Characterizing the Neurodevelopmental Pesticide Exposome in an Agricultural Children’s Cohort

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In Washington State, apples are the number one commodity produced in the $49 billion food and agriculture industry. Apple growers often rely on pesticides to control insect, plant, and fungal pests. Many of these pesticides have known and well characterized health effects, including several that are known neurotoxicants. Occupational exposure to these pesticides is especially concerning given that many farmworkers carry pesticide residues home and can subsequently expose their children. With the multiple pesticides that farmworkers and their children are exposed to, it is valuable to study the pesticide “exposome,” which accounts for environmental exposures across the lifespan. This thesis uses an exposome framework to study how pesticide exposure changed in a children’s agricultural cohort between 2005 and 2011. The specific aims are to: 1. broadly evaluate how the pesticide exposome changed over the study period, and 2. examine how the neurodevelopmental pesticide exposome changed over the study period. Additionally, this work will consider the impact of changing regulations on the pesticide exposome.
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BACKGROUND

Agriculture in Washington State

Agriculture is a highly important industry in Washington State. Not only is agriculture a long-standing part of the state’s culture, but the food and agriculture industry collectively makes up approximately 13% of the state’s economy and employs around 160,000 people. (State of Washington Department of Commerce; Washington State Department of Agriculture 2015). Additionally, there are farms located in all 39 counties of the state, and agriculture and food manufacturing has been identified as one of the top eight key sectors with high growth by the State of Washington Department of Commerce. Washington Governor Jay Inslee has outlined specific goals to continue to grow the industry, including efforts to eliminate regulatory barriers that may restrict sector growth (State of Washington Department of Commerce). Washington State currently leads the nation in the production of eleven different crops, including hops, pears, sweet cherries, and apples (Washington State Department of Agriculture 2015). Apples represent the state’s top commodity and account for approximately 70% of US production (State of Washington Department of Commerce). Yakima County, located in central Washington, is the largest apple-producing county (by acres in production) in the US (USDA-NASS 2015).

Pesticide Use in Washington’s Agricultural Sector

In order to maintain and grow agricultural productivity in Washington State, the majority of farmers rely on the use of pesticides to control insect, plant, and fungal pests (Slattery et al. 2011). Herbicides help control weeds, which minimizes competition for soil nutrients, water, and physical space; insecticides control insect pests that physically damage crops; and fungicides deter fungal growth, which can allow crops to be grown in wetter conditions than they could otherwise (Cooper and Dobson 2007). For apples, the primary pest concerns include species such
as codling moth, apple maggot, canker, and mildew (Washington State University Extension 2017). Additionally, weed control in apple production can improve water and nutrient uptake by the trees and deter rodents and certain insects from living in orchards (Smith 2017). The type of compounds used to control these pests varies depending on the agricultural system. Organic apple growers are restricted in their pest management options and are prohibited from using most synthetic pesticides (Electronic Code of Federal Regulations 2016). Conventional apple growers, which represent approximately 92% of harvested acres in Washington State, are not subject to the same restrictions (Slattery et al. 2011; USDA-NASS 2015). Commonly used pesticides in Washington State conventional apple production include organophosphate (OP), neonicotinoid, or carbamate insecticides;azole, dithiocarbamate, or anilide fungicides; and herbicides like glyphosate, 2,4-dichlorophenoxyacetic acid (2,4-D), and pendimethalin (USDA-NASS 2011). Many of these pesticides have known or suspected adverse health effects. OPs are among the most well studied in terms of human health outcomes and have historically been the most widely used pesticides in pome fruit production. OPs are neurotoxicants that can cause acute symptoms such as blurred vision, abdominal cramps, tachycardia, flaccid paralysis, convulsions and coma (Costa 2013). Chronic exposure to OPs, though less well understood, has been linked with neurobehavioral disorders and certain types of cancers (Bouchard et al. 2010; Engel et al. 2011; McCauley et al. 2006; Rauh et al. 2011). Farmworkers responsible for growing and maintaining crops that have been sprayed with these pesticides are at a much greater risk of being exposed and potentially suffering these adverse health effects than the general population.

**Pesticides and the Occupational Take-home Pathway**

Occupational exposure to pesticides and other agricultural chemicals does not only affect farmworkers. Previous studies with a cohort of pome fruit farmworkers in the Yakima Valley of
Washington State have provided evidence that agricultural workers carry OP pesticide residues home with them. OPs have been detected in both house and vehicle dust of agricultural workers to a much higher degree than non-farmworkers, and children of agricultural workers have higher levels of OP metabolites in their urine than children of non-agricultural households (Coronado et al. 2006; Curl et al. 2002; Thompson et al. 2014). This OP “take-home” exposure pathway is of particular concern because a child’s brain continues to develop long after birth and exposure to neurotoxicants like OPs at a young age can have lasting behavioral and neurological effects (Grandjean and Landrigan 2014; National Research Council 1993).

**The Case of Azinphos-methyl**

Historically, azinphos-methyl (AZM) was among the most commonly used OPs in agriculture, particularly in apple production, but due to concerns regarding worker health and ecological impacts, a phase-out of all AZM uses was proposed by the EPA in 2006 (Goldberger et al. 2011; US EPA 2012). This phase-out concluded with AZM sales being prohibited in 2012 and the use of any existing stocks prohibited in 2013 (US EPA 2012). Pome fruits (apples and pears) were among the last allowable uses for AZM, in part because of the heavy reliance on AZM for control of codling moth (CM), one of the major apple pests (Doerr et al. 2012). One of the major reasons for the incremental phase-out of AZM was to minimize the negative economic impacts of eliminating a major method of pest control by allowing sufficient time for growers to transition to alternative chemicals and methods of insect control (Doerr et al. 2012; Goldberger et al. 2011; US EPA 2012). In Washington State, where apples are the major agricultural commodity produced, this was a particularly concerning issue for the Washington State Department of Agriculture (Washington State Department of Agriculture 2015). Throughout the duration of the AZM phase-out, Washington State University (WSU) Extension issued reports on
suitable alternatives to AZM to help control CM. The alternatives to AZM suggested by WSU Extension include other OPs such as phosmet and diazinon, neonicotinoids such as acetamiprid and thiacloprid, the N-methyl carbamate carbaryl, and the insect growth regulators methoxyfenozide and pyriproxyfen (Doerr et al. 2012; Washington State University Extension 2008, 2011). To date, there have been very few studies (Pouzou 2016) directly detailing how apple growers in the state shifted pesticide usage in response to the ban on AZM, and there have been no studies exploring the potential impact of the AZM phase-out on pesticide exposure.

**Regulation of Pesticide Exposure**

The primary institution that manages pesticide exposure is the US EPA, largely under the authority set forth in the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) and the Federal Food, Drug, and Cosmetic Act (FFDCA) (US EPA 2016a). FIFRA requires all pesticides (including antimicrobials, biopesticides, conventional pesticides, and inert ingredients) sold or distributed in the US to go through a registration process. This registration process broadly requires that pesticides not cause any “unreasonable adverse effects” to humans or the environment (FIFRA 2012; U.S. EPA 1996). Through this process, the EPA evaluates the risks and benefits of using the product, establishes the label directions for use, and provides limits on use (including suspending a product). FIFRA has also been amended through the Food Quality Protection Act (FQPA) to require “reasonable certainty of no harm” before a pesticide can be registered (FIFRA 2012; US EPA 2016a). The FFDCA gives the EPA authority to set tolerance levels of pesticide residue on foods. These tolerances are levels at which there is “reasonable certainty” of no harm from aggregate exposure to the pesticide both in food and other likely sources of exposure. The FFDCA also includes special protections for infants, children, and other sensitive subpopulations (FFDCA 1938; US EPA 2002b, 2016a). Historically, under the
FIFRA and FFDCA, the US EPA set pesticide tolerances and use restrictions based on aggregate exposure, or exposure to the same pesticide from multiple sources (e.g., residues on food and residential exposure). Under the amendments put in place under the FQPA, the EPA must now also consider the cumulative health effects of exposure to pesticides with a common mechanism of toxicity (FQPA 1996; US EPA 2002a). The EPA defines a Common Mechanism Group (GMG) as “two or more pesticide chemicals or other substances that cause a common toxic effect to human health by the same, or essentially the same, sequence of major biochemical events” (US EPA 1999). To date, the EPA has conducted formal cumulative risk assessments on the OPs, N-methyl carbamates, triazines, chloroacetanilides, and the pyrethrins/pyrethroids (US EPA 2016b). The OPs in particular have been subject to a great deal of regulation under the provisions set forth in FIFRA, FFCDA, and the FQPA amendments. The EPA has taken action to restrict or eliminate the use of several OPs, including azinphos-methyl, chlorpyrifos, and malathion due to concerns about human exposure to these toxic pesticides (US EPA 2001, 2012, 2017).

The Role of the Exposome

Although evaluating cumulative exposures to pesticides with a common mechanism of toxicity can be more protective than evaluating single pesticides alone, these evaluations may be insufficient, especially in regards to children’s health. The exposome (Wild 2005) provides a conceptual model to better identify and characterize child pesticide exposures and any resultant health effects. The role genes play in human health has been studied in depth since the discovery of DNA and the advent of new, high throughput sequencing techniques (Jones 2016; Miller and Jones 2014). However, genes only account for a small portion of total disease risk (Miller and Jones 2014; Rappaport and Smith 2010). Environmental exposures, including the diet, behavior,
exogenous, and endogenous (e.g., hormones) factors account for the majority of disease risk (Miller and Jones 2014). The wide variety of environmental factors, biological endpoints, and gene-environment interactions makes the task of defining the human exposome difficult (Jones 2016; Miller and Jones 2014; Rappaport and Smith 2010; Slama and Vrijheid 2015; Wild 2005, 2012). Wild (2012) suggested there were three broad exposure categories: internal (e.g., hormones, microflora), specific external (e.g., infectious disease, toxicants), and general external (e.g., social, psychological), and posited that measuring in any one area can reflect certain aspects of the overall exposome (Wild 2012). Several methods for evaluating the exposome have been proposed, including what Rappaport and Smith (2010) termed “bottom-up” environmental monitoring and “top-down” biomonitoring, the latter of which has received much attention for its potential for revealing both internal and external components of the exposome (Rappaport and Smith 2010). However, biomonitoring alone can be difficult to connect to specific exposures, which can make risk assessment and intervention, including regulatory decision making, difficult (Wild 2012). Thus, for the purposes of protecting human health, it may be advantageous to focus the exposome to particular classes of exposures such as pesticides as a way to apply broader exposome concepts within a risk assessment framework.

**THESIS GOALS**

**Specific Aim 1: Examine how the pesticide exposome changed between 2005 and 2011**

Using pesticide residues detected in household dust, establish the pesticide exposome of an agricultural community, and quantitatively evaluate how that exposome changed between the 2005 and 2011 agricultural seasons for farmworker (FW) and non-farmworker (NFW) households.
Hypothesis 1: Organophosphate use will decrease between 2005 and 2011, and the use of other insecticides (e.g., neonicotinoids, insect growth regulators, macrocyclic lactones) will increase.

Specific Aim 2: Examine how the exposome for pesticides with demonstrated neurotoxicity changed between 2005 and 2011

Using EPA Registration Eligibility Decisions (REDs), establish which pesticides detected in household dust have demonstrated evidence of neurotoxicity (human or animal models), and evaluate how the exposome for these pesticides changed between the 2005 and 2011 agricultural seasons.

Hypothesis: The proportion of pesticides with neurotoxic potential will decrease between 2005 and 2011, and that decrease will largely be driven by the decrease in OP use resulting from the US EPA’s ban on azinphos-methyl (AZM).

INTRODUCTION TO THESIS

Chapter 1 establishes the big-picture role that an exposome framework can play in exposure assessments. In particular, we compare the pesticide exposome between the 2005 and 2011 agricultural seasons for both farmworkers (FWs) and non-farmworkers (NFWs) living in the agricultural Yakima Valley. The 46 pesticides detected in over 5% of household dust samples were grouped into 18 distinct pesticide chemical classes, and the levels of each class detected were compared between years and occupational group. In particular, given increased regulation on the OPs over the study period (2005 – 2011), we focus on changes in the OPs as compared to other pesticide classes that were less regulated over the same period. This chapter is the first study to look at longitudinal pesticide exposome data for such a wide variety of pesticides.
Chapter 2 is a more in-depth exposome study that focuses particularly on the health endpoint of neurodevelopment. Building off the exposome framework applied in Chapter 1, this study groups pesticides by their neurotoxicity rather than by their pesticide chemical class. As our cohort is an agricultural children’s cohort, one of our primary concerns is children’s exposure to neurotoxicants that could affect their neurodevelopment and lead to life-long health consequences. Over half of the pesticides detected in household dust have some degree of potential neurotoxicity. Using an exposome framework, we assess how children’s exposure to these potentially neurotoxic pesticides changed between study years and examine whether or not FW or NFW households are more affected.

CHAPTER 1: LONGITUDINAL VARIABILITY IN THE PESTICIDE EXPOSOME:
CHARACTERIZING SEASONAL AND OCCUPATIONAL TRENDS OF PESTICIDES IN HOUSE DUST

Abstract
Agriculture requires a number of chemical inputs such as pesticides, many of which have known adverse health effects. Children of farmworkers are especially vulnerable, and early childhood pesticide exposure can have lasting health effects. Because children of farmworkers are potentially exposed to a variety of both agricultural and residential pesticides, we propose using an exposome framework explore the full extent of longitudinal child pesticide exposure. Using household dust samples from a children’s agricultural cohort in central Washington State, we examine snapshots of the pesticide exposome from the 2005 and 2011 agricultural seasons. In particular, we analyze how the pesticide exposome changes over time and the differences in the
pesticide exposome between farmworker and non-farmworker households. Dust samples were collected from floor areas where children played and analyzed by LC-MS. Individual pesticides were grouped into pesticide classes using the EPA’s chemical classification, and trends in pesticide concentrations were analyzed at the class level. Across the entire cohort, levels of organophosphates, macrocyclic lactones, quinolines, pyridazinones, and phenols significantly decreased in household dust samples between 2005 and 2011, whereas levels of guanidines, anilides, 2,6-dinitroanilines, and chlorophenols significantly increased. Among farmworkers, there were significantly lower levels of N-methyl carbamates and neonicotinoids in household dust in 2011. In 2005, a higher proportion of farmworker households had high (>4.63 nM/g dust) pesticide levels in household dust than non-farmworker households for the OP, neonicotinoid, pyrethroid, macrocyclic lactone, and dodine pesticides. This study provides critical longitudinal data on the changes in the pesticide exposome for a number of pesticides.

Introduction

Agriculture in Washington State

Agriculture is a highly important industry in Washington State. The food and agriculture industry as a whole makes up approximately 13% of the state’s economy and employs around 160,000 people (Washington State Department of Agriculture 2015). Washington State leads the nation in production of eleven different crops including hops, apples, sweet cherries, and pears. Apples and pears (pome fruits), as of 2013, accounted for 57.0% and 49.5% of total US production, respectively (USDA National Agricultural Statistics Service (NASS) 2015).

Chemical Inputs in Agriculture
Apples are susceptible to a number of pests and diseases, so growers often turn to the use of synthetic pesticides to maintain agricultural productivity (Slattery et al. 2011). Commonly used pesticides in Washington State apple production include organophosphate (OP), neonicotinoid, or carbamate insecticides;azole, dithiocarbamate, or anilide fungicides; and herbicides like glyphosate, 2,4-dichlorophenoxyacetic acid (2,4-D), and pendimethalin (USDA-NASS 2011). Many of these pesticides have known or suspected adverse health effects. OPs are among the most well studied in terms of human health outcomes and have historically been the most widely used pesticides in pome fruit production. OPs are neurotoxicants that can cause acute symptoms such as blurred vision, abdominal cramps, tachycardia, flaccid paralysis, convulsions and coma (Costa 2013). Chronic exposure to OPs, though less well understood, has been linked with neurobehavioral disorders and certain types of cancers (Bouchard et al. 2010; Engel et al. 2011; McCauley et al. 2006; Rauh et al. 2011). Farmworkers responsible for growing and maintaining crops that have been sprayed with these pesticides are at a much greater risk of being exposed and potentially suffering these adverse health effects than the general population.

*Occupational Take-home Pathway*

Occupational exposure to pesticides and other agricultural chemicals does not only affect farmworkers. Previous studies with a cohort of pome fruit farmworkers in the Yakima Valley of Washington State have provided evidence that agricultural workers carry OP pesticide residues home with them. OPs have been detected in both house and vehicle dust of agricultural workers to a much higher degree than non-farmworkers, and children of agricultural workers have higher levels of OP metabolites in their urine than children of non-agricultural households (Coronado et al. 2006; Curl et al. 2002; Thompson et al. 2014). This “take-home” exposure pathway is of
particular concern because a child’s brain continues to develop long after birth and exposure to neurotoxicants like OPs at a young age can have lasting behavioral and neurological effects (Engel et al. 2011, 2016; Grandjean and Landrigan 2014; Marks et al. 2010; National Research Council 1993).

Residential Pesticide Exposure

In addition to the various agricultural pesticides that may be brought home via the agricultural take-home pathway, children can also be exposed to pesticides through residential pesticide usage. Many common household pesticides include 2,4-D, glyphosate, carbaryl, malathion, and several pyrethroids, which are all commonly used in agriculture as well (Atwood and Paisley-Jones 2017; Grube et al. 2011; Guha et al. 2013). Thus, the agricultural take-home pathway coupled with residential pesticide exposure may put children living with farmworkers at a particularly high risk of developing adverse health outcomes, such as neurodevelopmental effects, from pesticide exposure.

The Pesticide Exposome

With the number of different pesticides children of farmworkers may be exposed to, it becomes critical to evaluate the impact that these cumulative exposures have on their health. The concept of the exposome (Wild 2005) provides a conceptual model to better identify and characterize child pesticide exposures and any resultant health effects. Environmental exposures, including the diet, behavior, and exogenous and endogenous factors, account for the majority of disease risk (Miller and Jones 2014). The wide variety of environmental factors, biological endpoints, and gene-environment interactions makes the task of defining the human exposome
difficult (Jones 2016). Thus, it becomes advantageous to narrow the focus of the exposome to a particular class of exposures such as pesticides to begin to understand the totality of the human exposome.

In the present study, we have used household dust as a method of measuring the pesticide exposome in an agricultural children’s cohort. By looking across the 2005 and 2011 agricultural seasons, we can use pesticide levels detected in household dust to demonstrate how the pesticide exposome changes over time. Furthermore, we can discern differences in the pesticide exposome between farmworker and non-farmworker households. In particular, we expect to see decreases in the OPs, as azinphos-methyl, one of the most commonly used OPs in Washington apple production was being phased-out during the study period (US EPA 2012).

Methods

Cohort Description and Subject Recruitment

University of Washington Center for Child Environmental Health Risks Research (CHC) Cohort is located in the lower Yakima Valley, one of Washington State’s major agricultural regions. In 2005, a cohort of approximately 200 households was recruited as previously described in Thompson et al (2014). The cohort was predominantly Hispanic (>95%) and split approximately evenly between farmworker (FW) and non-farmworker (NFW) families, each with a referent child aged 2-6 years. FWs were defined as individuals who worked in pome fruit crops (apples and pears), as the initial study focus was on orchard crops that received OP applications. The same CHC cohort participated in a follow-up study conducted during the 2011 growing season.
Sample Collection

Household dust samples were collected from each residence between April-July of 2005 and June-August of 2011. These seasons represent the “thinning season,” where buds and small fruit are removed from the trees to promote the growth of larger fruit. Previous studies have indicated that OP exposure, both in household dust and urine, is highest during this thinning season (Smith et al. 2016; Thompson et al. 2014). In 2005, household dust samples were collected using a Nilfisk GM-80 vacuum cleaner unit as previously described (Smith et al. 2016). Briefly, dust was collected from areas identified as frequent child play areas. Dust collection was standardized using 0.5x0.5m$^2$ collection templates, and the floor surface (i.e., plush carpet, thin carpet, hard floors) was accounted for by the number of templates collected. The hose, nozzle, and lower container of the vacuum was cleaned between each use, and a new polyliner and vacuum bag were used for each sample collection. Dust samples from 2011 were collected in the same manner as 2005, however a Metropolitan VM-500 High-Powered hand-led vacuum was used instead of the Nilfisk. One Metropolitan vacuum was used per household, and the nozzle and stainless steel sections were washed thoroughly before use.

Dust Analysis

For all collected dust, samples were transferred to 150µm metal sieves (VWR, West Chester, PA) and sieved for 10 minutes. Dust passing through the sieve was weighed, and the bulk sample was partitioned into two 1g aliquots. If less than 2g of dust was sieved, the second aliquot contained whatever dust remained after the first 1g aliquot. All dust samples were stored at -10°C until sample analysis. Of the initial cohort of 200 households, 75 had dust samples collected and analyzed for both 2005 and 2011. Dust samples were analyzed for 86 different
pesticides (described below) using acetonitrile extraction with liquid-liquid partitioning and liquid chromatography-tandem mass spectrometry (LC-MS) using electrospray ionization. Samples were analyzed in 2007, 2011, and 2012.

There were initially 305 pesticides used in Washington State considered for analysis. Fifty-five of those 305 were screened out because of either analytical difficulties including low stability in dust, unavailable LC-MS calibrates, or incompatibility with LC-MS methodology. Compounds selected for analysis were prioritized based on their toxicity, agricultural use, and availability of LC-MS standards. From the 250 remaining compounds, 145 were selected for further analysis, and 86 were successfully detected in household dust.

**Characterization of Pesticide Exposome in House Dust**

All pesticides with greater than 5% detection in household dust were initially included in the analysis. Individual pesticides were then grouped into their chemical classes using the EPA chemical classification system (see Table 1-1). Any pesticides that were in a class by themselves (e.g., triclosan, dodine) were listed with the pesticide name in parenthesis (e.g., guanidine (dodine)).

**Statistical Analysis**

**Heat Map Generation**

A heat map was generated to show the overall pesticide exposome. This was accomplished by using pesticide levels detected in household dust. Each column represents a class of pesticides (e.g., organophosphate), and each row represents an individual household. The coloring of each box represents the summed concentration of all the pesticides in each class in
that household for the given study year (2005 or 2011), reported as nanomolar concentrations per gram of dust (exact pesticide levels detected in each household available upon request). A tricolor (red, yellow, green) scoring was devised to relate each pesticide class to the 2005 levels of OPs. Red coloring defines pesticide concentrations in the 75th percentile of 2005 OPs (4.63nM), yellow represents the 50th percentile of 2005 OPs (1.13nM), and green represents the 25th percentile (0.032nM). This coloring was applied to each of the eighteen pesticide classes.

**Farmworker vs. Non-farmworker Comparisons**

A binomial comparison was used to assess the difference between the levels of pesticides detected in household dust between farmworker (FW) and non-farmworker (NFW) households. Using the heat map color scheme, described above, the fraction of red households (i.e., greater than 4.63nM pesticide per gram of dust) was determined for each pesticide class among FWs and NFWs. This generated two categories, from which a nonparametric proportions test was used to compare the “redness” of FWs vs. NFWs. This allows for the determination of whether FWs or NFWs have a higher proportion of households with the highest levels of pesticides detected in household dust for each pesticide class, which provides an indication of how the pesticide exposome differs between FW and NFW households.

**Comparisons Across Time**

For each household, it was determined whether the levels of each pesticide class increased, decreased, or stayed the same between 2005 and 2011 (e.g., [OP]_{CHC3} - [OP]_{CHC2}). Then, using a nonparametric proportions test, we compared the proportion of FW vs NFW households that had decreased pesticide levels between 2005 and 2011. This allowed for the determination of how the exposome has changed over time and whether those changes are different between FW and NFW households.
Additionally, a mixed effects model was used to examine the broader trend in pesticide levels between 2005 and 2011. Using household as the random effect, subsequent models were compared using time as a fixed effect, time and occupation (FW vs. NFW) as fixed effects with interaction, and time and occupation as fixed effects without interaction. This allows us to determine whether time had an effect on pesticide levels, whether those changes in time were affected by occupation, and whether or not there was truly an interaction between time and occupation. An additional model within occupation (FW and NFW) compared a household random effect only model with a model using time as a fixed effect, which allows us to see whether pesticide levels changed with time for each occupation independently.

Results

Dust Analysis

Eighty-six of the initial 145 pesticides were successfully analyzed in the dust. Of these 86 pesticides, 46 were detected in over 5% of samples, and 18 were detected in over 50% of samples (see Table 1-1). In 2005, OPs and pyrethroids had mean household dust concentrations greater than 1 nM, and in 2011, pyrethroids, dodine, and triclosan all had mean concentrations greater than 1 nM. Table 1-1 details the full list of pesticides that were successfully analyzed by class. Overall, we detected nine OPs, 6 pyrethroids, 5 azoles, 3 N-methyl carbamates, and a variety of other compounds including neonicotinoids, chlorophenoxy acids/esters, and insect growth regulators, among others, that met our inclusion criteria.

Pesticide Exposome Analysis
Figure 1-1 shows the concentration of pesticides, by class, detected in each household dust sample for 2005 and 2011. The red coloring indicates pesticide levels that are greater than the 75th percentile of OPs in 2005 (4.63nM per g dust), which was the pesticide class with the highest mean household dust concentration (1.17nM) for that year.

Organophosphate Insecticides in Household Dust

In 2005, 48% of FW households had OP levels higher than the 75th percentile (4.63nM/g dust; colored red in Figure 1-1). This was significantly higher than NFW households, of which none had OP levels greater than the 75th percentile (Table 1-2, p < 0.001). Although fewer FW households were “red” in 2011 (13%), there was still a significantly greater proportion of red FW households than the proportion of NFW households (p = 0.05). Throughout the whole cohort, OP concentrations in household dust were significantly lower in 2011 than in 2005 (Table 1-3, p = 0.003). This decrease in OP concentrations, however, was largely driven by FW households. Among FWs alone, OP levels were significantly lower in 2011 (Table 1-3, p < 0.001), whereas among NFWs, the decrease in OP levels between 2005 and 2011, while present, was not significant (p = 0.55). The proportion of individual FW households that had a decrease in OP pesticide levels between 2005 and 2011 was 83.3%, which is significantly greater than the 56% of NFW households that had decreased OP levels (Table 1-2, difference = 0.28 (95% CI: 0.05 – 0.51), p = 0.02).

N-Methyl Carbamate Insecticides in Household Dust

In 2005, 32% of FW households had N-methyl carbamate levels that were higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1), which was significantly greater than 9% of NFW households (Table 1-2, difference = 0.23 (95% CI: 0.05 – 0.42), p = 0.02). By 2011, however, there was no statistically significant difference in the “redness” of FW households.
and NFW households (p = 0.10). Across the entire cohort, the concentration of N-methyl carbamates in the household dust was significantly lower in 2011 than in 2005 among FWs (Table 1-3, p = 0.02), but there was no significant change among NFWs (p = 0.40). However, the proportion of individual FW households that had a decrease in N-methyl carbamate levels between 2005 and 2011 was not significantly greater than the proportion of NFW households (Table 1-2, p = 0.30).

Neonicotinoid Insecticides in Household Dust

In both 2005 and 2011, there were no households with neonicotinoid levels higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group. Across the entire cohort, the concentration of neonicotinoids in household dust was significantly lower in 2011 than in 2005 among FWs (Table 1-3, p = 0.03), but not among NFWs (Table 1-3, p = 0.40). Additionally, 71% of FW households had decreased neonicotinoid levels between 2005 and 2011, which is significantly greater than the 37% of NFW households with decreased levels over the same time period (Table 1-2, difference = 0.34 (95% CI: 0.08 – 0.60), p = 0.02).

Pyrethroid Insecticides in Household Dust

In 2005 there was no significant difference in the proportion of “red” (75th percentile of OPs, Figure 1-1) households between FWs and NFWs for the pyrethroids (Table 1-2, p = 0.83). Likewise, in 2011, the proportion of red FW households was not significantly different from the proportion of red NFW households (Table 1-2, p = 0.59). Across the entire cohort, the concentration of pyrethroids was not significantly different between 2005 and 2011, regardless of occupation (Table 1-3). However, 77% of FW households had lower pyrethroid levels in
2011, which is significantly greater than the 48\% of NFW households that had decreased pyrethroid levels (Table 1-2, difference = 0.29 (95\% CI: 0.03 – 0.55), p = 0.04).

**Insect Growth Regulator Insecticides in Household Dust**

In 2005, there were no households with insect growth regulator levels higher than the 75\textsuperscript{th} percentile for OPs (4.63nM/g dust, colored red in Figure 1-1) in either occupational group. In 2011, there were still no red NFW households, but 3\% of FW households were “red.” However, this was not significantly different from the proportion of red NFW households (Table 1-2, p = 0.34). Across the entire cohort, the levels of IGRs detected in household dust were not significantly different between 2005 and 2011, regardless of occupation (Table 1-3). Additionally, there was no significant difference between the proportion of FW and NFW households that had decreased IGR levels between 2005 and 2011 (Table 1-2, p = 0.92).

**Macrocyclic Lactone Insecticides in Household Dust**

In both 2005 and 2011, there were no households with macrocyclic lactone levels higher than the 75\textsuperscript{th} percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group. Across the entire cohort, the levels of macrocyclic lactones were significantly lower in 2011 than in 2005 among NFWs (Table 1-3, p < 0.001), but not among FWs (Table 1-3, p = 0.73). Additionally, 59\% of FW households had macrocyclic lactone concentrations that were lower in 2011 than in 2005, which is significantly lower than the 85\% of NFW households with decreased macrocyclic lactone concentrations (Table 1-2, difference = 0.26 (95\% CI: 0.02 – 0.51), p = 0.04).

**Other Insecticides in Household Dust**

In 2005, 17\% of FW households had insecticides in the “other” category that were detected at levels greater than the 75\textsuperscript{th} percentile of OPs (4.63nM/g dust, colored red in Figure 1-
1), which is not significantly different from the 6% of “red” NFW households (Table 1-2, difference = 0.11 (95% CI: -0.05 – 0.26), p = 0.18). In 2011, 13% of FW households were red, which is not significantly different from the 15% of red NFW households (Table 1-2, p = 0.87). Across the entire cohort, the levels of other insecticides were not significantly different between 2005 and 2011, regardless of occupation (Table 1-3). Furthermore, there was no significant difference between the proportion of FW and NFW households that had decreased levels of other insecticides between 2005 and 2011 (Table 1-2, p = 0.41).

Azole Fungicides in Household Dust

In both 2005 and 2011, there were no households with azole levels higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group (Table 1-2). Across the entire cohort, the levels of azoles were not significantly different between 2005 and 2011, regardless of occupation (Table 1-3). Furthermore, there was no significant difference between the proportion of FW and NFW households that had decreased levels of azoles between 2005 and 2011 (Table 1-2, p = 0.95).

Strobin Fungicides in Household Dust

In both 2005 and 2011, there were no households with strobin levels higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group (Table 1-2). Across the entire cohort, the levels of strobins were not significantly different between 2005 and 2011, regardless of occupation (Table 1-3). Furthermore, there was no significant difference between the proportion of FW and NFW households that had decreased levels of strobins between 2005 and 2011 (Table 1-2, p = 0.36).

Guanidine Fungicides (Dodine) in Household Dust
In 2005, 13% of FW households had dodine levels greater than the 75th percentile for OPs (4.63nM/g dust, colored red in Figure 1-1), which is not significantly different from the 3% of NFW households (Table 1-2, difference = 0.10 (95% CI: -0.03 – 0.24), p = 0.13). In 2011, there was likewise no statistically significant difference in the proportion of “red” households between FWs and NFWs (Table 1-2, p = 0.82). Across the entire cohort, the levels of dodine were significantly higher in 2011 than in 2005 for FWs (Table 1-3, p = 0.02), NFWs (p < 0.001), and across both occupation groups combined (p < 0.001). Although dodine levels increased across the cohort, 36% of FW households had a decrease in dodine levels between 2005 and 2011, which is significantly greater than the 11% of NFW households that had decreased dodine levels (Table 1-2, decrease = 0.25 (95% CI: 0.02 – 0.49), p = 0.04).

Anilide Fungicides (Boscalid) in Household Dust

In 2005, 3% of FW households had boscalid levels greater than the 75th percentile of OPs (4.63nM/g dust, colored red in Figure 1-1), which is not significantly different from NFW households, none of which were colored red (Table 1-2, p = 0.29). By 2011, 17% of FW households were red, which is significantly greater than the proportion of red NFW households, of which there were none (Table 1-2, p = 0.03). Across the entire cohort, the levels of boscalid detected in household dust were significantly higher in 2011 than in 2005 for FWs (Table 1-3, p < 0.001), NFWs (p = 0.001), and for both occupational groups combined (p < 0.001). The proportion of FW households that had decreased boscalid levels between 2005 and 2011 was 27%, which was not significantly different from the 15% of NFW households that had a decrease over the same period (Table 1-2, p = 0.28).

Quinoline Fungicides (Quinoxyfen) in Household Dust
In both 2005 and 2011, there were no households with quinoxyfen levels higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group (Table 1-2). Across the entire cohort, the levels of quinoxyfen detected in household dust were significantly lower in 2011 than in 2005 for FWs, NFWs, and for both occupational groups combined (Table 1-3, p < 0.001). The proportion of FW households that had decreased quinoxyfen levels between 2005 and 2011 was 92%, which was not significantly different from the 100% of NFW households that had a decrease over the same period (Table 1-2, p = 0.31).

Urea Herbicides in Household Dust

In 2005, there was no significant difference between the proportion of FW households that had urea levels greater than the 75th percentile for OPs (4.63nM/g dust, colored red in Figure 1-1), of which there were none, and the 3% of “red” NFW households (Table 1-2, p = 0.31). By 2011, there were no red households among either occupational group. Across the entire cohort, there was no significant difference in the urea concentrations in household dust between 2005 and 2011, regardless of occupational group (Table 1-3). Additionally, the proportion of FW households that had lower urea concentrations in 2011 was not significantly different from the proportion of NFW households that had decreased urea levels over the same period (Table 1-2, p = 0.69).

Chlorophenoxy Acid/Ester Herbicides in Household Dust

In 2005, there was no significant difference between the proportion of FW households that had chlorophenoxy acid/ester levels greater than the 75th percentile for OPs (4.63nM/g dust, colored red in Figure 1-1), of which there were none, and the 3% of “red” NFW households (Table 1-2, p = 0.34). This was likewise the case in 2011 (Table 1-2, p = 0.29). Across the entire cohort, there was no significant difference in the chlorophenoxy acid/ester concentrations in
household dust between 2005 and 2011, regardless of occupational group (Table 1-3). Additionally, the proportion of FW households that had lower chlorophenoxy acid/ester concentrations in 2011 was not significantly different from the proportion of NFW households that had decreased chlorophenoxy acid/ester levels over the same period (Table 1-2, p = 0.10).

2,6-Dinitroaniline Herbicides (Pendimethalin) in Household Dust

In both 2005 and 2011, there were no households with pendimethalin levels higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group (Table 1-2). Across the entire cohort, the levels of pendimethalin in household dust were significantly greater in 2011 than in 2005 for FWs (Table 1-3, p < 0.001), NFWs (p = 0.01), and for both occupations combined (p < 0.001). Additionally, the proportion of FW households that had lower pendimethalin levels in 2011 than 2005 was 17%, which was not significantly different from the 33% of NFW households (Table 1-2, p = 0.17).

Pyridazinone Herbicides (Norflurazon) in Household Dust

In both 2005 and 2011, there were no households with norflurazon levels higher than the 75th percentile for OPs (4.63nM/g dust; colored red in Figure 1-1) in either occupational group (Table 1-2). Across the entire cohort, the levels of norflurazon were significantly lower in 2011 than in 2005 for FWs (Table 1-3, p < 0.001), NFWs (p = 0.001), and for both occupations combined (p < 0.001). Additionally, the proportion of FW households that had lower pendimethalin levels in 2011 than 2005 was 83%, which was not significantly different from the 78% of NFW households (Table 1-2, p = 0.44).

Chlorophenol Microbiocides (Triclosan) in Household Dust

In 2005, there was no significant difference between the proportion of FW households that had triclosan (TCS) levels greater than the 75th percentile for OPs (4.63nM/g dust, colored
red in Figure 1-1) and the proportion of “red” NFW households (Table 1-2, p = 0.45). This was also the case in 2011 (Table 1-2, p = 0.23). Across the entire cohort, the levels of TCS were significantly higher in 2011 than in 2005 for FWs, NFWs, and for both occupations combined (Table 1-3, p < 0.001). Additionally, only 14% of FW households had lower TCS levels in 2011 in 2005, which was not significantly different from the 7% of NFW households (Table 1-2, p = 0.44).

Phenol Microbiocides (Sodium Ortho-Phenylphenate) in Household Dust

In 2005, there was no significant difference between the proportion of FW households that had sodium ortho-phenylphenate (Na OPP) levels greater than the 75th percentile for OPs (4.63nM/g dust, colored red in Figure 1-1) and the proportion of “red” NFW households (Table 1-2, p = 0.31). In 2011, there were no red households, regardless of occupation. Across the entire cohort, the levels of Na OPP were significantly lower in 2011 than in 2005 for FWs (Table 1-3, p = 0.02), NFWs (p < 0.001), and for both occupations combined (p < 0.001). Additionally, 71% of FW households had lower Na OPP levels in 2011 in 2005, which was not significantly different from the 74% of NFW households (Table 1-2, p = 0.84).

Discussion

Using household dust as a window into the pesticide exposome

Our analysis has revealed how the pesticide exposome changed in household dust for an agricultural cohort between the 2005 and 2011 agricultural seasons. It is exceedingly rare to have longitudinal exposure data for such a wide spectrum of pesticides. Additionally, the level of granularity has allowed us to explore changes in the pesticide exposome at the household,
occupational, and community level for our cohort. This has allowed us to define the longitudinal exposure profiles for a children’s agricultural cohort.

*Farmworker households showed greater declines in pesticide concentrations in dust than non-farmworker households*

In 2005, FWs had a greater proportion of “red” households (households with pesticide levels greater than 4.63nM/g dust) than NFWs for the organophosphates and the N-methyl carbamates, and in 2011 FWs had a greater proportion of red households for the organophosphates and boscalid. Non-farmworker households did not have a greater proportion of red households for any pesticide class. This is consistent with previous findings in this cohort that support an occupational take-home pathway for OPs (Coronado et al. 2006; Curl et al. 2002; Thompson et al. 2014). Most of the pesticides that were analyzed are used in agriculture, particularly in pome fruit production. Thus it follows that FW households would have a higher proportion of households with higher levels of these pesticides detected in their dust. Between 2005 and 2011, FW households overall experienced a decrease in OPs, n-methyl carbamates, neonicotinoids, quinolines (quinoxyfen), pyridazinones (norflurazon), and phenols (Na OPP). NFW households only decreased in the latter three pesticide classes. Furthermore, there was a greater proportion of FW households that had a decrease in OPs, neonicotinoids, pyrethroids, and guanidines (dodine) than NFW households. Taken together, this suggests that while FWs are more likely to have high (i.e., greater than the 75th percentile of OPs) pesticide levels in their household dust, they are also more likely to have decreased pesticide levels over time.
**Trends in agricultural pesticide usage align with the farmworker pesticide exposome detected in household dust**

In FW households, we observed a significant decrease in pesticide levels between 2005 and 2011 for OPs, N-methyl carbamates, neonicotinoids, and norflurazon, and an increase in pendimethalin and boscalid. All of these pesticides classes are used in apple production in Washington State, and the changes in the use of these 7 pesticide classes aligns with the trends observed in household dust (Figure 1-2). This suggests that FW households may be directly impacted by changes in agricultural pesticide usage. This is altogether consistent with our previous findings regarding the importance of the occupational take-home pathway (Coronado et al. 2006; Curl et al. 2002; Thompson et al. 2014). These findings further demonstrate the utility of using household dust as a way to measure the pesticide exposome. The connection between agricultural pesticide usage and the FW pesticide exposome suggests that household dust could be used to confirm the effectiveness of pesticide regulations and link pesticide use with the potential for direct population exposures.

**Residential pesticide exposure plays a complex role in the pesticide exposome**

While the use patterns in several classes of agricultural pesticides are seemingly detected in the household dust of FWs, the connection between residential pesticide use and the pesticide exposome is more complex. Several agricultural pesticides, such as 2,4-D, malathion, and carbaryl are also used in residential settings (Atwood and Paisley-Jones 2017; Grube et al. 2011; Guha et al. 2013), which makes visualizing the trends in household dust difficult. Furthermore, the usage of residential pesticides is not monitored like agricultural pesticide usage, and so sales estimates of active ingredient are the best available approximation (Atwood and Paisley-Jones
2017; Grube et al. 2011). Additionally, some pesticides registered for use by the EPA such as the antimicrobial triclosan (TCS) are incorporated into consumer products (US EPA 2008), which makes tracking usage even more difficult. Thus, without detailed household data, fully linking residential pesticide use to the pesticide exposome in household dust is impossible. Nevertheless, in our cohort we have observed interesting trends in residential pesticide use, particularly among the microbiocides. TCS levels in household dust increased significantly between 2005 and 2011 across the entire cohort. This finding is particularly notable given that urinary TCS in the US population (according to the National Health and Nutrition Examination Survey (NHANES)) decreased from a geometric mean concentration of 18.5μg/L to 11.8μg/L. Among Mexican Americans, which more closely represents the ethnicity of our cohort, the decrease is even larger – from 26.7μg/L in 2005/06 to 12.6μg/L in 2011/12 (CDC 2017). Although household dust and urine are not equivalent mediums, the fact that we observe the opposite trend as NHANES is notable. Because we see the levels of TCS in household dust increase between 2005 and 2011, we hypothesize that the presence of TCS in those households must have increased as well. It is possible that the integration of TCS in consumer products (e.g., toys, toothbrushes, textiles, garbage bags, etc.) increased over this period, and the degradation of these products leads to a higher TCS signal in house dust than in urine, which could explain the discrepancy between the trend observed in NHANES and the trend observed in the present study.

Guanidine fungicides (dodine) present another interesting case. While dodine is an agricultural fungicide, its use in pome fruit production is negligible (no available USDA chemical use data), and its use across all of Yakima County agriculture is minimal (less than 575lbs applied annually in 2011) (Baker and Stone 2015; Thelin and Stone 2013; USDA-NASS 2011). Additionally, the use of dodine in agriculture declined between 2005 and 2011 (Baker and
Stone 2015; Thelin and Stone 2013). However, regardless of occupation, we observe a significant increase in dodine within our cohort. Dodine has very similar chemical structure and properties as dodecylguanidine hydrochloride (DGH), a microbiocide that the EPA has grouped with dodine for registration purposes. DGH can be found in papers that come into contact with food, paint, and diapers (US EPA 2005). Given the minimal use of dodine in the community and the fact that the general pattern observed for TCS in household dust over time matches the pattern of dodine, it is quite possible that we are detecting a combination of DGH and dodine in household dust in our cohort.

The increase of TCS and dodine/DGH in our cohort presents an interesting case. While we observe a decrease in many agricultural pesticides in household dust, there is a concurrent increase in many pesticides with uses in consumer products. The reasons for these opposing trends is uncertain, but they present a compelling case for using an exposome framework for evaluating pesticide exposure. While agricultural pesticide use has declined overall, there are still pesticide exposures occurring through other (i.e., residential) routes, some of which may be increasing in magnitude. From a regulatory perspective it may be prudent evaluate these multiple and varied exposures collectively to fully capture the spectrum of pesticide exposure. In this analysis alone, the health impact of these multiple exposures on children’s health is uncertain. Nevertheless, we demonstrate the complexity of the pesticide exposome and highlight a need for longitudinal life-stage monitoring, as the pesticide exposome is dynamic and cannot be fully evaluated under the traditional exposure-monitoring paradigm.

Conclusion
We have defined the pesticide exposome in a children’s agricultural cohort and defined how that exposome changed between two agricultural seasons six years apart. This is the first instance of any such longitudinal effort to explore the changes in exposure to this many pesticides. There is a demonstrated link between reported agricultural chemical use and the levels of pesticides detected in the household dust of farmworkers. In 2005, FWs had higher concentrations of organophosphates and N-methyl carbamates, two major agricultural pesticides used in apple production, in their household dust than NFWs. In 2011, FWs had higher levels of organophosphates and the anilide fungicide boscalid than NFW households, further demonstrating the linkage between agricultural pesticide use and the levels detected in the household dust of farmworkers. Additionally, this work highlights the importance of detailed longitudinal exposure monitoring, especially for the purposes of capturing the changes in residential pesticide use, which may not follow the same trends as agricultural pesticide usage and for which use data is much less readily available. The observed increase in DGH and TCS suggests that the presence of microbiocide-containing consumer products may have increased over the study period. This hypothesis is difficult to verify, however, because residential pesticide use is not as well monitored as agricultural pesticide use. Although this analysis does not offer any insight into the potential health impacts at this point, we do lay a foundation for the use of the pesticide exposome to examine how cumulative pesticide exposures may affect the health of children in an agricultural cohort.
CHAPTER 2: CHARACTERIZING THE NEURODEVELOPMENTAL PESTICIDE EXPOSOME IN A CHILDREN’S AGRICULTURAL COHORT

Abstract

The exposome provides a conceptual model for identifying and characterizing lifetime environmental exposures and resultant health effects. Several methods for evaluating the exposome have been proposed. In this study, we look specifically at the neurodevelopmental pesticide exposome, which focuses on exposures to pesticides that have the potential to cause an adverse neurodevelopmental affect. Using household dust samples from a children’s agricultural cohort located in the Yakima Valley of Washington State, we identified 86 individual pesticides, 47 of which have evidence of neurotoxicity included in the EPA (re)registration materials. Over the two study years (2005 and 2011), we demonstrate a significant decrease in the neurodevelopmental pesticide exposome across the cohort, but particularly among farmworker households. Additional analysis that weighted the levels of potentially neurotoxic pesticides detected in household dust revealed that the decrease in potentially neurotoxic pesticides was largely a result of decreases in some of the most potent neurotoxicants. Overall, this study provides evidence that the neurodevelopmental pesticide exposome framework could be a useful tool in assessing the effectiveness of specific interventions in reducing exposure to neurotoxicants as this framework can evaluate changes in multiple pesticides at the same time.

Introduction

*The Exposome*
The concept of the exposome (Wild 2005) provides an ambitious conceptual model for identifying and characterizing lifetime environmental exposures and any resultant health effects. Environmental exposures, including the diet, behavior, exogenous, and endogenous (e.g., hormones) factors, account for the majority of disease risk (Miller and Jones 2014), thus the exposome is an important complement to the genome in terms of understanding human health. However, the wide variety of environmental factors, biological endpoints, and gene-environment interactions makes the task of defining the human exposome difficult (Jones 2016; Miller and Jones 2014; Rappaport and Smith 2010; Slama and Vrijheid 2015; Wild 2005, 2012). Wild (2012) suggested there were three broad exposure categories: internal (e.g., hormones, microflora), specific external (e.g., infectious disease, toxicants), and general external (e.g., social, psychological), and measuring in any one area can reflect certain aspects of the overall exposome (Wild 2012). Several methods for evaluating the exposome have been proposed, including what Rappaport and Smith (2010) termed “bottom-up” environmental monitoring and “top-down” biomonitoring, the latter of which has received much attention for its potential for revealing both internal and external components of the exposome (Rappaport and Smith 2010). However, biomonitoring alone can be difficult to connect to specific exposures, which can make risk assessment and intervention, including regulatory decision making, difficult (Wild 2012). Thus, for the purposes of protecting human health, it may be advantageous to narrow the focus of the exposome to a particular class of exposures, such as pesticides, as a way to apply broader exposome concepts in a risk assessment framework. Additionally, focusing the exposome on specific life-stages (e.g., specific periods of development) can both narrow down the compounds of interest and the window of exposure monitoring (Buck Louis et al. 2013; Shaffer et al. 2017),
especially for episodic exposures, such as agricultural pesticides that are typically used on a seasonal basis.

**Aggregate Exposure Pathways (AEPs) and the Exposome**

Teeguarden et al. (2016) have proposed using an Aggregate Exposure Pathway (AEP) as an organizational framework connecting exposure science to environmental health. The AEP builds on the concept of the Adverse Outcome Pathway (AOP), which is a framework that links molecular perturbations to an adverse outcome of regulatory relevance. Using the same basic concept, the AEP links the introduction of a stressor into the environment to its ultimate concentration at a biologically relevant site of action. In this framework, Teeguarden et al. define exposure as the actual amount of stressor reaching the environmental area of interest (e.g., buildings, soil, human tissues) without determining when that exposure becomes a dose (Teeguarden et al. 2016). This AEP framework provides a possible avenue for narrowing the focus of the broader exposome concept. By looking at a class of stressors, such as pesticides, and how they accumulate at a specific site of action, the exposome can be more directly applied in a framework that is better suited for regulatory decision making and other related public health interventions.

**The Neurodevelopmental Exposome**

Neurodevelopment is a critical endpoint to examine in children’s health (Selevan et al. 2000). Although the majority of neurogenesis and neuron migration occurs during the prenatal period, there are still several key processes in brain development that occur postnatally (Stiles and Jernigan 2010). As a result, there is an extended “window of susceptibility” for children’s
neurodevelopment, and exposure to environmental neurotoxicants throughout early adolescence can have significant impacts on a child’s learning, behavior, and attention (Grandjean and Landrigan 2014; Selevan et al. 2000; Stiles and Jernigan 2010; Tyler et al. 2008). There are a number of well characterized environmental toxicants that are known to have an adverse impact on children’s neurodevelopment, including lead, methylmercury, tetrachloroethylene, and the OP pesticide chlorpyrifos (Grandjean and Landrigan 2014). As children can be exposed to a number of environmental neurotoxicants at any given time, it is important to consider the neurodevelopmental exposome when assessing the potential adverse health effects of child exposures, especially to pesticides.

Many pesticides share similar modes of action, which can exacerbate the impacts on human health when co-exposures occur. For example, OP and N-methyl carbamate pesticides both act by inhibiting acetylcholinesterase, thus co-exposures can lead to an even greater accumulation of acetylcholine and subsequent neurotoxic effects. The neurodevelopmental exposome follows on this concept by considering multiple pesticide classes that may act by different modes of action, but share similar target systems such as the neurological system. In 2011, a trio of child health studies observed significant neurodevelopmental delays associated with prenatal and early childhood exposure to OPs (Bouchard et al. 2010; Engel et al. 2011; Rauh et al. 2011). Other pesticide groups, such as the neonicotinoids have also been associated with impaired neurodevelopment in animal models (Crosby et al. 2016). Both OPs and neonicotinoids are commonly used in agriculture, and so exploring the neurodevelopmental pesticide exposome is particularly important when considering the health of children living in agricultural communities.
Using a Neurodevelopmental Exposome Framework to Characterize Pesticide Exposure in a Children’s Agricultural Cohort

In the present study, we have combined the AEP and exposome frameworks to characterize the neurodevelopmental pesticide exposome in a longitudinal children’s agricultural cohort located in the Lower Yakima Valley of Washington State. By using household dust as a representative exposure medium, we have determined the levels of prevalent pesticides used in pome fruit (apples and pears) production in Yakima for two agricultural seasons six years apart (2005 and 2011).

Methods

Cohort Description and Sample Collection

The University of Washington Center for Child Environmental Health Risks Research (CHC) Cohort is an agricultural children’s cohort located in the Lower Yakima Valley. This cohort consists of approximately 200 households split approximately evenly between farmworker (FW) and non-farmworker (NFW) families, each with a referent child aged 2-6 years as previously described in Thompson et al (Thompson et al. 2014). A subset of approximately 100 households of this cohort had dust samples collected between April-July of 2005 and again in June-August of 2011. Dust samples were collected by vacuuming 0.5m$^2$ x 0.5m$^2$ square areas of floor surface as previously described in Chapter 1 (Smith et al. 2016).

Dust Analysis

Dust samples were analyzed as previously described (see Chapter 1). Briefly, samples were sieved through 150μm metal sieves for 10 minutes and partitioned into 1g aliquots. Dust
samples were analyzed for pesticide concentrations using acetonitrile extraction with liquid-liquid partitioning and liquid chromatography-tandem mass spectrometry (LC-MS) using electrospray ionization. 86 pesticides out of 145 initial candidates were successfully analyzed.

Assessment of Potential Pesticide Neurotoxicity

All 86 pesticides that were detected in household dust samples were included in the preliminary neurotoxicity assessment. For each pesticide, neurotoxicity potential was scored by a simple “yes/no” system based on data available in US EPA Registration Eligibility Decisions (REDs). All pesticide REDS were searched for using the EPA Pesticide Chemical Search Database ([https://iaspub.epa.gov/apex/pesticides/f?p=chemicalsearch:1]/). For pesticides without a RED available at the time of analysis (e.g., boscalid), the most recent human health risk assessment or other relevant health assessment material available in the pesticide reregistration (or registration review) docket was used instead. Pesticides were scored as “potentially neurotoxic” if the RED (or other available material) provided a specific dose (e.g., LOAEL, NOAEL, RfD, etc.) associated with a neurotoxic endpoint. Neurotoxic endpoint assessment followed the EPA guidelines for Neurotoxicity Risk Assessment (US EPA 1998). Briefly, five broad endpoints are used to identify neurotoxic effects: structural (e.g., morphological changes in the brain), neurophysiological (e.g., alterations in nerve conduction), neurochemical (e.g., alterations in synthesis/breakdown of neurotransmitters), behavioral (e.g., changes in motor activity), and developmental (e.g., delayed behavior appearance) endpoints (US EPA 1998). Pesticides were scored as “non-neurotoxic” if the RED or other available reregistration documents did not conduct a neurotoxicity evaluation or include any neurotoxic endpoints. Dose
information was used from the “Summary of Toxicology Endpoint Selection” for non-
occupational exposures available in the RED or human health risk assessment.

Statistical Analysis

Heat Map Generation

A neurodevelopmental exposome heat map was generated using pesticide levels detected
in household dust. This method of heat map generation was adapted from the method described
in Chapter 1. Briefly, the columns represent the grouped neurotoxic or non-neurotoxic pesticides
for each of the study years (2005 and 2011), and each row represents an individual household.
The each box indicates the summed concentration of each pesticide group (neurotoxic or non-
neurotoxic) for the corresponding year. A tri-color (red, yellow, green) scoring system was used
to relate each group of pesticides to the concentration of neurotoxic pesticides detected in 2005.
The red coloring represents pesticide levels greater than the 75th percentile for 2005 neurotoxic
pesticides (17.60 µM/g dust), yellow represents the 50th percentile (7.03 µM/g dust) and green
represents the 25th percentile (2.16 µM/g dust). This same coloring system was applied to both
groups of pesticides for both study years to allow for comparisons across time.

Farmworker vs. Non-farmworker Comparisons

A binomial comparison was used to assess the difference between the levels of
neurotoxic and non-neurotoxic pesticides detected in household dust between FW and NFW
households, as previously described in (dust exposome paper). Using the heat map coloring
(described above), the proportion of “red” households (i.e., greater than 17.60 µM/g dust) was
determined for each pesticide grouping. This generated two categories (high vs. low
concentration). A nonparametric proportions test was then used to compare the redness of FW
versus NFW houses. This allowed us to determine whether FWs or NFWs have a higher proportion of household dust samples with the highest levels of potentially neurotoxic pesticides, and whether those differences are consistent between 2005 and 2011.

**Comparisons Across Time**

A mixed effects model was used to examine the trends in potentially neurotoxic and non-neurotoxic pesticides between the two study years (2005 and 2011). All models used household as a random effect (null model), and subsequent models used time as a fixed effect (model 1), time and occupation (FW vs. NFW) as fixed effects with interaction (model 2), and time and occupation as fixed effects without interaction (model 3). An ANOVA was used to compare each model with the subsequent model (e.g., model 1 with the null model, model 2 with model 1, etc.). This allowed us to determine whether there were differences in the levels of potentially neurotoxic/non-neurotoxic pesticides detected in household between study years, and whether those differences were related to occupation. To further explore the role that occupation may have in the changes in potentially neurotoxic pesticide concentrations over time, additional mixed effects modeling was used to examine the pesticide trends over time within an occupation. In this secondary analysis, the null model of random household effect alone was compared to a model that included time (2005 vs. 2011) as a fixed effect within each individual occupational group.

An additional binomial test was used to assess whether the change in potentially neurotoxic and non-neurotoxic pesticides between 2005 and 2011 was significantly different between FWs and NFWs. For each household, we determined whether the levels of potentially neurotoxic/non-neurotoxic pesticides increased, decreased, or stayed the same between 2005 and 2011 (e.g., \([\text{neurotoxic}]_{2005} - [\text{neurotoxic}]_{2011}\)). Then, the proportion of households that decreased over time
was determined for each occupation (FW vs. NFW), and a nonparametric proportions test was used to determine whether the proportion of households with decreased pesticide levels was significantly different between FWs and NFWs.

**Results**

*Categorization of Potentially Neurotoxic Pesticides*

Table 2-1 details which pesticides have neurotoxic potential, as determined by EPA REDs, human health risk assessments, or other available pesticide (re)registration information. Of the 86 initial pesticides, 47 had some evidence of neurotoxicity in animal models used in the EPA registration decision. The remaining 39 pesticides either had no neurotoxicity assessments conducted in the registration process, or had no neurotoxic endpoint in the toxicology endpoint summary table. For pesticides that did have a neurotoxic endpoint provided in the registration materials, the associated dose information is provided in Table 2-1. When available, the acute reference dose (RfD) or population adjusted dose (PAD) was selected. Otherwise, the most sensitive acute dose (e.g., NOAEL used over LOAEL) was selected, and the RfD was calculated using standard uncertainty factors of 100x for NOAELs and 1000x for LOAELs.

*Neurodevelopmental Exposome Heat Map*

Figure 2-1 shows the concentration of potentially neurotoxic and non-neurotoxic pesticides for both study years (2005, left, and 2011, right) among farmworkers (top) and non-farmworkers (bottom). The red coloring indicates pesticide levels that are greater than the 75th percentile of potentially neurotoxic pesticides in 2005 (17.60 µM/g dust).
Trends in Potentially Neurotoxic Pesticides

Unweighted Analysis

Figure 2-2A shows that in 2005, 38% of FW households had potentially neurotoxic pesticide levels greater than the 75th percentile (17.60 µM/g dust, colored red in Figure 1), which is significantly greater than the 11% of “red” NFW households (Table 2-2, difference = 0.27 (95% CI: 0.08 – 0.44), p = 0.01). By 2011, the proportion of red households was not significantly different between FWs and NFWs (Table 2-2, p = 0.57). Across the entire cohort, the levels of potentially neurotoxic pesticides detected in household dust were not significantly different between 2005 and 2011 (Table 2-2, p = 0.57). However, when occupation was added into the model (with interaction), the difference between 2005 and 2011 was statistically significant (Table 2-2, p > 0.001). This suggests that the levels of potentially neurotoxic pesticides for FWs and NFWs change differently over the study period. Indeed, there is a higher proportion of NFW households that have high levels of potentially neurotoxic pesticides in 2011 than in 2005, although this difference is not statistically significant (p = 0.28). Additionally in 2011, there is a higher proportion of NFW households with high potentially neurotoxic pesticide levels than FW households, but again, this is not a significant difference.

Weighted Analysis

Figure 2-2 B shows that in the weighted neurodevelopmental pesticide exposome analysis, 43% of FWs had households with potentially neurotoxic pesticide levels greater than the 75th percentile (weighted), which is significantly greater than the 6% of NFW households (Table 2-3, difference = 0.37 (95% CI: 0.20 – 0.54), p < 0.001). In 2011, the proportion of households above the 2005 75th percentile was not significantly different between FWs and NFWs (Table 2-3, p = 0.62). Across the entire cohort, the levels of potentially neurotoxic
pesticides decreased between 2005 and 2011 (Table 2-3, p = 0.004), but this decrease was only significant among FWs (p = 0.001), and not among NFWs (p = 0.38). Additionally, the trend of increasing potentially neurotoxic pesticide levels among NFW households that was present in the unweighted analysis does not appear in the unweighted analysis.

**Trends in Non-Neurotoxic Pesticides**

Figure 2-2C shows that for both 2005 and 2011, the proportion of FW households that had non-neurotoxic pesticide levels greater than the 75th percentile of potentially neurotoxic pesticides (17.60 µM/g dust, colored red in Figure 2-1) was not significantly different than the proportion of red NFW households (Table 2-2, p = 0.46 for 2005, p = 0.28 for 2011). Across the entire cohort, the levels of non-neurotoxic pesticides in household dust were significantly higher in 2011 than in 2005 (p < 0.001). This same trend was observed among FWs and NFWs individually as well (Table 2-2, p < 0.001). Additionally, there was no significant difference between the proportions of FW vs. NFW households with decreased non-neurotoxic pesticide levels between 2005 and 2011 (p = 0.36).

**Discussion**

*The neurodevelopmental exposome of farmworkers and non-farmworkers became more similar over time*

In 2005, FW households tended to have significantly higher levels of potentially neurotoxic pesticides than NFW households in both the weighted and unweighted analyses. However, by 2011, there was no significant difference between the two occupational groups for both analyses. These differences between FW and NFW households is likely largely driven by
the OPs, all of which have a neurotoxic endpoint (AChE inhibition) and decreased significantly between 2005 and 2011 (see Chapter 1). Most OPs are restricted to agricultural uses, which is consistent with the observation that the decline in potentially neurotoxic pesticides mirrors the decline in OPs for FWs, but not for NFWs. Furthermore, there is an increase in the levels of non-neurotoxic pesticides detected in household dust for both occupational groups. This suggests that there may be a shift in pesticide use away from potentially neurotoxic pesticides towards non-neurotoxic alternatives. Indeed, the regulatory efforts of the EPA reflect a movement away from neurotoxic pesticides in apple production (Goldberger et al. 2011; US EPA 2001, 2012, 2017).

It should be noted, however, that in the present study neurotoxicity was assessed by the health endpoints highlighted in the EPA REDs. The “non-neurotoxic” pesticides may still have an effect on the nervous system that has either not been well characterized or was not sensitive enough to be incorporated into the EPA registration decision. Additionally, this analysis did not include pesticides that may affect the neuroendocrine system in the potentially neurotoxic classification. For example, according to the EPA Human Health Assessment Scoping Document, the anilide fungicide boscalid does not directly affect nervous system, but has been reported to affect the thyroid (US EPA 2014). For this analysis, these pesticides were categorized as “non-neurotoxic,” because it is difficult to assess whether alterations to the endocrine system will ultimately affect the nervous system. Instead, this analysis focused on pesticides that have overt neurotoxicity to allow for clearer endpoint definition. Future studies may include potential neuroendocrine effects in the neurodevelopmental exposome, but that assessment is beyond the scope of this analysis.
Weighted analysis reveals a greater decline in the levels of more potent potentially neurotoxic pesticides with time

Although the neurodevelopmental exposome of FWs and NFWs became more similar over time, a mixed effects analysis suggests that these exposomes are changing differently with time. In the unweighted analysis, the FW households tended towards having decreased levels of potentially neurotoxic pesticides (non-significant), whereas the NFW households tended towards increasing levels (non-significant). The trend of decreasing levels of potentially neurotoxic pesticides among FWs is consistent with decreasing OP use in apple production (see Chapters 1), but the increasing trend among NFWs was unexpected. When the potentially neurotoxic pesticides were weighted by their relative potencies, the trend of decreasing potentially neurotoxic pesticides does become significant for FWs, and, while still non-significant, the trend towards increasing potentially neurotoxic pesticide levels disappears among NFWs. These differences between the weighted and unweighted analysis suggest that the pesticides that are decreasing the most between 2005 and 2011 are those that are more potent (i.e., have lower RfDs). This is consistent with the significant decrease observed in the OPs across the entire cohort over this study period (see Chapter 1). The OPs are among some of the more potent neurotoxicants (see Table 2-1) and were present in in very high levels in 2005, but decreased significantly by 2011 (see Chapter 1). The phase-out of AZM that was occurring during this study period is likely contributing to these changes. By using both the weighted and unweighted analyses, we can demonstrate that not only are potentially neurotoxic pesticide levels decreasing over this study period, but the levels of some of the most potent potentially neurotoxic pesticides are also decreasing. This provides evidence that the changes in pesticide use, whether driven by regulatory or other pressures, were largely focused on the more potent potential neurotoxicants.
Conclusion

This study is the first of its kind to look at aggregate pesticide exposures in an exposome framework. By looking at a wide variety of pesticides with a common target organ system, we can gain a better understanding of how changes in exposure may affect the risk of specific health outcomes. Although there were no significant changes in the neurodevelopmental pesticide exposome between 2005 and 2011 in the unweighted analysis, weighting each potentially neurotoxic pesticide by its RfD reveals a significant decrease in the levels of potentially neurotoxic pesticides detected in household dust across the entire cohort. This trend was largely driven by FW households, and is largely due to the significant decrease in OPs over this study period. These findings are consistent with regulatory efforts by the US EPA to reduce exposures to OPs during the timeframe of this study. Furthermore, this study provides evidence that the more potent potentially neurotoxic pesticides were reduced more so than some of the less potent potentially neurotoxic pesticides over the study period. This observation provides evidence that using a neurodevelopmental pesticide exposome framework could be a useful tool in assessing the effectiveness of specific interventions in reducing exposure to neurotoxicants.

CONCLUSION TO THE THESIS

This work has revealed the utility of using an exposome framework to evaluate how pesticide levels change over time. In particular, we demonstrate significant decreases in the organophosphate, N-methyl carbamate, neonicotinoid, quinoline, and pyridazinone pesticide classes and an increase in the anilide and 2,6-dinitroaniline pesticide classes among FW households. These changes in agricultural pesticide levels detected in household dust are
consistent with changes in the use of these pesticides in apple production in Washington State. The consistency between pesticide levels in the household dust of FWs and the use of these pesticides in apple production suggests that household dust can accurately reflect large-scale changes in pesticide use. Additionally, this study revealed that the levels of the microbiocide triclosan and signal from dodine (or DGH) increased in household dust during the study years, which may be consistent with increasing use of these compounds in consumer products with time.

Human health is one of the primary drivers behind pesticide regulation and efforts to reduce exposure to pesticides in general. By looking specifically at the neurodevelopmental pesticide exposome, we have added additional relevancy to using a pesticide exposome framework to look at changes in pesticide levels over time. In 2005, the proportion of FW households with high levels of potentially neurotoxic pesticides detected in their household dust were significantly greater than the proportion of NFW households with very high levels. However, by 2011, there was no significant difference between FW and NFW households. Additionally, by weighting the analysis, we demonstrate that the levels of potentially neurotoxic pesticides decrease significantly between 2005 and 2011. This finding is consistent with efforts, both by the US EPA and within the community, to reduce exposure to OPs, one of the more potent neurotoxic pesticides. The consistency between the levels of pesticides detected in household dust, changes in pesticide use, and changes pesticide regulation during this period provides compelling evidence that the pesticide exposome can be a useful tool in studying the effect of future regulatory changes on pesticide exposure.
REFERENCES

food-manufacturing/ [accessed 4 November 2017].


Chapter 1

<table>
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<th>Class</th>
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Table 1-1 Full List of Compounds Detected in Household Dust. Table 1-1 lists the compounds detected in household dust, grouped by class, that were detected in over 5% of collected samples.
Figure 1-1 – Heat Map for Pesticides Detected in Household Dust. The 46 pesticides detection in over 5% of samples were grouped into 18 different pesticide classes. The above figure shows the relative concentrations of pesticides, by class, detected in over 5% of household samples over the two sampling periods (2005 and 2011). The coloring is relative to the 25th (0.032nM/g), 50th (1.13nM/g), and 75th (4.63nM/g) percentiles of organophosphates in 2005, with red representing higher pesticide concentrations.
Table 1-2 Summary of Proportional Statistical Tests. For each pesticide class, we compared the proportion of farmworker (FW) households that were “red” in Figure 1-1 (had pesticide concentrations greater than 4.63 nM/g dust) to the proportion of red non-farmworker (NFW) households. This table provides the proportion of red FW households, red NFW households, the difference between those two proportions, and the p-value associated with those differences. Additionally, for each pesticide class, we compared the proportion of FW households that had a decrease in pesticide concentrations between 2005 and 2011 to the proportion of NFW households that decreased in pesticide concentrations. * p < 0.05; ** p < 0.01; *** p <0.001.

<table>
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<tr>
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<th>Organophosphate</th>
<th>N-Methyl Carbamate</th>
<th>Neonicotinoid</th>
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<td>FW NFW Difference (95% CI)</td>
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<td>FW NFW Difference (95% CI)</td>
<td>FW NFW Difference (95% CI)</td>
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<tr>
<td>Proportion Red, 2005</td>
<td>0.48 0.00 0.48 (0.32 - 0.63) &lt;0.001***</td>
<td>0.32 0.09 0.23 (0.05 - 0.42) 0.02*</td>
<td>0.27 0.24 0.03 (-0.19 - 0.24) 0.83</td>
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<tr>
<td>Proportion Red, 2011</td>
<td>0.13 0.00 0.13 (0.01 - 0.25) 0.05*</td>
<td>0.23 0.07 0.16 (-0.02 - 0.34) 0.10</td>
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<td>Proportion Decrease ('05-'11)</td>
<td>0.83 0.56 0.28 (0.05 - 0.51) 0.02*</td>
<td>0.63 0.48 0.14 (-0.123 - 0.41) 0.30</td>
<td>0.71 0.37 0.34 (0.08 - 0.60) 0.02*</td>
<td>0.77 0.48 0.29 (0.03 - 0.55) 0.04*</td>
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<table>
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<tr>
<th>Insect Growth Regulator</th>
<th>Macrocyclic Lactone</th>
<th>Other</th>
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<td>FW NFW Difference (95% CI)</td>
<td>FW NFW Difference (95% CI)</td>
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<td>Proportion Red, 2005</td>
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<td>0.17 0.15 0.02 (-0.20 - 0.17) 0.87</td>
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<td>Proportion Red, 2011</td>
<td>0.59 0.85 -0.26 (-0.51 - -0.02) 0.04*</td>
<td>0.64 0.52 0.12 (-0.16 - 0.39) 0.41</td>
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<td>Proportion Decrease ('05-'11)</td>
<td>0.73 0.74 -0.01 (-0.26 - 0.24) 0.92</td>
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<th>Bosacil (Anilide)</th>
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<td>FW NFW Difference (95% CI)</td>
<td>FW NFW Difference (95% CI)</td>
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<td>0.92 1.00 -0.08 (-0.24 - 0.07) 0.31</td>
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<tr>
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<th>Norflurazon (Pyridazinone)</th>
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<td>FW NFW Difference (95% CI)</td>
<td>FW NFW Difference (95% CI)</td>
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<td>FW NFW Difference (95% CI)</td>
<td>FW NFW Difference (95% CI)</td>
<td></td>
</tr>
<tr>
<td>Proportion Red, 2005</td>
<td>0.07 0.03 0.04 (-0.07 - 0.15) 0.45</td>
<td>0.07 0.15 -0.08 (-0.24 - 0.07) 0.31</td>
</tr>
<tr>
<td>Proportion Red, 2011</td>
<td>0.43 0.59 -0.16 (-0.42 - 0.01) 0.23</td>
<td>0.71 0.74 -0.03 (-0.28 - 0.23) 0.84</td>
</tr>
</tbody>
</table>
Table 1 shows the models used for the mixed effects analysis of the cohort-level changes in pesticide levels detected in household dust between 2005 and 2011. The models were compared as listed above, and the resultant p-values are reported. *p < 0.05; **p < 0.01; ***p < 0.001

<table>
<thead>
<tr>
<th>Mixed Effects Tests</th>
<th>Models</th>
<th>p-value for model comparisons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Null vs. Time Fixed</td>
<td>( \log(\text{pest}) \sim 1 + (1</td>
<td>\text{house}) )\n( \log(\text{pest}) \sim \text{tm} + (1</td>
</tr>
<tr>
<td>Time Fixed vs. Time*Occupation</td>
<td>( \log(\text{pest}) \sim \text{tm} + (1</td>
<td>\text{house}) )\n( \log(\text{pest}) \sim \text{tm} + (1</td>
</tr>
<tr>
<td>Null vs. Time Fixed w/in Occupation</td>
<td>( \log(\text{pest}) \sim 1 + (1</td>
<td>\text{house}) )\n( \log(\text{pest}) \sim \text{tm} + (1</td>
</tr>
<tr>
<td>FW</td>
<td>( \log(\text{pest}) \sim 1 + (1</td>
<td>\text{house}) )\n( \log(\text{pest}) \sim \text{tm} + (1</td>
</tr>
<tr>
<td>NFW</td>
<td>( \log(\text{pest}) \sim 1 + (1</td>
<td>\text{house}) )\n( \log(\text{pest}) \sim \text{tm} + (1</td>
</tr>
</tbody>
</table>

Table 1-3 - Mixed Effects Analysis Models and Results. Table 1-3 shows the models used for the mixed effects analysis of the cohort-level changes in pesticide levels detected in household dust between 2005 and 2011. The models were compared as listed above, and the resultant p-values are reported. *p < 0.05; **p < 0.01; ***p < 0.001
Figure 1-2 – Changes in pesticide use between 2005 and 2011 for select agricultural pesticides. Pesticide use statistics (USDA-NASS 2011) in total pounds applied for six agricultural pesticides in 2005 (dark gray) and 2011 (light gray). Organophosphates, N-methyl carbamates, neonicotinoids, and norflurazon decrease in use between 2005 and 2011; pendimethalin and boscalid increase in use.
### Table 2-1 – Classification of Pesticides Detected in Household Dust

Pesticides were identified as potentially neurotoxic based on available health endpoint data in US EPA Registration Eligibility Decisions (REDs), Human Health Risk Assessments, or other health assessment data available in (re)registration dockets. Table 2-1 identifies which pesticides were identified as potentially neurotoxic and provides a reference dose (RfD) for each.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Identified as Neurotoxic</th>
<th>Reference Dose (mg/kg/day)</th>
<th>Compound</th>
<th>Identified as Neurotoxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>2_4D</td>
<td>Y</td>
<td>0.067</td>
<td>2_4DB</td>
<td>N</td>
</tr>
<tr>
<td>Acetamiprid</td>
<td>Y</td>
<td>0.1</td>
<td>2_4DP</td>
<td>N</td>
</tr>
<tr>
<td>Aldicarb</td>
<td>Y</td>
<td>0.001</td>
<td>Boscalid</td>
<td>N</td>
</tr>
<tr>
<td>Azinphosmethyl</td>
<td>Y</td>
<td>0.0033</td>
<td>Carfenrazone-ethyl</td>
<td>N</td>
</tr>
<tr>
<td>Azoxystrobin</td>
<td>Y</td>
<td>0.67</td>
<td>Clofentezine</td>
<td>N</td>
</tr>
<tr>
<td>Bifenazate</td>
<td>Y</td>
<td>0.134*</td>
<td>Diuron</td>
<td>N</td>
</tr>
<tr>
<td>Carbaryl</td>
<td>Y</td>
<td>0.01</td>
<td>Dodeine</td>
<td>N</td>
</tr>
<tr>
<td>Carbofuran</td>
<td>Y</td>
<td>0.00006</td>
<td>Etoxazole</td>
<td>N</td>
</tr>
<tr>
<td>Chlorpyrifos</td>
<td>Y</td>
<td>0.005</td>
<td>Fenamid</td>
<td>N</td>
</tr>
<tr>
<td>Clothianidin</td>
<td>Y</td>
<td>0.025</td>
<td>Fenhexamid</td>
<td>N</td>
</tr>
<tr>
<td>Coumaphos</td>
<td>Y</td>
<td>0.007</td>
<td>Fenoxycarb</td>
<td>N</td>
</tr>
<tr>
<td>Cyphenothrin</td>
<td>Y</td>
<td>0.23*</td>
<td>Fenpyroximate</td>
<td>N</td>
</tr>
<tr>
<td>Deltamethrin</td>
<td>Y</td>
<td>0.01</td>
<td>Flumoxazin</td>
<td>N</td>
</tr>
<tr>
<td>Diazinon</td>
<td>Y</td>
<td>0.0025</td>
<td>Gibberelic Acid</td>
<td>N</td>
</tr>
<tr>
<td>Dicamba</td>
<td>Y</td>
<td>1</td>
<td>Hexasafox</td>
<td>N</td>
</tr>
<tr>
<td>Dichlorvos</td>
<td>Y</td>
<td>0.008</td>
<td>Imazamox</td>
<td>N</td>
</tr>
<tr>
<td>Dimethoate</td>
<td>Y</td>
<td>0.013</td>
<td>Imazapic</td>
<td>N</td>
</tr>
<tr>
<td>Ethionoprop</td>
<td>Y</td>
<td>0.00025</td>
<td>Imazapyr</td>
<td>N</td>
</tr>
<tr>
<td>Etofenprox</td>
<td>Y</td>
<td>0.57*</td>
<td>Imazethapyr</td>
<td>N</td>
</tr>
<tr>
<td>Imidacloprid</td>
<td>Y</td>
<td>0.14</td>
<td>Linuron</td>
<td>N</td>
</tr>
<tr>
<td>Imiprothrin</td>
<td>Y</td>
<td>0.33**</td>
<td>Mefenoxam</td>
<td>N</td>
</tr>
<tr>
<td>Malathion</td>
<td>Y</td>
<td>0.14</td>
<td>Metribuzin</td>
<td>N</td>
</tr>
<tr>
<td>MCPA</td>
<td>Y</td>
<td>0.04</td>
<td>Myclobutanol</td>
<td>N</td>
</tr>
<tr>
<td>MCPP</td>
<td>Y</td>
<td>1.75</td>
<td>Na_o_Phenylphenate</td>
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<tr>
<td>Methamidophos</td>
<td>Y</td>
<td>0.003</td>
<td>Norflurazon</td>
<td>N</td>
</tr>
<tr>
<td>Methidathion</td>
<td>Y</td>
<td>0.002</td>
<td>Norvaluron</td>
<td>N</td>
</tr>
<tr>
<td>Methomyl*</td>
<td>Y</td>
<td>0.05*</td>
<td>Pendimethalin</td>
<td>N</td>
</tr>
<tr>
<td>Methyl Parathion</td>
<td>Y</td>
<td>0.0011</td>
<td>Piperonyl Butoxide</td>
<td>N</td>
</tr>
<tr>
<td>Naled</td>
<td>Y</td>
<td>0.01</td>
<td>Propargite</td>
<td>N</td>
</tr>
<tr>
<td>Oxamyl</td>
<td>Y</td>
<td>0.001</td>
<td>Pyrimethanil</td>
<td>N</td>
</tr>
<tr>
<td>Permethrin</td>
<td>Y</td>
<td>0.25</td>
<td>Pyriproxifen</td>
<td>N</td>
</tr>
<tr>
<td>Phorate</td>
<td>Y</td>
<td>0.0025</td>
<td>Quinclorac</td>
<td>N</td>
</tr>
<tr>
<td>Phosmet</td>
<td>Y</td>
<td>0.045</td>
<td>Quinosylfen</td>
<td>N</td>
</tr>
<tr>
<td>Pirimicarb</td>
<td>Y</td>
<td>0.04*</td>
<td>S-Metolachlor</td>
<td>N</td>
</tr>
<tr>
<td>Propiconazole</td>
<td>Y</td>
<td>0.3</td>
<td>Spinosyn A/D (spinosad)</td>
<td>N</td>
</tr>
<tr>
<td>Propoxur</td>
<td>Y</td>
<td>0.005</td>
<td>Triclopyr</td>
<td>N</td>
</tr>
<tr>
<td>Pyridaben</td>
<td>Y</td>
<td>0.44</td>
<td>Triclosan</td>
<td>N</td>
</tr>
<tr>
<td>S-Bioallethrin**</td>
<td>Y</td>
<td>0.0013***</td>
<td>Trifloxystrobin</td>
<td>N</td>
</tr>
<tr>
<td>Sumithrin</td>
<td>Y</td>
<td>0.03</td>
<td>Tetramethrin</td>
<td>N</td>
</tr>
<tr>
<td>Tebuconazole</td>
<td>Y</td>
<td>0.016#</td>
<td>Triflumizole</td>
<td>Y</td>
</tr>
<tr>
<td>Terbufos</td>
<td>Y</td>
<td>0.0003</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Tetrachlorvinphos</td>
<td>Y</td>
<td>0.067</td>
<td>Triflnizole</td>
<td>Y</td>
</tr>
<tr>
<td>Thiachlorprid</td>
<td>Y</td>
<td>0.01</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>Thiamethoxam</td>
<td>Y</td>
<td>0.35</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>Thiophanate methyl</td>
<td>Y</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triadimefon</td>
<td>Y</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* RfDs marked by asterisks were calculated from NOAELs reported in the available docket information. Standard UFs of 100 were applied in each case.

** RfD was calculated from the reported NOAEL of 10 mg/kg/day, and divided by the UFs of 1000, as reported in the RED

*** RfD was calculated from the reported NOAEL of 1 mg/kg/day and divided by standard UFs of 1000

# RfD was calculated from the reported LOAEL of 16.3 mg/kg/day and divided by standard UFs of 1000

^ The only route of entry with a neurotoxic endpoint was dermal

^^The only route of entry with a neurotoxic endpoint was inhalation

Acute dietary exposure was the route of exposure, unless otherwise indicated.

For pesticides with a Population Adjusted Dose (PAD), the additional Food Quality Protection Act (FQPA) safety factor was multiplied back out for consistency with reported reference doses.

In cases where different doses were given for males and females, the more sensitive population was used.

* RfDs marked by asterisks were calculated from NOAELs reported in the available docket information. Standard UFs of 100 were applied in each case.

** RfD was calculated from the reported NOAEL of 10 mg/kg/day, and divided by the UFs of 1000, as reported in the RED

*** RfD was calculated from the reported NOAEL of 1 mg/kg/day and divided by standard UFs of 1000

^ The only route of entry with a neurotoxic endpoint was dermal

^^The only route of entry with a neurotoxic endpoint was inhalation

### Table 2-1 – Classification of Pesticides Detected in Household Dust

Pesticides were identified as potentially neurotoxic based on available health endpoint data in US EPA Registration Eligibility Decisions (REDs), Human Health Risk Assessments, or other health assessment data available in (re)registration dockets. Table 2-1 identifies which pesticides were identified as potentially neurotoxic and provides a reference dose (RfD) for each.
Figure 2-1 Neurodevelopmental Exposome Heat Map. Using the pesticide categorizations provided in Table 2-1, the concentration of potentially neurotoxic and non-neurotoxic pesticides (in µM/g dust) were summed for each household (row). Figure 2-1 shows how the levels of potentially neurotoxic and non-neurotoxic pesticides changed over the two sampling periods (2005 and 2011). The red coloring indicates pesticide levels greater than the 75th percentile for potentially neurotoxic pesticides in 2005 (17.6µM/g); yellow indicates the 50th percentile (7.03µM/g); and green indicates the 25th percentile (2.16µM/g).
### Table 2-2 Statistical Summary Table for Unweighted Neurodevelopmental Exposome Analysis

**Proportions Tests:** For both neurotoxicity groupings, the proportion of “red” FW households in Figure 2-1 (levels greater than 17.6µg/g) was compared to the proportion of red NFW households for both study years (2005 and 2011). Table 2-2 provides the proportion of FW red households, the proportion of red NFW households, the difference between the two, and the associated p-value. Additionally, the proportion of FW vs. NFW households with decreased pesticide levels was compared for both study years.

**Mixed Effects Tests:** Table 2-2 also provides the mixed effects models used to examine cohort-wide trends in potentially neurotoxic and non-neurotoxic pesticides over time, which models were compared in the analysis, and whether those models were significantly different from one another. * p < 0.05; ** p < 0.01; *** p < 0.001.

<table>
<thead>
<tr>
<th></th>
<th>Potentially Neurotoxic Pesticides</th>
<th>Non-neurotoxic Pesticides</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proportions Tests</strong></td>
<td>FW</td>
<td>NFW</td>
</tr>
<tr>
<td>Proportion Red 2005</td>
<td>0.38</td>
<td>0.11</td>
</tr>
<tr>
<td>Proportion Red 2011</td>
<td>0.15</td>
<td>0.20</td>
</tr>
<tr>
<td>Proportion Decreased (2005-2011)</td>
<td>0.70</td>
<td>0.52</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Potentially Neurotoxic Pesticides (Weighted)</th>
<th>Potentially Neurotoxic Pesticides (Unweighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mixed Effects Tests</strong></td>
<td>FW</td>
<td>NFW</td>
</tr>
<tr>
<td>Null vs. Time Fixed</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
<tr>
<td>Time Fixed vs. Time*Occupation</td>
<td>log(pest) ~tm + (1</td>
<td>house)</td>
</tr>
<tr>
<td>Null vs. Time Fixed w/in Occupation</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
<tr>
<td>FW</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
<tr>
<td>NFW</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
</tbody>
</table>

### Table 2-3 – Statistical Summary Table for Weighted Neurodevelopmental Exposome Analysis

Table 2-3 includes the results of the proportions tests and the mixed effects tests for the unweighted (left, for comparison) and weighted (right) neurodevelopmental exposome analysis. The unweighted analysis is the same as presented above in Table 2-2. The weighted analysis provides the proportion of FW and NFW households that had potentially neurotoxic pesticide levels greater than the 2005 75th percentile, the difference between FWs and NFWs, and the associated p-value. Additionally, the mixed effects analysis shows which models were compared in the weighted analysis, and whether those models were significantly different from one another. * p < 0.05; ** p < 0.01; *** p < 0.001.

<table>
<thead>
<tr>
<th></th>
<th>Potentially Neurotoxic Pesticides (Unweighted)</th>
<th>Potentially Neurotoxic Pesticides (Weighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proportions Tests</strong></td>
<td>FW</td>
<td>NFW</td>
</tr>
<tr>
<td>Proportion Red 2005</td>
<td>0.38</td>
<td>0.11</td>
</tr>
<tr>
<td>Proportion Red 2011</td>
<td>0.15</td>
<td>0.20</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Potentially Neurotoxic Pesticides (Unweighted)</th>
<th>Potentially Neurotoxic Pesticides (Weighted)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mixed Effects Tests</strong></td>
<td>FW</td>
<td>NFW</td>
</tr>
<tr>
<td>Null vs. Time Fixed</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
<tr>
<td>Time Fixed vs. Time*Occupation</td>
<td>log(pest) ~tm + (1</td>
<td>house)</td>
</tr>
<tr>
<td>Null vs. Time Fixed w/in Occupation</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
<tr>
<td>FW</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
<tr>
<td>NFW</td>
<td>log(pest) ~ 1 + (1</td>
<td>house)</td>
</tr>
</tbody>
</table>
Figure 2-2 Proportion of Households with High Pesticide Concentrations, 2005 – 2011. Figure 2-2 shows the proportion of FW and NFW households that had high (>17.6µM/g) potentially neurotoxic (unweighted) (A) or non-neurotoxic (B) pesticide levels in 2005 and 2011. Dark grey bars represent FW households, and light grey bars represent NFW households. * p < 0.05; ** p < 0.01; *** p < 0.001