



Exposure to Organophosphates through Agricultural Work in the San  
Joaquin Valley: Poison at Low Doses through  
An Extended Period of Time

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**Abstract:**

Organophosphates (OPs), toxic insecticides, attack the nervous system by high-jacking the acetylcholinesterase mechanism. This attack results in an accumulation of Acetylcholine in the nervous system, which impairs breathing among other health issues. We still don't know the extent of the damage caused by (OPs), which is troubling since more than 1 billion pounds of pesticides are sprayed each year in the United States of America. Our objective for this research is to study the long-term effects of OPs on the neurodevelopment of children. While previous research was conducted on animal models, we will focus on the children of farm workers. Children are more susceptible to harm from OPs because they are exposed starting in utero and continuing as infants and children as they interact with their environment. Our methodology includes taking urine samples from pregnant women in order to check DAP metabolites and examining children with the Bayley Scales to assess children neurodevelopment and motor skills, and with the Wechsler Intelligence Scale for children (WISC-IV) for the cognitive examination of eight year old children. We will determine the impact OPs have on children.



## Exposure to Organophosphates through Agricultural Work in the San Joaquin Valley: Poison at Low Doses through An Extended Period of Time

Organophosphates<sup>1</sup> are a type of toxin used as insecticides for pest control in Agriculture (Barr, 2003). Currently more than one billion pounds of pesticides are employed annually in the United States, 75 % of which are for agriculture purposes (Eskenazi, 2007). For example in Kern County, which is part of the San Joaquin Valley, in the year 2009 alone, about 21,997,483 pounds of pesticides were applied to crops (California Department of Pesticide Regulation, 2011). Consequently, we are exposed to synthetic family of insecticides that acts upon entering the body by interacting with the acetylcholinesterase mechanism at high concentrations it inhibits the acetylcholine sterase mechanism; or at low concentrations through long term periods of exposure, it affects brain areas dealing with cognition (Slotkin, 2008)). OPs can be absorbed through contact with the skin, ingestion, or inhalation (Eskenazi, 2007). Once in a human system it works by breaking down acetylcholinesterase-- an enzyme needed to break down acetylcholine (Eskenazi, 2007). When acetylcholine is not broken down, it accumulates in the nervous system, which impairs breathing (Young, 2006).

However, we still don't know the extent of damage OPs have on humans in utero and on the development of children. In order to understand the effects of these toxins, several studies with rats as model organisms have tried to establish a correlation between OPs and neurodevelopment ((Slotkin, 2008 & Vidair, 2004). Rat brains were affected when exposed to OPs in utero (Slotkin, 2008 & Vidair, 2004). Moreover, the pesticide parathion organophosphate was administered to postnatal rats, and after examining their brains, it was determined that postnatal rats' exposure produces numerous and persistent deficiencies in cholinergic synaptic

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<sup>1</sup>OPs are composed of several subfamilies: parathion, dialkylphosphate, chlorpyrifos, diazinon



function (Slotkin, 2008). These deficiencies can continue into adolescence and adulthood (Slotkin, 2008). Even at low doses, doses that are barely detectable, OPs affect cholinergic pathways in the brain (Slotkin, 2008). Potentially, this effect, in humans, can also last through adolescence and into adulthood (Slotkin, 2008).

To date, Eskenazi et al. is one of the few teams who have done research in an effort to find a correlation between exposure in utero to organophosphates and neurobehavioral disorders with humans (Eskenazi, 2007 & Eskenazi, 2010). Similar to their method, we also plan to collect urine samples and examine the children at key developmental stages. To measure development, we will use the Bayley Scales and the Wechsler Intelligence Scale for Children (WISC-IV) to determine level of exposure, and we will observe children's behavior for potential disorders since they were exposed in utero (Engel, 2011 & Young, 2005). This exposure is accidental and occurs, for example, when babies crawl on ground infected by residue that falls from heavy soiled clothing worn by their farm working parents and other relatives (Eskenazi, 2007 & Eskenazi, 2010). In addition, children tend to put everything in their mouths while crawling and playing (Eskenazi, 2007). Though our study is similar to the Eskenazi et al's. study, in that both are longitudinal and both use the Bayley Scales and collect urine samples, our study will, in addition to the urine sample that Eskenazi et al. collected at the third trimester and postpartum, we will collect urine samples from the neonates at 6, 12, and 24 months and another one from the 8 year olds. It is important to collect samples at these times during pregnancy because this is when their nervous system continues to develop. Furthermore, we test 8 year old participants for any neurodevelopment using WISC, to see whether the problem of organophosphates and their detrimental effects on children's Neurodevelopment gets worse with



long-term exposure or if it is unaffected. Therefore more urine samples are required in this study from both the mothers and the children.

After gathering and analyzing data about organophosphates and their potential detrimental effects on humans, we share our findings with the scientific community and especially with the Environmental Protection Agency. If, as we fear, the effects on children are profound, our data could be used as evidence for policy makers to regulate and hopefully reduce Organophosphate spraying in agriculture: where the fruits and vegetables we consume are grown, and where farmworkers are exposed while doing their jobs.

### **Specific Aims**

#### **Recruitment and Participants**

We will recruit 50 pregnant women at less than 20 weeks of gestation 18 years or older, and 25 eight-year old children for study. Recruitment, interview, and Data collection will take place at the Earlimart Health Center in Earlimart and California and Delano Regional Medical Center. Because pregnant women and children are vulnerable communities special precautions will be taken and are detailed in the methods section.

#### **Collecting Urine samples**

We will collect 3 urine samples during the second and third trimester from the pregnant women, and also postpartum. Also, will collect three urine samples from the newborn one at 6 months, second one at 12 months, and the third at 24 months. The urine collection will to measure the Dialkylphosphate (DAP) metabolites and will observe how it affects the mother, fetus, and infant.

#### **Testing methods for children**



In order to assess the impact of organophosphates on neurodevelopment in children, we will employ the Bailey's Scales and the Wechsler Intelligence Scale for Children (WISC-IV). The results from these tests will be compared against the Diagnostic and Statistical Manual of Mental Disorders (DSM).

### **Research Design and Methods**

#### **Participants and recruitment:**

Fifty pregnant women initiating prenatal care at Earlimart Health Center, a county clinic located in the town Earlimart, and at Delano Regional Medical Center located in the San Joaquin Valley, will be recruited to participate in a longitudinal birth cohort study assessing the impact of pesticides and other environmental factors on the health of pregnant women and their children. In addition to the recruitment of the 50 women, we're recruiting 25 eight-year-old children to assess the impact of pesticides in their lives as well. The recruitment sites primarily serve a low-income population of which a vast number are agricultural workers.

Women considered eligible for the study are required to be at less than 20 weeks of gestation, 18 years of age or older, Medi-Cal eligible during pregnancy, fluent in Spanish and/ or English, and planning to deliver at Delano Regional Medical Center (Eskenazi, 2007). A total of 50 women will be enrolled in the first two months of the study. Any mother that is sick, or has a child born with a disease such as down syndrome will be excluded from the study as it could potentially skew the results. We will seek Institutional Review board approval from the University of California Merced, Delano Medical Center, and Earlimart Health Center before any recruitment. Moreover, all participants will sign an informed consent written in and explained in their primary language. Parents and/ or guardians will sign the informed consent form after the children give a verbal consent. Furthermore, to make sure we are following human





and just treatment, regarding their privacy we will also follow closely with HIPPA regulations.

### **Collect urine samples to measure DAP metabolites:**

We will collect urine samples of the participating women when they go for a routine check up or a physical. The urine samples collected will be immediately tested due to OPs less than 24 hours life span. We will collect samples from each participant at their second and third trimester, and a third sample postpartum. Once the children are born, we will also collect samples from them at specific times: at birth, and 6, 12, and 24 months. The urine samples from the infants will be taken during a regular check up with his/her pediatrician for convenience as well as not asking the families to make extra trips. We will also collect a urine sample from the 25 eight-year-old children recruited separately at the beginning and end of the study.

The urine samples collected will be kept at -80 degrees Celsius for transport to Quest Laboratory Company in order to keep costs down, who will test urine samples for traces of organophosphates (Barr, 2003). The Metabolite levels in the urine will be measured by gas chromatography-tandem mass spectrometry and quantified by isotope dilution calibration. These tests will provide the type and quantity of OPs present in our participants' urine samples. Ultimately, these urine samples will be key in determining which contamination is worse for child development: exposure through the mother in utero or long-term exposure as an infant and child. We expect to find that in the long-term exposure is far more harmful than in utero exposure. We expect our results to reinforce the findings of Eskenazi et al.

### **Children's Intelligence Testing:**

#### *Infant Cohort.*

Once the children are born, they will be examined using the Bayley Scale and compare the results the DSM guide in order to detect potential neurodevelopmental disorders. At 6





months these same infants will be evaluated with the Mental Development Index (MDI), which characterizes cognitive abilities. Then, at 12 months, we will examine the children with Psychomotor Development Index (PDI) to examine large muscle and fine motor coordination. Both scales will be administered in Spanish and/or English by child development blind to exposure. Finally, at 24 months, we will test the infants with the Child Behavior Check List (CBCL) designed for children of ages 1.5-5 yrs (Eskenazi, 2007 & Eskenazi, 2010) and is widely used in cross-cultural research. CBCL collects data on wide range of behavioral problem, yielding scores for several syndrome scales consistent with DSM diagnoses (Eskenazi, 2010). It's composed of 99-items and it will be administered to mothers to assess their 2-year-old's emotional/behavioral problems and competencies (Eskenazi, 2007). A child psychologist specialist will be trained using standardized protocols and will be supervised for quality assurance by a clinical Neuropsychologist from the University of California Merced (Eskenazi, 2007). Moreover, mothers in our study will be trained by a psychologist to recognize behavioral problems following the Attention Deficit Hyperactive Disorder (ADHD)-DSM oriented which includes "can't sit still," "can't concentrate," and the DSM-oriented Pervasive Development Disorder (PDD) which includes "rocks head, body," and "unresponsive to affection." These symptoms are rated as very consistent with Asperger's syndrome and Autistic disorder (Eskenazi, 2007).

#### *Eight-Year Old Cohort*

For the eight-year old children, the WISC-IV test will be administered by a child psychologist blinded to exposure in a private room without the mother present but with the mothers' written consent and children's verbal consent. The WISC-IV includes cognitive testing methods such as: Block design, Similarities, Digit span, Picture concepts, Coding, Matrix





Reasoning, Vocabulary, Letter-Number Sequence, Comprehension, and Symbol Search subsets. The test methods are designed to measure each child’s Verbal Composite, Perceptual Reasoning, Working Memory, and Processing speed (Engel, 2011). We will derive Full Scale Intelligence Quotient (FSIQ) scores from the WISC-IV scores, an established method of producing an IQ score (Engel, 2011). We will compare the FSIQ scores with WISC-IV national averages in order to determine whether the child presents with neurological disorders such as Autism, ADHD, and Asperger’s syndrome.

In summary, we propose to do a similar study to Eskenazi et al. by pregnant farm working women on how organophosphates impact their life and their lives of their children in a longitudinal study. Furthermore, we plan to collect three urine samples to determine level of exposure: one at second trimester, second at 3<sup>rd</sup> trimester, and the last one postpartum. After the children are born they are going to be examined using the Bayley scales at critical developmental stages.

In our study, however, we are introducing a new variable. We are examining children eight years of age whose mothers work in agriculture to determine the level of exposure to OPs and the neurological effects of that exposure. The effects of exposure will be assessed by comparing our WISC-IV results against U.S. and Latin American averages. Demonstrating a relationship between organophosphates and neurodevelopment is crucial not only for other researches, but for EPA and other regulatory agencies so that we, as a society, can make informed decisions about pesticides.

**Project Timeline**

<b>Objective</b>	Aug 16 Feb-16	Sept-16	Mar-17	Mar-18	Jun-18	Sep-18
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<b>Take urine samples and measure Dialkylphosphate (DAP) metabolite levels- 2 prenatal 1 postpartum</b>	<b>XXX</b>					
<b>At 6 month examination with Mental Development Index (MDI)</b>		<b>X</b>				
<b>At 12 month examination with Pervasive Development Index (PDI)</b>		<b>X</b>				
<b>At 24 moth examination with Child Behavior Checklist (CBCL)</b>		<b>X</b>				
<b>Examine the eight year old children with (WISC-IV)</b>	<b>X</b>					<b>X</b>
<b>Analysis</b>		<b>X</b>	<b>X</b>	<b>X</b>	<b>X</b>	
<b>Publication</b>						<b>X</b>

### Budget

For our study we need to pay for a principal investigator along with two graduate students from the University of California Merced. Additionally a child development specialist will be hired and trained to examine the children. Also, the mothers will be compensated for participating with the examination of their infants and children. Next, a miscellaneous part was added for transportation, food, and other supplies needed. Finally, we added a section to pay for the urine samples to be analyze

<b>Item/or professional</b>	<b>Time</b>	<b>Cost</b>
<b>Quest urine creatine sample test</b>	<b>\$16 per sample</b>	<b>\$3,600</b>
<b>1 Child Development Specialist</b>	<b>2 years</b>	<b>\$92,000</b>
<b>Compensation to the Pregnant women and the parents of the 8 yr. old children</b>	<b>2 years</b>	<b>\$15,000</b>
<b>Principal Investigator salary</b>	<b>2 years</b>	<b>\$201,072</b>





<b>2 graduate students</b>	<b>2 years</b>	<b>\$140,272</b>
<b>Miscellaneous</b>	<b>2 years</b>	<b>\$10,000</b>
<b>Total</b>	<b>2 years</b>	<b>\$448,684</b>

### Public Health Impact Statement

Organophosphate based insecticides are toxic. OPs impair breathing and can potentially cause long-lasting effects on the neurodevelopment of children. Our study could help the general population make an informed decision about pesticides, and Environmental Protection Agency and other policy makers to regulate and potentially ban pesticides.

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