	56	56
				2	12	33	<LOD	33	43	43
Tetrachlorvinphos	DMP	10	Agricultural	1	14	14	<LOD	<LOD	252	252
				2	15	7	<LOD	<LOD	271	271
			Urban	1	13	8	<LOD	<LOD	16	16
				2	12	0	--	--	--	--
Malathion	DMP, DMTP, DMDTP	10	Agricultural	1	14	7	<LOD	<LOD	52	52
				2	15	7	<LOD	<LOD	71	71
			Urban	1	13	15	<LOD	<LOD	877	877
				2	12	8	<LOD	<LOD	1,158	1,158
Diazinon-oxon[c]	DEP	4	Agricultural	1	14	0	--	--	--	--
				2	15	0	--	--	--	--
			Urban	1	13	8	<LOD	<LOD	5	5
				2	12	0	--	--	--	--

a. Organophosphate (OPs) pesticides not detected in any samples included: chlorpyrifos-oxon, methidathion, methyl parathion, and phorate. b. Number of samples analyzed for OP pesticides in each location was dependent on the amount of sample volume remaining after initial analyses for DAPs: 29 samples from 15 Salinas agricultural homes and 25 samples from 13 urban homes. Summary statistics reported are for the number of samples. c. One molecule of the OP pesticide diazinon can either breakdown into DETP and the specific metabolite, isopropyl-methyl-hydroxypyrimidine (IMHP), or it may undergo activation into diazinon-oxon subsequently breaking down into DEP and IMHP. Abbreviations and notation: LOD=Limit of detection; DF=Detection frequency; "--"statistic not reported when analyte was not detected.

Table 5. Spearman rank order correlations between DEP residues in dust and the two most frequently detected OPs in dust by collection time point.[a,b]

Collection time point	Homes	Diazinon & DEP in dust		Chlorpyrifos & DEP in dust	
		n†	Spearman rho (pvalue)	n†	Spearman rho (pvalue)
1	Agricultural	14	0.02 (0.95)	14	-0.03 (0.91)
	Urban	13	0.01 (0.97)	13	---
	All homes	27	-0.02 (0.92)	27	---
2	Agricultural	15	-0.02 (0.94)	15	-0.18 (0.53)
	Urban	12	0.07 (0.83)	12	---
	All homes	27	0.07 (0.74)	27	---

a. Correlations were only conducted for DEP since it was detected in >50% of the samples and for respective parent OP pesticides for which detection frequency was also >50%.
b. OP pesticides were only measured in a subset of samples analyzed for DAPs (15 agricultural homes and 13 urban homes).
"--" Correlations not provided given the low detection frequency of chlorpyrifos in urban homes (DF<50% at both collections).
† Sample size indicated is based on number of homes for which OP pesticides.

Table 6. Summary statistics for dialkylphosphate metabolites in children's urine samples collected on the same day as dust samples by location and collection time point (nmol/L).[a,b,c]

					Metabolite concentrations in children's urine, nmol/L				
Dialkylphosphate	**Children**	**Collection time point**	**n[c]**	**%DF**	**p25**	**p50**	**p75**	**p95**	**Max**
DEP	Agricultural	1	20	45	<LOD	<LOD	32	142	205
		2	20	45	<LOD	<LOD	121	307	401
	Urban	1	20	65	<LOD	16	47	121	181
		2	19	79	7	20	33	118	119
DETP	Agricultural	1	20	65	<LOD	19	36	103	104
		2	20	50	<LOD	4	42	78	81
	Urban	1	20	60	<LOD	6	13	139	169
		2	19	32	<LOD	<LOD	12	49	49
DEDTP	Agricultural	1	20	5	<LOD	<LOD	<LOD	<LOD	4
		2	20	0	--	--	--	--	--
	Urban	1	20	15	<LOD	<LOD	<LOD	37	63
		2	19	5	<LOD	<LOD	<LOD	90	90
DMP	Agricultural	1	20	40	<LOD	<LOD	62	192	206
		2	20	45	<LOD	<LOD	34	188	227
	Urban	1	20	80	8	20	46	149	223
		2	19	63	<LOD	15	40	57	57
DMTP	Agricultural	1	20	100	47	119	199	426	525
		2	20	90	24	54	151	669	993
	Urban	1	20	95	24	51	134	527	777
		2	19	84	9	19	98	259	259
DMDTP	Agricultural	1	20	25	<LOD	<LOD	2	36	39
		2	20	20	<LOD	<LOD	<LOD	57	69
	Urban	1	20	40	<LOD	<LOD	11	97	98
		2	19	11	<LOD	<LOD	<LOD	10	10

a. Detection limits: DEP=0.2 µg/L, DETP=0.1µg/L, DEDTP=0.1µg/L, DMP=0.6 µg/L, DMTP= 0.2 µg/L, and DMDTP=0.1 µg/L. b. Concentrations reported are not creatinine-adjusted. c. One participant in the urban cohort (Oakland, CA) was lost to follow up prior to collection of the second urine sample.
Abbreviations and notation: LOD=Limit of detection; DF=Detection frequency; "---"statistic not reported when analyte was not detected.

Table 7. Level of detection agreement between frequently detected analyte residues in dust and respective urinary metabolites by collection time point.

Analyte(s) detected in dust	Agreement with detection of DEP in urine					Agreement with detection of DEP + DETP in urine				
	Collection time point	n[a]	Kappa coefficient[b]	(SE)	p-value[c]	Collection time point	n[a]	Kappa coefficient[b]	(SE)	p-value[c]
DEP	1	40	-0.13	(0.15)	0.81	1	--	--	--	--
	2	39	0.11	(0.16)	0.24	2	--	--	--	--
Chlorpyrifos	1	27	-0.33	(0.19)	0.96	1	27	-0.27	(0.19)	0.93
	2	27	0.05	(0.19)	0.40	2	27	0.05	(0.17)	0.38
Diazinon	1	27	-0.24	(0.18)	0.91	1	27	-0.01	(0.15)	0.51
	2	27	-0.07	(0.19)	0.64	2	27	0.16	(0.14)	0.12
DEP+Chlorpyrifos + Diazinon	1	27	-0.27	(0.16)	0.95	1	27	-0.27	(0.19)	0.93
	2	27	-0.19	(0.15)	0.89	2	27	-0.07	(0.19)	0.64

a. Indicates number of samples included in the calculation; children may have up to 2 dust samples. b. Kappa coefficient: 1= perfect agreement, 0=no agreement above that expected by chance, −1=perfect disagreement. c. High p-values (p>0.05) indicate that Kappa coefficients are not significantly greater than 0 (i.e., no agreement in detection between media above that expected by chance). Abbreviations and notations: SE=standard error of kappa statistic. "--" Because DEP in dust could not lead to detection of DETP in urine no kappa statistic is provided.

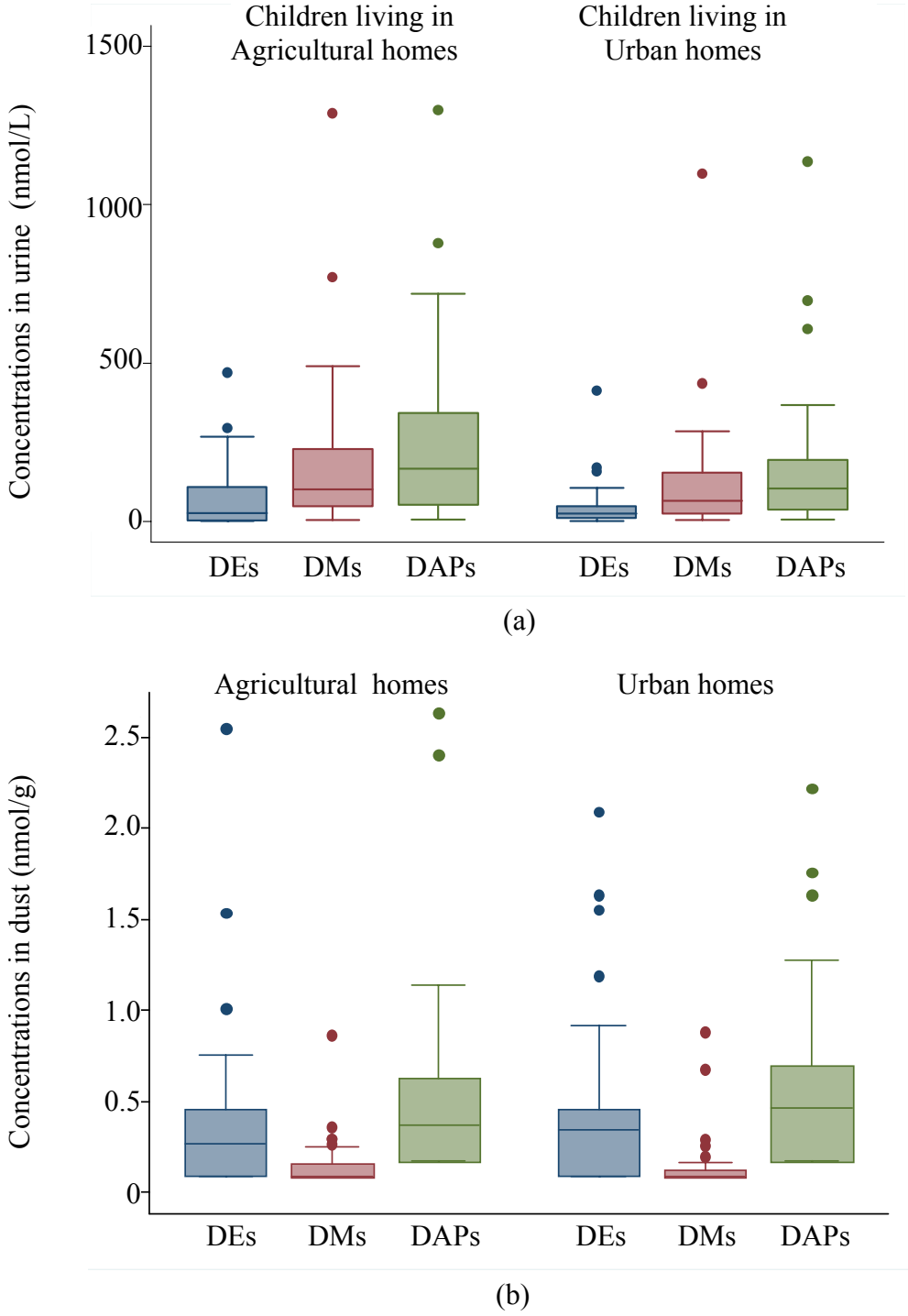


Figure 1. Distribution of total DE, DM and DAP levels in children's urine samples collected on the same day as dust collection (a) and in dust samples (b) by location. †

†Data presented is for 20 children and 20 homes in each location and each participant contributed up to two urine and two house dust samples, except for two agricultural homes and one urban home for which we did not include their dust concentrations for one collection for graphical representation (dust concentration, >6 nmol/g). Results and interpretation of data remain with or without omission of these data points.

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Chapter 4:
Prenatal and postnatal organophosphate pesticide exposures and their association with children's autonomic function

CHAPTER SUMMARY

Autonomic dysregulation (concomitant sympathetic activation and parasympathetic withdrawal) may be one the earliest and most sensitive measures of long-term systemic physiologic effects of organophosphate (OP) pesticide exposure in infants and young children (Eskenazi, Bradman et al. 1999) given acetylcholinesterase inhibition is central to the toxic effects of these pesticides. We conducted a study to assess the effects of *in utero* and postnatal OP pesticide exposures on children's autonomic nervous system (ANS) regulation using resting and reactivity measures for respiratory sinus arrhythmia (RSA), pre-ejection period (PEP), and heart rate (HR). Pesticide exposures were assessed by measuring urionary dialkylphosphates (DAPs, total diethyls-DEs, and total dimethyls-DMs) in maternal and children's spot urine samples. The main objectives of this study were to: (1) examine the cross-sectional effects of prenatal and postnatal OP pesticide exposure on children's ANS at four time points: 6 months, 1 year, 3 ½ and 5 years of age; (2) determine whether cumulative measures of prenatal and postnatal pesticide exposure were associated with ANS function at 5 years; and (3) determine whether children with consistently high versus consistently low OP pesticide exposures had significantly different mean ANS responses at 5 years. We hypothesized that OP pesticide exposure could lead to low resting HR (i.e., high resting RSA and/or PEP) and/or ANS dysregulation in children (i.e., more parasympathetic withdrawal concomitant with more sympathetic activation during challenge tasks compared to rest). Results from cross sectional models indicate that total dimethyls and DAPs (unadjusted urinary concentrations) were negatively associated with resting RSA in 6-month olds [for every 10-fold increase in child DMs we observed a -0.24 decrease (95%CI: -0.42 to -0.05; p=0.02) and for every 10-fold increase in DAPs we observed a -0.27 decrease (95%CI: -0.48 to -0.06; p=0.01) in resting RSA]. We also found that for every 10-fold increase in child DEs, we observed a 4.33 (95%CI: 1.24 to 7.42; p=0.01) increase in resting PEP in the 1 year-olds. Prenatal DMs, were also associated with a 3.77 (95%CI: 0.21 to 7.33; p=0.01) increase in resting PEP in this age group. For reactivity measures, we found a positive and borderline association between prenatal DMs and DAPs and PEP reactivity in the 6-month-olds. For every 10-fold increase in prenatal DMs we observed a 1.21 increase (95%CI: 0.03 to 2.40; p=0.05) in PEP difference scores, while for every 10-fold increase in prenatal DAPs we observed a 1.23 increase (95%CI: -0.07 to 2.54; p=0.06) in PEP difference scores. We also observed a positive association between prenatal DMs and DAPs and RSA difference scores in 1 year-olds [for every 10-fold increase in prenatal DM and DAP concentrations we observed a 0.25 (95%CI: 0.05 to 0.45; p=0.01) and 0.24 (95% CI: 0.03 to 0.46; p=0.03) respective increase in the RSA reactivity measures]. No associations were observed in 3 ½- or 5-year-old children and most of the previous associations observed were not in the direction hypothesized. When cumulative OP pesticide exposures were used to assess ANS dysregulation at 5 years we observed a -3.19 decrease in resting heart rate (95% CI: -6.29 to -0.09, p=0.04) with increasing cumulative prenatal total DEs (unadjusted concentrations). If we adjust for multiple comparisons, none of the associations observed would be significant. Results suggest that OP pesticide exposure is not associated with ANS dysregulation. Limitations of both exposure and outcome measures are discussed in depth. Future investigations in this population will involve evaluating what factors predict ANS regulation

and whether ANS resting and reactivity measures are related to physical and mental problems in this cohort as observed in previous studies.

INTRODUCTION

Organophosphate (OP) pesticides are known neurotoxins widely used in the United States for agriculture and, previously, for residential purposes (Barr, Bravo et al. 2004). Acute OP pesticide exposures affect the central nervous system and the autonomic nervous system (ANS) (Eskenazi, Bradman et al. 1999). The ANS consists of two branches, the parasympathetic and sympathetic nervous systems, which act in complementary ways to respond to and recover from environmental stressors. These pesticides exhibit their pharmacological effects through the inhibition of acetylcholinesterase, leading to the accumulation of acetylcholine in neuronal junctions. Acetylcholine is critical to the normal functioning of the ANS because it is the preganglionic neurotransmitter for both ANS branches, as well as the postganglionic neurotransmitter of parasympathetic neurons. Accumulation of acetylcholine (ACh) could lead to systemic effects in the ANS (e.g., by disrupting cholinergic receptor functions essential to smooth and cardiac muscle function). Acute OP toxicity can affect muscarinic and nicotinic receptors which can then lead to autonomic dysregulation and interfere with smooth muscle contraction, regulation of cardiac muscle and/or stimulation or inhibition of glandular secretions (Appendzeller O and Oribe E. 1997; Eskenazi, Bradman et al. 1999; Rosas and Eskenazi 2008). Some of the muscarinic effects that could result from acute OP pesticide toxicity include lower heart rate, lacrimation, and sweating while nicotinic effects may include respiratory compromise and cardiac arrest (Costa 2006). In low-level exposures, inhibition of acetylcholinesterase may be cumulative, and there may be no predictable correlation between the degree of cholinesterase inhibition and the severity of symptoms (Eskenazi, Bradman et al. 1999).

There has been increasing public health concern about OP pesticides given that low-level *in utero* exposures have been associated with altered neurodevelopment in young children (e.g., abnormal neonatal behavior and reflexes, altered mental development, and pervasive developmental problems) (Young, Eskenazi et al. 2005; Rauh, Garfinkel et al. 2006; Engel, Berkowitz et al. 2007; Eskenazi, Marks et al. 2007). Critical nervous system developments of the ANS occur in the first years of life (e.g., maturation of the central regulatory nuclei of cardiovascular activity and myelination of the nervous system) (Pereyra, Zhang et al. 1992). OP inhibition of AChE during this period can impact the cholinergic receptors (nicotinic and muscarinic receptors) and adrenergic receptors of the parasympathetic and sympathetic nervous systems, respectively, possibly leading to short- and long-term effects on the regulation of ANS-controlled functions. Because AChE inhibition is so central to the toxic effects of OP pesticides, autonomic dysregulation may be one the earliest and most sensitive measures of low-level cumulative or long-term systemic physiologic effects of OP pesticide exposure in infants and young children (Eskenazi, Bradman et al. 1999).

To assess ANS regulation in children, researchers have relied on measures of the parasympathetic nervous system, such as respiratory sinus arrhythmia (RSA), and the sympathetic nervous system, such as pre-ejection period (PEP) (Alkon, Goldstein et al. 2003).

RSA measures the periodic oscillation in sinus rhythm occurring in synchrony with respiration. RSA accounts for the decrease in heart rate during expiration and an increase during inspiration. PEP measures the duration of isovolumetric ventricular contraction and is a noninvasive and indirect measure of the sympathetic nervous system's influence on the cardiac cycle. Heart rate (HR) is also used as a measure of ANS regulation; it is an integrated measure of the parasympathetic and sympathetic nervous systems (Berntson, Cacioppo et al. 1993; Sherwood 1993; Beauchaine 2001).

Children's autonomic resting, challenging and reactivity measures for RSA, PEP, and HR are important indices of individual psychobiology. ANS resting measures indicate a child's physiology during a calm state such as listening to a lullaby; challenging measures indicate a child's physiologic response to stressors or challenging conditions; and reactivity measures indicate the physiologic response to a discrete environmental stimulus or challenge compared to resting state (Alkon, Lippert et al. 2006).

Measures of ANS functioning during childhood are related to physical and psychological health problems (Porges, Doussard-Roosevelt et al. 1996; Doussard-Roosevelt, Porges et al. 1997). Several studies have shown that children with lower resting heart rates and high ANS reactivity are at risk for behavior problems (Raine, Venables et al. 1997; Boyce, Quas et al. 2001; Pearson, Alkon et al. 2005). For example, higher resting RSA measures (indicating parasympathetic activation and resulting in a decrease in resting HR) in children have been associated with greater suppression in response to stressors (El-Sheikh 2005; Calkins, Graziano et al. 2008), while diminished RSA reactivity scores (indicating more parasympathetic withdrawal during a challenging condition compared to rest and resulting in an increase in resting HR) have been observed in adolescents with conduct disorder and attention-deficit hyperactivity disorder (Beauchaine, Katkin et al. 2001). Low resting HR in 3-year-old children has been associated with predisposition of aggression at age 11 years (Raine, Venables et al. 1997); and children with more sympathetic activation (i.e., low PEP reactivity scores indicating more sympathetic activation and low PEP during the challenging conditions compared to resting) display more externalizing behavior problems compared to children who have less sympathetic activation (Boyce, Quas et al. 2001; Pearson, Alkon et al. 2005). While we have summarized some studies showing a relationship between low resting heart rates and negative outcomes, other studies have also shown that positive behavioral and developmental outcomes are associated with low resting heart rate (Fox and Porges 1985; Fox N.A. 1989; Doussard-Roosevelt, Porges et al. 1997).

Although no studies have assessed the effects of chronic low-level OP pesticide exposure on children's ANS regulation using RSA and PEP measures, limited studies in animals and one in adults examined resting RSA in response to OP pesticide exposure (Dellinger 1985; Dellinger, McKiernan et al. 1987; Dellinger, Taylor et al. 1987; Dellinger and Mostrom 1988; Dellinger, Jansen et al. 1988). Though the sample size in the animal studies was small ($n \leq 15$ in animal studies), subacute OP pesticide exposures did not significantly alter resting RSA and treatment with atropine (an OP antidote which is a muscarinic receptor antagonist used to inhibit the effects of excessive vagal activation on the heart) with or without prior exposure to an OP pesticide was associated with decreased resting RSA. In the small human study (n=4, 2 exposed and 2 controls) resting RSA was found to decrease in both controls and one exposed aerial pesticide applicator, while it was found to increase in one aerial pesticide applicator. OP pesticide exposure in this human study was not

directly assessed; but was postulated given that application of an OP pesticide made up 85% of the total acreage treated by the pilots. Results on the direction of resting RSA in humans after OP pesticide exposure is thus inconclusive from this small study. Nonetheless, this research suggests that RSA, as a measure of the parasympathetic nervous system, deserves further investigation as it may serve as a non-invasive indicator of cholinergic effects and may be a sensitive marker of chronic low-level OP pesticide exposure.

Measures of ANS dysregulation (concomitant sympathetic activation and parasympathetic withdrawal) may offer insights into the neurobehavioral effects of OP pesticide exposure. We conducted a study to assess the effects of OP pesticide exposure on ANS regulation in children living in an agricultural region. We employed resting and reactivity measures for RSA, PEP and HR by having children complete a series of standardized tasks designed to elicit ANS responses. The main objectives of this study were to: (1) examine the cross-sectional effects of prenatal and postnatal OP pesticide exposure on children's ANS at four time points: 6 months, 1 year, 3 ½ and 5 years of age; (2) determine whether cumulative measures of prenatal and postnatal pesticide exposure were associated with ANS function at 5 years; and (3) determine whether children with consistently high versus consistently low OP pesticide exposures had significantly different mean ANS responses at 5 years. We hypothesized that OP pesticide exposure could lead to low resting HR (i.e., high resting RSA and/or PEP) and/or ANS dysregulation in children (i.e., more parasympathetic withdrawal concomitant with more sympathetic activation during challenge tasks compared to rest; See Supporting Information Tables S1-S2 for further details).

STUDY DESIGN AND METHODS

Study Population and participant selection criteria for ANS protocols

Participating children were part of the Center for the Health Assessment of Mothers and Children of Salinas (CHAMACOS) longitudinal birth cohort study, which is evaluating the potential health effects of pesticides and other environmental chemicals on pregnant women and their children living in the agricultural community of Salinas Valley, CA. The Salinas Valley is a region in central California with intense agricultural OP pesticide use (California Department of Pesticide Regulation). Population recruitment details have been described elsewhere (Eskenazi, Harley et al. 2004). Briefly, expectant mothers were screened for eligibility over a one-year period (October 1999 - October 2000). Eligibility criteria included women who were: ≥ 18 years old, < 20 weeks gestation at the time of screening, fluent in Spanish or English, qualified to receive poverty-based government health insurance, receiving prenatal care, and planning to deliver at the county hospital. The University of California Center for the Protection of Human Subjects approved all study procedures and written informed consent was obtained from eligible mothers upon enrollment. A total of 531 women (536 children) were followed to delivery of a liveborn neonate.

Technical and logistical problems, particularly at the earlier time points, prevented us from administering the ANS reactivity protocols to all of the children enrolled in the study at each time point. Thus, for this study, infants at the six month visit with an even identification number and who were enrolled between April and October 2000 were eligible to participate in

the ANS reactivity protocols at 6 months, 1 year and 3 ½ years of age. When children were five years of age, additional funding sources made it possible to offer the ANS reactivity protocol to all of the children enrolled at this time. Table S3 outlines the selection process of our population for the analyses presented herein. Briefly, of the children enrolled at 6 months (n=433), 228 children were eligible to participate in the ANS protocols and of those eligible children, 178 participated in the ANS assessment at 6 months. Of the 228 children eligible at 6 months, 212 and 203 children were eligible to participate in the ANS protocols at 1 and 3 ½ years, respectively. Of those eligible, 172 and 160 children participated in ANS protocols at the 1 and 3 ½ year assessments, respectively. Finally, at 5 years, 350 children were enrolled and eligible to participate in the ANS reactivity protocols and, of those eligible, 316 children participated. For our analyses, we excluded children who:

(1) did not have scoreable and/or sufficient data on resting or reactivity ANS measures at each time point (n=17 children at 6 months, n=14 children at 1 year, n=21 children at 3 ½ years, and n=15 children at 5 years);
(2) were not singletons (n=8 children at 6 months and 5 years, n=2 children at 1 year, and n=6 children at 3 ½ years);
(3) had a diagnosed medical condition; i.e., Down syndrome, deafness, hydrocephalus, or had seizures in the first year of life (n=2 children at 6 months and at 1 and 3 ½ years, and n=5 children at 5 years);
(4) did not have complete pesticide exposure information on the mother; i.e., no creatinine concentrations in urine samples (n=1 child at the 5 year assessment); and
(5) did not have complete postnatal urinary pesticide metabolite data at each assessment (n=2 children at 6 months, n=5 children at 1 year, n=34 children at 3 ½ years, and n=13 children at 5 years).

After implementing this exclusion criteria, a total of 149 children provided data at 6 months and 1 year, and 97 and 274 children provided data at 3 ½ and 5 years of age, respectively. These children had prenatal and postnatal pesticide exposure measurements. A total of 179, 89, and 68 children had both ANS and pesticide (prenatal and postnatal) measurements exclusively at one, two, and three time points, respectively. Only 27 children had ANS and pesticide measurements at all four time points (i.e., at 6 months, and 1, 3 ½ and 5 years). Overall, there were no significant demographic differences between the subsample of children who completed the ANS protocols at 6 months, 1 year and 3 ½ years compared to the larger sample of children that completed the ANS protocol at 5 years.

Collection of demographic and medical information. We conducted maternal interviews during pregnancy (i.e. at approximately 14 and 26 weeks gestation), and when children were 6 months, 1, 3 ½, and 5 years old to obtain demographic information on the families and participating children. Select health information on children was obtained from medical records by a registered nurse; this information was used to exclude children with a diagnosed medical condition as described above.

ANS Reactivity Protocols and ANS Data Acquisition. We administered a previously validated ANS reactivity protocol to the 3 ½- and 5-year-old children and used a modified version of this protocol for the 6-month- and 1-year-old children (Boyce, Quas et al. 2001; Alkon, Goldstein et al. 2003; Alkon, Lippert et al. 2006). ANS reactivity protocols consisted of age-appropriate social, physical, emotional, and for the 3 ½ and 5 year olds, cognitive challenge tasks designed to represent common stressors experienced by infants and young children and to elicit sympathetic and parasympathetic autonomic responses. Resting conditions were also included in the protocol to collect children's resting physiologic measures. Due to differences in children's attention span, the protocol lasted 7 minutes for the 6-month-old and 1-year-old children and 15 minutes for the 3½- and 5-year-old children. Details on the ANS protocol have been described in detail elsewhere (Boyce, Quas et al. 2001; Alkon, Goldstein et al. 2003; Alkon, Lippert et al. 2006) and summarized in Table 1. Protocols were administered in the child's native language (English or Spanish) by trained bilingual staff in a recreational vehicle (RV), at the research field office, and/or at the participants' home (for 17 participants at the 5 year assessment).

To acquire children's sympathetic and parasympathetic autonomic responses during the protocol, three physiological response parameters were measured during the resting conditions and challenge tasks: RSA, HR, and PEP. Continuous measures of these parameters were obtained by placing four band electrodes (for 6-month and 1-year olds) (Alkon, Lippert et al. 2006) or seven spot electrodes (for 3 ½-and 5-year olds) on the child's neck and thoracic region as detailed previously (Boyce, Quas et al. 2001; Alkon, Goldstein et al. 2003; Alkon, Lippert et al. 2006). Electrodes were attached to a Minnesota Impedance Cardiograph HIC-2000 (Bio-Impedance Technology, Inc., Chapel Hill, NC) for the younger children and to a Biopac MP150 (BIOPAC Systems, Inc. Goleta, CA) for the older children. RSA scores were computed using interbeat intervals on the ECG readings, respiratory rates derived from the impedance signal (dZ/dt), and a bandwidth range of 0.24-1.04 Hz for the 6-month- and 1-year-olds and 0.15 to 0.80 Hz for the 3 ½- and 5-year-olds (Bar-Haim, Marshall et al. 2000; Alkon, Lippert et al. 2006). Heart rate was ascertained as the number of R-waves on the ECG. PEP was measured in milliseconds as the interval between the ECG's Q-wave and the B notch on the dZ/dt signal. After acquiring RSA, HR, and PEP measurements, data were then filtered, extracted, and scored using the ANS Suite software (National Instruments, Austin, TX) for the 6-month- and 1-year-olds and Mindware (www.mindware.org) (Alkon, Goldstein et al. 2003; Alkon, Lippert et al. 2006) for the 3 ½- and 5-year-old children.

ANS Response Measures & Data Reduction

For the present analyses, we calculated nine summary measures to get the six ANS measures to be used in our analyses. These nine measures comprised of mean scores for the resting and challenge tasks and reactivity scores (hereafter referred to as difference scores) for each of the three summary ANS response measures for RSA, HR, and PEP. The mean scores for the challenge tasks were used to derive the difference scores which were calculated for each child as the mean of the challenge tasks minus the mean of the first resting condition (Alkon, Goldstein et al. 2003). Thus, the six measures in our analyses included mean resting and difference scores for RSA, HR, and PEP. Difference scores allowed us to evaluate the child's individual response to challenge tasks compared to their individual resting state. A

negative RSA difference score indicates parasympathetic withdrawal (inhibition) during the challenge tasks compared to resting, while a negative PEP difference score indicates sympathetic activation during the challenge tasks compared to resting (Pearson, Alkon et al. 2005). A negative RSA or PEP difference score also corresponds to an increase in HR; and a positive mean HR difference score indicates a higher HR during the challenge task compared to resting.

We also generated ANS profiles to summarize the direction and magnitude of the reactivity scores and the inhibition and/or activation of the parasympathetic and sympathetic nervous systems. ANS profiles combined both RSA and PEP difference scores and were used to classify children into one of four profiles: (1) coactivation (activation of both sympathetic and parasympathetic nervous systems during the challenge tasks compared to rest) (2) coinhibition (inhibition of both sympathetic and parasympathetic nervous systems during challenge tasks compared to rest), (3) reciprocal parasympathetic activation and sympathetic withdrawal, or (4) reciprocal sympathetic activation and parasympathetic withdrawal (Berntson, Cacioppo et al. 1991; Salomon, Matthews et al. 2000). ANS profile four has been defined in the psychobiology literature as the most highly reactive profile (Alkon, Goldstein et al. 2003). Children in this profile are at risk of exhibiting more mental health symptoms compared to children categorized in other ANS profiles (Treadwell MJ, Alkon A et al. 2010: In press).

Pesticide exposure assessment

To assess children's exposure to OP pesticides, we collected spot urine samples and measured non-specific urinary dialkylphosphate (DAPs) metabolites when children were approximately 6 months, 1, 2, 3½ and 5 years of age, respectively. Spot urine samples were generally collected on the same day or within seven days of conducting the ANS assessments. Although no ANS assessments were conducted when children were 2 years old, urinary metabolite measurements collected at this time were used to assess the effects of potential cumulative exposure on 5 year ANS measures as detailed in the data analysis section. To assess *in utero* OP pesticide exposure, we also collected maternal spot urine samples during the prenatal interviews [i.e., at approximately (Mean $\pm$ SD) 14.0 $\pm$ 5.0 and 26.7 $\pm$ 2.7 weeks gestation, respectively]. All urine specimens were aliquoted and stored at -80°C until shipment for analysis to the laboratory at the Centers for Disease Control and Prevention (CDC) (National Center for Environmental Health, Atlanta, Georgia). Further details on the specimen collection methods and quality control procedures have been described elsewhere (Bradman, Whitaker et al. 2007; Eskenazi, Marks et al. 2007).

Six DAP metabolites were measured in maternal and child urine samples including three diethylphosphates: diethylphosphate (DEP), diethylthiophosphate (DETP), diethyldithiophosphate (DEDTP); and three dimethylphosphates: dimethylphosphate (DMP), dimethylthiophosphate (DMTP), dimethyldithiophosphate (DMDTP). These metabolites represent the by-products of over 70% of the OP pesticides registered for use in the U.S. by the U.S. Environmental Protection Agency (U.S. EPA March 2010) and approximately 81% of the OP pesticides used in the Salinas Valley (Bradman, Whitaker et al. 2007). DAP metabolite concentrations were measured using isotope dilution gas chromatography–tandem mass spectrometry (GC-MS/MS) as detailed previously (Bravo et al., 2004). Creatinine

concentrations (mg/dL) in urine samples were also determined using a commercially available diagnostic enzyme method (Vitros CREA slides, Ortho Clinical Diagnostics, Raritan, NJ, USA). Limits of detection (LOD) for each DAP metabolite was as follows: 0.2 μg/L, 0.1μg/L, and 0.1μg/L for DEP, DETP, and DEDTP, respectively; and 0.6 μg/L, 0.2 μg/L, and 0.1 μg/L for DMP, DMTP, and DMDTP, respectively.

Data analysis

Individual DAP metabolite concentrations below the LOD were imputed to LOD/√2 and then summed to create total DE (molar sum of DEP+DETP+DEDTP), total DM (molar sum of DMP+DMTP+DMDTP) and total DAP (molar sum of total DEs + total DMs) concentrations for our analyses. We generated our prenatal pesticide exposure measure for each child by taking the mean concentration of DAP metabolites for the two pregnancy measurements; the two prenatal measurements of total DAPS were did not significantly differ (paired t-test = -0.6916, p=0.50). Twenty-two mothers had only one prenatal pesticide measurement. For these mothers, their one measure was considered the child's prenatal pesticide measurement (results of subsequent cross sectional analyses were similar whether we included or excluded urinary metabolite concentrations for these mothers).

For each time point for which the ANS reactivity protocol was conducted and for which children had pesticide metabolite measurements, we summarized demographic characteristics and calculated descriptive statistics for the ANS outcomes (both mean resting and difference scores for RSA, HR, and PEP, respectively); and prenatal and postnatal pesticide metabolite concentrations (total DEs, DMs and DAP concentrations).

Lowess smoothers were used to determine the shape of the relationship between each ANS outcome measure and pesticide metabolite concentrations. Lowess carries out a locally weighted regression of the dependent variable on the independent variable and displays the graph allowing one to observe the relationship between these variables (NIST/SEMATECH). These analyses revealed a log-linear relationship between urinary pesticide concentrations and ANS outcome measures. Thus, log-10 transformed DAP metabolite concentrations were used in subsequent statistical analyses in which continuous measures of DAPs were used unless otherwise noted.

Cross Sectional Analyses between ANS measures and pesticide exposures at each time point. To investigate the association between each of the six ANS measures (i.e., resting and difference scores for RSA, HR, and PEP scores), and prenatal and postnatal OP pesticide exposures, we constructed separate linear regression models for each ANS outcome at each of the four time points (i.e., at 6 months, and 1, 3 ½ and 5 years of age). Because prenatal and postnatal DAP metabolite concentrations were not correlated at any time point (r=-0.04 to 0.03, p>0.60), we placed both of these measures into each model for each ANS outcome. Coefficients from these models were similar to those obtained in models containing either prenatal or postnatal exposures alone. Covariates in cross sectional models (and subsequent regression models) were selected if they were related to testing conditions [i.e., exact age of the child at the time of the ANS assessment, (continuous variable, measured in months);

location of the assessment (RV or field office (6 months to 3 ½ years), and RV, field office or participant's home at 5 years), and psychometrician (n=2 to 4)]; and related ($p<0.10$) to more than one ANS outcome and DAP metabolite concentrations at more than one time point as assessed in univariate analysis [i.e., breastfeeding duration]. An association between breastfeeding duration and ANS outcomes was also reported previously (Bensadoun JA 2009). The breastfeeding duration variable was coded as a continuous variable truncated at the time of the ANS assessment (months breastfed). For example, if the child was breastfed for a total of 9 months and their "6 month" ANS assessment took place at 6.2 months of age then the child was assigned 6.2 for this variable in the 6 month cross sectional models. For models at 3 ½ and 5 years we used total duration of breastfeeding. We also adjusted for sex of the child (boy=0, girl=1) *a priori* in each model. For consistency, we included the same covariates in all regression models.

In secondary analyses, we ran all regression models (cross sectional models and models only using 5-year ANS measures) controlling for factors potentially on the causal pathway [birthweight and gestational age, both considered as continuous variables and then as categorical variables: $<$ or $\geq$ 37 weeks gestation and $<$ or $\geq$ 2500 grams)]. However, because inclusion of these covariates did not change our results we excluded them from our final models.

Additionally, to determine if children's mean prenatal or postnatal DAP concentrations significantly differed among the four ANS profiles, we conducted a one-way analysis of variance (ANOVA) at each time point using total DAP metabolite concentrations.

Assessment of ANS responses at 5 years using all prenatal and postnatal DAP metabolite concentrations. We examined the relationship of exposure and ANS in more detail for a subset of the 5-year-old children (n=223 out of 274 children who were assessed at 5 years) since the sample size was largest and more measures of exposure were available. These analyses allowed us to use data from 223 children to determine if long term exposure, as assessed using all prenatal and postnatal DAP measures, would have an association with ANS measures at 5 years. To be included in these analyses, children had to meet the following criteria: (1) have at least one ANS measure at 5 years, (2) have both prenatal DAP measurements (i.e., at 14 and 26 weeks gestation); (3) have the six month and five year postnatal DAP measurements; and (4) have at least two more postnatal DAP metabolite measurements (i.e., missing no more than one of those taken at 2 and 3 ½ years). For those children with four postnatal DAP measurements (i.e., missing at 2 or 3 ½ years) we imputed their missing value by taking the average of the preceding and subsequent untransformed DAP concentrations. Thus, every child contributed a total of five postnatal pesticide measurements. We assessed whether prenatal and postnatal pesticide exposures were associated with each of the six ANS outcomes at 5 years in separate linear regression models using 5-year ANS function measures as outcomes, and prenatal and postnatal DAP exposure measures as separate covariates in our models. To get an estimate of cumulative exposure during the prenatal and postnatal periods we used an area-under-the-concentration–time-curve (AUC) methodology. The AUC method is usually applied in pharmacology in cases where a drug has variable bioavailability and unpredictable elimination characteristics to estimate total

exposure to the drugs given they are often correlated with pharmacodynamic effects (Kaplan, Meier-Kriesche et al. 1997). This method was chosen given that excretion of pesticide metabolites during pregnancy is not well understood (Bradman, Eskenazi et al. 2005). An alternative method would be to sum doses across all time points to estimate an overall cumulative dose. Doses at each time point could be calculated by multiplying urine concentrations by the urine volume excreted in 24 hours and the potential time exposed; however, data on urine volume excretion in a 24-hour period was not collected or available. Thus, our prenatal exposure variable consisted of the AUC for the untransformed DAP concentrations observed at approximately 14 and 26 weeks gestation, while the postnatal exposure variable consisted of the AUC for the untransformed DAP concentrations observed between 6 months and 5 years. Prenatal and postnatal AUC values were log-normally distributed; and were log-10 transformed prior to inclusion in regression models. Covariates in these models included sex, location, psychometrician, and exact age at the 5 year assessment.

We repeated the method above using logistic regression models to determine if prenatal and/or postnatal DAP measures predicted whether a child would be classified into ANS profile four at 5 years. This was done since we expected children with higher OP pesticide exposures to display more reciprocal sympathetic activation and parasympathetic withdrawal (ANS profile four) based on the cholinergic effects of OP pesticides on the ANS.

Furthermore, using data from the 223 children with 5-year ANS function measures, we used t-tests to evaluate whether children with consistently high DAP concentrations had significantly different mean ANS scores compared to children who had consistently low DAP concentrations. These analyses were done using prenatal DAPs only, postnatal DAPs only, and all DAP concentrations (prenatal and postnatal). For each of these analyses, we first created a variable that categorized exposure at each time point by quartiles. We then created scores for each child by summing the quartile categories over the time points. There were few children who were in the lowest or highest quartiles at all time points. Thus, we compared ANS scores for children whose scores fell in the highest 10% of DAP scores (representing children with "consistently high DAP levels") with those children with the lowest 10% of DAP scores (representing children with "consistently low DAP levels").

All regression models were conducted using unadjusted urinary metabolite concentrations and repeated using creatinine-adjusted urinary metabolite concentrations. We also ran cross-sectional regression models using creatinine concentrations as covariates; however because results were similar to those models using creatinine-adjusted concentrations these models are not presented. With the exception of univariate analyses for covariate selection ($p<0.10$) for our regression models, statistical significance was considered at $p<0.05$. All statistical analyses were done using Stata 10 for Windows (StataCorp LLC, College Station, TX).

RESULTS

Population Characteristics

Similar demographic characteristics were observed at each time point (Table 2). Few children weighed < 2500 grams at birth (≤5%) and few were born at ≤ 37 weeks gestation (≤7%). The majority of children lived with at least one farmworker in the home (≥65%) and the median total breastfeeding duration for children was six months. Mothers were primarily young (average age ± SD of mothers ranged from 25.7 ± 4.8 to 26.6 ± 5.2 years), and relatively few had completed high school. As reported during the first prenatal visit, most mothers were born in Mexico (>86%) and had lived in the U.S. an average of approximately 8 years. Additionally, over 60% of the participating families lived below the federal poverty threshold. The majority of mothers also reported abstaining from smoking (≥95%) and from consuming alcohol (≥74%) during pregnancy.

ANS Function Measures

Mean ANS resting measures and difference scores are presented in Table 3. In general, mean RSA resting measures (mean ± SD) were higher for 3 ½- and 5-year-olds (6.3 ± 1.1 and 6.8 ± 1.2, respectively) compared to the 6 month-old and 1 year-olds (3.3 ± 0.8 and 3.7 ± 0.9, respectively), while mean HR resting measures were higher in the 6 month-olds and 1 year-olds (139.8 ± 10.8 and 133.2 ± 12.3, respectively) compared to the 3 ½- and 5 year-olds (100.9 ± 9.8 and 93.8 ± 10.8, respectively). Mean PEP resting measures were found to be higher in the older children (i.e., 3 ½ and 5-year-olds). In general, all mean resting measures were similar between the two younger age groups (i.e., 6 month-olds and 1-year-olds) and between the two older age groups (i.e., 3 ½ and 5-year-olds). We observed negative mean HR difference scores for 6-month-old and 1-year-old children (-0.8 ± 5.4 and -2.7 ± 6.0, respectively) and positive mean HR difference scores for the 3½- and 5-year-old children (4.9 ± 3.5 and 5.7 ± 4.4, respectively). No clear trend was observed for mean RSA and PEP difference scores.

Pesticide Metabolite Measurements

Table 4 presents the geometric means (GM) and respective confidence intervals (CIs) for maternal (*in utero*) DAP concentrations (unadjusted and creatinine adjusted) for children assessed at each time point. Total DM metabolite concentrations were higher than total DE concentrations at each time point. The ranges observed for GM unadjusted and creatinine-adjusted metabolite concentrations were comparable (unadjusted concentrations ranged from 23.1 nmol/L to 166.6 nmol/L and creatinine-adjusted concentrations ranged from 25.5 to 181.9 nmol/gCre).

Table 5 shows the children's (postnatal) urinary OP pesticide metabolite concentrations (unadjusted and creatinine-adjusted) at each timepoint. As observed for prenatal DAP metabolite concentrations, we found that children's GM concentrations for total DMs were higher than total DEs at each time point and that concentrations for total DEs, DMs and DAPs were comparable between 6-month-olds and 1-year-olds and between 3 ½- and 5 years-olds (Table 5). These trends were observed regardless of whether concentrations were

unadjusted or creatinine-adjusted. GM concentrations for total DEs, DMs, and DAPs did not necessarily increase with age. Additionally, unadjusted maternal DAPs were uncorrelated with unadjusted child DAPs (r= -0.08 to 0.05, p>0.45) at each time point; the same trend was observed between creatinine-adjusted maternal and child DAPs (r= -0.04 to 0.06, p>0.46).

Cross-sectional Analyses. Tables 6a-d present the change (and 95% confidence intervals, CIs, and pvalues) in ANS response measures associated with a 10-fold increase in metabolite concentrations (unadjusted and creatinine-adjusted) at each time point, controlling for covariates.

Resting Measures: We found a statistically significant negative association between resting RSA and total child DMs and DAPs at 6 months. Specifically, we found that for every 10-fold increase in child DMs we observed a -0.24 (95%CI: -0.42 to -0.05; p=0.02) decrease and for every 10-fold increase in DAPs we observed a -0.27 decrease (95%CI: -0.48 to -0.06; p=0.01) in the 6 month-olds' mean resting RSA score. However, at other ages, there were no associations between pesticide metabolite concentrations and mean resting RSA.
We also found that for every 10-fold increase in child DEs, we observed a 4.33 (95%CI: 1.24 to 7.42; p=0.01) increase in resting PEP score in the 1 year-olds. Prenatal DMs, were also associated with a 3.77 (95%CI: 0.21 to 7.33; p=0.01) increase in resting PEP in this age group. No other associations were observed between DAPs and mean PEP at other ages. When we creatinine-adjusted urinary metabolite concentrations, we found similar results although some associations were attenuated. For example, we found that for every 10-fold increase in creatinine-adjusted child DEs there was a 3.36 (95%CI: 0.14 to 6.58, p=0.04) increase in resting PEP in 1-year-olds and the association between concentrations of DMs and PEP in 1 year-olds became borderline significant when we creatinine adjusted (β=3.51, 95%CI: -0.26 to 7.28, p=0.07).

Difference Scores: In general, results did not differ substantially when we evaluated associations between difference scores and unadjusted and creatinine-adjusted urinary metabolite concentrations. Using unadjusted metabolite concentrations, we found a positive and borderline association between prenatal DMs and DAPs and PEP difference scores in the 6-month-olds. For every 10-fold increase in prenatal DMs we observed a 1.21 increase (95%CI: 0.03 to 2.40; p=0.05) in PEP difference scores, while for every 10-fold increase in prenatal DAPs we observed a 1.23 increase (95%CI: -0.07 to 2.54; p=0.06) in PEP difference scores. We also observed a significant and positive association between prenatal DMs and DAPs and RSA difference scores in 1 year-olds. Specifically, for every 10-fold increase in prenatal DM and DAP concentrations we observed a 0.25 (95%CI: 0.05 to 0.45; p=0.01) and 0.24 (95% CI: 0.03 to 0.46; p=0.03) respective increase in the RSA difference scores. Associations between PEP difference scores and creatinine-adjusted prenatal DMs and DAPs for 6 month-olds were significant and in the same direction as those observed using unadjusted concentrations. Additionally, associations between creatinine-adjusted prenatal DMs and DAPs and RSA difference scores in 1 year-olds were similar to those obtained using unadjusted urinary metabolite concentrations. Similar to resting measures, associations

observed between difference scores and urinary metabolite concentrations (unadjusted or creatinine-adjusted) at specified time points were not observed at other time points.

ANS profiles at each time point: Tables 7a-7d present GM total DAP concentrations by ANS profile for each time point. We found that prenatal DAP metabolite concentrations did not significantly differ among ANS profiles at each time point regardless of whether we considered unadjusted (p=0.21 to p=0.48; ANOVA) or creatinine-adjusted prenatal metabolite concentrations (p=0.11 to 0.58; ANOVA). We also observed no significant differences in unadjusted child DAP concentrations among profiles at each time point (p=0.15 to 0.63; ANOVA). However, we did observe a significant difference in creatinine-adjusted child DAP concentrations among ANS profiles in 6-month-olds (F=3.18, p=0.03; ANOVA); but not in any other age group. GM child DAP concentrations (creatinine-adjusted) were highest among children in ANS profile four (i.e., children with reciprocal sympathetic activation and parasympathetic withdrawal) (GM=510 nmol/gCre, CI: 234.9 to 1111.3).

Analyses at 5 years. Tables 8a-b present our regression models for ANS measures at 5 years using prenatal and postnatal AUC values. The only significant association observed was between prenatal DEs and resting heart rate. For every 10-fold increase in prenatal AUC values, we observed a -3.19 decrease in resting heart rate (95% CI: -6.29 to -0.09, p=0.04); however, this association was no longer present when we used creatinine-adjusted concentrations for AUC values. Additionally, using AUC prenatal and postnatal values did not increase the odds of a child being categorized into ANS profile four (i.e., reciprocal sympathetic activation and parasympathetic withdrawal) regardless of whether we used unadjusted or creatinine-adjusted AUC values (not shown).

Lastly, we did not observe any significant differences for each of the six ANS response measures (p>0.13, not shown) between children with consistently high versus consistently low DAP metabolite concentrations (prenatal only, postnatal only, or child and postnatal).

DISCUSSION

Previous research demonstrates that children's psychobiologic reactivity is a valid measure of the autonomic nervous system's ability to regulate under resting and challenging conditions (Matthews, Woodall et al. 1990; Alkon, Goldstein et al. 2003). Studies have shown that individual psychobiologic measures are associated with children's physical and psychological health, and may affect how children interact with their family members, adults and peers (Kagan, Reznick et al. 1988; Boyce, Frank et al. 1998). Exposure to OP pesticides has also been linked to neurodevelopmental and behavioral problems in young children (Rauh, Garfinkel et al. 2006; Eskenazi, Marks et al. 2007). In a few, small animal studies acute OP pesticide exposures did not significantly alter resting RSA and in one human study, the direction of resting RSA as a result of postulated OP pesticide exposure was inconclusive

(Dellinger, McKiernan et al. 1987; Dellinger and Mostrom 1988). This is the first study to evaluate the association between prenatal and postnatal OP pesticide exposures in young children and ANS regulation. The use of RSA, HR, and PEP as outcome measures of early OP pesticide exposure represents a unique departure from previous research, as these measures have traditionally been used as predictors of psychobiology, behavioral, and cardiovascular outcomes.

In general, results from our cross-sectional models were similar when we used prenatal and postnatal unadjusted and creatinine-adjusted urinary pesticide metabolite concentrations. We hypothesized that as OP pesticide exposures increased, we would observe less parasympathetic withdrawal and sympathetic activation during rest (i.e., higher resting RSA and resting PEP, and lower resting HR measures), but more parasympathetic withdrawal and sympathetic activation during challenge (i.e., negative RSA and PEP difference scores); however, we did not see these associations at all time points. Several of the associations observed were in the opposite direction of what we hypothesized. For example, we observed significant negative associations between resting RSA and child DMs and DAPs (i.e., more parasympathetic withdrawal) in 6-month-olds and positive associations between PEP difference scores and prenatal DMs and DAPs (i.e., less sympathetic activation during challenge compared to rest) in 1-year-olds. At 3 ½ and 5 years of age DEs, DMs, and DAPs were not associated with any ANS resting and reactivity measures.

Additionally, although creatinine-adjusted DAP concentrations were observed to be higher among 6-month-old children categorized into ANS profile four (i.e., reciprocal sympathetic activation and parasympathetic withdrawal), this was not observed at any other time points nor did we observe this finding using prenatal (unadjusted or creatinine-adjusted concentrations) or unadjusted child DAP concentrations at each time point. We also did not observe trends in DAP concentrations among ANS profiles (i.e., the highest DAP concentrations observed at each time point were not always observed among children categorized in the same profiles) suggesting that OP pesticide exposures are not associated with ANS dysregulation.

When we evaluated whether cumulative prenatal and/or postnatal DAPs (using AUC values) were associated with ANS function at 5 years, the only association observed was between resting HR and prenatal DEs (negative HR per 10-fold increase in prenatal DEs). This was not present when we used creatinine-adjusted concentrations. Additionally, although this finding was in the hypothesized direction, this association was not evident with child DEs or child or prenatal total DMs or DAPs and no significant associations were observed with RSA or PEP resting measures or any of the difference scores. This AUC methodology may not have been the most optimal to estimate cumulative exposure, but it was implemented in the absence of critical data needed to estimate potential doses (e.g., 24-hour urine volume data for pregnant women based on their gestational age and on children at all five time points). Nonetheless, when we compared children with consistently high versus consistently low urinary DAP concentrations (unadjusted and creatinine-adjusted concentrations) we confirmed that mean ANS response measures did not differ significantly between these two groups. Additionally, none of the cross-sectional analyses or those performed in the subset of children at 5 years was adjusted for all of the multiple comparisons performed. If we were to adjust our results for the multiple comparisons, then no significant findings are observed. All of this evidence further confirms that OP pesticide exposure, at the

levels observed in this population and as measured via urinary DAP metabolite concentrations, is not associated with children's resting and reactivity measures.

Studies that have evaluated the reliability and ontogenesis of ANS function measures used herein reported similar mean resting ANS measures in children (Fracasso M, Porges S et al. 1994; Bornstein and Suess 2000; Bazhenova, Plonskaia et al. 2001; Alkon, Goldstein et al. 2003). For example, two studies in primarily European-American 5-month-old infants reported similar mean resting RSA measures of 3.3 (Bazhenova, Plonskaia et al. 2001) and 3.0 (Fracasso M, Porges S et al. 1994) compared to that observed in our 6-month-olds (mean resting RSA= 3.3). A study by Bornstein and Suess (Bornstein and Suess 2000) reported identical mean resting RSA in 13-month-olds compared to our 1-year olds (mean resting RSA=3.7). Lastly, Alkon et al. (Alkon, Goldstein et al. 2003) reported comparable mean ($\pm$SD) RSA, PEP, and HR resting measures for 3 to 5-year-olds in a non-Latino population (mean resting RSA = 5.7 $\pm$1.2 versus 6.3 $\pm$ 1.1 and 6.8 $\pm$1.2 for the 3 ½ and 5-year-olds in this population; mean resting PEP= 77.5 $\pm$ 6.6 versus 74.0 $\pm$ 6.7 and 78.3 $\pm$7.4 for the 3 ½ and 5-year-olds in this population; mean resting HR = 98.7 versus 100.9 $\pm$ 9.8 versus 93.8 $\pm$ 10.8 for the 3 ½ and 5-year-olds in this population). No previous studies have reported PEP measures in 6-month-olds or 1-year-olds. Similarity in ANS resting measures between our study population and others suggests that the resting ANS measures observed in this population were not due to cohort effects. However, it is hard to compare the profile scores and/or reactivity scores, from which the profiles are derived, as there are no "norms" reported in the literature. Profiles and difference scores have been reported to differ by age and health status, and few studies have reported the distribution of profile scores in their populations overall or by ethnicity. In one pilot study of 4-9 year-old African American children with sickle-cell disease (Pearson, Alkon et al. 2005) 26% of the children were classified into profile four (classic reactive profile or reciprocal sympathetic activation and parasympathetic withdrawal) and in a study of 8-10 and 15-18 year-old European- and African American children 75% (Salomon, Matthews et al. 2000) were classified into profile four. In our population, the percentage of children classified into this profile differed by age; 20, 15, 41, and 37% of children at 6 months, 1, 3 ½ and 5 years, respectively were classified into this profile. It is not certain whether our children responded differently to the ANS reactivity protocols than other ethnic groups and because there are no other studies that stratified by ethnic group there is no comparison group to determine if there is a cohort effect for this sample of primarily Latino children for profile scores (i.e., difference scores).

Research on the effects of OP pesticide exposure on ANS regulation is limited and results are not consistent. For example, one study in dogs (n=6; three controls and three OP-treated dogs) by Dellinger et al. (Dellinger, McKiernan et al. 1987) found no overall change in resting RSA (parasympathetic withdrawal) in OP-exposed dogs (dose = 60 mg/kg) followed by a decrease in resting RSA after an atropine sulfate treatment (an OP pesticide antidote). Authors reported similar findings in another animal study (Dellinger and Mostrom 1988). In one human study (n=4; two exposed aerial pesticide applicators and two controls) (Dellinger 1985) researchers reported an increase in resting RSA (less parasympathetic withdrawal) as a result of postulated OP pesticide exposure in only one of the exposed subjects and decreasing resting RSA in both controls and one exposed subject. These studies used different protocols and equipment to assess RSA from ours, and were severely limited by the small sample sizes. Furthermore, the animals in the Dellinger et al. studies (Dellinger,

McKiernan et al. 1987; Dellinger and Mostrom 1988) and the exposed subjects in the human study experienced acute OP pesticide exposures (versus children in our population who experienced low-level chronic OP pesticide exposures as assessed via DAPs). Additionally, the human study only measured cholinesterase inhibition (biomarker of effect not specific to OP pesticide exposure) rather than using biomarkers of OP pesticide exposure and/or environmental monitoring to confirm OP pesticide exposure. The human study also used the dark-adapted pupil dilation test to evaluate ChE inhibition; however, previous research suggests that this is not a sensitive indicator for systemic exposure to OP pesticides (Elkner, Fenske et al. 1991). No sympathetic measures (e.g., PEP) were obtained in any of these studies. These factors limit comparability to our study.

Although DAP metabolites have been extensively used in epidemiologic and exposure studies as biomarkers of OP pesticide exposure (Duggan, Charnley et al. 2003; Engel, Berkowitz et al. 2007; Eskenazi, Marks et al. 2007), there are limitations to this biomarker. Recent studies suggest that DAP metabolites in urine may also represent exposure to preformed DAPs present in a person's environment (Lu, Bravo et al. 2005; Quirós-Alcalá, Bradman et al. Submitted, 2010). Additionally, because OP pesticide exposure may vary substantially from day to day and OP pesticides have short biological half-lives, DAP metabolites in spot urine samples may not reflect long-term OP pesticide exposure throughout periods of critical neurologic development (Eskenazi, Marks et al. 2007). Preliminary results from our Center (personal communication) indicate that 24-hour urine samples from 3 to 6 year-old children obtained three days apart are not significantly correlated ($r=0.14$, $p=0.50$ for unadjusted total DAP concentrations) confirming the rapid metabolism of these pesticides. The variability in metabolite excretion is further confirmed by our finding that children's prenatal and postnatal DAP metabolite concentrations did not consistently fall into the same exposure quartiles at each time point. In addition to the short biological half-lives of OP pesticides, exposure sources and pathways are likely to vary over time explaining, in part, some of this variability. Thus, it is possible that some misclassification of OP pesticide exposure occurred in our population. Moreover, while adjustment for creatinine was conducted to account for dilution of metabolite concentrations, this practice may not be appropriate for populations undergoing rapid physiologic changes, such as pregnant women and young children, due to the high intraindividual variability in creatinine concentrations. For example, urinary creatinine levels change depending on the time of gestation; creatinine levels are approximately 20% lower in the third trimester compared to the first trimester. Moreover, urine volume may increase approximately 25% during pregnancy (Ye, Pierik et al. 2008). The extent to which these and other changes affect pesticide metabolite clearance remains unclear.

Other study limitations include the fact that we conducted multiple cross-sectional analyses and did not adjust for multiple comparisons as mentioned previously. Additionally, we focused on a relatively demographically and ethnically homogeneous study population; thus, our results may not be generalizable to other populations. It is also not known whether genetic differences between our population and others would result in different findings. This study also has a number of strengths. Because we focused on children living in an agricultural region with intense OP pesticide use and children lived with at least one farmworker we were able to focus on a vulnerable group which has been found to exhibit higher OP pesticide exposures than the general population (McKone, Castorina et al. 2007). Additionally, we had

relatively large sample sizes at each time point and were able to assess exposure and ANS regulation at multiple time points. Having a demographically and ethnically homogenous population at each time point may limit generalizability of our findings; however, this allowed us to account for unmeasured confounders in our population. Another study strength involved our use of previously validated ANS protocols (Boyce, Quas et al. 2001; Alkon, Goldstein et al. 2003; Alkon, Lippert et al. 2006) and use of reliable ANS response measures (i.e., indices capable of measuring parasympathetic and sympathetic response).

In summary, we report no detrimental effects of prenatal and postnatal OP pesticide exposure, as measured by urinary DAPs, on children's individual psychobiologic measures including resting and reactivity measures for HR, RSA, and PEP. This is the first study to use ANS response measures as outcomes to investigate the association between OP pesticide exposures in children and ANS regulation. Future investigations in this population will involve evaluating what factors predict ANS regulation and whether ANS resting and reactivity measures of HR, RSA, and PEP are related to later physical and mental problems as observed in prior studies.

Supporting Information (SI)

Supporting information is provided for this chapter on the direction of ANS measures with increasing OP pesticide exposure, interpretation on PEP and RSA difference scores by reactivity status, and on the selection of the population for analyses. This information is available in the Appendix.

Chapter 4: Tables

Table 1. Description of ANS reactivity protocols.

	Description of task	
Challenge Task	*6 month-old and 1- year-old participants*[a]	*3½- and 5-year-old participants*[b]
Social	Child watched a jack-in-the box wound up and jumping out of the box three times. (1 min.)	Child was asked a series of questions derived from the Gessell School Readiness Screening Test (Carlson RD 1985).(3 mins)
Cognitive	No cognitive task administered.	Child was asked to repeat a series of numbers up to six digits in length (Kaufman & Kaufman, 1983). (2 mins.)
Emotional	The child listened to a digital recording of a sick baby crying. (1 min.)	Child watched two short emotion-evoking videoclips selected to elicit fear (a child having a nightmare during a thunderstorm) and sadness (a child whose pet bird has dies) (Eisenberg, Fabes et al. 1988). (4 minutes)
Physical	The child felt a vibrator on his/her legs, one at a time. (1 min.)	Child was asked to identify a drop of concentrated lemon juice placed on the child's tongue. (1 min.)

a. Protocol was preceded and followed by 2-min resting periods to obtain resting physiologic measures. For this age group, resting periods consisted of children listening to digitally recorded lullabies.
b. Age-appropriate relaxing stories (2 minutes each) were read to the child before and upon conclusion of the protocol to obtain resting physiologic measures. In addition, a 1 min. recovery period was included after the cognitive task.

Table 2. Demographic characteristics of study participants at each ANS assessment.[a]

	6 month visit (n=149)		1 year visit (n=149)		3½ year visit (n=97)		5 year visit (n=274)	
Characteristic								
Child	**mean**	**(SD)**	**mean**	**SD**	**mean**	**SD**	**mean**	**SD**
Average age (months)	6.8	(1.1)	12.8	(1.4)	43.7	(2.3)	60.9	(2.4)
Average years of family living in the U.S.[b]	7.6	(7.3)	7.8	(7.0)	7.8	(6.7)	8.1	(7.5)
Sex	**n**	**%**	**n**	%	**n**	%	**n**	%
Male	84	(56.4)	72	(48.3)	45	(46.4)	132	(48.2)
Female	65	(43.6)	77	(51.7)	52	(53.6)	142	(51.8)
Birth weight (g)								
<2,500	7	(4.7)	8	(5.4)	4	(4.1)	8	(2.9)
2,500-3,500	73	(49.0)	80	(53.7)	58	(59.8)	145	(52.9)
>3,500	69	(46.3)	61	(40.9)	35	(36.1)	121	(44.2)
Gestational age (weeks)								
≥37	140	(94.0)	139	(93.3)	91	(93.8)	254	(92.7)
<37	9	(6.0)	10	(6.7)	6	(6.2)	20	(7.3)
Mother								
Age at delivery (years)								
18-24	70	(47.0)	61	(40.9)	39	(40.2)	106	(38.7)
25-29	49	(32.9)	47	(31.5)	35	(36.1)	94	(34.3)
30-34	22	(14.8)	30	(20.1)	16	(16.5)	48	(17.5)
35-45	8	(5.4)	11	(7.4)	7	(7.2)	26	(9.5)
Education								
≤6th grade	64	(43.0)	64	(43.0)	43	(44.3)	117	(42.7)
7th-12th grade (no diploma)	57	(38.3)	54	(36.2)	33	(34.0)	99	(36.1)
High school diploma or more	28	(18.8)	31	(20.8)	21	(21.7)	58	(21.2)
Mother's country of birth								
US	16	(10.7)	18	(12.1)	9	(9.3)	35	(12.8)
Mexico	132	(88.6)	129	(86.6)	86	(88.7)	236	(86.1)
Other	1	(0.7)	2	(1.3)	2	(2.1)	3	(1.1)
Family income above poverty threshold[c,d]								
No	104	(69.8)	98	(65.8)	60	(61.9)	168	(61.3)
Yes	45	(30.2)	51	(34.2)	37	(38.1)	106	(38.7)
Tobacco use during pregnancy								
No	142	(95.3)	143	(96.0)	95	(97.9)	263	(96.0)
Yes	7	(4.7)	6	(4.0)	2	(2.1)	11	(4.0)
Any alcohol consumption during pregnancy[c]								
No	110	(75.9)	112	(76.2)	75	(78.1)	199	(74.3)
Yes	35	(24.1)	35	(23.8)	21	(21.9)	69	(25.8)

a. Data presented for each time point is for children who participated in the ANS reactivity assessment and provided a urine specimen at the specified time point, and had at least one of the two prenatal DAP measurements available. b. Information obtained at the first prenatal visit. c. Information was missing for some mothers so numbers may not add to the total number of participants at specified time point. d. Information is based on U.S. Census Bureau data for the year in which the assessment took place.

Table 3. Summary statistics for children's ANS resting measures and difference scores at each time point.[a,b]

	Resting Measures			Difference Scores		
Collection time point	**n**	**Mean**	**(SD)**	**n**	**Mean**	**(SD)**
6 months						
RSA	147	3.3	(0.8)	146	0.01	(0.5)
HR	148	139.8	(10.8)	147	-0.8	(5.4)
PEP	139	66.4	(9.0)	138	0.2	(3.6)
1 year						
RSA	149	3.7	(0.9)	147	0.1	(0.6)
HR	147	133.2	(12.3)	145	-2.7	(6.0)
PEP	141	66.6	(10.7)	137	0.6	(4.1)
3½ years						
RSA	97	6.3	(1.1)	97	-0.4	(0.6)
HR	97	100.9	(9.8)	97	4.9	(3.5)
PEP	96	74.0	(6.7)	96	0.1	(1.8)
5 years						
RSA	273	6.8	(1.2)	273	-0.2	(0.5)
HR	274	93.8	(10.8)	274	5.7	(4.4)
PEP	272	78.3	(7.4)	272	-0.2	(1.7)

a. Abbreviations: SD= standard deviation; RSA= resting respiratory sinus arrhythmia; HR= heart rate; PEP= pre-ejection period. b. Values may vary slightly depending on the number of children with available information at the collection time point.

Table 4. Geometric Mean (GM) for mother's (prenatal) DAP concentrations and respective Confidence Intervals (CI) for those children with ANS and DAP measurements at each time point.[a]

Sample size[b] and respective metabolites	Unadjusted Concentrations (nmol/L)		Creatinine adjusted Concentrations (nmol/gCre)[b]	
6 months (n=149)	**GM**	**(95% CI)**	**GM**	**(95% CI)**
Total DEs	23.1	(19.7 ,27.2)	25.5	(21.5 ,30.2)
Total DMs	123.4	(102.5 ,148.5)	133.6	(111.4 ,160.2)
Total DAPs	159.8	(134.9 ,189.3)	174.3	(147.5 ,205.9)
1 year (n=149)				
Total DEs	25.6	(21.7 ,30.2)	28.5	(24.1 ,33.6)
Total DMs	126.7	(104.9 ,153.0)	137.6	(114.7 ,165.0)
Total DAPs	166.6	(139.8 ,198.4)	181.9	(153.8 ,215.1)
3 1/2 years (n=97)				
Total DEs	22.2	(18.1 ,27.3)	23.3	(18.8 ,28.8)
Total DMs	116.1	(94.7 ,142.2)	117.0	(94.7 ,144.6)
Total DAPs	148.4	(122.4 ,179.9)	150.7	(123.1 ,184.4)
5 years (n=274)				
Total DEs	21.5	(19.2 ,24.2)	25.8	(22.9 ,29.1)
Total DMs	100.2	(88.6 ,113.3)	116.2	(102.4 ,131.9)
Total DAPs	134.8	(120.8 ,150.4)	157.3	(140.2 ,176.5)

a. Arithmetic mean prenatal DAPs were first calculated by taking the average of the mother's DAP concentrations at approximately 14 and 26 weeks gestation. The geometric means reported in this table are for the arithmetic mean metabolite concentrations.
b. Sample sizes indicated are for the number of mother's whose children completed ANS protocols at each time point.

Table 5. Geometric mean (GM) concentrations and respective confidence intervals (CIs) for children's (postnatal) urinary DAP metabolite concentrations at each ANS assessment.[a,b]

	Unadjusted Metabolite Concentrations (nmol/L)		Creatinine-adjusted Metabolite Concentrations (nmol/gCre)	
Metabolite	**GM**	**(95% CI)**	**GM**	**(95% CI)**
6 months (n=149)				
Total DEs	10.4	(8.2,13.1)	53.9	(42.4, 68.6)
Total DMs	26.2	(20.1, 34.2)	135.9	(103.1, 179.2)
Total DAPs	48.6	(38.5, 61.3)	252.0	(199.0, 319.0)
12 months (n=149)				
Total DEs	10.7	(8.5, 13.3)	46.7	(37.6, 58.1)
Total DMs	30.2	(22.5, 40.4)	132.4	(98.2, 178.6)
Total DAPs	50.4	(38.9, 65.2)	221.0	(170.8, 285.9)
3½ years (n=97)				
Total DEs	6.6	(4.8, 9.1)	12.9	(9.3, 17.8)
Total DMs	79.9	(57.8, 110.4)	154.5	(113.0, 211.2)
Total DAPs	96.5	(70.9, 131.5)	186.7	(138.7, 251.4)
5 years (n=274)				
Total DEs	7.6	(6.3, 9.3)	11.1	(9.1, 13.5)
Total DMs	78.5	(65.6, 93.9)	114.1	(95.8, 136.0)
Total DAPs	100.8	(85.0, 119.6)	146.6	(124.2, 173.0)

a. Individual metabolites were converted to molar concentrations and then summed to produce total DEs, DMs, and DAPs. Abbreviations: 95% CI= 95% Confidence interval for the geometric mean; DEs= Diethyls; DM=Dimethyls; LOD= limit of detection. LODs for individual metabolites were as follows: DEP=0.2 µg/L, DETP=0.1µg/L, DEDTP=0.1µg/L, DMP=0.6 µg/L, DMTP=0.2 µg/L, and DMDTP=0.1 µg/L.
b. Sample size indicated reflects subset of children with at each time point with available ANS and DAP data.

Table 6a. Cross sectional models using both unadjusted child and prenatal DAPs (Resting Measures).[a, b,c]

	6 months					1 year					3 1/2 years					5 years				
	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p
Resting Scores																				
RSA																				
DEs(child)	142	-0.13	(-0.34,	0.09)	0.23	149	-0.03	(-0.29,	0.24)	0.84	95	-0.05	(-0.37,	0.27)	0.76	270	0.03	(-0.17,	0.23)	0.77
DEs (PN)		-0.06	(-0.37,	0.25)	0.70		-0.13	(-0.47,	0.21)	0.44		0.22	(-0.30,	0.74)	0.40		0.17	(-0.17,	0.51)	0.34
DMs (child)	142	-0.24	(-0.42,	-0.05)	0.02**	149	-0.06	(-0.26,	0.13)	0.52	95	-0.15	(-0.46,	0.17)	0.35	270	-0.13	(-0.34,	0.09)	0.26
DMs (PN)		-0.07	(-0.34,	0.19)	0.58		-0.10	(-0.39,	0.20)	0.52		0.13	(-0.39,	0.64)	0.63		0.02	(-0.30,	0.34)	0.91
DAPs(child)	142	-0.27	(-0.48,	-0.06)	0.01**	149	-0.06	(-0.28,	0.16)	0.58	95	-0.13	(-0.46,	0.20)	0.45	270	-0.11	(-0.34,	0.12)	0.33
DAPS (PN)		-0.05	(-0.33,	0.24)	0.75		-0.11	(-0.43,	0.21)	0.50		0.19	(-0.35,	0.73)	0.49		0.14	(-0.22,	0.49)	0.46
HR																				
DEs(child)	143	0.62	(-2.23,	3.47)	0.67	147	-0.02	(-3.57,	3.53)	0.99	95	1.58	(-1.38,	4.53)	0.29	271	-0.14	(-1.92,	1.65)	0.88
DEs (PN)		-0.38	(-4.51,	3.75)	0.86		-1.63	(-6.12,	2.86)	0.47		-3.78	(-8.51,	0.95)	0.12		-0.77	(-3.87,	2.33)	0.63
DMs (child)	143	1.41	(-1.11,	3.94)	0.27	147	0.44	(-2.14,	3.02)	0.74	95	2.28	(-0.58,	5.14)	0.12	271	-1.13	(-3.09,	0.84)	0.26
DMs (PN)		-1.19	(-4.71,	2.33)	0.51		-2.48	(-6.39,	1.43)	0.21		-3.11	(-7.81,	1.60)	0.19		1.96	(-0.93,	4.85)	0.18
DAPs(child)	143	1.39	(-1.43,	4.21)	0.33	147	0.38	(-2.58,	3.34)	0.80	95	2.17	(-0.83,	5.16)	0.15	271	-1.14	(-3.21,	0.93)	0.28
DAPS (PN)		-1.09	(-4.96,	2.78)	0.58		-2.50	(-6.72,	1.73)	0.25		-3.82	(-8.74,	1.10)	0.13		1.36	(-1.89,	4.60)	0.41
PEP																				
DEs(child)	136	-0.59	(-3.15,	1.98)	0.65	141	4.33	(1.24,	7.42)	0.01**	94	-0.96	(-2.87,	0.94)	0.32	269	0.70	(-0.53,	1.93)	0.26
DEs (PN)		1.25	(-2.46,	4.96)	0.51		2.74	(-1.20,	6.68)	0.17		1.03	(-2.01,	4.07)	0.50		0.39	(-1.75,	2.53)	0.72
DMs (child)	136	-0.06	(-2.27,	2.16)	0.96	141	0.34	(-1.99,	2.68)	0.77	94	0.74	(-1.11,	2.59)	0.43	269	0.27	(-1.10,	1.63)	0.70
DMs (PN)		-0.77	(-3.90,	2.35)	0.62		3.77	(0.21,	7.33)	0.04**		1.04	(-2.01,	4.09)	0.50		-1.18	(-3.18,	0.83)	0.25
DAPs(child)	136	-0.31	(-2.83,	2.22)	0.81	141	1.27	(-1.39,	3.92)	0.35	94	0.57	(-1.38,	2.51)	0.56	269	0.35	(-1.09,	1.79)	0.63
DAPS (PN)		-0.67	(-4.11,	2.76)	0.70		3.72	(-0.09,	7.53)	0.06*		1.27	(-1.92,	4.47)	0.43		-0.86	(-3.11,	1.39)	0.45

a. Cross sectional models adjusted for sex, exact age at time of ANS assessment (years), breastfeeding duration at the time of ANS assessment (months), location of ANS assessment, psychometrician conducting ANS assessment, average prenatal creatinine concentration and child creatinine concentration. b. To obtain a measure of prenatal DAPs exposure we calculated an average of DAPs collected at approximately 14 and 26 weeks of gestation and then log10 transformed the average to generate prenatal averages. c. Child DAP concentrations were log-10 transformed in models. Beta coefficients are provided for child and prenatal (PN) DAPs. ** $p<0.05$, * $p<0.10$.

Table 6b. Cross sectional models using both unadjusted child and prenatal DAPs (Difference Scores).[a, b,c]

	6 months					1 year					3 1/2 years					5 years				
	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p
Difference Scores																				
RSA																				
DEs(child)	141	0.00	(-0.14,	0.15)	0.96	147	0.09	(-0.09,	0.27)	0.33	95	0.01	(-0.16,	0.18)	0.89	270	-0.02	(-0.11,	0.07)	0.67
DEs (PN)		-0.09	(-0.31,	0.12)	0.38		0.01	(-0.22,	0.24)	0.96		0.02	(-0.25,	0.29)	0.89		-0.01	(-0.17,	0.14)	0.86
DMs (child)	141	-0.08	(-0.21,	0.05)	0.22	147	-0.01	(-0.14,	0.12)	0.88	95	-0.02	(-0.19,	0.14)	0.80	270	0.06	(-0.04,	0.16)	0.21
DMs (PN)		-0.15	(-0.33,	0.03)	0.10*		0.25	(0.05,	0.45)	0.01**		0.07	(-0.21,	0.34)	0.63		-0.04	(-0.18,	0.11)	0.63
DAPs(child)	141	-0.10	(-0.24,	0.05)	0.19	147	0.01	(-0.14,	0.16)	0.91	95	-0.03	(-0.20,	0.14)	0.74	270	0.06	(-0.04,	0.16)	0.26
DAPS (PN)		-0.17	(-0.36,	0.03)	0.09*		0.24	(0.03,	0.46)	0.03**		0.06	(-0.23,	0.34)	0.70		-0.08	(-0.25,	0.08)	0.33
HR																				
DEs(child)	142	1.23	(-0.24,	2.71)	0.10*	145	-1.09	(-2.89,	0.71)	0.23	95	0.00	(-1.07,	1.08)	0.99	271	-0.30	(-1.00,	0.41)	0.41
DEs (PN)		0.44	(-1.69,	2.57)	0.68		0.13	(-2.16,	2.42)	0.91		-0.69	(-2.41,	1.02)	0.42		0.22	(-1.01,	1.45)	0.73
DMs (child)	142	0.78	(-0.54,	2.09)	0.25	145	-0.13	(-1.46,	1.19)	0.84	95	-0.02	(-1.07,	1.02)	0.96	271	0.08	(-0.71,	0.86)	0.85
DMs (PN)		0.52	(-1.31,	2.36)	0.57		-0.32	(-2.34,	1.70)	0.75		-0.44	(-2.16,	1.27)	0.61		0.19	(-0.96,	1.35)	0.74
DAPs(child)	142	1.20	(-0.26,	2.67)	0.11	145	-0.36	(-1.88,	1.15)	0.64	95	0.08	(-1.01,	1.17)	0.89	271	-0.09	(-0.91,	0.73)	0.83
DAPS (PN)		0.62	(-1.37,	2.62)	0.54		-0.20	(-2.38,	1.98)	0.86		-0.51	(-2.31,	1.28)	0.57		0.42	(-0.87,	1.72)	0.52
PEP																				
DEs(child)	135	-0.11	(-1.10,	0.88)	0.83	137	0.75	(-0.50,	2.00)	0.24	94	-0.21	(-0.77,	0.36)	0.47	269	0.23	(-0.05,	0.50)	0.11
DEs (PN)		0.07	(-1.37,	1.51)	0.92		-0.08	(-1.66,	1.49)	0.92		0.18	(-0.72,	1.07)	0.70		-0.26	(-0.74,	0.22)	0.28
DMs (child)	135	0.05	(-0.80,	0.89)	0.91	137	0.37	(-0.53,	1.28)	0.41	94	-0.20	(-0.74,	0.35)	0.47	269	0.15	(-0.16,	0.46)	0.35
DMs (PN)		1.21	(0.03,	2.40)	0.05*		-1.00	(-2.39,	0.38)	0.16		0.23	(-0.67,	1.13)	0.61		-0.32	(-0.77,	0.14)	0.17
DAPs(child)	135	-0.03	(-1.00,	0.93)	0.95	137	0.64	(-0.40,	1.67)	0.22	94	-0.23	(-0.80,	0.34)	0.42	269	0.18	(-0.14,	0.50)	0.28
DAPS (PN)		1.23	(-0.07,	2.54)	0.06*		-1.07	(-2.56,	0.41)	0.16		0.27	(-0.67,	1.21)	0.57		-0.35	(-0.85,	0.16)	0.18

a. Cross sectional models adjusted for sex, exact age at time of ANS assessment (years), breastfeeding duration at the time of ANS assessment (months), location of ANS assessment, psychometrician conducting ANS assessment, average prenatal creatinine concentration and child creatinine concentration. b. To obtain a measure of prenatal DAPs exposure we calculated an average of DAPs collected at approximately 14 and 26 weeks of gestation and then log10 transformed the average to generate prenatal averages. c.Child DAP concentrations were log-10 transformed in models. Beta coefficients are provided for child and prenatal (PN) DAPs. ** $p<0.05$, * $p<0.10$

Table 6c. Results from cross sectional models using creatinine adjusted child and prenatal DAPs (Resting Measures).[a, b,c]

	6 months					1 year					3 1/2 years					5 years				
	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p
Resting Scores																				
RSA																				
DEs(child)	142	0.01	(-0.21,	0.22)	0.96	149	0.00	(-0.27,	0.27)	0.98	95	-0.05	(-0.37,	0.26)	0.75	270	0.07	(-0.13,	0.27)	0.49
DEs (PN)		-0.11	(-0.40,	0.19)	0.47		-0.11	(-0.45,	0.23)	0.52		0.17	(-0.32,	0.67)	0.49		0.08	(-0.24,	0.41)	0.61
DMs (child)	142	-0.12	(-0.31,	0.07)	0.21	149	-0.05	(-0.25,	0.14)	0.59	95	-0.16	(-0.49,	0.16)	0.32	270	-0.09	(-0.31,	0.13)	0.44
DMs (PN)		-0.07	(-0.34,	0.20)	0.60		-0.11	(-0.42,	0.19)	0.47		0.11	(-0.39,	0.60)	0.67		-0.01	(-0.32,	0.29)	0.93
DAPs(child)	142	-0.14	(-0.35,	0.08)	0.21	149	-0.05	(-0.28,	0.17)	0.65	95	-0.14	(-0.49,	0.20)	0.41	270	-0.07	(-0.30,	0.16)	0.55
DAPS (PN)		-0.04	(-0.34,	0.25)	0.77		-0.12	(-0.45,	0.21)	0.47		0.15	(-0.36,	0.67)	0.56		0.09	(-0.25,	0.43)	0.60
HR																				
DEs(child)	143	0.37	(-2.42,	3.17)	0.79	147	0.10	(-3.52,	3.71)	0.96	95	1.22	(-1.67,	4.11)	0.40	271	-0.45	(-2.27,	1.37)	0.63
DEs (PN)		0.88	(-3.01,	4.76)	0.66		-2.48	(-6.93,	1.97)	0.27		-3.47	(-7.99,	1.05)	0.13		-0.33	(-3.31,	2.65)	0.83
DMs (child)	143	1.32	(-1.16,	3.79)	0.29	147	0.47	(-2.08,	3.01)	0.72	95	2.16	(-0.78,	5.10)	0.15	271	-1.51	(-3.51,	0.48)	0.14
DMs (PN)		-0.68	(-4.26,	2.91)	0.71		-2.75	(-6.80,	1.30)	0.18		-3.95	(-8.43,	0.53)	0.08**		1.93	(-0.85,	4.71)	0.17
DAPs(child)	143	1.31	(-1.52,	4.15)	0.36	147	0.43	(-2.55,	3.40)	0.78	95	2.03	(-1.06,	5.13)	0.20	271	-1.57	(-3.69,	0.54)	0.14
DAPS (PN)		-0.45	(-4.38,	3.47)	0.82		-2.91	(-7.30,	1.48)	0.19		-4.45	(-9.11,	0.21)	0.06**		1.18	(-1.90,	4.25)	0.45
PEP																				
DEs(child)	136	-0.91	(-3.36,	1.55)	0.47	141	3.36	(0.14,	6.58)	0.04**	94	-0.90	(-2.75,	0.95)	0.34	269	0.60	(-0.65,	1.86)	0.34
DEs (PN)		0.06	(-3.38,	3.50)	0.97		1.40	(-2.63,	5.42)	0.49		1.84	(-1.04,	4.72)	0.21		0.43	(-1.63,	2.49)	0.68
DMs (child)	136	-0.36	(-2.48,	1.77)	0.74	141	-0.22	(-2.55,	2.10)	0.85	94	0.79	(-1.12,	2.69)	0.41	269	0.11	(-1.28,	1.50)	0.88
DMs (PN)		-1.58	(-4.78,	1.61)	0.33		3.51	(-0.26,	7.28)	0.07*		1.73	(-1.19,	4.65)	0.24		-0.93	(-2.86,	1.00)	0.34
DAPs(child)	136	-0.69	(-3.15,	1.77)	0.58	141	0.58	(-2.13,	3.29)	0.67	94	0.61	(-1.39,	2.62)	0.54	269	0.18	(-1.30,	1.65)	0.82
DAPS (PN)		-1.56	(-5.05,	1.92)	0.38		3.40	(-0.66,	7.46)	0.10*		2.05	(-0.99,	5.08)	0.18		-0.63	(-2.76,	1.51)	0.56

a. Cross sectional models adjusted for sex, exact age at time of ANS assessment (years), breast feeding duration at the time of ANS assessment (months), location of ANS assessment and psychometrician conducting ANS assessment. b.To obtain a measure of prenatal DAPs exposure we calculated an average of DAPs collected at approximately 14 and 26 weeks of gestation and then log10 transformed the average to generate prenatal averages. c. Child DAP concentrations were log-10 transformed in models. Beta coefficients are provided for child and prenatal (PN) DAPs. ** $p<0.05$, * $p<0.10$

Table 6d. Results from cross sectional models using creatinine adjusted child DAPs and prenatal DAPs (Difference Scores).[a, b,c]

	6 months					1 year					3 1/2 years					5 years				
	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p	n	β	(95%	CI)	p
Difference Scores																				
RSA																				
DEs(child)	141	-0.03	(-0.17,	0.12)	0.72	147	0.06	(-0.12,	0.25)	0.49	95	0.01	(-0.15,	0.18)	0.88	270	-0.02	(-0.12,	0.07)	0.60
DEs (PN)		-0.10	(-0.30,	0.10)	0.32		0.02	(-0.21,	0.24)	0.88		-0.04	(-0.30,	0.22)	0.74		-0.02	(-0.17,	0.13)	0.78
DMs (child)	141	-0.10	(-0.22,	0.03)	0.13	147	-0.02	(-0.15,	0.11)	0.72	95	-0.02	(-0.19,	0.15)	0.81	270	0.06	(-0.04,	0.16)	0.24
DMs (PN)		-0.16	(-0.34,	0.02)	0.08*		0.26	(0.05,	0.46)	0.01**		0.06	(-0.20,	0.32)	0.63		-0.02	(-0.17,	0.12)	0.73
DAPs(child)	141	-0.12	(-0.26,	0.02)	0.10*	147	-0.01	(-0.16,	0.14)	0.91	95	-0.03	(-0.21,	0.15)	0.75	270	0.06	(-0.05,	0.16)	0.31
DAPS (PN)		-0.18	(-0.37,	0.02)	0.08*		0.26	(0.04,	0.48)	0.02**		0.05	(-0.22,	0.32)	0.73		-0.07	(-0.22,	0.09)	0.41
HR																				
DEs(child)	142	1.22	(-0.22,	2.67)	0.10*	145	-1.11	(-2.96,	0.74)	0.24	95	-0.10	(-1.14,	0.95)	0.86	271	-0.28	(-1.00,	0.44)	0.45
DEs (PN)		-0.18	(-2.18,	1.82)	0.86		-0.10	(-2.36,	2.16)	0.93		-0.56	(-2.19,	1.08)	0.50		0.05	(-1.13,	1.24)	0.93
DMs (child)	142	0.70	(-0.59,	1.99)	0.29	145	-0.10	(-1.41,	1.21)	0.88	95	-0.13	(-1.21,	0.94)	0.80	271	0.11	(-0.69,	0.91)	0.79
DMs (PN)		-0.16	(-2.03,	1.71)	0.87		-0.59	(-2.68,	1.50)	0.58		-0.70	(-2.34,	0.93)	0.40		0.08	(-1.03,	1.20)	0.88
DAPs(child)	142	1.19	(-0.28,	2.66)	0.11	145	-0.32	(-1.84,	1.21)	0.68	95	-0.04	(-1.17,	1.09)	0.95	271	-0.06	(-0.90,	0.78)	0.89
DAPS (PN)		-0.24	(-2.27,	1.79)	0.81		-0.59	(-2.84,	1.66)	0.61		-0.76	(-2.47,	0.94)	0.38		0.33	(-0.90,	1.56)	0.60
PEP																				
DEs(child)	135	-0.24	(-1.18,	0.71)	0.62	137	1.08	(-0.21,	2.37)	0.10*	94	-0.15	(-0.70,	0.40)	0.59	269	0.18	(-0.10,	0.47)	0.20
DEs (PN)		0.23	(-1.11,	1.57)	0.74		0.04	(-1.51,	1.59)	0.96		-0.18	(-1.04,	0.67)	0.67		-0.12	(-0.58,	0.35)	0.62
DMs (child)	135	-0.11	(-0.92,	0.69)	0.79	137	0.50	(-0.39,	1.39)	0.27	94	-0.16	(-0.72,	0.41)	0.59	269	0.09	(-0.22,	0.41)	0.56
DMs (PN)		1.49	(0.28,	2.70)	0.02**		-1.07	(-2.51,	0.37)	0.14		0.16	(-0.70,	1.03)	0.71		-0.15	(-0.59,	0.29)	0.51
DAPs(child)	135	-0.23	(-1.17,	0.70)	0.63	137	0.82	(-0.22,	1.86)	0.12	94	-0.19	(-0.78,	0.40)	0.53	269	0.12	(-0.21,	0.45)	0.48
DAPS (PN)		1.50	(0.18,	2.82)	0.03**		-1.10	(-2.64,	0.44)	0.16		0.15	(-0.75,	1.05)	0.75		-0.14	(-0.62,	0.34	0.56

a. Cross sectional models adjusted for sex, exact age at time of ANS assessment (years), breast feeding duration at the time of ANS assessment (months), location of ANS assessment and psychometrician conducting ANS assessment. b.To obtain a measure of prenatal DAPs exposure we calculated an average of DAPs collected at approximately 14 and 26 weeks of gestation and then log10 transformed the average to generate prenatal averages. c. Child DAP concentrations were log-10 transformed in models. Beta coefficients are provided for child and prenatal (PN) DAPs. ** $p<0.05$, * $p<0.10$

Table 7a. Geometric mean OP urinary metabolite levels (unadjusted; total child DAPs) by ANS profiles at each assessment.

	Profile	SNS	PNS	n	GM	95% CI	
6 months							
	Coactivation	+	+	22	46.3	(28.2,	76.2)
	Coinhibition	-	-	43	58.8	(39.5,	87.3)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	38.8	(24.9,	60.5)
	Reciprocal SNS activation & PNS withdrawal	+	-	20	88.4	(40.3,	193.9)
12 months							
	Coactivation	+	+	35	34.5	(20.4,	58.2)
	Coinhibition	-	-	33	58.0	(32.2,	104.5)
	Reciprocal PNS activation & SNS withdrawal	-	+	43	68.6	(42.2,	111.8)
	Reciprocal SNS activation & PNS withdrawal	+	-	21	46.9	(21.0,	105.0)
3 1/2 years							
	Coactivation	+	+	11	93.3	(23.2,	376.0)
	Coinhibition	-	-	26	77.2	(45.2,	131.8)
	Reciprocal PNS activation & SNS withdrawal	-	+	14	73.6	(37.1,	146.3)
	Reciprocal SNS activation & PNS withdrawal	+	-	40	132.1	(78.9,	221.3)
5 years							
	Coactivation	+	+	47	99.4	(66.2,	149.4)
	Coinhibition	-	-	75	110.0	(79.2,	153.0)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	124.8	(73.7,	211.4)
	Reciprocal SNS activation & PNS withdrawal	+	-	99	90.4	(68.7,	119.0)

a. Total n at each time point may not add to the total number of children sampled at each designated time point as age categories at each time point were truncated as follows for the statistics reported in this table: 6 month time point (included children 5-8 months of age), 1 year time point (included children between 9 and 15 months of age), 3 1/2 year time point (included children 2 1/2 to 4 years of age), and 5 year time point (4.5-5.5 years of age). For cross sectional and longitudinal models, age at the time of assessment was included as a covariate so more children were included at each time point. b. "+" indicates activation and "-" indicates withdrawal or inhibition.

Table 7b. Geometric mean OP urinary metabolite levels (creatinine adjusted; total child DAPs) by ANS profiles at each assessment.

	Profile	SNS	PNS	n	GM	95% CI	
6 months							
	Coactivation	+	+	22	199.6	(120.6,	330.4)
	Coinhibition	-	-	43	338.5	(228.4,	501.5)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	188.7	(121.8,	292.3)
	Reciprocal SNS activation & PNS withdrawal	+	-	20	510.9	(234.9,	1111.3)
12 months							
	Coactivation	+	+	35	140.2	(82.6,	238.1)
	Coinhibition	-	-	33	265.9	(140.6,	502.8)
	Reciprocal PNS activation & SNS withdrawal	-	+	43	313.9	(196.4,	501.6)
	Reciprocal SNS activation & PNS withdrawal	+	-	21	229.1	(115.7,	453.8)
3 1/2 years							
	Coactivation	+	+	11	172.7	(46.8,	637.0)
	Coinhibition	-	-	26	156.2	(94.2,	259.1)
	Reciprocal PNS activation & SNS withdrawal	-	+	14	147.6	(79.4,	274.4)
	Reciprocal SNS activation & PNS withdrawal	+	-	40	243.3	(143.3,	413.0)
5 years							
	Coactivation	+	+	47	141.1	(95.7,	208.0)
	Coinhibition	-	-	75	165.6	(120.0,	228.5)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	154.9	(92.3,	260.0)
	Reciprocal SNS activation & PNS withdrawal	+	-	99	135.1	(103.3,	176.7)

a. Total n at each time point may not add to the total number of children sampled at each designated time point as age categories at each time point were truncated as follows for the statistics reported in this table: 6 month time point (included children 5-8 months of age), 1 year time point (included children between 9 and 15 months of age), 3 1/2 year time point (included children 2 1/2 to 4 years of age), and 5 year time point (4.5-5.5 years of age). For cross sectional and longitudinal models, age at the time of assessment was included as a covariate so more children were included at each time point. b. "+" indicates activation and "-" indicates withdrawal or inhibition.

Table 7c. Geometric mean OP urinary metabolite levels (unadjusted; total prenatal DAPs) by ANS profiles at each assessment.

	Profile	SNS	PNS	n	GM	95% CI	
6 months							
	Coactivation	+	+	22	147.7	(97.2,	224.6)
	Coinhibition	-	-	43	198.3	(143.6,	273.8)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	160.8	(113.8,	227.3)
	Reciprocal SNS activation & PNS withdrawal	+	-	20	110.0	(69.9,	173.1)
12 months							
	Coactivation	+	+	35	216.4	(157.0,	298.4)
	Coinhibition	-	-	33	141.8	(100.8,	199.5)
	Reciprocal PNS activation & SNS withdrawal	-	+	43	173.4	(125.2,	240.3)
	Reciprocal SNS activation & PNS withdrawal	+	-	21	143.1	(77.4,	264.6)
3 1/2 years							
	Coactivation	+	+	11	198.0	(101.9,	384.7)
	Coinhibition	-	-	26	185.9	(117.0,	295.4)
	Reciprocal PNS activation & SNS withdrawal	-	+	14	128.3	(72.4,	227.5)
	Reciprocal SNS activation & PNS withdrawal	+	-	40	121.2	(96.1,	152.9)
5 years							
	Coactivation	+	+	47	138.0	(102.0,	186.6)
	Coinhibition	-	-	75	132.2	(106.3,	164.3)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	115.0	(85.8,	154.1)
	Reciprocal SNS activation & PNS withdrawal	+	-	99	149.7	(126.4,	177.3)

a. Total n at each time point may not add to the total number of children sampled at each designated time point as age categories at each time point were truncated as follows for the statistics reported in this table: 6 month time point (included children 5-8 months of age), 1 year time point (included children between 9 and 15 months of age), 3 1/2 year time point (included children 2 1/2 to 4 years of age), and 5 year time point (4.5-5.5 years of age) and depends on the number of children with PN DAPs. For cross sectional and longitudinal models, age at the time of assessment was included as a covariate so more children were included at each time point. b. "+" indicates activation and "-" indicates withdrawal or inhibition.

Table 7d. Geometric mean OP urinary metabolite levels (creatinine adjusted; total prenatal DAPs) by ANS profiles at each assessment.

	Profile	SNS	PNS	n	GM	95% CI	
6 months							
	Coactivation	+	+	22	147.4	(103.8,	209.4)
	Coinhibition	-	-	43	212.7	(155.2,	291.6)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	182.1	(124.7,	266.1)
	Reciprocal SNS activation & PNS withdrawal	+	-	20	110.5	(74.2,	164.6)
12 months							
	Coactivation	+	+	35	214.8	(155.6,	296.6)
	Coinhibition	-	-	33	145.3	(104.4,	202.2)
	Reciprocal PNS activation & SNS withdrawal	-	+	43	203.2	(143.5,	287.6)
	Reciprocal SNS activation & PNS withdrawal	+	-	21	162.0	(94.7,	277.2)
3 1/2 years							
	Coactivation	+	+	11	195.0	(98.0,	387.7)
	Coinhibition	-	-	26	173.8	(113.9,	265.2)
	Reciprocal PNS activation & SNS withdrawal	-	+	14	122.8	(65.5,	230.2)
	Reciprocal SNS activation & PNS withdrawal	+	-	40	128.9	(98.9,	168.1)
5 years							
	Coactivation	+	+	47	169.1	(119.6,	239.1)
	Coinhibition	-	-	75	151.6	(120.7,	190.4)
	Reciprocal PNS activation & SNS withdrawal	-	+	41	136.6	(104.2,	179.1)
	Reciprocal SNS activation & PNS withdrawal	+	-	99	171.5	(143.4,	205.2)

a. Total n at each time point may not add to the total number of children sampled at each designated time point as age categories at each time point were truncated as follows for the statistics reported in this table: 6 month time point (included children 5-8 months of age), 1 year time point (included children between 9 and 15 months of age), 3 1/2 year time point (included children 2 1/2 to 4 years of age), and 5 year time point (4.5-5.5 years of age) and depends on the number of children with PN DAPs. For cross sectional and longitudinal models, age at the time of assessment was included as a covariate so more children were included at each time point. b. "+" indicates activation and "-" indicates withdrawal or inhibition.

Table 8a. ANS responses at 5 years as predicted by cumulative exposure proxies (unadjusted) [a,b]

	Resting Measures						Difference Scores				
	n	β	(95%	CI)	p		n	β	(95%	CI)	p
RSA						**RSA**					
Total DEs(child)	218	-0.03	(-0.33,	0.28)	0.87	Total DEs(child)	218	-0.03	(-0.17,	0.12)	0.73
Total DEs(PN)		0.16	(-0.18,	0.51)	0.35	Total DEs(PN)		-0.06	(-0.23,	0.10)	0.45
Total DMs(child)	218	0.05	(-0.28,	0.39)	0.77	Total DMs(child)	218	-0.10	(-0.26,	0.06)	0.22
Total DMs(PN)		-0.02	(-0.33,	0.29)	0.90	Total DMs(PN)		-0.03	(-0.18,	0.12)	0.69
Total DAPs(child)	218	0.15	(-0.22,	0.52)	0.43	Total DAPs(child)	218	-0.08	(-0.26,	0.10)	0.36
Total DAPs(PN)		0.03	(-0.30,	0.37)	0.85	Total DAPs(PN)		-0.04	(-0.20,	0.12)	0.65
HR						**HR**					
Total DEs(child)	218	2.43	(-0.33,	5.20)	0.09	Total DEs(child)	218	0.15	(-1.05,	1.35)	0.81
Total DEs(PN)		-3.19	(-6.29,	-0.09)	0.04	Total DEs(PN)		0.62	(-0.72,	1.96)	0.36
Total DMs(child)	218	0.67	(-2.36,	3.70)	0.66	Total DMs(child)	218	0.65	(-0.65,	1.95)	0.33
Total DMs(PN)		2.37	(-0.42,	5.17)	0.10	Total DMs(PN)		0.18	(-1.01,	1.38)	0.76
Total DAPs(child)	218	-2.93	(-6.27,	0.42)	0.09	Total DAPs(child)	218	0.75	(-0.69,	2.19)	0.31
Total DAPs(PN)		2.19	(-0.83,	5.21)	0.15	Total DAPs(PN)		0.13	(-1.17,	1.43)	0.85
PEP						**PEP**					
Total DEs(child)	216	-0.54	(-2.58,	1.51)	0.60	Total DEs(child)	216	-0.32	(-0.78,	0.14)	0.17
Total DEs(PN)		0.40	(-1.89,	2.69)	0.73	Total DEs(PN)		0.16	(-0.35,	0.68)	0.53
Total DMs(child)	216	1.33	(-0.89,	3.54)	0.24	Total DMs(child)	216	0.37	(-0.12,	0.87)	0.14
Total DMs(PN)		-0.49	(-2.53,	1.55)	0.64	Total DMs(PN)		-0.30	(-0.76,	0.15)	0.19
Total DAPs(child)	216	0.55	(-1.91,	3.01)	0.66	Total DAPs(child)	216	0.30	(-0.25,	0.86)	0.28
Total DAPs(PN)		-0.24	(-2.47,	1.98)	0.83	Total DAPs(PN)		-0.30	(-0.80,	0.19)	0.23

a. Models have been adjusted for sex, location of assessment, exact age and psychometrician at at the 5 year assessment. b. To estimate prenatal and postnatal cumulative exposure, we used an area-under-the-concentration–time-curve methodology (AUC). The AUC for the two prenatal pesticide measurements and the AUC for the five postnatal measurements were used as separate covariates in each model. Unadjusted metabolite concentrations were used to estimate the AUC. Prenatal and postnatal AUC values were log-normally distributed so log-10 AUC values were used in regression models.

Table 8b. ANS responses at 5 years as predicted by cumulative exposure proxies (creatinine-adjusted)[a,b]

	Resting Measures						Difference Scores				
	n	β	(95%	CI)	p		n	β	(95%	CI)	p
RSA						**RSA**					
Total DEs(child)	218	-0.09	(-0.45,	0.28)	0.64	Total DEs(child)	218	-0.05	(-0.23,	0.12)	0.53
Total DEs(PN)		0.12	(-0.19,	0.44)	0.45	Total DEs(PN)		0.02	(-0.13,	0.17)	0.81
Total DMs(child)	218	0.13	(-0.24,	0.50)	0.48	Total DMs(child)	218	-0.06	(-0.24,	0.11)	0.49
Total DMs(PN)		0.04	(-0.26,	0.35)	0.78	Total DMs(PN)		0.00	(-0.15,	0.14)	0.96
Total DAPs(child)	218	0.08	(-0.31,	0.48)	0.68	Total DAPs(child)	218	-0.08	(-0.26,	0.11)	0.43
Total DAPs(PN)		0.11	(-0.22,	0.44)	0.51	Total DAPs(PN)		-0.01	(-0.17,	0.14)	0.85
HR						**HR**					
Total DEs(child)	218	1.04	(-2.27,	4.34)	0.54	Total DEs(child)	218	0.91	(-0.49,	2.30)	0.20
Total DEs(PN)		-0.04	(-2.90,	2.83)	0.98	Total DEs(PN)		-0.43	(-1.64,	0.78)	0.48
Total DMs(child)	218	-2.07	(-5.40,	1.26)	0.22	Total DMs(child)	218	0.76	(-0.66,	2.18)	0.30
Total DMs(PN)		1.79	(-0.99,	4.56)	0.21	Total DMs(PN)		0.00	(-1.19,	1.18)	1.00
Total DAPs(child)	218	-1.60	(-5.16,	1.97)	0.38	Total DAPs(child)	218	0.91	(-0.61,	2.42)	0.24
Total DAPs(PN)		1.31	(-1.68,	4.31)	0.39	Total DAPs(PN)		0.01	(-1.26,	1.28)	0.99
PEP						**PEP**					
Total DEs(child)	216	1.07	(-1.32,	3.45)	0.38	Total DEs(child)	216	0.53	(-0.00,	1.06)	0.05
Total DEs(PN)		0.46	(-1.61,	2.53)	0.66	Total DEs(PN)		-0.02	(-0.48,	0.44)	0.92
Total DMs(child)	216	-0.01	(-2.44,	2.41)	0.99	Total DMs(child)	216	0.40	(-0.14,	0.94)	0.14
Total DMs(PN)		-0.31	(-2.34,	1.71)	0.76	Total DMs(PN)		-0.17	(-0.62,	0.28)	0.46
Total DAPs(child)	216	0.15	(-2.43,	2.74)	0.91	Total DAPs(child)	216	0.55	(-0.02,	1.13)	0.06
Total DAPs(PN)		-0.05	(-2.22,	2.13)	0.97	Total DAPs(PN)		-0.13	(-0.62,	0.35)	0.59

a. Models have been adjusted for sex, location of assessment, exact age and psychometrician at at the 5 year assessment. b. To estimate prenatal and postnatal cumulative exposure, we used an area-under-the-concentration–time-curve methodology (AUC). The AUC for the two prenatal pesticide measurements and the AUC for the five postnatal measurements were used as separate covariates in each model. Creatinine-adjusted metabolite concentrations were used to estimate the AUC. Prenatal and postnatal AUC values were log-normally distributed so log-10 AUC values were used in regression models.

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Chapter 5: Conclusions

CONCLUSIONS

In recent years, there has been increased interest in environmental factors affecting children's health. Children have unique vulnerabilities to environmental toxicants due to their immature and developing physiological systems. Children also may have higher exposure because they eat, drink and breathe more per unit volume compared to adults and because of their unique exploratory behaviors that predispose them to environmental contaminant exposures (Bearer 1995; Roberts, Wallace et al. 2009). Proper exposure assessment of environmental contaminants is necessary to identify vulnerable populations and understand the sources and pathways of exposure. Furthermore, the use of biomarkers of exposure is a common practice in epidemiologic and biomonitoring studies. Biomarkers are used to track exposure trends, identify vulnerable populations, and to link environmental exposures to health outcomes. Because of the widespread use of biomarkers in exposure assessment, it is important to understand what these biomarkers represent and their limitations.

This dissertation focused on children's residential exposures to pesticides and flame retardants (polybrominated diphenyl ethers or PBDEs), the presence of pesticide degradation products in the environment and their relation to urinary pesticide metabolite concentrations, and the relationship between organophosphate (OP) pesticide exposures and children's autonomic nervous system functioning. Below is a summary of the major findings, public health implications and future directions for each of the studies conducted.

Chapter 2:

Major findings. In this study, concentrations for 24 pesticides, one pesticide synergist, and three PBDE congeners (major constituents of the pentaBDE flame retardant commercial mixture commonly used on furniture) were measured in house dust samples from low-income farmworker and urban homes in California. Pesticides frequently detected in most homes included two organophosphates (chlorpyrifos and diazinon) (voluntarily phased-out for residential use by the urguing of the U.S. EPA prior to this study), permethrins, allethrin, cypermethrin (pyrethroids), and the synergist piperonyl butoxide. Chlorthal-dimethyl was detected solely in farmworker homes, suggesting contamination due to regional agricultural use. The latter finding indicates that the presence of farmworkers in the home and/or proximity of homes to nearby fields in agricultural communities is associated with higher indoor concentrations of some pesticides as reported in prior studies (Lu, Fenske et al. 2000; Harnly M., Bradman A. et al. 2009). All three PBDE congeners were detected in house dust from all homes and maximum concentrations for each of these are the highest reported to date (BDE-47: 125 632 ng/g, BDE-99: 218 768 ng/g, BDE-100: 41 149 ng/g). It is possible that the PBDE concentrations observed in this study resulted from California's stringent flammability standards which had previously required the use of these flame retardants on furniture. It is also possible PBDE levels observed in these home resulted from poorly constructed or deteriorating furniture treated with PBDEs as previously hypothesized by other researchers (Zota A.R., Rudel R. et al. 2008).

Concentrations for frequently detected analytes (both pesticides and PBDEs) were generally strongly correlated within homes (Spearman ρ=0.70-1.00, $p<0.01$). Additionally, diazinon and chlorpyrifos concentrations in Salinas farmworker homes were 40-80% lower than concentrations reported in samples from Salinas farmworker homes studied between 2000-2002, suggesting a temporal reduction after the U.S. EPA's call for a voluntary residential phase-out. Lastly, for some resident children, estimated non-dietary PBDE, but not pesticide, intake exceeded U.S. EPA recommended chronic reference doses (RfDs).

Public Health Implications & Future Directions. Studies of environmental contaminants in low-income residential environments, including this study, have been limited in sample size and often selection of participants has not been random. Collection methods, analytical techniques, and timing of data collection also differ making comparisons between studies difficult. Nonetheless, these studies indicate that low-income children are exposed to a mixture of pesticides and PBDEs in their residential environments. It is also evident that children living in agricultural communities may be exposed to additional contaminants used in the area for agriculture (e.g., chlorthal-dimethyl) and that historical or current residential pesticide use is also likely to contribute to ongoing exposures. Most families also reported frequent pesticide use, particularly pyrethroids for which the long-term health effects in children are unknown, suggesting that there is a need to promote and implement community-level education efforts on the impacts of pesticide use and effective integrated pest management strategies (Williams, Barr et al. 2006).

A recent study (Meeker, Johnson et al. 2009) found that PBDE dust concentrations at substantially lower concentrations than those observed in this study were associated with altered hormone levels in men. This raises a concern as PBDE non-dietary ingestion exposure estimates alone for some resident children exceeded the U.S. EPA's recommended RfD. It is also possible that children in these homes may encounter higher cumulative PBDE exposures resulting from other sources and pathways (e.g., dermal absorption or dietary consumption); however, this was not assessed in this study. Results from recent studies and those presented herein suggest that PBDEs should be a high priority for future exposure and health research and that exposure mitigation may be warranted.

Although estimated non-dietary intakes for select pesticides were below EPA RfDs, it should not be concluded that intakes below these government guidelines are "acceptable" or free of any health risks. Recent research has identified mechanisms of OP pesticide toxicity that were not considered in defining current U.S. EPA RfDs (Slotkin and Seidler 2008). Moreover, RfDs do not account for differences in vulnerability to pesticides due to genetic factors (Holland, Furlong et al. 2006). Additionally, the intake calculations for pesticides (and PBDEs) do not account for other exposure pathways (e.g., inhalation, dermal absorption or diet); nor do they take into account the fact that some children could have pica or other behaviors that could increase intake. RfDs were also not available for some of the compounds we measured and the lack of an RfD does not imply there are no potential health risks associated with exposure. Lastly, it is clear that the children in these homes are exposed to multiple indoor contaminants and the hazard evaluation in this study does not account for exposure to complex mixtures.

Additional research is needed to quantify exposures and the potential health effects from these compounds. Finally, such research should consider the complex mixture of chemicals found in indoor environments and include both environmental and biomonitoring measurements to assess cumulative exposures.

Chapter 3:

Major findings. This is the first study to report the detection of dialkylphosphates (DAPs) in house dust from homes located in both agricultural and urban communities. DEP was the most frequently detected DAP in homes ($\geq$60% detection frequency at each collection). Other DAPs were detected less frequently or not detected at all. Concentrations for the most frequently detected DAP, DEP, did not differ between or within communities. Additionally, DEP concentrations in dust were not correlated with concentrations of the two most frequently detected diethyl-devolving parent OP pesticides, chlorpyrifos and diazinon. Furthermore, there was no agreement in detection between the most frequently detected analytes in dust (DEP, chlorpyrifos, and diazinon) and the respective metabolite(s) in urine (kappa coefficients=-0.33 to 0.16). Finally, estimated children's non-dietary ingestion intake from DEP in dust was $\leq$ 5% of the dose calculated from DEP levels in urine, suggesting that dust through the non-dietary ingestion pathway is not a significant source of DAPs in urine.

Public Health Implications & Future Directions. Urinary DAP metabolites have been widely used to assess human OP pesticide exposure in multiple epidemiologic and biomonitoring studies (Loewenherz, Fenske et al. 1997; Fenske, Kissel et al. 2000; Castorina, Bradman et al. 2003; Duggan, Charnley et al. 2003; Barr, Bravo et al. 2004). Their use was first introduced in occupational studies to assess OP pesticide exposures. However, the validity of their use as biomarkers of exposure, particularly in non-occupational populations, has been questioned due to recent studies reporting detection of a specific OP urinary metabolite in environmental and food media (Morgan, Sheldon et al. 2005) and preformed DAPs in fruit juices and produce (Lu, Bravo et al. 2005; Zhang C, Driver JH et al. 2008).

One of the most interesting findings from this study was the absence of major differences in DAP dust concentrations between homes located in urban and agricultural communities. This could have resulted from a common source such as historical indoor use of OP pesticides indoors. Information on the environmental fate of OP pesticide degradation products is scarce; research in this area could help inform future pesticide exposure studies. Additionally, we found that the distribution of diethyl and dimethyl DAP concentrations in dust did not reflect the distribution of concentrations observed in children's urine indicating that DAPs may behave differently in the environment than in the human body.

At this time the biological fate of DAPs in humans is not known and data from animal studies on the pharmacokinetics of DAPs are inconclusive (Imaizumi H, Nagamatsu K et al. 1993; Timchalk, Busby et al. 2007). However, if humans excrete DAPs unchanged then it is possible for urinary DAPs to reflect exposure to OP pesticides as well as DAPs present in someone's environment and/or food. Results from this study indicate that DAPs in dust, through the non-dietary ingestion pathway, is not a significant contributor of urinary DAPs in

children but DAPs in food and other sources may still impact urinary DAP levels (Krieger, Dinoff et al. 2003; Lu, Bravo et al. 2005; Morgan, Sheldon et al. 2005). More research is needed on the pharmacokinetics and toxicodynamics of preformed DAPs and other specific OP metabolites to determine the extent of their contribution to urinary biomarkers in humans. Future analysis in this study population will include more in-depth assessment of the effects of diet on urinary pesticide metabolite concentrations.

Chapter 4:

Major findings. This is the first study to have evaluated the relationship between OP pesticide exposures in children and autonomic dysregulation. Because AChE inhibition is central to the toxic effects of OP pesticides, autonomic dysregulation may be one the earliest and most sensitive measures of long-term physiologic effects of OP pesticide exposure in infants and young children (Eskenazi, Bradman et al. 1999). Given their mechanism of toxicity, we hypothesized that OP pesticide exposure could lead to low resting HR (i.e., high resting RSA and/or PEP) and/or ANS dysregulation in children (i.e., more parasympathetic withdrawal concomitant with more sympathetic activation during challenge tasks compared to rest), however, these associations were not observed at all time points and most associations were observed in the opposite direction of the original hypotheses. Additionally, although creatinine-adjusted DAP concentrations were observed to be higher among 6-month-old children categorized into the ANS profile with reciprocal sympathetic activation and parasympathetic withdrawal, this was not observed at any other time points nor was this finding observed using prenatal (unadjusted or creatinine-adjusted concentrations) or unadjusted child DAP concentrations at each time point. No trends in urinary DAP concentrations were observed among ANS profiles at each time point suggesting that OP pesticide exposures are not associated with ANS dysregulation.

Similar findings were observed when cumulative exposures were used to assess the relation between OP pesticide exposures and ANS functioning in 5-year-old children. Furthermore, no differences in mean ANS response measures at 5 years were observed between children with consistently high versus consistently low urinary DAP concentrations. If we were to adjust our results for the multiple comparisons performed, then no significant findings are observed. These results indicate that OP pesticide exposure, at the levels observed in this population and as measured via urinary DAP metabolite concentrations, is not associated with children's resting and reactivity measures.

Public Health Implications & Future Directions. Previous studies have shown that children's psychobiologic reactivity is a valid measure of the ability of the ANS to regulate bodily functions under resting and challenging conditions (Matthews, Woodall et al. 1990; Alkon, Goldstein et al. 2003). Research also shows that individual psychobiologic measures are associated with children's physical and psychological health, and may affect how children interact with their family members, other adults and peers (Kagan, Reznick et al. 1988; Boyce, Frank et al. 1998). The use of RSA, HR, and PEP as outcome measures of early OP pesticide exposure represents a unique departure from previous research, as these measures

have conventionally been used as predictors of psychobiology, behavioral, and cardiovascular outcomes.

Research on the effects of OP pesticide exposure on ANS regulation is limited in number and sample size, and restricted to measuring resting RSA as a measure of ANS dysregulation (Dellinger 1985; Dellinger, McKiernan et al. 1987; Dellinger, Taylor et al. 1987; Dellinger and Mostrom 1988; Dellinger, Jansen et al. 1988). Most of these studies have been conducted in animals, results are not consistent, and different protocols and equipment were used to assess RSA. Furthermore, the animals in the Dellinger et al. studies (Dellinger, McKiernan et al. 1987; Dellinger and Mostrom 1988) and the exposed subjects in the human study experienced subacute OP pesticide exposures (versus children in our population who experienced low-level chronic OP pesticide exposures as assessed via DAPs). Additionally, the human study only measured cholinesterase inhibition (biomarker of effect not specific to OP pesticide exposure) rather than using biomarkers of OP pesticide exposure and/or environmental monitoring to confirm OP pesticide exposure. No sympathetic measures (e.g., PEP) were obtained in any of these studies. All of these factors limit comparability with this study.

Although results indicate that OP pesticide exposure is not associated with ANS dysregulation, the study focused on a relatively demographically and ethnically homogeneous study population; thus, the results may not be generalizable to other populations. As described previously in Chapter 3, DAP metabolites possess their own limitations as biomarkers of pesticide exposure.

In summary, we report no detrimental effects of prenatal and postnatal OP pesticide exposure, as measured by urinary DAPs, on children's individual psychobiologic measures, including resting and reactivity measures for HR, RSA, and PEP. This is the first study to use ANS response measures as outcomes to investigate the association between OP pesticide exposures in children and ANS regulation. Future investigations in this population will involve evaluating what factors predict ANS regulation and whether ANS resting and reactivity measures of HR, RSA, and PEP are related to later physical and mental problems as observed in prior studies.

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Appendix

Chapter 2: Supporting Information

Table S1. Select analyte chemical and physical properties and amounts applied in 2006 for agricultural and non-agricultural purposes in the counties where our homes were sampled.†

Analyte	CAS no.	Molecular Weight (g/mol)	log K_{ow} (octanol:water partition coefficient)	Vapor Pressure (Pa)	Henrys Law Constant (Pa-m^3/mol)	Amount applied for agricultural and non-agricultural purposes in 2006 (kgs) [a,b]	
						Monterey County (agricultural community) *Ag (Non-ag) use*	Alameda County (urban community) *Ag (Non-ag) use*:
Organophosphates							
Chlorpyrifos‡	2921-88-2	350.6	5.0	2.7×10^{-03}	2.94×10^{-01}	28,335 (3)	65 (3)
Diazinon‡	333-41-5	304.4	3.8	1.2×10^{-02}	1.11×10^{-02}	65,872 (6)	3 (<2)
Diazinon-oxon§	--	--	--	--	--	NA	NA
Malathion	121-75-5	330.4	2.4	2.4×10^{-02}	4.96×10^{-04}	16,900 (56)	<1 (581)
Methidathion	950-37-8	302.3	2.2	4.5×10^{-04}	7.30×10^{-04}	3,861 (NA)	NA
Methyl Parathion	298-00-0	263.2	2.9	2.0×10^{-04}	$8.51 \times 10^{+01}$	94 (NA)	NA (11)
Phorate	298-02-2	260.4	3.6	8.5×10^{-02}	4.46×10^{-01}	276	NA
Tetrachlorvinphos	22248-79-9	366.0	3.5	5.6×10^{-06}	1.82×10^{-04}	NA	NA
Pyrethroids							
Allethrin‡	584-79-2	302.4	5.0	1.6×10^{-04}	6.18×10^{-02}	NA	NA (<1)
Bifenthrin	82657-04-3	422.9	>6	2.4×10^{-05}	1.01×10^{-01}	400 (29)	1 (212)
Cypermethrin‡	52315-07-8	416.3	6.6	4.1×10^{-07}	4.26×10^{-02}	618 (38)	NA (404)
Deltamethrin	52919-63-5	505.2	6.2	2.0×10^{-06}	5.07×10^{-01}	<1 (16)	NA (67)
Esfenvalerate	66230-04-4	419.9	6.2	2.0×10^{-07}	4.15×10^{-02}	1,317 (<1)	1 (<1)
Imiprothrin	72963-72-5	318.4	2.9	1.8×10^{-06}	not found	NA	NA
Permethrin‡	52645-53-1	391.3	6.5	2.9×10^{-06}	1.93×10^{-01}	19,486 (308)	15 (478)
Prallethrin	23031-36-9	300.4	4.5	1.3×10^{-05}	1.00×10^{-02}	NA	NA
Sumithrin	26002-80-2	350.5	7.5	1.9×10^{-05}	6.89×10^{-01}	<1(<1)	NA (<1)
Others							
Chlorthal-dimethyl‡	1861-32-1	332.0	4.4	3.3×10^{-04}	2.23×10^{-01}	33,969 (53)	NA
Iprodione	36734-19-7	330.0	3.0	5.0×10^{-07}	3.14×10^{-04}	17,095 (249)	2 (121)
Piperonyl Butoxide‡	51-03-6	207.3	4.8	3.5×10^{-05}	9.02×10^{-06}	165 (16)	NA (71)
PBDEs[d]							
BDE-47‡	5436-43-1	485.8	6.8	1.9×10^{-04}	$1.52 \times 10^{+00}$	--	--
BDE-99‡	60348-60-9	564.7	7.3	1.7×10^{-05}	2.33×10^{-01}	--	--
BDE-100‡	189084-64-8	564.7	7.2	2.9×10^{-05}	6.99×10^{-02}	--	--

† Unless otherwise specified by a footnote, main source used was HSDB(2001); Available at:http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB ‡ Analyte detected in more than 50% of the samples in at least one location. § This analyte is an environmental breakdown product of diazinon, so no chemical/physical properties are provided. a. Agricultural usage is reported followed by non-agricultural usage in parentheses. Usage reported is at the county level; Salinas farmworker homes are located in Monterey County, and Oakland urban homes are located in Alameda County. Source: California Department of Pesticide Regulation Pesticide Use Reporting (PUR) database (2006): Monterey county (Salinas, CA): http://www.cdpr.ca.gov/docs/pur/pur06rep/chemcnty/monter06_ai.pdf; Alameda county (Oakland, CA): http://www.cdpr.ca.gov/docs/pur/pur06rep/chemcnty/alamed06_ai.pdf. b. Non-agricultural uses include applications for landscape maintenance, rights of way, commodity fumigation, structural pest control by licensed applicators, and public health.
c. Source: IUPAC Pesticides Database; Available at: http://sitem.herts.ac.uk/aeru/iupac/1474.htm. d. PBDEs are flame retardants and are not used for agricultural purposes in the locations sampled. NA: Indicates that pesticide was not applied in the respective county.

Table S2. Demographic characteristics for farmworker homes (n=15) in Salinas, CA and urban homes (n=13) in Oakland, CA for Mexican American families.[a]

	Salinas farmworker homes		Oakland urban homes	
	n	**(%)**	**n**	**(%)**
Maternal education (highest grade completed)				
< completed 9th grade or lower	8	(53.3)	8	(61.5)
Grades 10-12 (no diploma)	3	(20.0)	1	(7.7)
High school diploma/GED or technical school	2	(13.3)	4	(30.8)
College graduate	2	(13.3)		---
Paternal education (highest grade completed)				
< completed 9th grade or lower	12	(85.7)	10	(83.3)
Grades 10-12 (no diploma)	1	(7.1)	1	(8.3)
High school diploma/GED or technical school	1	(7.1)	1	(8.3)
College graduate		---		---
Family income relative to federal poverty level[b]				
≤ Poverty level	10	(66.7)	9	(69.2)
>Poverty level but <200% poverty level	5	(33.3)	4	(30.8)
Number of household members				
3-5	8	(53.3)	5	(38.5)
>6	7	(46.7)	8	(61.5)
Reported rodent sighting in the home				
Yes	2	(13.3)	3	(23.1)
No	13	(86.7)	10	(76.9)
Reported cockroach sighting in the home				
Yes	3	(20.0)	5	(38.5)
No	12	(80.0)	8	(61.5)
Reported pesticide application in the last 3 months				
Yes	10	(66.7)	11	(84.6)
No	5	(33.3)	2	(15.4)
Farmworkers wore work clothing indoors[c]				
Yes	12	(80.0)		---
No	2	(20.0)		
Farmworkers wore work shoes indoors[c]				
Yes	7	(50.0)		---
No	7	(50.0)		
Farmworkers living in the home (past 3 months)				
0		---	11	(84.6)[d]
1-3	15	(100.0)	2	(15.4)
Farmworkers currently living in the home				
0	1	(6.7)		---
1-3	11	(73.3)		---
4-7	3	(20.0)		---
Distance of home to nearest field/orchard				
50-20 feet	1	(6.7)		---
>200 feet-1/4 mile	3	(20.0)		---
>1/4 mile	11	(73.3)		---

a. No statistically significant differences were observed between locations for demographic factors unrelated to farmworker status. b. Families' poverty levels were based on U.S. Department of Health and Human Services thresholds for 2006. Source: http://aspe.hhs.gov/POVERTY/06poverty.shtml. c. One participant in the Salinas group reported that the father was a farmworker during eligibility screening; however, the father was not living in the home during the sample collection period so information is only available for 14 of the 15 farmworker households for this demographic characteristic. d. Two participants reported having a parent or parent's sibling working in a field/golf course doing maintenance/landscaping work potentially involving pesticide use; however, they were not doing this work during sample collection.

Table S3. Summary statistics for dust loadings (ng/m^2) in samples collected in 2006 from low-income urban and farmworker homes.[a]

	Salinas farmworker homes (n=29 samples collected from 15 homes)							Oakland urban homes (n=25 samples collected from 13 homes)						
Analyte	**DF**	**min**	**p25**	**p50**	**p75**	**p95**	**max**	**DF**	**min**	**p25**	**p50**	**p75**	**p95**	**max**
Organophosphates														
Diazinon	79	--	9	23	73	358	742	52	--	--	9	185	1,470	2,195
Chlorpyrifos	55	--	--	28	69	2,312	2,639	36	--	--	--	76	877	2,693
Malathion	7	--	--	--	--	50	70	12	--	--	--	--	1,869	2,804
Tetrachlorvinphos	10	--	--	--	--	846	3316	4	--	--	--	--	--	112
Diazinon-oxon	ND	--	--	--	--	--	--	4	--	--	--	--	--	701
Methidathion	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
Methyl Parathion	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
Phorate	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
Pyrethroids														
cis-permethrin	100	89	375	816	4,109	14,164	14,466	100	24	348	768	7,197	32,215	69,025
trans-permethrin	100	187	721	1,566	6,549	20,251	21,540	100	47	624	1,616	12,606	56,352	116,313
Allethrin[b]	83	--	34	128	565	1,020	1,122	80	--	68	129	523	1,744	2,983
Cypermethrin[c]	55	--	--	438	2,345	16,305	42,317	64	--	--	1,649	6,715	19,117	24,423
Bifenthrin	14	--	--	--	--	37	83	44	--	--	--	71	16,023	39,186
Sumithrin	24	--	--	--	--	4,134	8,857	8	--	--	--	--	204	550
Deltamethrin	17	--	--	--	--	7,286	9,496	12	--	--	--	--	140,424	207,551
Imiprothrin	7	--	--	--	--	210	7,687	4	--	--	--	--	--	6,326
Prallethrin	ND	--	--	--	--	--	--	4	--	--	--	--	--	362
Esfenvalerate	3	--	--	--	--	--	223	ND	--	--	--	--	--	--
Other														
Piperonyl butoxide	86	--	101	359	1,243	10,649	30,145	96	--	144	678	14,033	48,589	148,873
Chlorthal-dimethyl	97	--	23	46	72	175	537	ND	--	--	--	--	--	--
Iprodione	ND	--	--	--	--	--	--	ND	--	--	--	--	--	--
PBDEs														
BDE-47	100	797	4,125	10,773	19,336	60,949	62,732	100	621	2,141	10,725	79,188	219,773	263,549
BDE-99	100	1,582	7,363	19,498	37,470	91,031	93,460	100	1,554	2,734	19,064	131,247	454,014	457,216
BDE-100	100	552	1,490	3,807	7,348	17,076	19,279	100	257	640	4,001	26,018	79,906	85,667

a. Two samples were collected from each home except for one home in each location due to inadequate sample volume.

b. Loading calculated after summing two isomers (cis-/trans-) isomers.

c. Loading calculated after summing four isomers.

Abbreviations and notation: DF=detection frequency, ND=not detected. '---' Indicates that concentration used to calculate loading was below the limit of detection or not detected thus, a loading is not reported.

Table S4. Consistency and variability of dust concentrations in low-income farmworker and urban homes sampled in 2006 for the most frequently detected analytes.[a]

	Salinas farmworker homes (n=14)[b]		Oakland urban homes (n=12)[b]		All homes (n=26)	
Analyte	**Spearman ρ**	**ICC**	**Spearman ρ**	**ICC**	**Spearman ρ**	**ICC**
Organophosphates						
Diazinon	0.88**	0.82	0.97**	0.96	0.92*	0.92
Chlorpyrifos	0.83**	0.85	--	--	--	--
Pyrethroids						
cis-permethrin	0.78**	0.85	1.00**	0.99	0.91**	0.93
trans-permethrin	0.70**	0.83	1.00**	0.99	0.90**	0.92
Allethrin	0.49	0.61	0.18	0.03	0.36	0.37
Cypermethrin	0.87**	0.83	0.89**	0.88	0.89**	0.85
Synergist Ingredient						
Piperonyl butoxide	0.77*	0.79	0.97**	0.91	0.87**	0.84
Phthalate Herbicide						
Chlorthal-dimethyl	0.78*	0.33	--	--	--	--
PBDEs						
BDE-47	0.94**	0.94	0.91**	0.95	0.93**	0.95
BDE-99	0.92**	0.92	0.90**	0.95	0.92**	0.94
BDE-100	0.92**	0.92	0.90**	0.95	0.91**	0.94

a. Spearman correlation coefficients (ρ) and intraclass correlation coefficients (ICCs) are provided when analyte DF≥50% in respective locations at each collection. Overall correlation coefficient and ICC are provided when analyte DF≥50% in both locations and collections. The ICC is the ratio of the analyte concentration variance between-homes to total variance. It is a measure of reproducibility of analyte concentrations sampled from the same group of homes over time. The ICC ranges from 0-1, where 0 indicates no reproducibility and 1 indicates perfect reproducibility (i.e., an ICC=1 indicates that 100% of the concentration variability is due to between-home differences, while an ICC=0 indicates that 100% of the variance is due to within-home differences.) b. Two samples were collected from each home except for one home in each location due to inadequate sample volume. *p<0.01, **p<0.0001.

Supplemental notes for Figure 1 and information on previous Salinas farmworker studies.

a. Reported agricultural and non-agricultural pesticide use data.

By law, all agricultural pesticide use is required to be reported in the state of California. Farmers must submit reports to the California Department of Pesticide Registration (CDPR) and include the following information: month and year of the application(s); county in which work was done; geographic location including the section, township, range, base, and meridian; field location; operator ID/permit number; operator name and address; applicator name and address; site ID; commodity/crop/site treated; acres or units planted; acres or units treated; date and time of application; application method (air, ground, other); U.S. EPA/California pesticide registration number of the pesticide product applied; pesticide product name and manufacturer; amount of product applied; and person who prepared the report. In addition, commercial/licensed applicators are required to submit monthly reports detailing information on their pesticide use and amounts applied for "non-agricultural" purposes including: structural, landscape maintenance, rights-of-way, public health, vertebrate, commodity fumigation, and regulatory pest control. Agricultural and "non-agricultural" uses are reported to the County Agricultural Commissioner's office who then reports this information to the CDPR of the California Environmental Protection Agency (EPA). Information on pesticide uses including agricultural/non-agricultural applications is available to the public in the Pesticide Use Reporting (PUR) database; available online at: http://www.cdpr.ca.gov/docs/pur/purmain.htm.

We used the PUR database to summarize Monterey County (location of Salinas, CA) and Alameda County (location of Oakland, CA) pesticide use for the years of interest (1999-2007 for chlorpyrifos and diazinon). We totaled pesticide amounts used for agricultural and non-agricultural purposes separately. For more information on the PUR program please refer to: http://www.cdpr.ca.gov/docs/pur/purovrvw/tabofcon.htm.

b. Reported agricultural and non-agricultural uses between 1999 and 2007.

Figure 1 in the manuscript reports kilograms of chlorpyrifos and diazinon applied for agricultural purposes from 1999-2007 in the county were our farmworker homes were sampled (Monterey County). Non-agricultural applications from 1999-2007 were negligible compared to agricultural applications as observed in Table S5 below.

Table S5. Approximate kilograms of diazinon and chlorpyrifos applied in Monterey county from 1999-2007 for agricultural and non-agricultural applications.[a,b]

	Diazinon (kgs)		Chlorpyrifos (kgs)	
Year	Agricultural	Non-Agricultural	Agricultural	Non-Agricultural
1999	46,644	717	25,196	1,519
2000	54,503	841	24,448	678
2001	60,052	1,301	24,581	355
2002	63,860	1,094	23,315	760
2003	71,294	1,076	26,325	101
2004	77,673	217	27,509	18
2005	71,468	96	30,018	54
2006	65,866	6	28,332	3
2007	65,165	2	28,141	<0.5

a. Pounds applied at the county level are reported by year by the California Department of Pesticide Regulation in their Pesticide Use Reporting (PUR) database. Pounds reported in the database were converted to kilograms.
b. Non-agricultural uses refer to uses such as landscape maintenance, public health, commodity fumigation, rights-of-way, and structural pest control applications by licensed applicators.

c. Supplemental notes for Figure 1 and information on previous Salinas farmworker studies.

The first three median dust concentrations presented for each OP insecticide in Figure 1 represent a subsample of homes included in Harnly et al.(2009) for the CHAMACOS cohort--a longitudinal birth cohort study of farmworkers and their children living in the Salinas Valley, CA. Median dust concentrations reported in the present manuscript for CHAMACOS samples are limited to farmworker homes located in the same zip codes in the city of Salinas as those homes sampled in the present study conducted in 2006 and our previous study by Bradman et al.(2006) conducted in 2002.

Chlorpyrifos and diazinon dust concentrations for CHAMACOS participants did not differ significantly between years sampled (i.e. 2000 vs. 2001, 2000 vs. 2002, and 2001 vs. 2002). Additionally, no significant differences were observed for chlorpyrifos and diazinon concentrations between individual CHAMACOS (2000-2002) studies and our farmworker study conducted in the latter part of 2002 by Bradman et al. Table S6 summarizes information from previous Salinas farmworker studies including: number of homes sampled in the same zip codes as homes in the present study, median dust concentrations for chlorpyrifos and diazinon, and percent decrease in median concentration between our 2006 study and previous studies indicated.

Table S6. Median dust concentrations for Salinas farmworker studies and percent decrease in median concentrations compared to the present study conducted in 2006.

			Chlorpyrifos			Diazinon	
Study	**Sampling dates**	**N**	**Median (ng/g)**	**% decrease[a]**	**N**	**Median (ng/g)**	**% decrease[b]**
CHAMACOS	Jan-Dec 2000	33	75.5	80.4*	33	24.7	42.5*
	Jan-Dec 2001	36	71.3	79.2*	36	27.0	47.5*
	Jan-May 2002	13	93.9	84.2*	13	45.5	68.8
Bradman et al. 2007	June-Sep 2002	20	48.8	69.7*	20	20.8	31.7
Present study	July-Sep 2006	15	14.8	--	15	14.2	--

a. Indicates percent decrease between the present study (conducted in 2006) and respective farmworker study conducted prior to 2006.
b. Indicates that concentrations in the present 2006 study are significantly lower ($p \leq 0.05$, Wilcoxon-Mann-Whitney Tests) than respective Salinas farmworker study.

Data Sources for Table S6:

Harnly M.; Bradman A.; Nishioka M.; McKone T.; Smith D.; McLaughlin R.; Baird-Kavannah G.; Castorina R.; Eskenazi B., Pesticides in Dust from Homes in an Agricultural Area. *Environ Sci Technol* 2009, *43*, (23), 8767-74.

Bradman, A.; Whitaker, D.; Quiros, L.; Castorina, R.; Henn, B. C.; Nishioka, M.; Morgan, J.; Barr, D. B.; Harnly, M.; Brisbin, J. A.; Sheldon, L. S.; McKone, T. E.; Eskenazi, B., Pesticides and their metabolites in the homes and urine of farmworker children living in the Salinas Valley, CA. *J Expo Sci Environ Epidemiol* 2006, *17*, (4), 331-49.

Table S7. Summary statistics on the estimated Hazard Quotients (HQ) for all study children.[a]

Analyte	RfD (mg/kg/dy)[b]	# samples with concentration >LOD	Range of Intake (mg/kg/day)[c]		Select Summary Statistics for HQs based on 54 dust samples from farmworker and urban children				# of children with HQ>1.0
			Min	Max	p50	p75	p95	Max	
Organophosphates									
diazinon	0.0002	36	--	0.4	--	2.6×10^{-04}	9.5×10^{-04}	2.1×10^{-03}	0
chlorpyrifos	0.00003	25	--	0.7	--	2.9×10^{-03}	1.4×10^{-02}	2.3×10^{-02}	0
malathion	0.07	5	--	2.9	--	--	1.7×10^{-06}	4.2×10^{-05}	0
tetrachlorvinphos	0.04	4	--	0.9	--	--	1.3×10^{-05}	2.3×10^{-05}	0
Pyrethroids									
cis-permethrin	0.25	54	0.03	76	5.8×10^{-06}	1.2×10^{-05}	5.0×10^{-05}	3.1×10^{-04}	0
trans-permethrin	0.25	54	0.05	134	1.0×10^{-05}	1.9×10^{-05}	9.6×10^{-05}	5.3×10^{-04}	0
cypermethrin	0.06	32	--	38	1.5×10^{-05}	5.1×10^{-05}	2.9×10^{-04}	6.4×10^{-04}	0
bifenthrin	0.015	15	--	6.8	--	2.7×10^{-06}	1.6×10^{-05}	4.5×10^{-04}	0
sumithrin	0.0007	9	--	2.2	--	--	1.9×10^{-03}	3.1×10^{-03}	0
deltamethrin	0.0033	8	--	54	--	--	4.0×10^{-03}	1.6×10^{-02}	0
prallethrin	0.025	1	--	0.1	--	--	--	4.5×10^{-06}	0
esfenvalerate	0.02	1	--	0.3	--	--	--	1.3×10^{-05}	0
Others									
chlorthal-dimethyl	0.01	28	--	0.1	1.8×10^{-06}	4.3×10^{-06}	8.7×10^{-06}	1.1×10^{-05}	0
piperonyl butoxide	0.16	49	--	133	3.0×10^{-06}	1.0×10^{-05}	1.5×10^{-04}	8.3×10^{-04}	0
PBDEs									
BDE-47	0.0001	54	0.6	359	0.1	0.2	2.9	3.6	2
BDE-99	0.0001	54	1.2	639	0.1	0.4	6.1	6.4	3

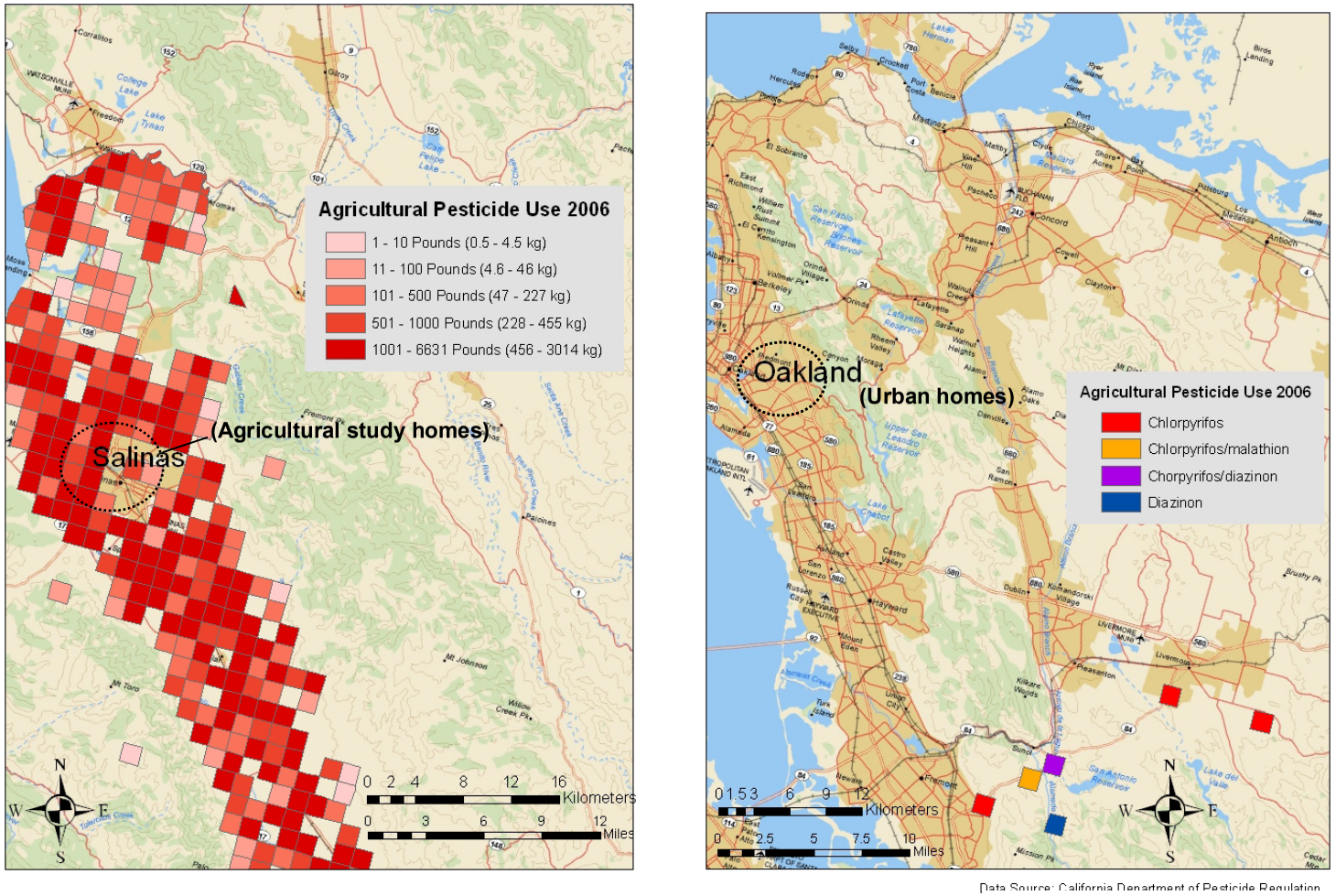
a. A hazard quotient (HQ) was calculated as the ratio of the potential dust intake to the respective analyte reference dose (RfD). The HQ was only calculated for those analytes for which an RfD was available.

b. Chronic population adjusted doses (cPADs) were used as the reference dose for chlorpyrifos (cPAD for children and females 13-50 years of age) and deltamethrin. Sources: IRIS database (http://cfpub.epa.gov/ncea/iris/index.cfm?fuseaction=iris.showSubstanceList) and EPA's Pesticide Registration Status: (http://www.epa.gov/opp00001/reregistration/status.htm).

c. Intake was calculated by multiplying the toxicant dust concentration by an ingestion rate of 60 mg/day then dividing by the child-specific body weight.

'--' Indicates that a percentile is not reported since dust concentrations were less than the limit of detection.

Chapter 3: Supporting Information



(a) **(b)**

Figure S1. Agricultural pesticide use in 2006 at the county level. (a) Agricultural pesticide use in Monterey County for 2006 for all DAP-devolving OP pesticides. Circled region represents area where agricultural homes sampled were located. (b) Agricultural pesticide use in Alameda County for 2006 for all DAP-devolving OP pesticides. Circled region represents area where urban homes sampled were located. Maps generated by Robert Gunier using data provided in the California Department of Pesticide Regulation's Pesticide Use Reporting system for 2006.

Table S1. Mole ratio of DEP to OP pesticide residues in house dust.[a,b]

	N	p25	p50	p75	p95	max
Mole Ratio						
Agricultural homes	29	0.5	2.2	4.0	23.2	24.1
Urban homes	25	1.1	1.6	6.4	21.4	70.0

a. Mole ratio of DEP in dust to OPs = DEP (nmol/g) / Σ OPs (nmol/g); where parent OP compounds were assumed to be diazinon and chlorpyrifos based on their detection frequency in dust samples and general use at the county level.
b. Values <LOD were imputed to LOD/√2 prior to calculating mole ratios.

Table S2. Overall level of detection agreement between frequently detected analyte residues in dust and respective urinary metabolites.

Analyte(s) in dust	Agreement with detection of DEP in urine				Agreement with detection of DEP + DETP in urine			
	n[a]	Kappa coefficient[b]	(SE)	p-value[c]	n[a]	Kappa coefficient[b]	(SE)	p-value[c]
DEP	79	-0.01	(0.11)	0.55		--	--	--
Chlorpyrifos	54	-0.14	(0.13)	0.85	54	-0.11	(0.13)	0.80
Diazinon	54	-0.15	(0.13)	0.88	54	0.09	(0.10)	0.20
DEP+Chlorpyrifos + Diazinon	54	-0.23	(0.11)	0.98	54	-0.17	(0.13)	0.90

a. Indicates number of samples included in the calculation; children may have up to 2 dust samples. b. Kappa coefficient: 1= perfect agreement, 0=no agreement above that expected by chance, −1=perfect disagreement. c. High p-values (p>0.05) indicate that Kappa coefficients are not significantly greater than 0 (i.e., no agreement in detection between media above that expected by chance). Abbreviations and notations: SE=standard error of kappa statistic. "--" Because DEP in dust could not lead to detection of DETP in urine no kappa statistic is provided.

Results from GEE Logistic Regression Models:

Table S3. Logistic regression models for detection of DAPs in urine based on detection of most frequently detected parent OPs and DAPs in dust samples (n=54 samples, 28 children).[a]

Dependent Variable (detection in urine collected on the same day as the dust sample; yes/no)	Independent Variable (Binary Predictor, detection of analyte(s) in dust)[b]	Odds Ratio	(95% CI)
DEP in urine	DEP	0.82	(0.3, 2.4)
DEP in urine	chlorpyrifos	0.72	(0.2, 2.2)
DEP in urine	diazinon	0.77	(0.2, 2.5)
Total DEs (DEP+DETP) in urine	chlorpyrifos	0.62	(0.2, 2.4)
Total DEs (DEP+DETP) in urine	diazinon	0.83	(0.2, 3.4)
Total DEs (DEP+DETP) in urine	diazinon + chlorpyrifos	0.57	(0.1, 2.3)
Total DEs (DEP+DETP) in urine	diazinon + chlorpyrifos+DEP	0.40	(0.1, 2.5)

a. Logistic regression models were controlled for location (Salinas=1, Oakland=0; referent group) and the diet children were following at the time of urine sample collection (1=conventional, 0=organic; referent group). b. Referent group for main predictor variable is those children for which we did not detect the indicated analyte or combinations of analytes in their house dust samples.
CI=Confidence Interval

Explanation of general GEE logistic regression models (Table S3) used to determine whether detection of an analyte or combination of analytes in dust increased the odds of detecting respective metabolite(s) in urine:

$$logit\,[P(Y_{ij}=1|X_{ij}=x_{ij})] = log\ \frac{P(Y_{ij}=1|\ X_{ij}=x_{ij})}{P(Y_{ij}=0|\ X_{ij}=x_{ij})} = \beta_o + \beta_{dust}x_{ij} + \beta_{location}\ell_i + \beta_{diet}\,d_{ij},\ where$$

i=individual "i"
j=time

Y_{ij} = Binary outcome variable representing the marginal odds ratio of detecting DEP in urine (yes/no) or DEP+DETP in urine (yes/no). Detection of DEP+DETP in urine was used when detection of either or both parent OP pesticides, chlorpyrifos and/or diazinon, was or when detection of all three frequently detected analytes (chlorpyrifos + diazinon + DEP) were used as the main predictors.

$e^{\beta_{dust}}$ = Odds ratio of detecting metabolite(s) in urine associated with comparing two subpopulations as follows:

(a) Main predictor variable was detection of only one analyte in dust (DEP or chlorpyrifos or diazinon):
- comparison groups were those children for which the specific analyte was detected in dust versus those children for which the analyte was not detected in dust;

(b) Main predictor variable in the model consisted of the detection of two parent OP pesticides- chlorpyrifos and diazinon:
- comparison groups were those children for which both OP pesticides were detected in dust versus those children for which only one or none of these OP pesticides were detected in dust;

(c) Main predictor variable consisted of detection of all three analytes in dust (chlorpyrifos + diazinon + DEP):
- comparison groups were those children for which both OP pesticides and DEP were detected in dust versus those children for which only two, one or none of these analytes were detected in dust;

$e^{\beta_{location}}$ = Odds ratio of detecting metabolite(s) in urine when location of residence changes from urban to rural (referent group is urban Oakland homes);

$e^{\beta_{diet}}$ = Odds ratio of detecting metabolite(s) in urine when diet changes between organic and conventional (referent diet is organic).

Chapter 4: Supporting Information

Table S1. Predicted direction of ANS resting (a) and reactivity (b) measures. As OP pesticide exposure increases we hypothesize the following:

(a)

Resting Measures	Hypothesis/Direction Expected
RSA	Less parasympathetic withdrawal during rest resulting in higher mean resting RSA.
HR	Lower resting HR
PEP	Less sympathetic activation during rest resulting in higher mean resting PEP.

(b)

Reactivity Measures (Difference scores)	Hypothesis
RSA	More parasympathetic withdrawal during challenge tasks compared to resting (i.e., higher mean resting RSA compared to mean challenge RSA) resulting in a negative RSA difference score.
HR	Positive mean HR difference score indicating a higher HR during the challenge task compared to resting
PEP	More sympathetic activation during challenge tasks compared to resting (i.e., higher mean resting PEP compared to mean challenge PEP) resulting in a negative PEP difference score.

Table S2. Interpretation of PEP and RSA Difference Scores by Reactivity Status

Mean Rest	Mean Challenge	Heart Rate	Difference/Reactivity Scores (challenge-rest)	Autonomic Response	Reactivity Status
High PEP score	Low PEP score	Increase	Negative	Sympathetic Activation	Sympathetic Reactivity
High RSA score	Low RSA score	Increase	Negative	Parasympathetic Withdrawal/Inhibition	Parasympathetic Reactivity
Low PEP score	High PEP score	Decrease	Positive	Sympathetic Inhibition	Not reactive
Low RSA score	High RSA score	Decrease	Positive	Parasympathetic Activation	Not reactive

Table S3. Selection of children for analyses.

					Exclusions				
Time point	**Children Enrolled**	**Children Eligible to participate in ANS reactivity protocols**	**Children who Actually Participated in ANS protocols**	**Children with scoreable ANS measures**	**Twins[c]**	**Had diagnosed medical condition[d]**	**Mother did not have complete urinary data**	**Child did not have complete urinary data**	**Total Number of Children included in our analyses**
	n	**n**	**n**	**n**	**n**	**n**	**n**	**n**	**n**
6 months	433	228	178	161	8	2	0	2	149
1 year	441[a]	212[b]	172	158	2	2	0	5	149
3 ½ years	365	203[b]	160	139	6	2	0	34	97
5 years	350	350	316	301	8	5	1	13	274

a. Mothers/children who did not complete the 6 month assessment were allowed to return to the study at the 1 year assessment; thus the number of participants enrolled at the 1 year assessment was slightly larger compared to the number enrolled at the 6 month assessment.
b. Number of children who were eligible at the 1 year and 3 ½ year assessments were drawn from the number of children who were eligible at the 6 month visit (n=228).
c. Twins: refers to the total number of children. To get the number of pairs of twins excluded divide the number provided by 2.
d. Children with Down's syndrome and/or experiencing seizures in the first year of life were excluded from our analyses.