THE IMPACT OF ENDOCRINE-DISRUPTING PESTICIDES ON
OVERWEIGHT/OBESITY

By

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To Samuel

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| 2,4-D | 2,4-dichlorophenol |
|---|---|
| 3PBA | 3-phenoxybenzoic acid |
| AHS | Agricultural Health Study |
| β-HCH | β-hexachlorocyclohexane |
| BMI | Body mass index |
| CDC | Centers for Disease Control and Prevention |
| CI | Confidence interval |
| DAP | Dialkyl phosphate |
| DDE | Dichlorodiphenyl-dichloroethylene |
| DDT | Dichlorodiphenyltrichloroethane |
| EDC | Endocrine disrupting chemicals |
| HCB | Hexachlorobenzine |
| LOD | Limit of detection |
| NHANES | National Health and Nutrition Examination Survey |
| OC | Organochlorine |
| OP | Organophosphate |
| OR | Odds ratio |
| PIR | Poverty-to-income ratio |
| PXR | Pregnane X receptor |
| TNFα | Tumor necrosis factor α |
| WHO | World Health Organization |

Abstract of Dissertation Presented to the Graduate School
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Obesity is an increasing epidemic throughout the world and has been proven to
be an important risk factor for a number of diseases including diabetes, cardiovascular
disease, and cancer. Though genetic and behavioral factors, such as lack of physical
activity and poor diet, are traditionally accepted as factors driving this epidemic, these
alone do not fully explain trends observed worldwide. Therefore, in recent years,
scientists have looked elsewhere for explanations and found that certain chemical and
environmental factors, collectively called obesogens, can disrupt the endocrine system
and affect weight gain. Several pesticides have been found to be endocrine disrupting
and have been thought of as putative obesogens. However, much is still unknown about
the role of pesticides as obesogens. The overall goal of this dissertation was to provide
longitudinal evidence on the impact of pesticides on weight, as well as to compare
pesticide levels and predictors between agricultural and non-agricultural groups. The
following specific aims were explored: 1. To evaluate the impact of endocrine disrupting
pesticides on BMI in the Agricultural Health Study. 2. To assess the exposure trends
between pesticide exposure and BMI outcome among farmers and commercial
applicators. 3. To investigate the differential influence of dietary and non-dietary factors

on biomarkers of endocrine-disrupting pesticides between agricultural workers and the general population.

To explore aims 1 and 2, I used data from the Agricultural Health Study, a longitudinal project focusing on agricultural populations in North Carolina and Iowa with over 89,000 participants. I used generalized linear mixed models to test the overall relationship between groups of endocrine-disrupting pesticides and overweight/obesity, as well as the impact of gender on this association. I further investigated whether this association increased with increasing exposure, and whether exposure to other groups of pesticides modified this association. For aim 3, I used data from the National Health and Nutrition Examination Survey, a cross-sectional study conducted in the general population by the Centers for Disease Control and Prevention with approximately 5000 individuals surveyed per year. With this dataset, I investigated whether agricultural work, dietary factors, and non-dietary factors impacted body burdens of endocrine-disrupting pesticides.

CHAPTER 1
INTRODUCTION

**Overview**

Obesity, defined as having a body mass index (BMI) greater than 30 kg/m$^2$, is an important risk factor for a number of diseases including diabetes, cardiovascular disease, and cancer.[1] It is estimated that over 250 million people live with diabetes[2] and upwards of 100 million have cardiovascular disease.[3] The World Health Organization (WHO) reports that overweight/obesity accounts for 35.8 million disability-adjusted life years.[4] In 1990, obesity was estimated to cost almost $70 billion in direct and indirect costs in the United States.[5] This figure is likely much higher today.

Currently, 68% of Americans are at least overweight, which is defined as having BMI greater than 25 kg/m$^2$, and 34% are considered to be obese.[6] The rates of obesity in the United States from 1995 until 2010 have changed dramatically. Data from the Behavioral Risk Factor Surveillance System show that in 1995, rates of obesity in United States adults were approximately 16%. In 2010, this number increased to over 27%.[7] If this trend continues, these numbers are expected to grow exponentially over the next few years. Researchers project that by 2020, the number people who are at least overweight will grow to 86% in the United States.[6] Worldwide rates are also showing these patterns. In 2005, it was estimated that approximately 23% of the world population was overweight, and almost 10% was considered obese. By 2030 it is projected that over 2 billion people will be overweight and over 1 billion will be obese if secular trends continue as they are.[8]

The rates among children are similarly worrisome. 17% children in the United States are considered obese.[9] Worldwide, 43 million children are overweight or obese,

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with an additional 92 million being at risk of becoming overweight.[10] In the United States, childhood obesity trends seem to be leveling off. The prevalence of infants and adolescents who are overweight or obese has not significantly changed from 1999 until 2008.[9] However, their worldwide rates are forecasted to increase by 36%.[10] Overweight/obese children are more likely to develop heart disease and diabetes later in life than normal weight children.[11] In many cases, obesity during childhood carries on to adulthood. Because of this, the life expectancy of children born between 1990 and the early 2000s will be the first to be shorter than their parents.[12]

Animals are also showing consistent increases in weight. A study done on tens of thousands animals of 8 species with data ranging from the late 1970s until 2005 showed that there was an increasing trend in body weight. This finding was consistent among all animals from various habitats, including research laboratories and domestic homes.[13]

Genetic factors are thought to play a significant role in the increasing trend among both humans and animals;[7,13] however, the rapid increase in obesity prevalence makes a purely genetic explanation unlikely. A more plausible explanation is due to an increase in calorie consumption and a decrease in physical activity.[1] Although this is widely accepted, it does not fully explain why this trend is seen in both children and adults worldwide and among animals,[13] and why there are such marked differences between individuals in amount of physical activity and weight loss.[6] Another explanation that has been proposed is that environmental factors, collectively called obesogens, can be triggers for weight gain.[6]

**Endocrine Disruptors and Obesity**

Endocrine disrupting chemicals (EDCs) are thought to be potentially obesogenic.[14] Since the discovery of leptin as an adipose-derived hormone,[15] fat cells

have been thought of as endocrine organs that release appetite and metabolism hormones.[15] Besides adipose hormones, other hormones have been found to affect fat cells. Studies show that receptors for progesterone, estrogen, and androgen exist in adipose tissues.[16] The exact mechanism by which this happens is still unclear, and appears to be complex and multifaceted. Illustrating this complexity, studies have found that estrogen and anti-androgen hormones administered to transsexual individuals increased their leptin levels, whereas testosterone decreased it.[17] In contrast, some researchers could not find an effect of estrogen and androgens in in vitro studies.[18] To further complicate matters, certain cases studies found it difficult to explore clear relationships between specific hormones and adipose tissue because of the aromatization of androgen to estrogen.[16] Additionally, studies have found that some EDCs can affect the number or size of fat cells, while others can interact with metabolism or appetite hormones.[19,20] Given this evidence, it follows that EDCs may affect weight gain by interacting with hormones and interfering with their normal function. Scientists have found evidence of several pesticides displaying endocrine-disrupting properties[21,22] and several studies have investigated their role on weight.

**Endocrine-Disrupting Pesticides**

Historically, organochlorine (OC) pesticides were among the first to have their endocrine disrupting properties identified.[23] This evidence, along with new evidence of their toxicity and persistence, resulted in their gradual ban beginning with dichlorodiphenyltrichloroethane (DDT) in the 1970s and the latest withdrawals occurring in the 2000s; though a few, such as lindane, are still allowed for restricted uses.[23] Other classes of pesticides, such as organophosphates and pyrethroids, have replaced OC pesticides because of their lower toxicities and for being less persistent in the

environment. However, many of these are now also being suspected of having endocrine-disrupting properties.[24]

**Evidence for the Role of Pesticides on Weight**

**Animal Evidence**

A few animal studies have attempted to look at the effect of pesticides on weight gain. A study done on Baltic Sea herring found that the condition factor, which is a marker for the stoutness of a fish, was positively associated with organochlorine pesticide concentrations.[25] However, this study could not ensure that fish that had more lipid content had so because of OC pesticides, or if they had greater levels of OC pesticides because they had more lipid content, and thus more fat. Primate research done on Rhesus monkeys found that those exposed to a single acute dose of DDT had increased fatty acid creation and triglycerides synthesis in the liver as well as increased triglycerides and cholesterol in both serum and adipose.[26] Studies that exposed rats to OC pesticide levels similar to those found in the food chain in vivo resulted in an inhibited uptake of insulin-dependent glucose.[27] Mice that were exposed to DDT and Lindane prenatally showed that after birth they had higher body weights than the control mice.[28,29]

Even fewer studies have looked at organophosphate (OP) pesticides and obesity or weight gain. Mice fed low doses of chlorpyrifos, diazinon, or parathion, all which are OP pesticides, neonatally and prenatally gained more weight in a high fat diet when compared to control mice in the same diet.[30,31] Rats exposed to parathion neonatally had disrupted lipid metabolism in adulthood, with suppressed adiponectin levels (a marker of prediabetes) and increased TNFα in white fat pads, which is also elevated in association with obesity and diabetes. Additionally, the usual associations between

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leptin levels and body weight were not seen in animals exposed to parathion, which indicates that this exposure is upsetting normal lipid metabolism later in life.[32] Several other pesticides were identified as associated with increases in body weight in the unpublished literature, which included malathion, dimethoate, and dichlorvos among others.[33] Other animal studies failed to find associations.[34,35]

**Human Evidence**

**Prenatal and postnatal exposure**

There have been several studies done on the effects of OC pesticides on weight gain in humans, which is a class of pesticides that have been largely banned in the United States since as early as the 1970s.[23] Several of these studies looked at prenatal exposure to these chemicals and weight later in life, but results are largely inconsistent. Two similar studies have found contrasting results, one reports an association between higher prenatal dichlorodiphenyl-dichloroethylene (DDE) exposure in adolescent boys only, but not girls,[36] but another finds no association at all in male adolescents.[37] Contrastingly, another study restricted to adult women only found daughters with DDE higher concentrations had higher BMI 20-30 years later.[38] Other studies focus on shorter time spans, and examine the effects of prenatal exposure on childhood weight. These have fairly consistent findings in that prenatal exposure to an organochlorine pesticide was significantly associated with overweight approximately six years after birth.[39,40] In one postnatal study, exposure to DDE through maternal milk consumption did not show to be associated with body weight in either adolescent boys or girls.[36] A cross-sectional study done on adolescents found no association for current exposure and BMI.[41] Only one study was done in an occupational population and with a large variety of pesticides. This study found that children born to women who worked in greenhouses and reported

use of a wide variety of pesticides had increased in BMI from birth to school age at a greater rate, had higher body fat, and higher skin fold measurements than those who were unexposed.[42] However, because more than 120 pesticides were investigated, the authors could not attribute results to any one pesticide in particular.[42] Collectively, these studies show that although there is evidence of some relationship between prenatal and perinatal exposure to pesticides and obesity, associations are unclear and seem to be modified by sex and age.

   **Adult exposure.** A few studies have investigated the relationship between weight and pesticides in adults. The primary issue with studies on adult exposure is that differences can exist in pesticide elimination between normal weight individuals and those with higher BMI making the association due to those with lower BMI simply having excreted more pesticides, thus leading to their lower serum levels.[43] This is especially true for cross-sectional studies of OC pesticides, which are stored in fat.[23] One such study looked at the relationship between levels of DDT, DDE, β-hexachlorocyclohexane (β-HCH), and HCB and BMI in healthy Spanish adults and found that all were correlated with BMI and age.[43] Cross-sectional analysis of a prospective study done on Swedish seniors that collected OC pesticide measurements at baseline that men with higher levels of DDE, trans-nonachlor, hexachlorobenzine (HCB) had 2 to 3 times higher odds of abdominal fat than those with the lowest levels of exposure.[44] Additionally, two cross-sectional studies have looked at other metabolic syndrome outcomes related to obesity in the general population. Results indicate that the odds of having insulin resistance and type 2 diabetes, of which obesity is a predictor, are higher among those with increased levels of OCs than those in the lower category.[45,46] Though compelling, results from

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these studies are unable to establish a temporal association due to the simultaneous measurement of both exposure and outcome.

Prospective studies have shown some evidence of the impact of pesticides on weight. A longitudinal study on Swedish seniors showed weak associations with organochlorine pesticides, and these were seen more clearly among women than men.[44] A study done by Lee and colleagues found that DDE predicted higher BMI 18 years later in a population of white and African American men and women from Birmingham, Chicago, Minneapolis, and Oakland.[47] These studies provide good prospective evidence that there may be a causal association between pesticides and obesity. In these instances, pesticide levels were measured prior to BMI measurement, eliminating the temporality issue. However, both have some limitations in that they focus only on organochlorine pesticides and have narrow populations. The Swedish senior study focuses on elderly individuals (ages 70 at baseline) only, and the second study uses only 90 individuals from a much larger cohort of 5000 participants.[44]

### Issues of Interest

**Lack of Data**

Most of the existing human evidence focuses on OC pesticides only. The lack of studies on other pesticide classes in the published literature implies that either these have not been well explored or that null effects are causing them to not get published or pursued further. Similarly, there is very limited prospective evidence on the effect of pesticides on weight. This is a particularly important issue given that these studies focus on OC pesticides. OC pesticides are lipophilic, and thus are stored in fat.[23] Those who have lower BMI may have lower levels of lipophilic chemicals due to the fact that they do not have as much fat storage, and may excrete these chemicals more.[43] Prospective

studies remedy this by measuring exposure prior to any gain in weight occurs, ensuring that a temporal association is established. To date there are only two studies that investigate the effect of pesticides on weight longitudinally. Both, however, focus on only OC pesticides which have been banned in the United States starting in 1972. Both studies also focus on a small population, Swedish seniors and a small subset from a non-representative population of white and African American young men and women from four cities in the U.S. This proposal plans to bridge this gap by focusing on prospective data of several classes of pesticides with evidence of endocrine disruption, as well as utilizing cross-sectional data from a nationally representative sample.

**Effect Modification**

The workshop on the "Role of Environmental Chemicals in the Development of Diabetes and Obesity" organized by the National Institute of Environmental Health Sciences' Division of the National Toxicology Program in 2011 recommended that further studies elucidate the role of effect modifiers and confounders on the association between environmental chemicals and obesity. Among the covariates mentioned were demographic variables such as sex.[33]

Some studies, especially animal studies, have found pesticides affect males and females differently. For instance, in rats postnatally exposed to chlorpyrifos, only males had elevated levels of plasma cholesterol and triglycerides, and males and females displayed different neurological outcomes. In humans, the relationship is not quite clear. One study among children prenatally exposed to DDE found boys, but not girls, had higher body weights in adolescence.[36] Yet another study found a baseline relationship between OC pesticides and BMI in men only, and a relationship between baseline measures and those five years later among women only.[44] Therefore, evidence does

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indicate that sex may modify the effect between pesticide exposure and obesity outcomes, and also that relationships vary by chemical. This study directly addresses the issue of whether sex modifies the relationship between pesticides and weight by statistically testing for an interaction and comparing results obtained for males and females.

**Interaction between Chemicals**

As previously mentioned, the exact mechanism by which hormones affect weight gain is complex and still under investigation. Leptin has been found to regulate appetite and energy storage and expenditure, and is known to be directly implicated in weight gain.[48] The sex hormones estrogen and androgen have both been found to affect leptin levels. [16-18] Additionally, leptin is thought to be a link in the association between thyroid hormone level changes and weight gain.[49] Endocrine disrupting pesticides can have a variety of effects on hormones[22]; however, there have been no studies that investigate the effect of exposure to more than one pesticide with similar endocrine disruption profiles. Additionally, no studies have investigated whether being exposed to pesticides with different endocrine disrupting profiles modifies its effect on weight. For instance, we do not know if exposure to two pesticides that are considered to be estrogenic affects weight gain differently than exposure to one. Similarly, there is no evidence of how simultaneous exposure to a pesticide with anti-estrogenic properties and one with estrogenic properties affects weight gain in an individual.

**Comparison across Populations**

There are little data regarding differences in pesticide levels between agricultural populations and the general population. Most of the studies that investigate pesticide exposure and obesity have focused on individuals in the general population who are

likely exposed to chronic low levels of pesticides.[16,36-41,43,44,46-48] Compared to the general population, agricultural populations have additional exposures to pesticides, which may lead to different body burdens of pesticides. However, there is no evidence to support this claim, and no evidence as to whether predictors of exposure differ between the two groups. Agricultural populations are thought to be at special risk for the endocrine-disrupting effects of pesticides due to their direct use.[22] In fact, the majority of studies investigating the health effects of pesticide use are in occupational populations.[50] However, the general population is also at risk for exposure through residential use as well as pesticide contamination of air, water, and soil.[51] Without this information, we cannot say that results found in the general population apply to agricultural populations, or vice-versa.

**Significance**

To our knowledge, this study is the first to prospectively investigate the effects of a wide range of endocrine-disrupting pesticides and weight gain in an occupational population. Though other studies have utilized prospective data, none have included organochlorine pesticides. This work is of public health importance as the prevalence of obesity is growing ever larger in the United States and abroad. Pesticides could be an important risk factor since exposure is wide and varied. Though some of these chemicals, such as many in the organochlorine group, have been banned starting in 1972  in the United States, they are still used in some other parts of the world.[23] In the United States, pesticides are found in several foods items, such as fruit, baked goods, and dairy as well as herbal supplements imported from other countries.[52-55] Organophosphates are still widely in use for agricultural and commercial purposes. For instance, approximately 10 million pounds of the OP pesticide chlorpyrifos is estimated

to be applied in the United States annually.[56] Additionally, pyrethroid pesticides are some the most widely used pesticides in the United States.[57]

## Specific Aims of Dissertation

The primary goal of this dissertation is to evaluate the longitudinal influence of endocrine disrupting pesticides on body weight. The secondary goal is to compare pesticide levels between agricultural and non-agricultural groups, as well as investigate predictors of exposure. In this dissertation, I used the Agricultural Health Study (AHS) and the National Health and Nutrition Examination Survey (NHANES) datasets using mixed models to explore longitudinal relationships and longitudinal models to compare populations.

I accomplished this via three specific aims. Aim 1 was to evaluate the impact of endocrine disrupting pesticides on BMI within the AHS cohort. Our primary objective in Aim 1 was to investigate this relationship in the entire AHS cohort (i.e. applicators and spouses). Our secondary objective was to then look at whether gender affects this relationship, and if so, whether menopause was an effect modifier for women.

Aim 2 sought to assess the exposure trends in the relationship between pesticide exposure and BMI outcome among farmers and commercial applicators. This aim had three objectives. First we investigated whether individuals with greater intensity-adjusted cumulative exposure scores (as defined by amount of pesticide and length of time used, as well as method of use and use of personal protective equipment) will have greater BMI than those with lower scores. We then investigated the whether exposure to other pesticides affected this association. Lastly, we compared whether this association would hold for number of pesticides, a less strict definition of pesticide exposure.

Finally, Aim 3 was to investigate whether being an agricultural worker was a significant predictor of body burden of pesticides, and how dietary and non-dietary factors were associated with biomarkers of endocrine-disrupting pesticides. We first identified whether levels of pesticides with evidence of endocrine disruption were higher among agricultural workers compared to the general population. We then evaluated the impact of agricultural work, dietary, and non-dietary among a sample representative of the United States population.

CHAPTER 2
# THE IMPACT OF GROUPS OF ENDOCRINE-DISRUPTING PESTICIDES ON BODY MASS INDEX

**Background**

Obesity is a chronic public health problem that is known to be associated with an increased risk of diseases such as diabetes, cardiovascular disease, and cancer.[1] In the United States, 68% of adults are considered at least overweight with a body mass index (BMI) greater than 25 kg/m$^2$, and 34% are considered obese with a BMI greater than 30 kg/m$^2$.[6] The obesity epidemic has been increasing for the past 30 years, doubling during that period.[58] Until recently, the most accepted reason for this increasing trend was insufficient exercise and poor diet.[1] However, the scientific community is beginning to believe that these factors may not be the only explanations why the increasing rates of obesity are seen among both adults and children worldwide.[13] Thus, environmental factors, such as endocrine disruptors, which may alter human metabolic processes, have emerged as potentially affecting weight.[6]

Many pesticides have been found to display endocrine disrupting properties[22] that are thought to affect weight. Studies investigating this association have generally focused on prenatal exposure to pesticides of the organochlorine variety.[36-40] A few studies have looked at adult exposure and obesity. Again, these have focused on organochlorine pesticides, and with few exceptions are cross-sectional in nature.[43,46,59] Results from these studies are inconsistent. Since weight gain is a gradual process, cross-sectional studies are ineffective in finding casual associations because they cannot ensure that pesticide exposure preceded the weight gain.

A few longitudinal studies have been conducted on organochlorine pesticides and these show positive associations with obesity later in life.[44,47] However, these studies

focus on only one type of pesticide, which has been banned in the US for some time,[23] and do not include individuals who are most at risk for exposure: pesticide applicators. Additionally, one study finds different associations between men and women, suggesting that gender may be an effect modifier.[44] The goal of this study is to investigate the impact of several different endocrine disrupting pesticides on BMI within the Agricultural Health Study (AHS), a cohort of pesticide applicators and their spouses. Additionally, we explored this association within different genders.

## Methods

### Study sample

Data came from the AHS, a large, prospective cohort of 89,655 commercial and private pesticide applicators and their spouses in Iowa and North Carolina recruited between 1993 and 1997.[60] Participants completed enrollment questionnaires and were given more detailed take-home questionnaires (Phase 1).[61] Over 80% of eligible applicators enrolled in the study (n=57,311). Of those enrolled, 25,291 returned the take-home questionnaire (44%). Spouses were only given the opportunity to complete the take-home portion,[60] and 32,345 spouses returned this questionnaire, equaling to approximately 75% of eligible married spouses. Applicators and their spouses were then followed up via telephone interviews for the first time beginning in 1999 and ending in 2005 (Phase 2, n=60,138), and for a second time beginning in 2005 and ending in 2010 (Phase 3, n=44,130).[61] Additionally, during Phase 2, participants were given a dietary questionnaire, which was completed by approximately 40% of the sample.[61] In Phase 3, commercial applicators were not interviewed.

We only included individuals who at Phase 1 did not have missing information on BMI, pesticide exposure, and the following covariates: age, gender, marital status, race,

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education, vital status, exercise, smoking status, kidney conditions, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat consumed. Our final sample consisted of 20,410 individuals, or 22.8% of the individuals who enrolled, 36.4% of the 56,030 individuals who completed the take home questionnaire, and 58.0% of the 35,164 individuals who completed the dietary questionnaire. Out of these, 48 had information for Phase 1 only, 5734 had information for Phases 1 and 2, 1 had information for Phases 1 and 3, and 14,627 had information for all phases.

**Variable description**

BMI was calculated from height information from the enrollment questionnaire and weight at each of the three time phases. For participants with missing height or weight data, we used BMI information that was calculated by AHS investigators for each phase of the study. We did not use this previously calculated variable for the entire study sample because it was not available for over 20,000 participants in Phase 1, for all participants in Phase 2, and 1,900 individuals in Phase 3. For individuals with BMI lower than 15 and greater than 70 at any of the three time points, we performed a visual check of the baseline and follow up weight data to see whether values were consistent across time and therefore plausible. For instance, if an individual had a reported weight of less than 80 pounds at Phase 1 and over 150 pounds in subsequent phases, we deemed that we could not verify whether this first value was correct and the participant was excluded. Similarly, if an individual had an implausible BMI (e.g. BMI <1), they were also excluded from analysis. We excluded 22 individuals from Phase 1, 16 individuals in Phase 2, and 3 individuals in Phase 3 because we could not ascertain the accuracy of their weight data. For all analyses, BMI was then separated into two categories: normal/underweight (BMI <25) and overweight/obese (BMI ≥25).

Pesticide information came from the following questions: "Have you ever personally mixed or applied [pesticide name]?" We selected the following pesticides that had evidence of endocrine disruption: 2.4-D, alachlor, aldicarb, aldrin, atrazine, benomyl, captan, carbaryl, chlordane, chlorothalonil, chlorpyrifos, DDT, diazinon, dieldrin, glyphosate, heptachlor, lindane, malathion, metribuzin, parathion, permethrin, toxaphene, trichlorfon, and trifluralin. Using the available knowledge on the mode of action of these individual pesticides, we separated pesticides into the following groups: estrogenic, anti-estrogen, anti-androgen, thyroid disruptor, PXR agonist, and other.[21,22] Table 2-1 lists the individual pesticides and their groupings. Each individual was categorized as ever used and never used for each pesticide and each group of pesticides. To be considered as having ever used a particular group of pesticides, an individual had to have responded to using at least one pesticide in that group. Individuals who reported never using any of the pesticides in a particular group were considered as never users. Individuals were not required to have complete information on all pesticides in a group to be considered having ever used or never used. In other words, only individuals with missing information on all pesticides within a particular category were assigned as having missing information for that category. Participants that had information on some pesticides, but not all, were categorized according to their available answers.

Potential confounders considered were based on associations between BMI and pesticides in the literature and included: age,[1] gender,[1] marital status,[62] race,[1] education,[63] vital status, doctor visit in the previous 12 months,[64] exercise,[1] smoking status,[65] chronic medical conditions (kidney,[66] thyroid,[67] respiratory,[68] diabetes,[1,59] cardiovascular disease,[1] and cancer[1]), and diet[1] (calories, total fat, saturated fat, and

cholesterol). Using medical history information asked in each of the three phases, we

created the following groups of chronic medical conditions: kidney (chronic kidney

infection/pyelonephritis, kidney disease, kidney failure, kidney stones, other kidney

disease), thyroid (thyrotoxicosis, Grave's disease, goiter, other thyroid disease),

respiratory (emphysema, farmer's lung disease, chronic bronchitis, other chronic lung

disease), and cardiovascular (heart disease, angina, high blood pressure, myocardial

infarction, arrhythmia, stroke). Similar to our coding of pesticides, individuals who

reported at least one of the conditions in each group were considered as having the

condition. Participants coded as not having the condition had to report not having

received a diagnosis for all of the individual diseases. For a condition to be coded as

missing, participants had to have missing information for all individual diseases within a

category.

Diet and exercise were also potential covariates created from the dietary

questionnaire given in Phase 2. Using Diet*Calc version 1.4.3 (National Cancer Institute,

Applied Research Program), we were able to obtain estimates for each individual's

calorie, total fat, saturated fat, and cholesterol intake. Diet, as well as cancer, data were

only present for one phase; therefore, we imputed this single value for all phases.

To adjust for exercise, we utilized the following question regarding an individual's

habits asked during Phases 1: "On average, how many hours per week do you spend

doing strenuous exercise (heart beat rapidly) during your leisure time?" In the third

phase, questions were asked regarding how many days per week an individual

exercised vigorously and how much time was spent per day. We combined these

variables to create a third variable describing how many hours per week were spent

exercising vigorously. For both phases 1 and 3, we re-categorized the variables into one

hour per week or less, or more than one hour per week.[69] Data for exercise was not

present in Phase 2, and Proc GLIMMIX, the procedure used for all models, deletes

observations with missing information. Therefore, we imputed Phase 1's exercise

information. By imputing exercise information from this Phase 3 into Phase 2, we would

be deleting all commercial applicators from this timepoint, as they were not sampled in

Phase 3. Results were similar when imputing and not imputing exercise (data not

shown).

**Statistical Analyses**

　　All statistical analyses were done using SAS version 9.3 (SAS Institute, Cary,

NC). We calculated frequencies for all potential covariates for each BMI group. For the

main analysis, we created a generalized linear mixed model with a binary distribution

and a random statement to account for the repeated nature of the data. We chose to

conduct a maximum likelihood estimation based on adaptive quadrature due to its

reduced potential for giving a biased estimate.[70] Because measures of BMI are more

likely to be similar between Phases 1 and 2 and Phases 2 and 3 than Phases 1 and 3,

we defined the covariance structure as a first-order autoregressive. First, we utilized all

potential covariates and then removed them one by one to assess their impact on the

model estimates. We left the following basic demographic covariates in the model

regardless of their impact: age, gender, race, marital status, and education. Additionally,

we did not remove the following variables that are known to be associated with BMI:

vigorous exercise, diet, thyroid conditions, and diabetes. Out of the dietary variables, we

chose total fat to be included in the model as it provided the smallest gradient value, and

thus, most stable estimate. The other dietary variables, calories, saturated fat, and

cholesterol produced similar estimates (data not shown). The final model was adjusted

for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.

These covariates were used in models for each of the pesticide groups. We then explored the possibility of gender as an effect modifier for each pesticide grouping. If an interaction was significant, we further examined this relationship by conducting a third analysis on females only and included the variable menopause as an effect modifier.

**Results**

In our sample, there were a total of 20,410 individuals. Of these, 63.2% (n=12,897) were overweight/obese and 36.8% (n=7513) were underweight/normal. Those who were overweight/obese at baseline responded more frequently to using all groups of pesticides (estrogenic: 57.1% vs 47.4%, anti-estrogenic: 68.3% vs 56.3%, anti-androgen: 58.4% vs 37.7%, thyroid disruptor: 57.5% vs 42.4%, PXR agonist: 61.0% vs 42.9%) than individuals who were underweight/normal (Table 2-2). Overweight/obese individuals were older with a mean age of 49.4 years old compared to underweight/normal individuals with a mean age of 46.6 years old. They also responded less frequently to exercising vigorously for more than one hour per week (58.3% vs 65.0%). Overweight/obese individuals more frequently responded to having received a diagnosis in all categories of co-morbidities, with the exception of thyroid conditions. Those who were overweight/obese during Phase 1 also reported higher mean calorie, total fat, saturated fat, and cholesterol intakes.

Figure 2-1 shows the average BMI in each phase by pesticide group for individuals in the study. Average BMI was higher in all three phases for those individuals who reported using pesticides in all categories when compared to those who did not report using pesticides. BMI changed more rapidly between the first and second phase

than between the second and third phase, though this was the same for both users and non-users. In the group "Other" average BMI was only slightly higher for users than non-users in the first phase, and this gap widened in Phases 2 and 3, but was still smaller than the difference for other pesticide groups.

Figure 2-2 shows the trajectory of average BMI across the phases by use of pesticide separately for men and women. Though males of each pesticide category have higher BMI than females across all phases, trajectory patterns are more similar for male users and non-users than for female users and non-users within each pesticide group; though estimates are more similar for females. The pesticide group "Other", again, shows a different pattern than other groups. Females who reported using pesticides in the "Other" category had lower average BMI than those who did not report use of pesticides in this category for phases 1 and 2, but this relationship reversed in Phase 3.

A statistically significant gender interaction was present for only two pesticide groups: estrogenic and PXR agonist (Table 2-3). For estrogenic pesticides, male users were statistically significantly less likely to be overweight/obese than non-users (OR: 0.84, 95% CI: 0.77, 0.92). Female users, however, were more likely to be overweight/obese than non-users (OR: 1.15, 95% CI: 1.07, 1.24). In the PXR agonist category, both male and female users were more likely to be overweight/obese, but male users had significantly higher odds than female users (OR: 1.35, 95% CI: 1.20, 1.52 vs OR: 1.12, 95% CI: 1.02, 1.22, respectively). We further investigated the role menopause plays for these two pesticide groupings; however, results show menopause was not a statistically significant effect modifier (data not shown).

Table 2-4 summarizes the relationship between pesticide use and BMI. Users of anti-estrogenic (OR: 1.08, 95% CI: 1.01, 1.15), anti-androgen (OR: 1.28, 95% CI: 1.18,

1.38), thyroid disruptors (OR: 1.13, 95% CI: 1.06, 1.20), and PXR agonist (OR: 1.20, 95% CI: 1.11, 1.28) were statistically significantly more likely than non-users to be overweight/obese.

## Discussion

We investigated the impact of endocrine-disrupting pesticide use on overweight and obese people in an occupational population. In our sample, 63.2% of individuals had BMIs over 25 at baseline, making them at least overweight. This figure is somewhat smaller than the current 2012 estimate of 69.5%.[71] However, comparing it to the 1994 general population estimate of 59.1% of individuals ages 40-49, the mean age of our sample, our sample had a slightly larger prevalence of people that were overweight.[72]

Overall, our results indicate that four out of the five categories of pesticides were positively associated with weight gain. Some cross-sectional studies looking at the impact of pesticides on BMI have had similar results.[43,44] Additionally, some studies have found greater odds of insulin resistance and type 2 diabetes in those with increased levels of certain pesticides.[45-47]

We found that for males, estrogenic pesticides were protective of overweight/obesity, and for females estrogenic pesticide users were more likely to be overweight/obese. The exact mechanism by which estrogen affects weight is not yet elucidated and is believed to be complex and multi-faceted.[73] Estrogen receptors are present in adipose tissue. Previous studies have found that chemicals with estrogenic properties could lead to a stimulation of mice cells into adipocytes.[73] It has also been theorized that estrogenic chemicals could disturb the energy metabolism pathways mediated by estrogen hormones and that, in turn, could lead to the development of obesity.[74] These theories, however, do not explain why in our study we saw the opposite

effects for males and females. One other study has seen similar patterns with DDT, an estrogenic pesticide, which was associated with a decrease in waist circumference in males but an increase in females.[75] Though estrogen has been known to affect adipose tissue in males in the same manner it affects females,[76] some researchers have proposed that perhaps in men there is a more complex relationship involving the conversion of androgens to estrogens and the resulting imbalance between the two hormones.[77,78]

A review by Chen and colleagues[74] describe the biological pathway for anti-estrogenic compounds as being the same as for estrogenic pesticides. In other words, anti-estrogenic compounds may affect weight by disrupting the glycolytic pathway and subsequently affecting energy metabolism and weight gain. Our results did not show a difference between males and females, which may indicate that the mechanism of action is not exactly the same as in estrogenic compounds.

We found that pesticides in the anti-androgenic group had the largest effect size compared to the other pesticide groups. This is in accordance with one cross-sectional study that reported an association between anti-androgenic compounds and abdominal obesity.[79] The existence of such a relationship is not biologically implausible: androgens have been found to be associated with increased muscle size and fat-free mass, as well as decreased fat mass.[80] Clinical trials have concluded that androgen therapy in healthy men resulted in decreased leptin levels[81] and visceral fat[80]. In women, however, the relationship is not as clear. Hormone therapy studies on post-menopausal women found that the inclusion of the androgen testosterone to the regimen negated the loss of fat associated with the administration of estrogen alone.[82] Several studies have been done on the administration of anti-androgenic oral contraceptives as therapy for

hyperandrogenicity in obese and non-obese women with polycystic ovarian syndrome. Though most have found a decrease in visceral fat and overall body fat distribution, one found that a decrease in androgen increased abdominal fat, which may be attributed to a simultaneous decrease in estrogen secretion.[82] In our study, we found no gender differences in the effect of anti-androgenic pesticides on BMI, which may indicate an interaction with other pesticide groups or the possibility of pesticides having additional effect on the endocrine system. One pesticide in this category, dieldrin, has been found to have estrogenic properties in addition to being an androgen antagonist.[83]

In our study, pesticides that were classified as thyroid disruptors were associated with an increase in the odds of obesity. Some theories support this association. Thyroid hormones are important in maintaining basal metabolic rate; however, the exact pathways are not clear.[49] Many studies of overweight and obese individuals have demonstrated that their thyroid levels are elevated in comparison to those with normal weight;[49] though, it is not known whether this is a cause or result of obesity. However, some animal studies have shown that thyroid hormones may be involved in the metabolism of the hormone leptin,[49] which affects appetite and energy storage.[48] In this sense, thyroid disruptors could alter this pathway, leading to weight gain.

It is thought that PXR, a nuclear receptor, affects the regulation of enzymes responsible for detoxification of xenobiotics.[84] Additionally, its signaling pathways can alter the presence of thyroid, estrogen, and androgen hormones.[21] Given the complex relationship between these hormones and the onset of obesity, pesticides that are thought to be PXR agonists have the potential to affect weight gain through similar pathways as pesticides in other categories without direct interaction with their receptors. Another method in which PXR agonists is thought to affect weight gain is through the

receptor's presence in the liver, one of the main organs for lipogenesis, lipid secretion, and fatty acid catabolism. Disruptions in lipid homeostasis have been associated with obesity and other weight-related conditions.[85]

Lastly, there was only one pesticide in the "other" category, chlorothalonil. It was put into its own category due to the lack of data on its exact endocrine-disrupting property. In one publication it is listed as an activator of the proliferation of androgen-sensitive cells.[86] Elsewhere, it is listed as unlikely to have any direct endocrine disruptive properties.[87] Studies on the mode of action of chlorothalonil show that in vitro assays are not entirely appropriate for studying chlorothalonil as some abnormal results can be attributed to its high cytotoxicity in the absence of other metabolic processes.[83] Therefore, accurately investigating its effects on the body is a difficult task. According to our results, this pesticide does not appear to affect BMI or have any significant gender interactions, which is in agreement with its questionable status as an endocrine disruptor.

Our study has several strengths. Firstly, we have a large sample, which means that even with a large number of missing data points, any non-significant findings are more likely to actually be insignificant and less likely because our study was underpowered to find differences. Secondly, the fact that our occupational population was sampled while getting licenses for pesticide use indicates that any exposure is unlikely to be misstated. In other words, those who use a pesticide would more accurately describe their exposure as opposed to someone in the general population who may not know whether or how their exposure occurred. Lastly, our exposure was whether an individual ever used pesticides, which was asked at enrollment during the license renewal process. Our outcome was BMI calculated at enrollment and in

subsequent phases. This ensured that the exposure preceded the outcome, and we are not subject to the problem of recall bias; though our Phase 1 measure of BMI, as well as pesticide exposure, are cumulative until the point of enrollment.

This study also has several limitations. Some questions, in our case diet, exercise, and cancer, were not present in all phases. Dietary exposures were only established in Phase 2; therefore, we had to make the assumption that this was the same for all phases. A previous national study on the stability of dietary patterns has shown that this assumption is valid, as changes in diet were modest for most nutrients over an 8-year period.[88] For exercise we only had information for Phases 1 and 3. We imputed data from Phase 1 into Phase 2 so as to not have the entire timepoint automatically deleted from our analysis. Though it may be incorrect to assume that exercise did not change from Phase 1 to Phase 2, it was the decision that yielded the least number of deleted observations. Additionally, we found no difference in overall results when imputing Phase 3's exercise data into Phase 2. Information on cancer status was only available for the first timepoint; therefore, we had to utilize this single instance to adjust for this variable.

We are also limited to the fact that data were collected for this study via self-report. A study done on the reliability of the AHS instrument found a very good agreement between repeated assessments, which ranged from 70-90% for questions regarding the ever use of pesticides.[89] Questions on medical conditions and smoking had equally high agreement.[89] We do not know the reliability of other variables such as diet and physical activity.

Another limitation is that we are subject to a potential misclassification of pesticide mode of action. There is a limited amount of literature on the endocrine-

disrupting properties of certain pesticides and we utilized two studies in our grouping of variables.[21,22] Some pesticides have been found to have more than one effect on the endocrine system. DDE, a metabolite of DDT for example, is classified as an estrogen in this study; however, previous work has also classified it as a potent anti-androgen.[90] It is entirely possible that new evidence may emerge in the future that can prove our groupings wrong. In this case, future research should explore different groupings and their relationship with our results. Similarly, in our groupings, we give equal weights to each pesticide within it. In other words, we do not account for how potent they are as endocrine disruptors.

Because we have an occupational population, results may not be generalizable to a non-agricultural worker population. The pesticides asked about in this study all require licenses to apply; thus, it is unlikely that a non-applicator will have the same types of exposure as our sample. Studies should investigate how similar or different these two populations are so that results can be translated to other groups. Lastly, for this study, we focused only on the effects of individual groups on BMI. This may not be appropriate, as it is unlikely that applicators utilize pesticides in one group alone, but rather a mixture of several pesticides across categories. Further studies should delve into the combined effects of different groups on BMI.

Lastly, there is a chance our study suffers from selection bias. We exclude a large number of individuals due to missing information at baseline. A brief analysis on information available for all individuals showed that those excluded were younger (average 48 years old included versus 46 years old excluded), more often males (51% included versus 66% excluded), private applicators (47% included versus 62% excluded), and deceased at last follow up (9% included versus 13% excluded) (data not

shown). Initial non-respondent analysis done by AHS researchers also showed that younger individuals were less likely to return questionnaires.[60] Our other exclusions may affect the representativeness of our sample in comparison to the AHS cohort. Initially, the AHS cohort was not a representative sample of the entire agricultural population in the United States, only of license-seeking applicators in North Carolina and Iowa.[60]

Our study has found that endocrine-disrupting pesticides may play a role in overweight/obesity. Though many of these have been banned in the United States, individuals exposed to pesticides that are still in use as well as to other chemicals that have endocrine-disrupting properties may be subject to the same effects. It is important that future studies build upon these findings and zero in on more exact pathways in which endocrine-disrupting chemicals affect weight gain.

Table 2-1. Potentially endocrine disrupting pesticides from the Agricultural Health Study by mode of action.

| Group | Name | Group | Name |
|---|---|---|---|
| Estrogenic | DDT[21,22] | Anti-androgen | Aldrin[22] |
| | Diazinon[22] | | Atrazine[22] |
| | Heptachlor[21,22] | | Chlordane[21,22] |
| | Permethrin[21,22] | | Chlorpyrifos[22] |
| | Toxaphene[22] | | Dieldrin[21,22] |
| | Aldicarb[22] | | Parathion[21] |
| | Benomyl[22] | Thyroid Disruptor | Malathion[22] |
| | Carbaryl[22] | | Metribuzin[22] |
| Anti-estrogen | Alachlor[21,22] | | Trichlorfon[22] |
| | Captan[22] | PXR Agonist | 2.4-D[21] |
| | Glyphosate[22] | | Trifluralin[21] |
| | Lindane[21,22] | Other* | Chlorothalonil |

*Chlorothalonil has been found to affect the proliferation of androgen-sensitive cells,[22] but no exact mechanism has been discovered.

Table 2-2. Baseline characteristics of Agricultural Health Study participants by baseline BMI categories.

| Variable | | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|---|
| | | | Underweight/ Normal n=7513 (36.8) | Overweight/ Obese n=12,897 (63.2) | |
| Estrogenic | | | | | |
| | Yes | 10,928 (53.5) | 3559 (47.4) | 7369 (57.1) | <0.0001 |
| | No | 9482 (46.5) | 3954 (52.6) | 5528 (42.9) | |
| Anti-estrogen | | | | | |
| | Yes | 13,180 (64.6) | 4233 (56.3) | 8947 (69.4) | <0.0001 |
| | No | 7230 (35.4) | 3280 (43.7) | 3950 (30.6) | |
| Anti-androgen | | | | | |
| | Yes | 10,361 (50.8) | 2834 (37.7) | 7527 (58.4) | <0.0001 |
| | No | 10,049 (49.2) | 4679 (62.3) | 5370 (41.6) | |
| Thyroid Disruptor | | | | | |
| | Yes | 10,607 (52.0) | 3186 (42.4) | 7421 (57.5) | <0.0001 |
| | No | 9803 (48.0) | 4327 (57.6) | 54.76 (42.5) | |
| PXR Agonist | | | | | |
| | Yes | 11,090 (54.3) | 3222 (42.9) | 7868 (61.0) | <0.0001 |
| | No | 9320 (45.7) | 4291 (57.1) | 5029 (39.0) | |
| Other | | | | | |
| | Yes | 792 (3.9) | 244 (3.3) | 548 (4.3) | 0.0004 |
| | No | 19,618 (96.1) | 7269 (96.7) | 12,349 (95.7) | |
| Age (mean) | | 48.4 | 46.6 | 49.4 | <0.0001 |
| Type | | | | | |
| Private Applicator | | 9634 (47.2) | 2567 (34.2) | 7067 (54.8) | <0.0001 |
| Commercial Applicator | | 1048 (5.1) | 284 (3.8) | 764 (5.9) | |
| Spouse | | 9728 (47.7) | 4662 (62.0) | 5066 (39.3) | |
| Gender | | | | | |
| | Male | 10,438 (51.1) | 2726 (36.3) | 7712 (59.8) | <0.0001 |
| | Female | 9972 (48.9) | 4787 (63.7) | 5185 (40.2) | |
| Marital Status | | | | | |
| Married/Living as Married | | 18,975 (93.0) | 6998 (93.2) | 11,977 (92.9) | 0.60 |
| Unmarried | | 985 (4.8) | 363 (4.8) | 622 (4.8) | |
| Divorced/Separated | | 335 (1.6) | 114 (1.5) | 221 (1.7) | |
| Widowed | | 115 (0.6) | 38 (0.5) | 77 (0.6) | |
| Race | | | | | |
| | White | 20,121 (98.6) | 7426 (98.8) | 12,695 (98.4) | 0.02 |
| | Other | 289 (1.4) | 87 (1.2) | 202 (1.6) | |

Table 2-2. Continued

| Variable | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|
| | | Underweight/ Normal n=7513 (36.8) | Overweight/ Obese n=12,897 (63.2) | |
| Education | | | | |
| Less than High School | 949 (4.7) | 256 (3.4) | 693 (5.4) | <0.0001 |
| High School Graduate or Equivalent | 8814 (43.2) | 2852 (38.0) | 5962 (46.2) | |
| Some College or Vocational School | 5740 (28.1) | 2246 (29.9) | 3494 (27.1) | |
| College or Beyond | 4907 (24.0) | 2159 (28.7) | 2748 (21.3) | |
| Vital Status at Last Follow-up | | | | |
| Alive | 18,552 (90.9) | 6952 (92.5) | 11,600 (89.9) | <0.0001 |
| Deceased | 1858 (9.1) | 561 (7.5) | 1297 (10.1) | |
| Doctor Visit in the Past 12 Months | | | | |
| Yes | 14,900 (73.0) | 5471 (72.8) | 9429 (73.1) | 0.65 |
| No | 5510 (27.0) | 2042 (27.2) | 3468 (26.9) | |
| Vigorous Exercise | | | | |
| Less than 1 hour/week | 8008 (39.2) | 2630 (35.0) | 5378 (41.7) | <0.0001 |
| More than 1 hour/week | 12,402 (60.8) | 4883 (65.0) | 7519 (58.3) | |
| Smoking Status | | | | |
| Never Smoked | 13,102 (64.2) | 5159 (68.7) | 7943 (61.6) | <0.0001 |
| Past Smoker | 5293 (25.9) | 1506 (20.0) | 3787 (29.4) | |
| Current Smoker | 2015 (9.9) | 848 (11.3) | 1167 (9.0) | |
| Kidney Conditions | | | | |
| Yes | 1793 (8.8) | 574 (7.6) | 1219 (9.5) | <0.0001 |
| No | 18,617(91.2) | 6939 (92.4) | 11,678 (90.5) | |
| Thyroid Conditions | | | | |
| Yes | 1040 (5.1) | 385 (5.1) | 655 (5.1) | 0.89 |
| No | 19,370 (94.9) | 7128 (94.9) | 12,242 (94.9) | |
| Respiratory Conditions | | | | |
| Yes | 1275 (6.3) | 367 (4.9) | 908 (7.0) | <0.0001 |
| No | 19,135 (93.7) | 7146 (95.1) | 11,989 (93.0) | |
| Diabetes | | | | <0.0001 |
| Yes | 667 (3.3) | 127 (1.7) | 540 (4.2) | |
| No | 19,743 (96.7) | 7386 (98.3) | 12,357 (95.8) | |

Table 2-2. Continued

| Variable | | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|---|
| | | | Underweight/ Normal n=7513 (36.8) | Overweight/ Obese n=12,897 (63.2) | |
| Cardiovascular Disease | | | | | |
| | Yes | 4573 (22.4) | 1160 (15.4) | 3413 (26.5) | <0.0001 |
| | No | 15,837(77.6) | 6353 (84.6) | 9484 (73.5) | |
| Cancer | | | | | |
| | Yes | 1935 (9.5) | 673 (9.0) | 1262 (9.8) | <0.0001 |
| | No | 18,475 (90.5) | 1262 (91.0) | 11,635 (90.2) | |
| Calories (kcal) | | 1996.3 | 1871.8 | 2068.8 | <0.0001 |
| Total Fat (g) | | 76.4 | 69.8 | 80.3 | <0.0001 |
| Saturated Fat (g) | | 25.4 | 23.2 | 26.6 | <0.0001 |
| Cholesterol (mg) | | 256.2 | 225.8 | 273.9 | <0.0001 |

Table 2-3. Adjusted* odds ratios for the interaction of pesticide groupings and gender among pesticide applicators and spouses in the Agricultural Health Study.

| | Overweight/obese Adjusted Odds Ratio (95% CI) | | p-value |
|---|---|---|---|
| | Males | Females | |
| Estrogenic | | | |
| No | 1.00 (reference) | 1.00 | <0.0001 |
| Yes | 0.84 (0.77, 0.92) | 1.15 (1.07, 1.24) | |
| Anti-estrogenic | | | |
| No | 1.00 | 1.00 | 0.39 |
| Yes | 1.13 (0.99, 1.30) | 1.06 (0.99, 1.14) | |
| Anti-androgen | | | |
| No | 1.00 | 1.00 | 0.73 |
| Yes | 1.26 (1.12, 1.41) | 1.30 (1.17, 1.44) | |
| Thyroid Disruptor | | | |
| No | 1.00 | 1.00 | 0.29 |
| Yes | 1.08 (0.98, 1.19) | 1.16 (1.07, 1.26) | |
| PXR Agonist | | | |
| No | 1.00 | | 0.01 |
| Yes | 1.35 (1.20, 1.52) | 1.12 (1.02, 1.22) | |
| Other | | | |
| No | 1.00 | | 0.65 |
| Yes | 0.95 (0.80, 1.12) | 0.87 (0.65, 1.18) | |

*Adjusted for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.

Table 2-4. Adjusted* odds ratios for overweight/obese and pesticide groupings among pesticide applicators and spouses in the Agricultural Health Study.

| | | Overweight/obese Adjusted Odds Ratio (95% CI) | p-value |
|---|---|---|---|
| Anti-estrogen | | | |
| | No | 1.00 | 0.03 |
| | Yes | 1.08 (1.01, 1.15) | |
| Anti-androgen | | | |
| | No | 1.00 | <0.0001 |
| | Yes | 1.28 (1.18, 1.38) | |
| Thyroid Disruptor | | | |
| | No | 1.00 | 0.0002 |
| | Yes | 1.13 (1.06, 1.20) | |
| Other | | | |
| | No | 1.00 | 0.32 |
| | Yes | 0.93 (0.80, 1.08) | |

*Adjusted for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.
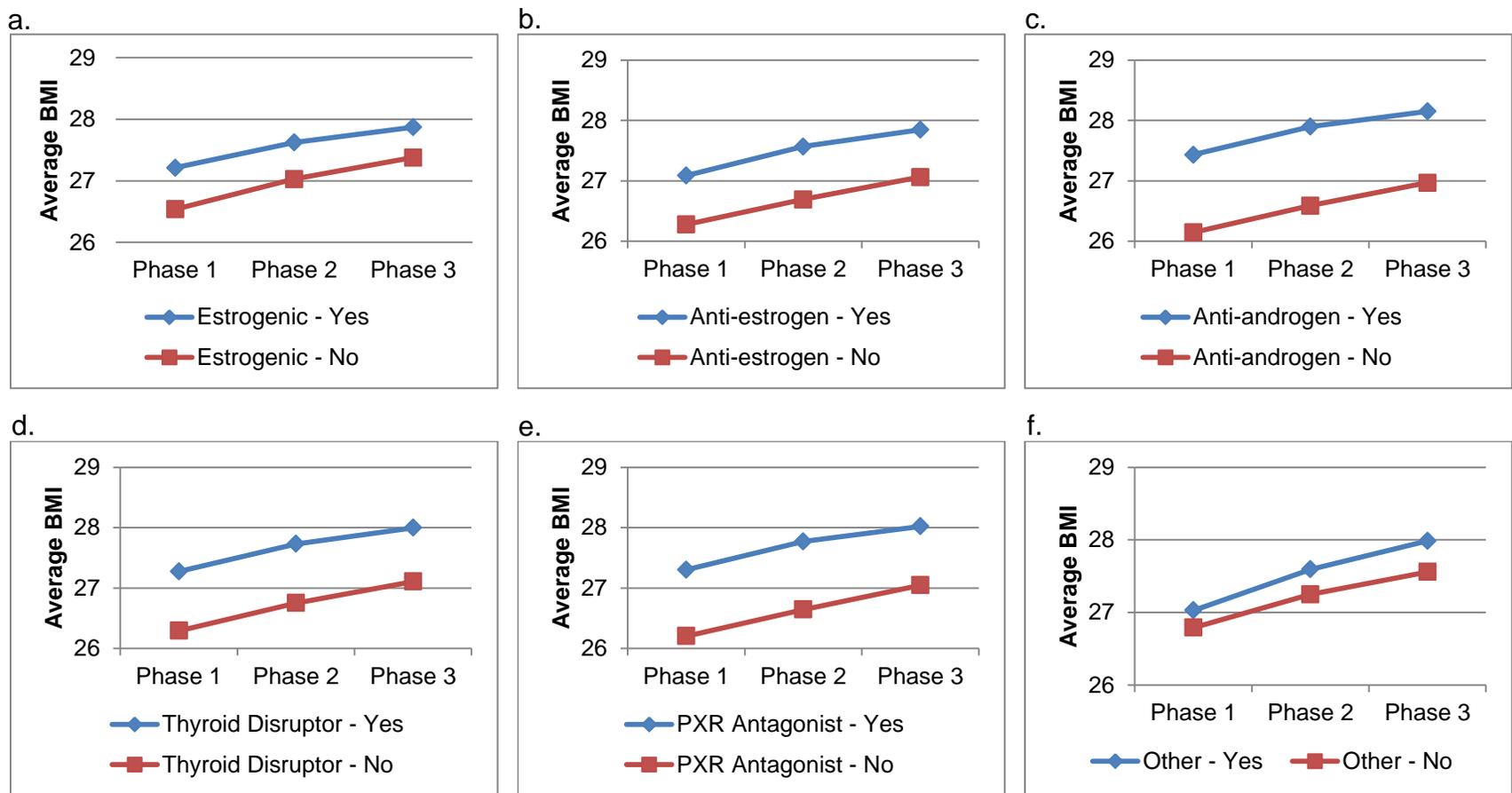
Figure 2-1. Trajectory of mean BMI across Phases 1, 2, and 3 in the Agricultural Health Study by pesticide groups: a) Estrogenic, b) Anti-estrogen, c) Anti-androgen, d) Thyroid Disruptor, e) PXR Agonist, and f) Other.
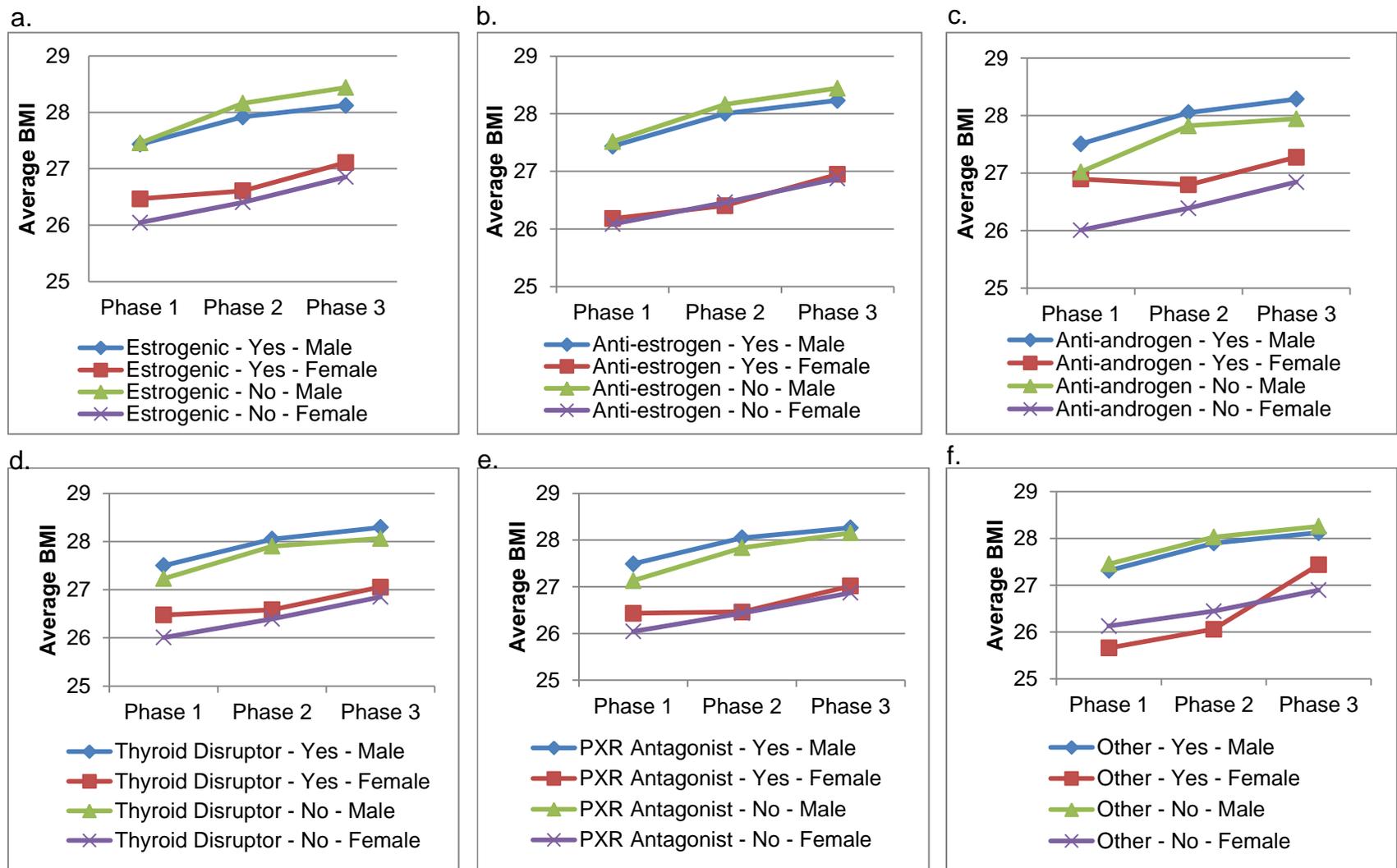
Figure 2-2. Trajectory of mean BMI for males and females across Phases 1, 2, and 3 in the Agricultural Health Study by pesticide groups: a) Estrogenic, b) Anti-estrogen, c) Anti-androgen, d) Thyroid Disruptor, e) PXR Agonist, and f) Other.

CHAPTER 3
EXAMINING DOSE-RESPONSE AND EFFECT-MODIFYING RELATIONSHIPS
BETWEEN ENDOCRINE-DISRUPTING PESTICIDES AND BODY MASS INDEX

**Background**

Obesity is widely believed to be a problem of excess energy balance: more calories consumed than are utilized results in weight gain.[1] Though in developed countries the increase in obesity has slowed down, figures are staggering nonetheless.[91] In the United States alone, almost 70% of adults are considered to be at least overweight with a BM of 25 and over.[71] Recently, the scientific community is beginning to consider that energy balance may not be the only component driving this pandemic.[92] Chemicals collectively called obesogens are one of the environmental factors thought to be causing obesity worldwide.[6] Many pesticides have endocrine disrupting properties and therefore are potential obesogens.

Previous research has found that many pesticides listed as endocrine-disrupting chemicals (EDCs) are associated with increased weight.[36-40,43,44,75] Other studies have found associations between pesticides and obesity-related metabolic conditions like diabetes and insulin resistance.[46,47,59,93,94] Though most studies have focused on prenatal exposure, the positive results found by some studies show that low-dose exposure for a prolonged period of time is also important in the development of obesity and other metabolic syndromes.[44,46,47,59,75] These studies do not include occupational populations, who are exposed to higher doses than the general population, and are potentially a higher risk population.

Two studies investigating the effects of a limited number of pesticides on diabetes in a longitudinal cohort of farmers show that these two issues may be important.[93,94] One investigates the impact of pesticide use on incident diabetes in pesticide applicators and found that aldrin, chlordane, heptachlor, dichlorvos, trichlorfon, alachlor, and cyanazine were positively associated with an increased odds of diabetes.[93] The second study looks at the same cohort, but focuses on women only and found that in this population, pesticides associated with incident diabetes were different than for the farmer subgroup. The authors found that women who reported use of fonofos, phorate, parathion, dieldrin, and 2,4-T were more likely to develop diabetes than nonusers.[94] Montgomery and colleagues further investigate a dose-response relationship for farmers and find that one exists for ten of the pesticides investigated.[93] We believe that these results suggest that increasing exposure to pesticides have an increasing relationship with overweight/obesity as the conditions are closely related.

The present study proposes to utilize data from the Agricultural Health Study to investigate whether the relationship between body mass index (BMI) and endocrine-disrupting pesticides is dose-dependent within an occupational cohort. Our secondary goal is to investigate interactions between pesticides.

**Methods**

**Study Sample**

We used data from the Agricultural Health Study as the source for our sample population. The AHS cohort was previously described in Chapter 2. Briefly, 57,311 pesticide applicators and 32,347 their spouses in Iowa and North Carolina were recruited between 1993 and 1997 at training and licensing

50

locations.[60] For Phase 1, the pesticide applicators were asked to complete

enrollment questionnaires, while their spouses were asked to complete a take-

home questionnaire that included more details on certain pesticide exposures

and medical history.[95] Then, this cohort was followed up via telephone interviews

for the first time beginning in 1999 and ending in 2005 (Phase 2, n=60,138), and

the second time beginning in 2005 and ending in 2010 (Phase 3, n=44,130 non-

commercial applicators only).[95] Additionally, 40% of participants (n=35,164)

completed the self-administered dietary questionnaire during Phase 2.[95]

We excluded individuals who did not have complete information for Phase

1 in the following covariates: BMI, pesticide exposure, and the following

covariates: age, gender, marital status, race, education, vital status, exercise,

smoking status, kidney conditions, thyroid conditions, diabetes, cardiovascular

disease, cancer, and total fat consumed. Our final sample consisted of 11,338

pesticide applicators. Spouses were not included as the information necessary to

create the intensity-adjusted cumulative exposure score was not collected for

them.

**Outcome Measurement**

We calculated BMI from height information asked in the enrollment

questionnaire and the weight questions asked at each of the three phases, as

described in detail in Chapter 2. For individuals who were missing height or

weight data, we used BMI that was previously calculated by AHS investigators.

We checked for errors by examining weights for individuals with BMIs that were

greater than 70 and lower than 15 for implausible BMIs (e.g. BMI < 1), and for

potential miscoding. We then created two categories for BMI:

normal/underweight (BMI <25) and overweight/obese (BMI ≥25).

**Pesticide Exposure Measurement**

Pesticide grouping was done in the same manner as in Chapter 2. Briefly,

pesticides were separated into the following groups using the available

knowledge on the mode of action of individual pesticides: estrogenic, anti-

estrogen, anti-androgen, thyroid disruptor, pregnane X receptor (PXR) agonist,

and other.[21,22] Table 2-1 lists the individual pesticides and their respective

groupings.

We used the intensity-adjusted cumulative exposure score variable

previously calculated by AHS researchers. Details on the algorithm and its

development have been previously published.[96,97] In short, the enrollment

questionnaire included questions regarding the intensity and duration of pesticide

exposures, such as the number of years and average number of days per year

an individual was exposed to a chemical and what type of protective equipment

was used. The answers to such questions were then used weights developed for

exposure scenarios to different chemicals. For instance, if an individual reported

never mixing a particular pesticide, he was assigned a weight of zero, but if he

reported mixing less than 50% of the time, he was assigned a weight of three.

Intensity scores are then calculated using these numerical factors via the

following equation:

Exposure Intensity Score = (MIX + APPLY + REPAIR) × PPE[96]

where MIX comes from questions regarding whether an individual mixes

pesticides and how often prior to usage, APPLY comes from questions regarding

whether and how an individual applies pesticides, REPAIR relates to questions on whether the participant repairs the equipment used application, and PPE is a factor used to account for reduced exposure due to use of protective equipment and practices. The exposure intensity score is then multiplied by the frequency and duration of pesticide use to create lifetime intensity-weighted days of pesticide exposure.[96,97]

We used the second version of this algorithm with updated weighting factors.[97] We added scores from each pesticide in a particular group to create one score for each pesticide grouping. Again, we assumed a score of zero for missing scores of individuals who a value of zero or above for any other pesticide in a particular group. Because these scores are more easily interpretable relative to other applicators, we chose to categorize this measure to facilitate comparisons between individuals. We then excluded individuals who had no exposure and calculated the median score. This was used to categorize individuals into having no exposure, being at or below the median or above the median for each pesticide group.

**Covariates**

Confounders were selected in Chapter 2 and included age,[1] gender (male, female),[1] marital status,[62] race (white, other),[1] education,[63] vital status, vigorous exercise,[1] smoking status (never smoker, former smoker, and current smoker),[65] thyroid conditions,[67] diabetes,[1,59] cardiovascular disease,[1] cancer,[1] and total fat intake.[1] The variable for vigorous exercise was created using questions on how many hours one exercised vigorously per week in Phase 1 and using questions on how many days per week an individual exercised vigorously and how much

time was spent per day in Phase 3. The following groups of conditions were created: thyroid (thyrotoxicosis, Grave's disease, goiter, other thyroid disease), and cardiovascular (heart disease, angina, high blood pressure, myocardial infarction, arrhythmia, stroke). Individuals who reported at least one of the conditions in each group were considered as having the condition, and those who were considered as not having he condition must have reported not having a diagnosis in any of the individual diseases. To be considered missing, individuals had to have all conditions missing within a group. As previously mentioned, during Phase 2, participants were given a self-administered dietary questionnaire. Using the Diet*Calc version 1.4.3 (National Cancer Institute, Applied Research Program), we obtained estimates for various nutritional variables and chose total fat to represent our adjustment for diet.

**Secondary Analysis Population**

The questions used to create the intensity-adjusted cumulative exposure score were not collected for spouses, the majority of whom were female. To assess whether the inclusion of females changed the results of our main analysis, we conducted a secondary analysis based on the number of pesticides to which an individual was exposed. Pesticide information came from the question: "Have you ever personally mixed or applied [pesticide name]?" We then calculated number of pesticides an individual had used in each group. Individuals who had not answered any of the questions for a particular group were categorized as missing for that category. However, if an individual had answered at least one question for a pesticide in a particular category regardless of whether it was "Yes" or "No", we assumed he did not use any of the other

pesticides with missing information. The number of pesticides in each group was then categorized as no use, one, and two or more for the estrogenic, anti-estrogen, anti-androgen, and thyroid disruptors groups. The group for PXR agonist included only two pesticides and was coded as no use, one, and two. We did not explore the category other as includes only one pesticide and no dose information can be gathered. For this part of the analysis, we also excluded individuals based on missing values for BMI, pesticide exposure, and the following covariates: age, gender, marital status, race, education, vital status, exercise, smoking status, kidney conditions, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat consumed in Phase 1. We included 21,528 individuals in this sample.

**Statistical Analyses**

All analyses were done using SAS version 9.3 (SAS Institute, Cary, NC). We calculated participant characteristics for all potential covariates for each BMI group separately for our main and secondary analyses. As mentioned previously in Chapter 2, Proc GLIMMIX deletes observations with missing information; therefore, we chose to impute the previous phase's information for the exercise variable, as this information was not available for Phase 2. Additionally, diet and cancer data was only present for Phases 2 and 1, respectively; therefore, we imputed this single value for all phases.

For all analyses, we used generalized linear mixed models with a binary distribution and a random statement to account for the repeated nature of the data. We conducted maximum likelihood estimations based on adaptive quadrature due to its reduced potential for giving biased estimates.[70] Because

measures of BMI are more likely to be similar for measures taken closer together than farther apart (i.e. between Phases 1 and 2 and Phases 2 and 3 than Phases 1 and 3), we defined the covariance structure as a first-order autoregressive. All models were adjusted for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.

For the main analysis, we assessed the impact of categorized intensity-adjusted cumulative exposure scores for each pesticide group on BMI. We then explored whether levels of intensity-adjusted cumulative exposure scores of one pesticide group modified the effect of another pesticide group. We tested each pesticide group with all other groups, individually. Our reference value was set to no use for both groups and odds ratios were obtained comparing each combination of levels to individuals who reported not using any pesticide in those groups. For instance, in the interaction between estrogenic and anti-estrogenic pesticides, individuals who reported above median levels of estrogenic (level 2), but below median levels of anti-estrogenic pesticides (level 1) were compared to individuals who had no use (level 0) in both groups of pesticides.

We repeated the first part of the analysis for the number of pesticides an individual was exposed to in a particular group, but we did no further explorations for pesticide group interactions.

**Results**

Our sample consisted of individuals who had information available to calculate the intensity-adjusted cumulative exposure score (n=11,338). The vast majority of these, 73.3% (n=8314) were overweight/obese with a BMI of over 25

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at baseline. Table 3-1 describes demographics and other covariates by BMI group. Mean age during Phase 1 was 49 years old for the entire sample, and those with underweight/normal BMI were statistically significantly younger, with a mean age of 47.8 years old. Because applicators are mostly male and this sample does not include spouses the majority for both overweight/obese and underweight/normal groups are male rather than females. When comparing both groups, females were less frequently overweight/obese than underweight/normal (1.8% vs 4.8%). Individuals who were married or living as married were more frequently overweight/obese than underweight/normal (88.3% vs 82.2%). Higher proportions of individuals who were overweight/obese reported a high school education or less. Individuals who were deceased at last follow up were more frequently overweight/obese at baseline than underweight/normal (12.1% vs 10.8%). While the majority of both groups of BMI reported more than one hour per week of vigorous exercise, a lesser proportion of those who were overweight/obese reported this behavior (57.4% vs 61.8% of underweight/normal individuals). Former smokers were more frequently obese/overweight at baseline than underweight/normal (36.6% vs 26.5%). When compared to participants with underweight/normal BMI, overweight/obese individuals more frequently reported ever being diagnosed with diabetes (3.8% vs 1.9%), and cardiovascular disease (26.1% vs 18.1%). The mean number of gram of total fat reported was also statistically significantly different between the two groups (90.4 g for overweight/obese BMIs vs 87.2 g for underweight/normal).

Overall there were statistically significant differences in intensity-adjusted cumulative exposure score category between BMI categories for four out of the six pesticide groupings (Table 3-2). For pesticides in the anti-estrogenic, anti-androgenic, thyroid disruptor and PXR agonist categories, individuals who were overweight/obese more frequently had exposure scores above median than those who were underweight/normal.

Table 3-3 displays the adjusted odds ratios for having a BMI considered overweight/obese for each pesticide group. Estrogenic pesticides were inversely associated with BMI with a reverse trend ($p_{trend}$= 0.004): below median an OR of 0.91 (95% CI: 0.83, 0.99) and above median with an OR of 0.86 (95% CI: 0.78, 0.94). Anti-estrogenic pesticides were statistically significantly associated with overweight/obese BMI for those in the above median category only (OR: 1.28, 95% CI: 1.13, 1.45). Both the anti-estrogenic group and the anti-androgenic pesticides showed a positive trend ($p_{trend}$<0.0001 for both). On average, those who had below average intensity-adjusted cumulative exposure scores were 1.18 times more likely to be overweight/obese than those who never used any anti-androgenic pesticides (95% CI: 1.06, 1.31). These odds increased to 1.47 times more likely for those who had above average scores (95% CI: 1.32, 1.63). For thyroid disruptors, those who had below average scores were 1.12 times more likely to be overweight/obese for those who had above average scores compared to those who did not use any thyroid-disrupting pesticides (95% CI: 1.02, 1.23).

For PXR agonists, those with below average scores had the highest odds of being overweight/obese (OR: 1.53, 95% CI: 1.37, 1.70). The odds decrease to

1.24 for those with above average intensity-adjusted cumulative exposure scores (95% CI: 1.12, 1.38). This group had a positive trend ($p_{trend}<0.0001$).

Figure 3-1 shows Forest plots of the interaction between pesticide groups comparing each combination of intensity-adjusted cumulative exposure score categories with the combination of no use for either pesticide group. Overall only one comparison of two groups had a significant interaction: PXR agonists and estrogenic pesticides. In the presence of estrogenic pesticides, the association between above median levels of PXR agonists and overweight/obesity weakens (Levels 2-2 and 2-1 compared to 2-0). When levels of PXR are below median, the association becomes non-statistically significant in the presence of above median levels of estrogenic pesticides (Level 1-2 compared to 1-1 and 1-0).

In our secondary analysis, we also investigated the impact of using number of pesticides in each category as our primary exposure variable. This sample was much larger and included spouses (n=13,640). Table 3-4 shows baseline characteristics for the sample available for secondary analysis. Demographically, the main and secondary samples were quite similar, with the exception of gender, marital status, smoking status, and thyroid conditions. Gender and marital status are intuitive since spouses were not included in the original sample, and the majority of the applicators are males. In our original sample, we had greater proportions of former and current smokers than in the expanded sample. Additionally, there were a much greater proportion of individuals with thyroid conditions in the expanded sample. Lastly, respondents had greater average total fat in the original sample.

Table 3-5 shows the results for the adjusted odds ratio for overweight/obesity and categorized number of pesticides. There was a reverse relationship for anti-androgens, the odds ratio of overweight/obese decreased slightly from 1.33 (95% CI: 1.22, 1.44) for one pesticide to 1.25 (95% CI: 1.15, 1.36) for two or more pesticides ($p_{trend}$<0.0001). In the thyroid disruptor group, use of one pesticide only resulted in an odds of 1.12 (95% CI: 1.05, 1.19), which increased to 1.21 for use of two or more pesticides (95%CI: 1.11, 1.33) ($p_{trend}$=0.002). No other pesticide group had significant trends.

The exposure trend analysis using number of pesticides as our main exposure variable showed that estrogenic and anti-estrogenic groups had a similar trend seen in our main analysis; however, these were not significant. For the thyroid disruptor groups there were also similar trends with odds ratios increasing with increasing number of pesticides as in the main analysis. Both the anti-androgenic and PXR agonist groups showed a similar positive association; however, their exposure trends were reversed compared to the primary analysis and only the anti-androgenic group had statistically significant trends.

**Discussion**

In our study, we found that there were positive trend with anti-estrogenic, anti-androgenic, and thyroid disrupting pesticides. We also found a reverse trend with estrogenic pesticides.

To date, only a few studies have investigated the effects of increasing pesticides exposure on obesity in adults. Researchers investigated whether organochlorine pesticides were associated with abdominal obesity in a Swedish cohort of elderly men and women ages 70 years old at baseline. In the cross-

sectional analysis for men only, the pesticide metabolites for DDT, chlordane, and hexachlorobenzene had significant p-values for trend.[44] Another study investigating serum levels of organochlorine pesticides in Spanish citizens found that increasing BMI categories had increasing mean concentrations of metabolites of lindane, DDT, and hexachlorbenzene.[43] These results are in agreement with our findings for the anti-androgen category, which includes chlordane, and the anti-estrogen category, which includes lindane. However, we found that estrogenic pesticides, which include DDT, had a reverse relationship with overweight/obesity, which was consistent with our results for men in Chapter 2. It is unclear why this result is inconsistent with the literature. One reason may be that our sample differs greatly from the Swedish and Spanish cohorts. In our study, data come from an occupational cohort in the United States. For this pesticide, level of exposure may be different between occupational and non-occupational populations, resulting in different exposure trends.

One study done on the AHS cohort has investigated whether pesticides had a dose-dependent relationship with incident diabetes, for which obesity is a risk factor.[93] Out of the pesticides tested, seven had a positive dose-response association, and of these six were also present in our study: diazinon, heptachlor, alachlor, atrazine, chlorpyrifos, and trichlorfon.[93] The direction of the dose-response relationship was consistent with the trends our study for all but diazinon and heptachlor, which are both part of the estrogenic group. This, along with the fact that our results on the exposure trend of estrogenic pesticides are also

contradictory to the literature on obesity is likely due to the fact that we found a protective effect of estrogen on weight.

To our knowledge, our study is the first to investigate potential interactions between pesticides. We found that only the interaction between the estrogenic group and the PXR agonist group was significant. Above median levels of pesticides in this group reversed the protective effect of estrogen. The signaling pathways of PXR have the ability of altering levels of estrogen hormones,[21] which may create additional competition for the estrogen receptor, a key player in the adipogenic pathway.[74] Thus the protective effects of estrogens will be attenuated in the presence of a chemical competing for the same receptor.[74]

Our secondary analysis using number of anti-androgenic pesticides showed a statistically significant exposure trend that was opposite what was seen for our main analysis. However, the effect size of the pesticide group was consistent for both analyses. It is unclear why there is a difference in the two measures. However, utilizing intensity-adjusted cumulative exposure score provides a much clearer picture of actual exposures. This score calculated by AHS investigators includes detailed information on method of application, type of equipment used, and PPE, among other measures, and can be a more reliable estimate of true cumulative exposure as opposed to a simpler measure, such as ever using a pesticide.[97] Similarly, using number of pesticides ever used does not capture information on frequency and duration of pesticide use, which are essential components for estimating dose.

Our study is subject to several limitations. First, we rely on self-report for the outcome and main exposure. At baseline, we had approximately 73% of individuals reporting height and weight equivalent to a BMI equal to or greater than 25, the cutoff between normal weight and overweight. This is greater than the age-adjusted prevalence of 59.3% estimated in the general population around the time of Phase 1.[72] Therefore, it is unlikely that BMI was underreported in this sample. Additionally, an evaluation of the instrument used in the Agricultural Health Study has shown decent reliability for questions used on the intensity-adjusted cumulative exposure score algorithm.[89] We do not believe that any misclassification that might occur would be different based on BMI status.

Second, we cannot ascertain that individuals who are not exposed to the pesticides we deemed endocrine disrupting were not exposed to other pesticides or chemicals that are obesogenic. In our interaction analysis, we see that groups could attenuate the positive effects of other groups or even strengthen weak associations. It is fair to assume that other chemicals have this same ability; however, we could not test for all pesticides and interactions.

Third, as with any longitudinal study, the Agricultural Health Study was subject to a fairly high dropout rate. In the overall study, 33% were lost to follow up by Phase 2, and an additional 27% did not complete Phase 3.[95] We have no reason to believe that those who have BMIs in the overweight range or higher were more likely to be lost to follow up than those who were normal weight. Though commercial applicators were not included in Phase 3, our analysis of

baseline characteristics did not show any significant differences between the two groups.

Lastly, we do not have any information on intensity-adjusted cumulative exposure scores for spouses. Spouses made up a large proportion of the female participants; therefore, we were unable to explore any interactions based on gender. Since three out of our six groups are based on activity on a sex hormone, we can hypothesize that gender plays a large role on these associations. Additionally, the results of our sensitivity analysis were not in complete accordance with our main results, which could be due to the small percentage of women in the main analysis.

Despite our limitations, our study had many strengths. First, the Agricultural Health Study is to our knowledge the largest study of occupational workers to date. Almost 90,000 individuals were enrolled, and even with the high amounts of missing information and high dropout rate, we ended up with a fairly large sample size. Second, participants that had information for all three phases were followed for at least 13 years, allowing us to get a picture of the long-term effects of pesticides on weight. Third, we also had detailed information on pesticide use and length of use via the exposure scores calculated by the AHS team.

In this cohort of occupational pesticide exposures, we found that exposure to an increasing amount of anti-estrogenic, anti-androgenic, and thyroid disrupting pesticides was associated with increasing BMI. We also found that estrogenic pesticides behave differently than other groups. Together these

results indicate that endocrine-disrupting pesticides may play a role in weight gain, and that this role is likely affected by cumulative exposures. Future research should investigate the effect of and interactions between pesticides and other endocrine-disrupting chemicals, with a focus on estrogenic pesticides. Additionally, because we only focus on an occupational population, future studies should focus in non-agricultural cohorts to fully understand the role endocrine-disruptors play on the increasing burden of obesity.

Table 3-1. Baseline characteristics of Agricultural Health Study farmers and commercial applicators by baseline BMI categories.

| Variable | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|
| | | Underweight/ Normal n= 3,024 (26.7) | Overweight/ Obese n= 8,314 (73.3) | |
| Age (mean) | 49.0 | 47.8 | 49.4 | <0.0001 |
| Type | | | | |
| Private Applicator | 10,259 (90.5) | 2,732 (90.3) | 7,527 (90.5) | 0.76 |
| Commercial Applicator | 1079 (9.5) | 292 (9.7) | 787 (9.5) | |
| Gender | | | | |
| Male | 11,041 (97.4) | 2,880 (95.2) | 8,161 (98.2) | <0.0001 |
| Female | 297 (2.6) | 144 (4.8) | 153 (1.8) | |
| Marital Status | | | | |
| Married/Living as Married | 9,829 (86.7) | 2,485 (82.2) | 7,344 (88.3) | <0.0001 |
| Unmarried | 1,033 (9.1) | 381 (12.6) | 652 (7.8) | |
| Divorced/Separated | 354 (3.1) | 120 (4.0) | 234 (2.8) | |
| Widowed | 122 (1.1) | 38 (1.3) | 84 (1.0) | |
| Race | | | | |
| White | 11,176 (98.6) | 2,988 (98.8) | 8,188 (98.5) | 0.20 |
| Other | 162 (1.4) | 36 (1.2) | 126 (1.5) | |
| Education | | | | |
| Less than High School | 683 (6.0) | 158 (5.2) | 525 (6.3) | <0.0001 |
| High School Graduate or Equivalent | 5,291 (46.7) | 1,281 (42.4) | 4,010 (48.2) | |
| Some College or Vocational School | 2,880 (25.4) | 787 (26.0) | 2,093 (25.2) | |
| College or Beyond | 2,484 (21.9) | 798 (26.4) | 1,686 (20.3) | |
| Vital Status at Last Follow-up | | | | |
| Alive | 10,005 (88.2) | 2,696 (89.2) | 7,307 (87.9) | 0.05 |
| Deceased | 1333 (11.8) | 326 (10.8) | 1,007 (12.1) | |
| Vigorous Exercise | | | | |
| Less than 1 hour/week | 4,695 (41.4) | 1,156 (38.2) | 3,539 (42.6) | <0.0001 |
| More than 1 hour/week | 6,643 (58.6) | 1,868 (61.8) | 4,775 (57.4) | |

Table 3-1. Continued

| Variable | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|
| | | Underweight/Normal n= 3,024 (26.7) | Overweight/ Obese n= 8,314 (73.3) | |
| Smoking Status | | | | |
| Never Smoked | 6,220 (54.9) | 1,809 (59.8) | 4,411 (53.1) | <0.0001 |
| Past Smoker | 3,848 (33.9) | 801 (26.5) | 3,047 (36.6) | |
| Current Smoker | 1,270 (11.2) | 414 (13.7) | 856 (10.3) | |
| Thyroid Conditions | | | | |
| Yes | 229 (2.0) | 49 (1.6) | 180 (2.2) | 0.07 |
| No | 11,109 (98.0) | 2,975 (98.4) | 8,134 (97.8) | |
| Diabetes | | | | |
| Yes | 373 (3.3) | 58 (1.9) | 315 (3.8) | <0.0001 |
| No | 10,965 (96.7) | 2,966 (98.1) | 7,999 (96.2) | |
| Cardiovascular Disease | | | | |
| Yes | 2,716 (23.9) | 546 (18.1) | 2,170 (26.1) | <0.0001 |
| No | 8,622 (76.1) | 2,478 (81.9) | 6,144 (73.9) | |
| Cancer | | | | |
| Yes | 1,226 (10.8) | 343 (11.3) | 883 (10.6) | 0.27 |
| No | 10,112 (89.2) | 2,681 (88.7) | 7,431 (89.4) | |
| Total Fat (g) | 89.6 | 87.2 | 90.4 | 0.001 |

Table 3-2. Categories of intensity-adjusted cumulative exposure score in six pesticide groups of Agricultural Health Study participants by baseline BMI categories.

| Variable | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|
| | | Underweight/ Normal | Overweight/ Obese | |
| Estrogenic | | | | |
| No Use | 3,805 (33.6) | 1,022 (33.8) | 2,783 (33.5) | 0.94 |
| Below Median | 3,666 (32.3) | 1,025 (33.9) | 2,689 (32.3) | |
| Above Median | 3,867 (34.1) | 977 (32.3) | 2,842 (34.2) | |
| Anti-estrogen | | | | |
| No Use | 1,281 (11.3) | 369 (12.2) | 912 (11.0) | <0.0001 |
| Below Median | 4,855 (42.8) | 1,393 (43.1) | 3,462 (41.6) | |
| Above Median | 5,202 (45.9) | 1,262 (41.7) | 3,940 (47.4) | |
| Anti-androgen | | | | |
| No Use | 1,881 (16.6) | 619 (20.5) | 1,262 (15.2) | <0.0001 |
| Below Median | 4,675 (41.2) | 1,299 (43.0) | 3,376 (40.6) | |
| Above Median | 4,782 (42.2) | 1,106 (36.5) | 3,676 (44.2) | |
| Thyroid Disruptor | | | | |
| No Use | 2,865 (25.3) | 818 (27.1) | 2,047 (24.6) | 0.005 |
| Below Median | 4,196 (37.0) | 1,131 (37.4) | 3,065 (36.9) | |
| Above Median | 4,277 (37.7) | 1,075 (35.6) | 3,202 (38.5) | |
| PXR Agonist | | | | |
| No Use | 1,734 (15.3) | 573 (18.9) | 1,161 (14.0) | <0.0001 |
| Below Median | 4,539 (40.0) | 1,266 (41.9) | 3,273 (39.3) | |
| Above Median | 5,065 (44.7) | 1,185 (39.2) | 3,880 (46.7) | |
| Other | | | | |
| No Use | 10,662 (94.0) | 2,829 (93.6) | 7,833 (94.2) | 0.38 |
| Below Median | 369 (3.3) | 109 (3.6) | 260 (3.1) | |
| Above Median | 307 (2.7) | 86 (2.8) | 221 (2.7) | |

Table 3-3. Adjusted* odds ratios for overweight/obese BMI and pesticide groupings among pesticide applicators in the Agricultural Health Study.

| | Overweight/Obese Adjusted Odds Ratio (95% CI) | p-value |
|---|---|---|
| Estrogenic | | |
| No Use | 1.00 (ref) | 0.004 |
| Below Median | 0.91 (0.83, 0.99) | |
| Above Median | 0.86 (0.78, 0.94) | |
| Anti-estrogen | | |
| No Use | 1.00 | <0.0001 |
| Below Median | 1.02 (0.91, 1.15) | |
| Above Median | 1.28 (1.13, 1.45) | |
| Anti-androgen | | |
| No Use | 1.00 | <0.0001 |
| Below Median | 1.18 (1.06, 1.31) | |
| Above Median | 1.47 (1.32, 1.63) | |
| Thyroid Disruptor | | |
| No Use | 1.00 | 0.06 |
| Below Median | 1.08 (0.98, 1.18) | |
| Above Median | 1.12 (1.02, 1.23) | |
| PXR Agonist | | |
| No Use | 1.00 | <0.0001 |
| Below Median | 1.53 (1.37, 1.70) | |
| Above Median | 1.24 (1.12, 1.38) | |
| Other | | |
| No Use | 1.00 | 0.13 |
| Below Median | 0.83 (0.68, 1.01) | |
| Above Median | 0.91 (0.73, 1.14) | |

*Adjusted for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.

Table 3-4. Baseline characteristics of Agricultural Health Study farmers, commercial applicators and spouses by baseline BMI categories.

| Variable | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|
| | | Underweight/ Normal n= 7,888 (36.6) | Overweight/ Obese n= 13,640 (63.4) | |
| Age (mean) | 48.5 | 46.8 | 49.5 | <0.0001 |
| Type | | | | |
| Private Applicator | 10,325 (48.0) | 2,744 (34.8) | 7,581 (55.6) | <0.0001 |
| Commercial Applicator | 1,085 (5.0) | 292 (3.7) | 793 (5.8) | |
| Spouse | 10,118 (47.0) | 4,852 (61.5) | 5,266 (38.6) | |
| Gender | | | | |
| Male | 11,151 (51.8) | 2,900 (36.8) | 8,251 (60.5) | <0.0001 |
| Female | 10,377 (48.2) | 4,988 (63.2) | 5,389 (39.5) | |
| Marital Status | | | | |
| Married/Living as Married | 20,012 (93.0) | 7,347 (93.1) | 12,665 (92.9) | 0.38 |
| Unmarried | 1,038 (4.8) | 383 (4.9) | 655 (4.8) | |
| Divorced/Separated | 355 (1.6) | 120 (1.5) | 235 (1.7) | |
| Widowed | 123 (0.6) | 38 (0.5) | 85 (0.6) | |
| Race | | | | |
| White | 21,219 (98.6) | 7,797 (98.8) | 13,422 (98.4) | 0.008 |
| Other | 309 (1.4) | 91 (1.2) | 218 (1.6) | |
| Education | | | | |
| Less than High School | 1,061 (4.9) | 289 (3.7) | 772 (5.7) | <0.0001 |
| High School Graduate or Equivalent | 9,353 (43.5) | 3,012 (38.2) | 6,341 (46.5) | |
| Some College or Vocational School | 6,014 (27.9) | 2,346 (29.7) | 3,668 (26.9) | |
| College or Beyond | 5,100 (23.7) | 2,241 (28.4) | 2,859 (20.9) | |
| Vital Status | | | | |
| Alive | 19,508 (90.6) | 7,287 (92.4) | 12,221 (89.6) | <0.0001 |
| Deceased | 2,020 (9.4) | 601 (7.6) | 1,419 (10.4) | |
| Vigorous Exercise | | | | |
| Less than 1 hour/week | 8,969 (39.3) | 2,773 (35.2) | 5,696 (41.8) | <0.0001 |
| More than 1 hour/week | 13,059 (60.7) | 5,115 (64.8) | 7,944 (58.2) | |

Table 3-4. Continued

| Variable | Overall n (%) | BMI n (%) | | p-value |
|---|---|---|---|---|
| | | Underweight/ Normal n= 7,888 (36.6) | Overweight/ Obese n= 13,640 (63.4) | |
| Smoking Status | | | | |
| Never Smoked | 13,786 (64.0) | 5,408 (68.6) | 8,375 (61.4) | <0.0001 |
| Past Smoker | 5,625 (26.1) | 1,597 (20.2) | 4,028 (29.5) | |
| Current Smoker | 2,120 (9.9) | 883 (11.2) | 1,237 (9.1) | |
| Thyroid Conditions | | | | |
| Yes | 1,086 (5.0) | 409 (5.2) | 677 (5.0) | 0.47 |
| No | 20,442 (95.0) | 7,479 (94.8) | 12,963 (95.0) | |
| Diabetes | | | | |
| Yes | 702 (3.3) | 131 (1.7) | 571 (4.2) | <0.0001 |
| No | 20,826 (96.7) | 7,757 (98.3) | 13,069 (95.8) | |
| Cardiovascular Disease | | | | |
| Yes | 4,866 (22.6) | 1,238 (15.7) | 3,626 (26.6) | <0.0001 |
| No | 16,662 (77.4) | 6,650 (84.3) | 10,012 (73.4) | |
| Cancer | | | | |
| Yes | 2,051 (9.5) | 705 (8.9) | 1,346 (9.9) | 0.03 |
| No | 19,477 (90.5) | 7,183 (91.1) | 12,294 (90.1) | |
| Total Fat (g) | 76.5 | 69.9 | 80.3 | <0.0001 |

Table 3-5. Adjusted* odds ratios for overweight/obese BMI and pesticide groupings among pesticide applicators and spouses in the Agricultural Health Study.

| | | Overweight/Obese BMI Adjusted Odds Ratio (95% CI) | p-value |
|---|---|---|---|
| Estrogenic | | | |
| | No Use | 1.00 (ref) | 0.01 |
| | 1 | 1.06 (0.99, 1.13) | |
| | 2 or More | 0.95 (0.89, 1.02) | |
| Anti-estrogen | | | |
| | No Use | 1.00 | <0.0001 |
| | 1 | 1.02 (0.96, 1.09) | |
| | 2 or More | 1.21 (1.12, 1.31) | |
| Anti-androgen | | | |
| | No Use | 1.00 | <0.0001 |
| | 1 | 1.33 (1.22, 1.44) | |
| | 2 or More | 1.25 (1.15, 1.36) | |
| Thyroid Disruptor | | | |
| | No Use | 1.00 | <0.0001 |
| | 1 | 1.12 (1.05, 1.19) | |
| | 2 or More | 1.21 (1.11, 1.33) | |
| PXR Agonist | | | |
| | No Use | 1.00 | <0.0001 |
| | 1 | 1.08 (1.00, 1.16) | |
| | 2 | 1.39 (1.28, 1.51) | |

*Adjusted for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.

Figure 3-1. Forest plots of adjusted * odds ratios for overweight/obese BMI and interactions between pesticides in the Agricultural Health Study.
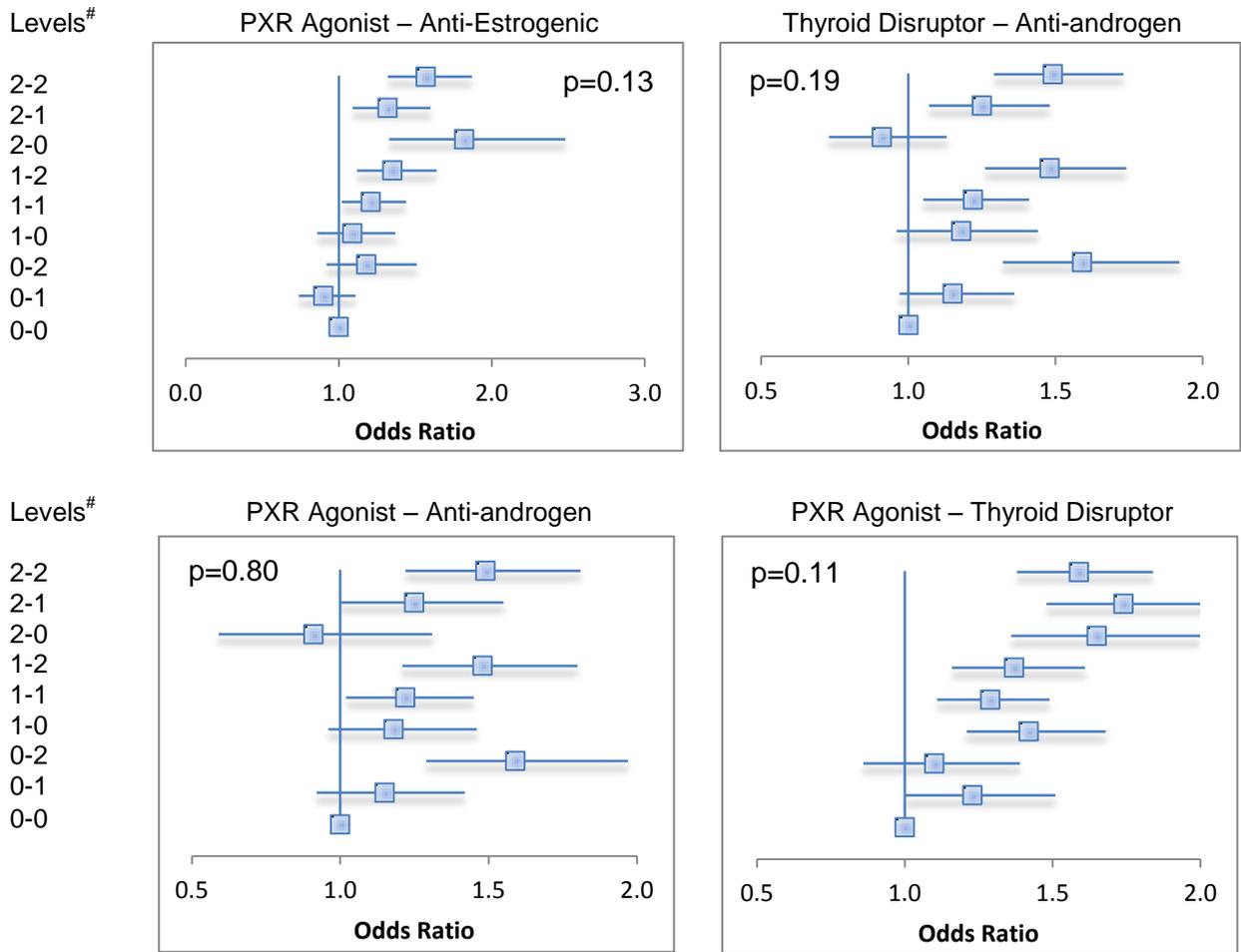
Figure 3-1. Continued

*Adjusted for age, gender, marital status, race, education, vital status, vigorous exercise, smoking status, thyroid conditions, diabetes, cardiovascular disease, cancer, and total fat intake.
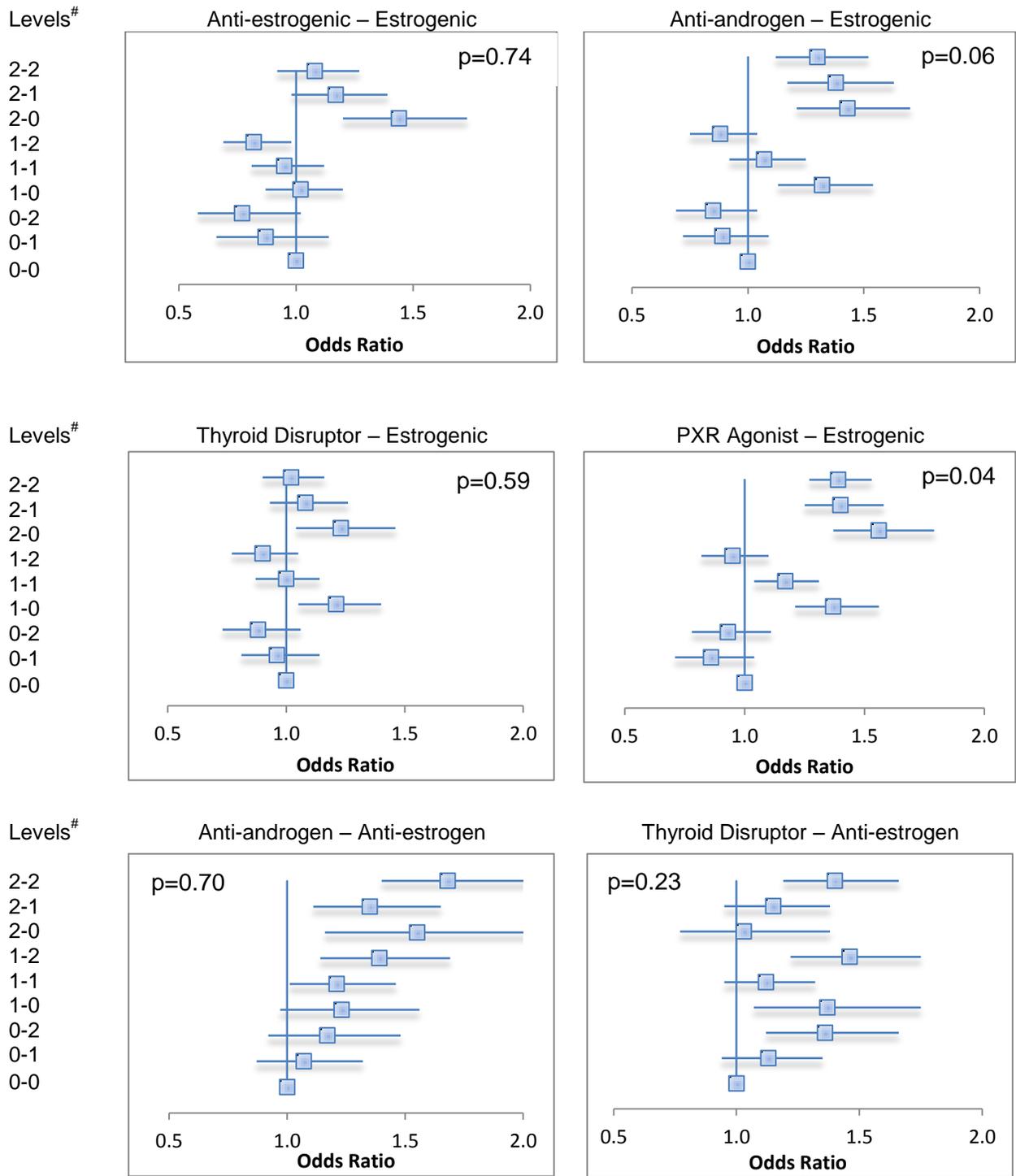[#] Levels: 2, Above Median; 1, Below Median; 0, No Use. For PXR Agonist – Anti-androgen for example, Level 2-2 is above median for both, 2-1 is above median for PXR Agonist and below median for Anti-androgen and 2-0 is above median for PXR Agonist and no use for anti-androgen.

CHAPTER 4
OCCUPATIONAL, DIETARY, AND DEMOGRAPHIC PREDICTORS OF EXPOSURE
TO ENDOCRINE-DISRUPTING PESTICIDE METABOLITES

**Background**

Obesogens are environmental chemicals that have the potential to affect adipogenesis and weight gain via a disruption of the endocrine system.[98] Current data shows that the rates of adult obesity in the United States from 1995 until 2010 have increased from 16% to over 27%.[7] Therefore, obesogens have come into focus in recent years as potentially helping drive these trends. Pesticides have been implicated in an increase in obesity,[44,47,75] as well as other weight-related metabolic conditions such as diabetes[59,93,94] and insulin resistance;[46,47] therefore, these chemicals have been found to be potential obesogens. Historically, organochlorine pesticides were the first class to be designated as endocrine disrupting leading to a gradual phase out of its use in the US.[23] However, these chemicals are persistent in the environment, and many other pesticides that have since replaced their use have also been found to have endocrine disrupting properties.[24]

Agricultural populations are thought to be at special risk for the endocrine-disrupting effects of pesticides due to their direct use.[22] In fact, the majority of studies investigating the health effects of pesticide use are in occupational populations.[50] However, the general population is also at risk for exposure through residential use as well as pesticide contamination of air, water, and soil.[51] Additionally, levels of pesticides have been found in a variety of foods, such as meats, fish, and dairy.[52,54] Thus, dietary exposure is another significant source that can affect both groups. Because of this, it is important that studies investigate the comparability of determinants of exposure between

the two groups so that conclusions from studies can be drawn in-between populations and appropriate interventions can be designed and targeted.

Few studies have directly compared non-occupational and occupational pesticide levels. One study on pregnant women from an agricultural cohort found that their pesticide levels were significantly higher compared to pregnant women in the general population. They also found that these women had less education, were poorer, and more often Mexican or Mexican-American compared to the general population.[99] Another study found that children of pesticide applicators had significantly higher pesticide levels than children from the general population.[100] In terms of exposure, children of pesticide applicators and pregnant women in an agricultural cohort who do not work on the field lie between occupational and general populations as they have a higher chance of exposure through proximity to where pesticides are applied as well as contact with pesticide residues brought into the home, though they are not in direct contact through mixing and applying these chemicals.[100] The only that study has compared to an occupational to a general population cohort found that levels were higher for the occupational cohort.[101]

To date, few studies have investigated predictors of pesticide exposure. Most studies focus on pregnant women and children from agricultural populations. In these studies, researchers have found that maternal age, race, birthplace, location of residence, and maternal smoking status were significant predictors.[102-105] One study that has investigated predictors of 3-phenoxybenzoic acid (3PBA) found that tobacco use, time spent gardening, and medication use were significant non-dietary predictors of 3-PBA. In this study, agricultural work was not a significant predictor of 3-PBA levels. The

authors also found that certain foods, such as bacon and raw spinach, were associated with 3-PBA levels; however, no patterns were apparent.[106]

Using data from the 1999-2010 National Health and Nutrition Examination Survey, our goal is to determine the predictors of the body burden of pesticide in the general adult population, primarily whether agricultural occupation is a significant predictor. Our secondary aim is to investigate the impact of specific food groups on body levels of pesticides.

## Methods

### Sample

Data for this study were collected by the Centers for Disease Control and Prevention (CDC) from 1999-2010 in six two-year cycles: 1999-2000, 2001-2002, 2003-2004, 2005-2006, 2007-2008, and 2009-2010. The NHANES utilizes a representative sample of the non-institutionalized population of the United States of all ages, recruiting around 5000 participants per year. Basic demographic information is collected along with data from medical assessments, laboratory tests, and questionnaires on health behaviors.[107] Information on pesticides was collected for a subsample of participants aged 6 years and older. For this study, we defined the eligible study population as participants aged 20 years old and older with available measurements on metabolites associated with endocrine disruption. This subsample was selected and weights were calculated in a manner that it remains representative of the national population. Weight formulas for each cycle combination are detailed in Figure 4-1. We created a domain variable to select for individuals aged 20 years old and older who had no missing information for the following variables: pesticide biomarker, agricultural worker status, age, gender, race, education, poverty index ratio, country of birth, home pesticide use,

and smoking status. Those who did have missing information were not deleted, but were placed in a separate domain category to utilize their weight information.

**Pesticide Measurements**

Several pesticide metabolites were measured in different years. For this study, we were interested in the pesticides also present in the Agricultural Health Study. We excluded pesticides that had over 40% of the sample with biomarkers below the limit of detection (LOD).[108] We made an exception for p,p'-DDT (45% below LOD). This metabolite, along with o,p'-DDT, p,p'-DDE, is indicative of DDT exposure. DDT has been banned since 1972, but the presence of p,p'-DDT metabolite indicates recent exposure; whereas the more persistent p,p'-DDE is indicative of historic exposures.[109] Table 4-2 shows the pesticides found in the AHS and their corresponding metabolites measured in the NHANES, as well as the cycles in which these metabolites were measured. We utilized four organochlorine pesticides measured from 1999-2010: heptachlor epoxide, p,p'-DDE, p,p'-DDT, and oxychlordane. We did not include trans-nonachlor in this study even though it did have a sufficient amount of individuals with levels above the limit of detection. Both oxychlordane and trans-nonachlor are products of exposure to the pesticide chlordane, but oxychlordane is the primary metabolite.[110] Dieldrin, another organochlorine pesticides was measured in two data cycles only, from 2001-2004. The organophosphate pesticide 3,5,6-trichloropyridinol and the non-specific organophosphate metabolites, dimethylphosphate, diethylphosphate, dimethylthiophosphate, diethylthiophosphate, dimethyldithiophosphate, and diethyldithiophosphate were measured 1999-2002 and 2007-2010. However, we only utilized data from the last two cycles due to our inability to properly calculate weights for data from non-adjacent years. Weights are constructed to be representative of the

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population at the midpoint of the combined period. Therefore, the midpoint of non-adjacent data cycles would include years in which information is missing. For the non-specific organophosphate metabolites, we created a composite measure: dialkyl phosphate (DAP). To create this variable, we first converted the six metabolites into nanomolar units by taking the concentration of each metabolite and dividing by its molecular weight, and multiplying by one thousand.[111] The resulting measure is in nanomoles per liter.

The pyrethroid 3-phenoxybenzoic acid is a product of an AHS pesticide, and sample levels had over 60% above the limit of detection. We chose to not include it in this study as a previous study has investigated its dietary and non-dietary predictors.[106]

Organochlorine pesticides were individually measured directly from serum samples. Details on handling, storage and handling of serum samples are detailed elsewhere.[112-114] Briefly, serum was collected from participants, stored at -20°C and analyzed using high-resolution gas chromatography/isotope dilution high-resolution mass spectrometry. Results were adjusted using concentrations of serum total cholesterol and triglycerides. Organophosphate pesticides were measured from urine samples. Spot urine samples were collected from participants, labeled, stored cold at 2-4°C or frozen until shipment for analysis.[57] Because urine was collected at different times of the day, pesticide levels may be affected by urine dilution. Therefore, we also used creatinine levels from the same urine samples to correct for this by adding it to each model containing a variable measured from urine samples.[111]

**Agricultural Occupation Status**

Occupation information was available from the questionnaire portion. The first question used was: "Thinking of all the paid jobs or businesses you ever had, what kind

of work were you doing the longest?" Participants provided job titles, which were then coded and grouped using the U.S. Census Bureau's Census Indexes of Industry and Occupations. We used the following occupation groups for definition of an agricultural occupation: "farm operators, managers, and supervisors, farm and nursery workers, and related agricultural, forestry, and fishing occupations" for data from 1999-2004 and "farming, fishing, forestry occupations" for data collected beginning with the 2005-2006 cycle. All others not belonging to these occupation groups were categorized as a non-agricultural worker.

**Covariates**

Data for potential covariates were collected during the interview portion of the NHANES.[107] Covariates considered for inclusion were age,[103,104] gender (male vs female),[115] race/ethnicity (non-Hispanic White, Mexican Americans, other), education (less than high school, high school graduate and above),[99] poverty-to-income ratio (PIR) (less than poverty level, 100-200% of poverty level, or 200% and above),[102,105] country of birth (United States, elsewhere),[99,102] smoking status (never smoker, former smoker, current smoker),[106] and home pesticide use (yes, no).[106] The variables race/ethnicity, education, PIR, and smoking status were recategorized because the small number of agricultural workers led to imprecise estimates in some categories.

**Dietary Variables**

Dietary data was collected during a 24-hour recall interview in which interviewers used measurement aids to collect information on all foods and beverages consumed in the previous day. Nutritional information, including grams consumed, was calculated for all reported foods[116]. We utilized each participant's individual food file to categorize consumption under the following food categories: "milk and milk products", "meat,

poultry, fish and mixtures", "legumes, nuts and seeds", "grain products", "fruits", and "vegetables". Foods were grouped via the first digit of each food's United States Department of Agriculture code.[117] We then calculated the total grams consumed within each group.

**Statistical Analysis**

For comparison of covariates, we utilized the entire sample from 1999-2010. Because we used different cycles and their number of missing information differed from each other, the number of individuals with information for each pesticide varied. The outcome variable, pesticide biomarker levels was skewed to the right; therefore, we utilized its natural log for all regression analyses. We calculated parameter estimates (PE) and 95% confidence intervals (95% CI) for both unadjusted models and adjusted model using linear regressions. We report the exponentiated parameter estimates that can be interpreted as the relative change in the ratio of the expected geometric mean of the pesticide biomarker for a one-unit increase in continuous predictor value, or relative change in ratio from the reference category for categorical predictors. For our continuous variable age, we report a rescaled parameter estimate to reflect the relative change for a ten-unit increase in age to ease in the interpretation of estimates. We then utilized this final model to test associations between individual dietary factors and above median levels in each pesticide group. Linear regression with a natural log-transformed outcome was also used for these analyses. Parameter estimates were rescaled to reflect a 100 gram relative change in the geometric mean.

Statistical analyses were conducted using SAS version 9.3. Because of the complex sampling design of the NHANES, analyses that accounted for clustering, weighting, and stratification were used (proc surveyfreq and proc surveyreg). We used

the weights to account for the subsample of total NHANES respondents who were tested for pesticide levels. Table 4-2 shows the calculation for sample weights for each group of data cycles used.

## Results

We had a total of 7,098 individuals available for inclusion in each group, of these only 177 were agricultural workers. Table 4-3 shows distribution for potential covariates in this group. In the agricultural worker group, the majority of respondents were male compared to non-agricultural workers (77.5% versus 47.9%). There was also a significantly higher proportion of Mexican American individuals in this group compared to non-agricultural workers (32.3% versus 7.7%). Individuals who reported a history of agricultural work more frequently reported not completing high school (48.7% versus 17.3%), were less frequently at the highest category of PIR (39.1% versus 68.1%), and were more frequently born outside of the United States (34.6% versus 10.7%) than non-agricultural workers. Agricultural workers reported home pesticide use in a higher proportion than non-agricultural workers (25.9% versus 13.8%).

Figure 4-1 to Figure 4-5 show geometric means of each metabolite for both agricultural and non-agricultural workers. Agricultural workers had statistically higher geometric means than non-agricultural workers for p,p'-DDT and p,p'-DDE only (Figure 4-1). Agricultural and non-agricultural workers had similar levels of all other pesticide biomarkers (Figures 4-2 and 4-3).

Home pesticide use was not associated with any of the pesticides; therefore, we did not include it in adjusted models. Table 4-4 shows the unadjusted and adjusted exponentiated parameter estimates for organochlorine pesticide metabolites. Agricultural work was associated with p,p'-DDT and p,p'-DDE levels only in unadjusted models, but

after adjustment associations became non-significant. After adjustment, a ten-year increase in age was statistically significantly associated with a 19% increase in heptachlor epoxide levels (95% CI: 1.16, 1.21), an 11% increase in metabolite levels (95% CI: 1.10, 1.13), 43% higher levels of p,p'-DDE (95% CI: 1.40, 1.46), 41% higher levels of oxychlordane (95% CI: 1.39, 1.44), and 16% higher levels of dieldrin (95% CI: 1.14, 1.17).

Being of Mexican American descent was associated with 1.20 times higher levels of heptachlor epoxide than non-Hispanic Whites (95% CI: 1.06, 1.35). For p,p'-DDT, Mexican Americans and other races had 47% (95% CI: 1.24, 1.75) and 31% (95% CI: 1.17, 1.47) higher levels of p,p'-DDT than non-Hispanic Whites, respectively. Being Mexican American and of other race/ethnicities was associated with 2.8 times (95% CI: 2.35, 3.34) and 1.64 times higher levels of p,p'-DDE than non-Hispanic Whites (95% CI: 1.46, 1.83), respectively. Individuals belonging to other races had 13% higher oxychlordane levels than non-Hispanic Whites (95% CI: 1.05, 1.21).

Those with less than a high school education had 11% higher levels of p,p'-DDT (95% CI: 1.01, 1.22) and 8% higher levels of oxychlordane (95% CI: 1.01, 1.15) than those with more than a high school education. Individuals who were below the poverty level had p,p'-DDT levels that were 1.16 times higher than those 200% above the poverty level (95% CI: 1.03, 1.32).

Those who were born elsewhere had 20% lower levels of heptachlor epoxide than those who were born in the United States (95% CI: 0.73, 0.87). Being born outside of the U.S. was also associated with a 27% decrease in oxychlordane levels  (95% CI: 0.66, 0.80), and a 9% decrease in dieldrin levels (95% CI: 0.84, 0.99). Foreign birth;

however, was associated with increased levels of both p,p'-DDT (PE: 1.48, 95% CI: 2.26, 1.73) and p,p'-DDE (PE: 1.65, 95% CI: 1.38, 1.97).

Individuals who reported being former smoker had 0.91 times the p,p'-DDT levels than those who never smoked (95% CI: 0.84, 0.97). Current smokers had 0.86 times the p,p'-DDT levels than nonsmokers (95% CI: 0.86, 0.93). Both former and current smokers had 10% lower levels of dieldrin than those who never smoked (95% CI: 0.84, 0.97 and 0.83, 0.99, respectively). Current smoking status was associated with an increase of 12% in oxychlordane levels (95% 1.07, 1.18).

Table 4-5 displays the unadjusted and adjusted parameter estimates for the organophosphate pesticides 3,5,6-trichloropyridinol and total dialkyl phosphate. Age was the only predictor associated with 3,5,6-trichloropyridinol after adjusting for all other covariates. A ten-year increase in age was associated with a 10% increase in metabolite levels (95% CI: 1.07. 1.14). Age also showed a 10% increase in total DAP levels for each ten year increase (95% CI: 1.07, 1.13). Female gender was associated with a 13% increase (95% CI: 1.02, 1.25) and foreign birth was associated with a 21% increase (95% CI: 1.05, 1.39) in total DAP. Current smoking status was associated with 0.81 times lower DAP levels than never smokers (95% CI: 0.72, 0.90).

Dietary variables were only associated with organophosphate pesticides (Table 4-7). For both 3,5,6-trichloropyridinol and DAP, a 100 gram increase in vegetable consumption was associated with 2% (95% CI: 1.01, 1.04) and 7% (95% CI: 1.07, 1.11) increase in metabolite levels, respectively.

## Discussion

In this study, we found that with the exception of DDT and DDE, agricultural work does not predict high levels of pesticide metabolites, and in fact, metabolite levels were

fairly similar between the two populations. Agricultural work was associated with both DDT and DDE in univariate models, but this association disappeared after adjusting for several demographic predictors, including age, race, and country of birth. This finding, though counterintuitive since agricultural populations are in direct contact with pesticides, is not necessarily surprising. The OC pesticides included in this study have been banned or discontinued since 1972.[23] This indicates that currently agricultural work may be a less important risk factor than being born outside of the U.S., where these pesticides are still in use, or race. OP pesticides are not persistent in the body, and a presence of DAP metabolites reflect exposure within a few days.[118] Therefore, it may be difficult in a survey such as the NHANES to measure the precise moment pesticides were applied. For all pesticides, however, we did find that some demographic and dietary factors did predict metabolite levels.

We found that age was positively associated with all organochlorine pesticides tested. Our results add to the growing body of evidence that age is perhaps one of the most consistent predictors of organochlorine pesticide use.[104,119,120] Organochlorine pesticides bioaccumulate in the body and are persistent.[23] Therefore it is logical that with increasing age, there is increasing body burdens of these pesticides. Additionally older individuals are more likely to have been exposed to increasing amount of these pesticides before their use was discontinued.[23] We found that country of birth was also associated with all OC pesticides, but direction of association was not consistent across pesticides. For heptachlor epoxide, oxychlordane, and dieldrin, non-U.S. country of birth was inversely associated with metabolite levels. This is in part consistent with a study that found that levels of chlordane were lower for Asian women, who were, for the most part, immigrants.[105] For DDT and DDE, foreign birth was associated with an increase in

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pesticide levels. The results for these two chemicals are consistent with the literature. One study done in a pregnant Latina population from a U.S. agricultural area found that risk factors for increase serum concentrations of DDT and DDE were time spent outside of the U.S. and coming from an area of Central American with recent OC pesticide use.[102] In another study of pregnant women in New York City, age and foreign birth were predictors of increased levels of DDE.[119]

Race/ethnicity was a significant predictor for heptachlor epoxide, DDT, and DDE even after adjusting for foreign birth. For all three pesticides, being Mexican Americans was associated with having higher levels of pesticide metabolites than non-Hispanic Whites. Studies on the CHAMACOS cohort in Salinas Valley have found that Mexican Americans have higher pesticide levels than women in the general population.[99,102] Additionally, we found that other race/ethnicities were associated with having higher levels of DDT and DDE.

Smoking status was associated with a decrease in the levels of DDT and dieldrin, but an increase in oxychlordane for current smokers only. Previous studies have found that smoking status was associated with an increase in DDT levels.[105] We believe that weight may play a role in the inverse patterns seen. OC pesticides are stored in fat, which become bioavailable and is excreted with weight loss.[23] Smokers tend to weigh less than nonsmokers.[121] Therefore, it is possible that historical exposure to pesticides has already been eliminated from the body for former and current smokers due to weight loss.

Our study did not find any dietary predictors associated with OC pesticides. This is in conflict with some previous studies that have found consumption of fish, fruit, and red meat was associated with increasing levels of OC pesticide.[119,120] Additionally,

studies have found measurable levels of OC pesticides in milk products. In Spain, hexachlorobenzene and chlordane were found in all samples of both organic and regular milk. DDE was also found in 80% of milk samples.[122] Composite food samples taken from supermarkets in Dallas, TX found low levels of DDEs in whole milk yogurt.[54] The absence of an association may be due to the fact that dietary data reflects recent eating patterns, which may not be relevant for historical organochlorine exposures.

To our knowledge, our study is the first to investigate predictors of organophosphate pesticides. In our study, we find that for organophosphate pesticides, age was a significant predictor for both 3,5,6-trichloropyridinol and total DAP. We also found that for DAP, female gender and foreign birth were associated with increases in DAP levels, but current smoking status was associated with a decrease in DAP. It is unclear why these predictors are associated with DAP levels, after adjusting for other variables. We also found that vegetable consumption was the only dietary variable associated with both organophosphate metabolites. This class of pesticides is frequently used in agriculture and in livestock facilities, and for most of the population, exposure comes from diet.[118,123] Our results indicate, that dietary consumption may be an important source of organophosphate exposure, and that individuals may want to take extra care to properly rinse and cook vegetables to reduce amount of pesticide consumed. We tested for any interactions between these predictors and diet, in an attempt to find an explanation seen for gender and foreign birth. There were no significant effect modifications (data not shown); therefore, associations cannot be explained by diet. Future studies should investigate if there are any health behaviors that can explain the increased exposure in these two groups.

Smoking status was associated with a decrease in DAP levels. There is no evidence in the literature to explain this association for this class of pesticides. One possible explanation is that smoking affects the expression of CYP enzymes involved in the metabolism of xenobiotics, which can lead to current smokers metabolizing these chemicals faster than nonsmokers.[124]

Our study has several limitations. First, and perhaps the most important, is that it is a cross-sectional dataset. This prevents us from ascertaining that certain exposures (diet, home pesticide use) preceded the outcome (pesticide body burden), and in the case of OC pesticides, this will most likely not be true. The inability to ascertain temporality is an issue for the examination of potential sources of pesticide exposure, as we cannot say with certainty that we believe a predictor could have led to the amount of pesticide found in the body because we do not know if it occurred before or after the pesticide exposure. For instance, OC pesticides are no longer in use but they persist in the body for many years. Thus, measured levels will be reflective of exposure that occurred quite some time before. Another limitation is that we rely on self-report for many variables. This is especially problematic for dietary variables. We were also unable to exclusively select agricultural workers who handle pesticides or who may be in direct contact with them. The occupational categories used also included workers from the forestry industry, who may not be exposed at all. Similarly, we had very small numbers of agricultural workers, which made us unable to investigate their predictors separately from the general population. Last, we performed several tests, which increase our chance of finding a significant relationship when there is none. However, because our goal is to explore predictors, it is recommended that no corrections are made to avoid erroneously missing important predictors.[125]

Our study had several strengths as well. First, we have biomarkers of pesticide exposure. Because non-occupational individuals may not realize or remember they are exposed to certain pesticides, biomarkers give us an objective measurement without relying on an individual's memory. Second, the NHANES oversamples Mexican Americans, which is an important population for pesticide exposure.[102] Last, the NHANES is representative of the general population of the United States, which means that all our results are generalizable to the entire country.

**Summary**

Our results show that agricultural workers were no more likely to have increased body burdens of pesticides than non-agricultural workers, with the exception of DDT and DDE. We also found that age, gender, race/ethnicity, country of birth, smoking status, and vegetable consumption were significantly associated with above median levels of endocrine-disrupting pesticides.

Future studies should focus on health behaviors associated with pesticide exposure, especially for the non-persistent organophosphates since they are still in use in US. Interventions can be targeted towards individuals who are at risk to mediate health effects associated with pesticide exposure, as well as to prevent future exposures, such as education to reduce exposure specifically targeted for Mexican Americans.

Table 4-1. Calculation of sample weights per groups of data cycle.

| Cycles | | Calculation |
|---|---|---|
| 1999-2004 | | |
| | 1999-2000 | WTSPO6YR = (2/3)*WTSPO4YR |
| | 2001-2002 | WTSPO6YR = (2/3)*WTSPO4YR |
| | 2003-2004 | WTSPO6YR = (1/3)* WTSB2YR |
| 2001-2004 | | |
| | 2001-2002 | WTSPO4YR = (1/2)*WTSPO2YR |
| | 2003-2004 | WTSPO4YR = (1/2)* WTSB2YR |
| 2007-2010 | | |
| | 2007-2008 | WTSC4YR = (1/2)* WTSC2YR |
| | 2009-2010 | WTSC4YR = (1/2)* WTSC2YR |
| 1999-2008 | | |
| | 1999-2000 | WTSPP10YR = (2/5)*WTSPP4YR |
| | 2001-2002 | WTSPP10YR = (2/5)*WTSPP4YR |
| | 2003-2004 | WTSPP10YR = (1/5)* WTSC2YR |
| | 2005-2006 | WTSPP10YR = (1/5)* WTSC2YR |
| | 2007-2008 | WTSPP10YR = (1/5)* WTSC2YR |
| 1999-2010 | | |
| | 1999-2000 | WTSPP12YR = (1/3)*WTSPP4YR |
| | 2001-2002 | WTSPP12YR = (1/3)*WTSPP4YR |
| | 2003-2004 | WTSPP12YR = (1/6)* WTSC2YR |
| | 2005-2006 | WTSPP12YR = (1/6)* WTSC2YR |
| | 2007-2008 | WTSPP12YR = (1/6)* WTSC2YR |
| | 2009-2010 | WTSPP12YR = (1/6)* WTSC2YR |

Table 4-2. Endocrine disrupting pesticides from the AHS and corresponding NHANES metabolites by cycle measured.

| AHS Pesticide | NHANES Pesticide/Metabolite Name | Cycle Measured | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1999-2000 | 2001-2002 | 2003-2004 | 2005-2006 | 2007-2008 | 2009-2010 |
| Lindane | Gamma-hexachlorocyclohexane | X | X | X | X* | X* | |
| Heptachlor | Heptachlor Epoxide (HPE)^ | X | X | X | X* | X* | |
| | o,p'-DDT | X | X | X | X* | X* | |
| DDT | p,p'-DDT | X | X | X | X* | X* | |
| | p,p'-DDE^ | X | X | X | X* | X* | |
| Chlordane | Oxychlordane^ | X | X | X | X* | X* | |
| | Trans-nonachlor^ | X | X | X | X* | X* | |
| Dieldrin | Dieldrin^ | | X | X | | | |
| Aldrin | Aldrin | | X | X | | | |
| 2,4-D | 2,4-D | X | X | X | | X | X |
| Chlorpyrifos | 3,5,6-trichloropyridinol^ | X | X | # | | X | X |
| | Dimethylphosphate+ | X | X | X | X | X | |
| Parathion, | Diethylphosphate+ | X | X | X | X | X | |
| Diazinon, | Dimethylthiophosphate+ | X | X | X | X | X | |
| Chlorpyrifos, | Diethylthiophosphate+ | X | X | X | X | X | |
| Malathion | Dimethyldithiophosphate+ | X | X | X | X | X | |
| | Diethyldithiophosphate+ | X | X | X | X | X | |
| | cis-chlorovinyl-dimeth prop carboacid | X | X | # | | | |
| Permethrin | trans-dichlorovnl-dimeth prop carboacid | X | X | # | | X | X |
| | 3-phenoxybenzoic acid^ | X | X | # | | X | X |

*Available for pooled samples only
^Percentage >LOD greater than or equal to 60%
+Percentage > LOD not available
#Recalled for the 2003-2004 cycle

Table 4-3. Weighted percentages and means of demographic characteristics of U.S. adults age 20 years and older by agricultural worker status from the NHANES 1999-2010.

| | Agricultural Worker % or mean (95% CI) | | p-value |
| --- | --- | --- | --- |
| | Yes (n=177) | No (n=6921) | |
| Age | 42.8 (40.4, 45.2) | 44.6 (44.0, 45.2) | 0.34 |
| Gender | | | <0.0001 |
| Male | 77.5 (69.6, 85.3) | 47.9 (46.7, 49.2) | |
| Female | 22.5 (14.7, 30.4) | 52.1 (50.8, 53.3) | |
| Race/Ethnicity | | | <0.0001 |
| Non-Hispanic White | 53.2 (42.3, 64.2) | 73.5 (71.0, 76.0) | |
| Mexican American | 32.3 (22.7, 42.1) | 7.7 (6.3, 9.0) | |
| Other | 14.3 (8.4, 20.2) | 18.8 (16.9, 20.7) | |
| Education | | | <0.0001 |
| Less than High School | 48.7 (37.2, 60.3) | 17.3 (16.1, 18.5) | |
| High School Graduate and Above | 51.3 (39.7, 62.8) | 82.7 (81.5, 83.9) | |
| PIR | | | <0.0001 |
| At or Less than Poverty Level | 35.3 (26.8, 43.8) | 12.3 (11.2, 13.3) | |
| 100-200% of Poverty Level | 25.6 (18.4, 32.9) | 19.7 (18.3, 21) | |
| 200% of Poverty Level and Above | 39.1 (27.8, 50.4) | 68.1 (66.3, 69.8) | |
| Country of Birth | | | <0.0001 |
| United States | 65.4 (55.8, 74.9) | 89.3 (87.8, 90.8) | |
| Elsewhere | 34.6 (25.1, 44.2) | 10.7 (9.2, 12.2) | |
| Home Pesticide Use | | | 0.0004 |
| No | 72.9 (63.9, 81.8) | 85.9 (84.7, 87.2) | |
| Yes | 27.1 (18.2, 36.1) | 14.1 (12.8, 15.3) | |
| Smoking Status | | | 0.19 |
| Never Smoker | 48.7 (38.1, 59.4) | 51.5 (49.8, 53.2) | |
| Former Smoker | 19.4 (10.8, 28) | 24.2 (22.9, 25.5) | |
| Current Smoker | 31.9 (23, 40.8) | 24.3 (22.8, 25.8) | |

Table 4-4. Unadjusted and adjusted exponentiated parameter estimates* for potential predictors by organochlorine pesticide metabolite in adults aged 20 years and older in the National Health and Nutrition Examination Survey 1999-2004.

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| Heptachlor Epoxide | | | | |
| | Agricultural Worker | Yes | 1.11 (0.94, 1.30) | 1.07 (0.89, 1.28) |
| | | No | 1.00 (referent) | 1.00 |
| | Age | 10-year increase | 1.18 (1.16, 1.21) | 1.19 (1.16, 1.21) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 1.01 (0.95, 1.07) | 0.97 (0.92, 1.03) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 0.96 (0.87, 1.06) | 1.20 (1.06, 1.35) |
| | | Other | 0.95 (0.86, 1.04) | 1.08 (0.98, 1.19) |
| | Education | Less than High School | 1.18 (1.10, 1.25) | 1.05 (0.98, 1.13) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 1.02 (0.90. 1.16) | 1.10 (0.98, 1.24) |
| | | 100-200% of Poverty Level | 1.09 (0.99, 1.19) | 1.06 (0.98, 1.16) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 0.83 (0.76, 0.92) | 0.80 (0.73, 0.87) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.02 (0.92, 1.13) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 1.12 (1.05, 1.20) | 0.98 (0.92, 1.04) |
| | | Current Smoker | 0.94 (0.87, 1.02) | 0.96 (0.89, 1.04) |

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Table 4-4. Continued

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| p,p'-DDT | | | | |
| | Agricultural Worker | Yes | 1.33 (1.09, 1.62) | 1.12 (0.96, 1.30) |
| | | No | 1.00 | 1.00 |
| | Age | 10-year increase | 1.09 (1.07, 1.11) | 1.11 (1.10, 1.13) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 1.07 (1.02, 1.13) | 1.04 (0.99, 1.09) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 1.82 (1.58, 2.09) | 1.47 (1.24, 1.75) |
| | | Other | 1.47 (1.32, 1.63) | 1.31 (1.17, 1.47) |
| | Education | Less than High School | 1.42 (1.29, 1.55) | 1.11 (1.01, 1.22) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 1.30 (1.14, 1.49) | 1.16 (1.03, 1.32) |
| | | 100-200% of Poverty Level | 1.22 (1.10, 1.35) | 1.09 (0.98, 1.21) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 1.82 (1.60, 2.09) | 1.48 (1.26, 1.73) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.01 (0.92, 1.10) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 0.94 (0.87, 1.02) | 0.91 (0.84, 0.97) |
| | | Current Smoker | 0.82 (0.75, 0.89) | 0.86 (0.79, 0.93) |

Table 4-4. Continued

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| p,p'-DDE | | | | |
| | Agricultural Worker | Yes | 1.43 (1.14, 1.78) | 1.14 (0.97, 1.35) |
| | | No | 1.00 | 1.00 |
| | Age | 10-year increase | 1.37 (1.35, 1.40) | 1.43 (1.4, 1.46) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 1.12 (1.03, 1.22) | 1.07 (1.00, 1.15) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 2.78 (2.4, 3.21) | 2.80 (2.35, 3.34) |
| | | Other | 1.62 (1.44, 1.82) | 1.64 (1.46, 1.83) |
| | Education | Less than High School | 1.77 (1.58, 1.98) | 1.11 (0.99, 1.25) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 1.14 (0.93, 1.39) | 0.97 (0.84, 1.12) |
| | | 100-200% of Poverty Level | 1.23 (1.07, 1.42) | 1.00 (0.90, 1.11) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 2.24 (1.88, 2.66) | 1.65 (1.38, 1.97) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.00 (0.90, 1.11) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 1.17 (1.04, 1.32) | 0.95 (0.85, 1.05) |
| | | Current Smoker | 0.86 (0.77, 0.97) | 1.02 (0.93, 1.12) |

Table 4-4. Continued

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| Oxychlordane | | | | |
| | Agricultural Worker | Yes | 1.01 (0.87, 1.16) | 1.05 (0.89, 1.22) |
| | | No | 1.00 | 1.00 |
| | Age | 10-year increase | 1.41 (1.38, 1.44) | 1.41 (1.39, 1.44) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 1.11 (1.05, 1.18) | 1.05 (1.00, 1.10) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 0.70 (0.62, 0.77) | 1.10 (0.99, 1.22) |
| | | Other | 0.87 (0.80, 0.95) | 1.13 (1.05, 1.21) |
| | Education | Less than High School | 1.26 (1.12, 1.41) | 1.08 (1.01, 1.15) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 0.84 (0.75, 0.93) | 0.96 (0.89, 1.04) |
| | | 100-200% of Poverty Level | 1.07 (0.95, 1.21) | 1.02 (0.94, 1.1) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 0.69 (0.62, 0.76) | 0.73 (0.66, 0.80) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.01 (0.90, 1.12) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 1.31 (1.2, 1.43) | 0.99 (0.93, 1.06) |
| | | Current Smoker | 1.03 (0.96, 1.10) | 1.12 (1.07, 1.18) |

Table 4-4. Continued

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| Dieldrin^ | | | | |
| | Agricultural Worker | Yes | 1.00 (0.83, 1.20) | 0.99 (0.83, 1.18) |
| | | No | 1.00 | 1.00 |
| | Age | 10-year increase | 1.15 (1.14, 1.17) | 1.16 (1.14, 1.17) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 0.88 (0.81, 0.96) | 0.86 (0.80, 0.93) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 0.83 (0.77, 0.90) | 1.01 (0.89, 1.13) |
| | | Other | 0.94 (0.85, 1.04) | 1.05 (0.94, 1.17) |
| | Education | Less than High School | 1.03 (0.96, 1.10) | 1.01 (0.94, 1.08) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 0.85 (0.77, 0.95) | 0.95 (0.85, 1.08) |
| | | 100-200% of Poverty Level | 0.94 (0.87, 1.02) | 0.95 (0.89, 1.02) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 0.88 (0.80, 0.96) | 0.91 (0.84, 0.99) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.00 (0.92, 1.08) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 1.03 (0.96, 1.12) | 0.90 (0.84, 0.97) |
| | | Current Smoker | 0.88 (0.80, 0.96) | 0.90 (0.83, 0.99) |

*Parameter estimates were exponentiated and are interpreted as the relative change in the ratio of the expected geometric mean of the pesticide biomarker for a one-unit increase in predictor value (continuous) or relative change in ratio from the reference category (categorical).
^2001-2004 cycles

Table 4-5. Unadjusted and adjusted exponentiated parameter estimates* for potential predictors by organophosphate pesticide metabolite in adults aged 20 years and older in the National Health and Nutrition Examination Survey 1999-2010.

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| 3,5,6-trichloropyridinol^ | | | | |
| | Agricultural Worker | Yes | 1.20 (0.88, 1.64) | 1.16 (0.85, 1.58) |
| | | No | 1.00 (referent) | 1.00 |
| | Age | 10-year increase | 1.11 (1.07, 1.14) | 1.10 (1.07, 1.14) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 1.04 (0.97, 1.12) | 1.05 (0.97, 1.14) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 0.94 (0.79, 1.12) | 1.01 (0.81, 1.28) |
| | | Other | 0.92 (0.79, 1.06) | 0.95 (0.82, 1.09) |
| | Education | Less than High School | 0.98 (0.83, 1.15) | 0.97 (0.82, 1.14) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 0.92 (0.79, 1.06) | 1.02 (0.86, 1.21) |
| | | 100-200% of Poverty Level | 0.96 (0.84, 1.1) | 0.97 (0.85, 1.11) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 0.95 (0.78, 1.16) | 1.00 (0.78, 1.26) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.14 (0.98, 1.32) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 1.07 (0.93, 1.23) | 0.99 (0.86, 1.15) |
| | | Current Smoker | 0.85 (0.76, 0.95) | 0.89 (0.80, 0.99) |

Table 4-5. Continued

| Metabolite | Variable | | Unadjusted Parameter Estimate (95% CI) | Adjusted Parameter Estimate (95% CI) |
|---|---|---|---|---|
| DAP$^{\#}$ | | | | |
| | Agricultural Worker | Yes | 0.94 (0.71, 1.25) | 0.96 (0.72, 1.29) |
| | | No | 1.00 | 1.00 |
| | Age | 10-year increase | 1.10 (1.08, 1.13) | 1.10 (1.07, 1.13) |
| | Gender | Male | 1.00 | 1.00 |
| | | Female | 1.12 (1.01, 1.23) | 1.13 (1.02, 1.25) |
| | Race | Non-Hispanic White | 1.00 | 1.00 |
| | | Mexican American | 1.05 (0.94, 1.16) | 1.03 (0.91, 1.17) |
| | | Other | 0.92 (0.83, 1.02) | 0.90 (0.81, 1.00) |
| | Education | Less than High School | 0.91 (0.82, 1.02) | 0.88 (0.79, 0.99) |
| | | High School Graduate and Above | 1.00 | 1.00 |
| | PIR | ≤ Poverty Level | 1.00 (0.88, 1.12) | 1.12 (1.00, 1.27) |
| | | 100-200% of Poverty Level | 0.95 (0.85, 1.05) | 0.99 (0.88, 1.11) |
| | | ≥ 200% of Poverty Level | 1.00 | 1.00 |
| | Country of Birth | United States | 1.00 | 1.00 |
| | | Elsewhere | 1.12 (1.00, 1.27) | 1.21 (1.05, 1.39) |
| | Home Pesticide Use | No | 1.00 | |
| | | Yes | 1.05 (0.92, 1.18) | |
| | Smoking Status | Never Smoker | 1.00 | 1.00 |
| | | Former Smoker | 1.10 (0.98, 1.23) | 1.04 (0.94, 1.16) |
| | | Current Smoker | 0.77 (0.69, 0.85) | 0.81 (0.72, 0.9) |

*Parameter estimates were exponentiated and are interpreted as the relative change in the ratio of the expected geometric mean of the pesticide biomarker for a one-unit increase in predictor value (continuous) or relative change in ratio from the reference category (categorical).
^2007-2010 data cycles
$^{\#}$1999-2008 data cycles

Table 4-6. Adjusted exponentiated parameter estimates* for the association between dietary predictors by organochlorine pesticide metabolite in adults aged 20 years and older in the National Health and Nutrition Examination Survey 1999-2010.

| Variable | Adjusted Parameter Estimate (95% CI) | | | | |
|---|---|---|---|---|---|
| | Heptachlor Epoxide | p,p'-DDT | p,p'-DDE | Oxychlordane | Dieldrin |
| Milk and Milk Products | 1.00 (0.99, 1.00) | 0.99 (0.98, 1.00) | 0.98 (0.97, 1.00) | 1.00 (0.99, 1.01) | 1.00 (0.99, 1.01) |
| Meat, Poultry, Fish and Mixtures | 1.01 (0.99, 1.02) | 1.00 (0.98, 1.02) | 1.00 (0.98, 1.02) | 1.01 (0.99, 1.02) | 1.00 (0.99, 1.02) |
| Legumes, Nuts, and Seeds | 1.01 (0.97, 1.05) | 0.99 (0.95, 1.03) | 1.01 (0.96, 1.07) | 1.04 (0.99, 1.09) | 1.01 (0.96, 1.06) |
| Grain Products | 0.98 (0.96, 1.01) | 0.99 (0.96, 1.02) | 0.99 (0.95, 1.02) | 0.99 (0.96, 1.01) | 0.98 (0.96, 1.00) |
| Fruits | 0.99 (0.98, 1.00) | 0.99 (0.98, 1.00) | 0.99 (0.98, 1.00) | 0.99 (0.98, 1.00) | 1.00 (0.99, 1.01) |
| Vegetables | 0.99 (0.98, 1.00) | 1.01 (1.00, 1.02) | 1.01 (1.00, 1.03) | 1.00 (0.99, 1.01) | 1.00 (0.99, 1.02) |

Table 4-7. Adjusted exponentiated parameter estimates* for the association between dietary predictors by organophosphate pesticide metabolite in adults aged 20 years and older in the National Health and Nutrition Examination Survey 1999-2010.

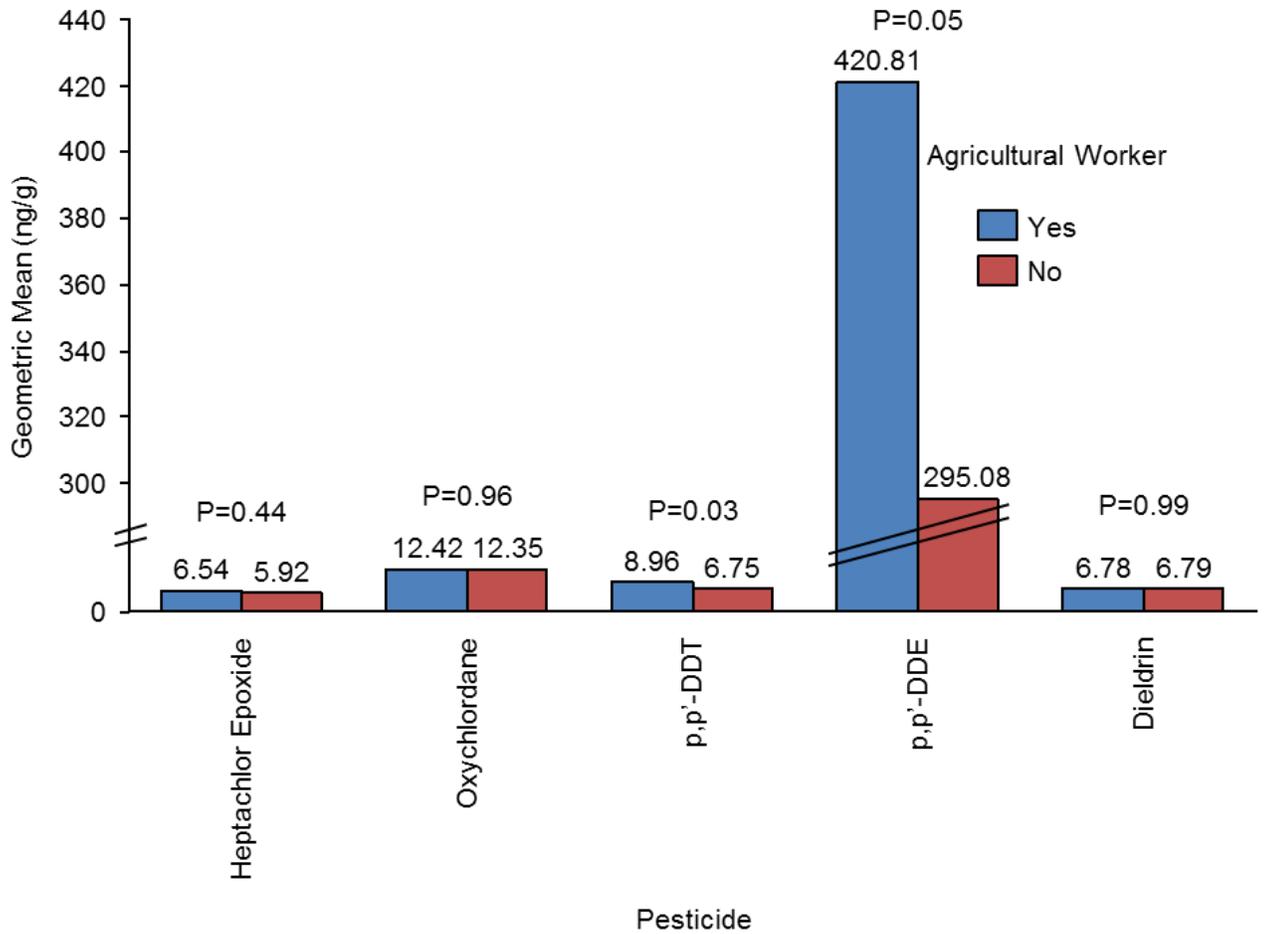| Variable | Adjusted Parameter Estimate (95% CI) | |
| --- | --- | --- |
| | 3,5,6-trichloropyridinol | DAP |
| Milk and Milk Products | 1.00 (0.98, 1.02) | 0.99 (0.98, 1.00) |
| Meat, Poultry, Fish and Mixtures | 0.97 (0.95, 0.99) | 0.99 (0.97, 1.01) |
| Legumes, Nuts, and Seeds | 0.92 (0.83, 1.03) | 1.05 (0.98, 1.14) |
| Grain Products | 1.03 (0.98, 1.08) | 1.04 (0.99, 1.09) |
| Fruits | 1.02 (0.99, 1.05) | 1.00 (0.99, 1.02) |
| Vegetables | 1.02 (1.01, 1.04) | 1.09 (1.07, 1.11) |

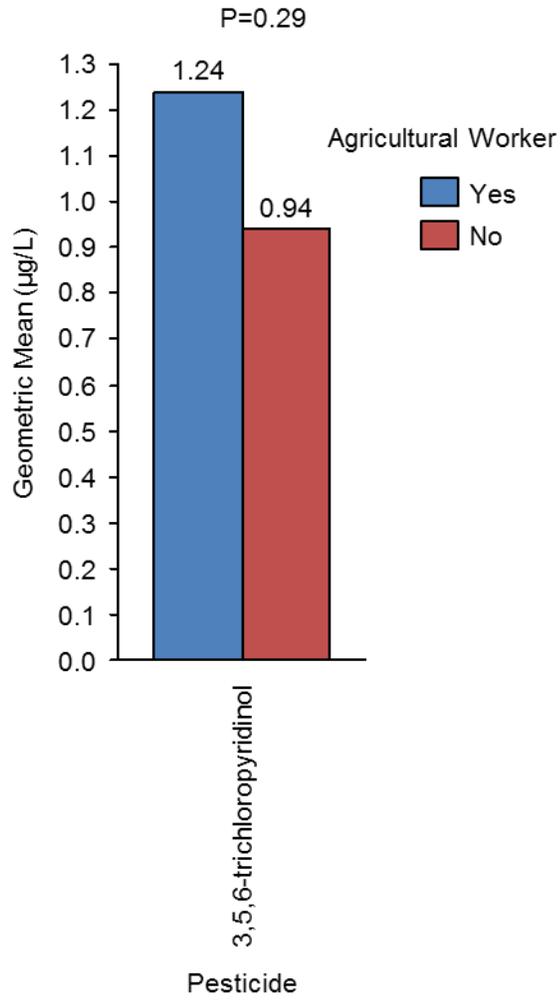Figure 4-1. Weighted geometric means of organochlorine pesticides by agricultural worker status (Yes/No)

Figure 4-2. Weighted geometric means of 3,5,6-trichloropyridinol by agricultural worker status (Yes/No)
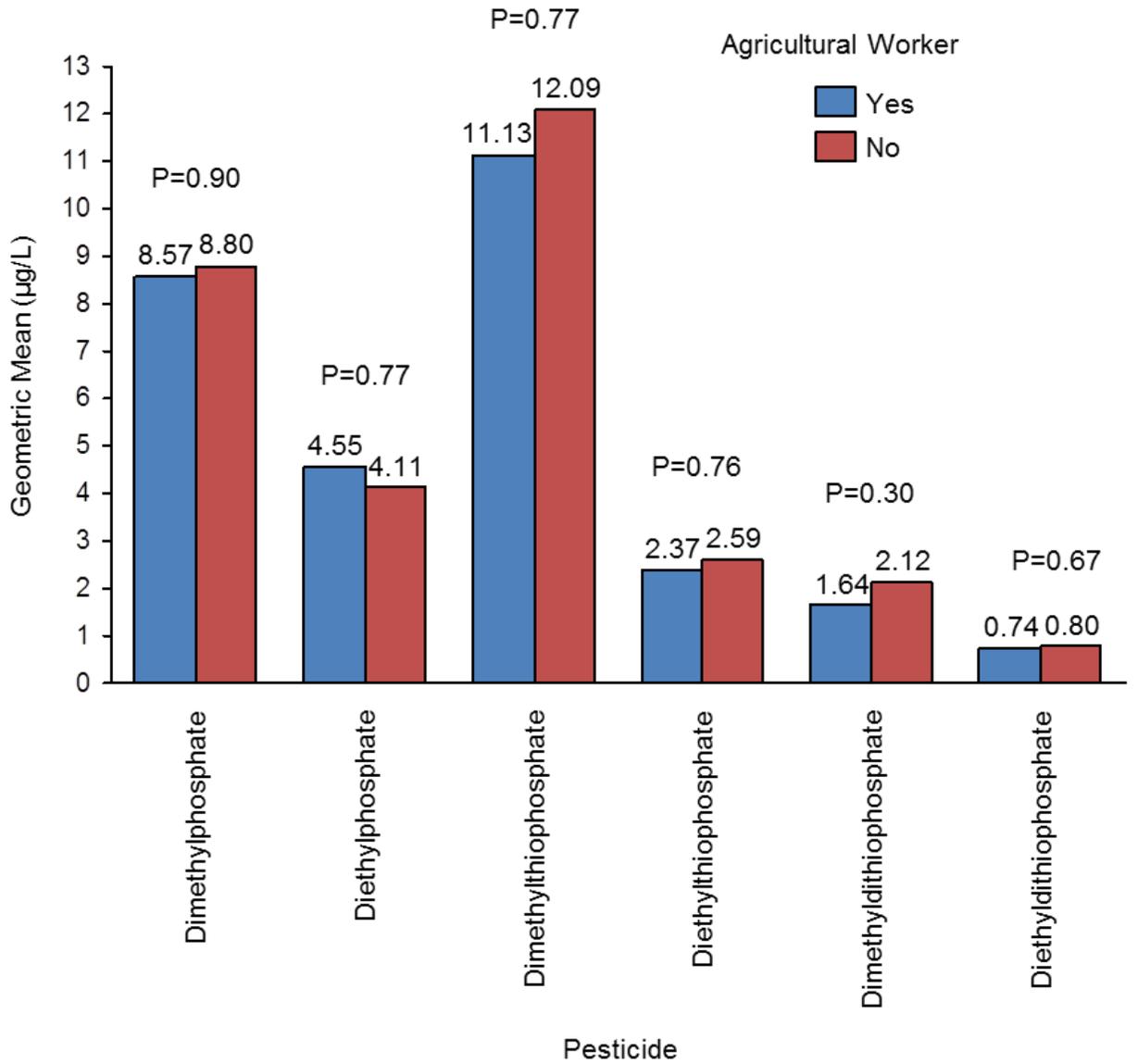
Figure 4-3. Weighted geometric means of dialkyl phosphate pesticides by agricultural
worker status (Yes/No)

CHAPTER 5
CONCLUSION

**Introduction**

Obesity is an increasing epidemic throughout the world and has been proven to be an important risk factor for a number of diseases including diabetes, cardiovascular disease, and cancer.[1] Though genetic and behavioral factors, such as lack of physical activity and poor diet, are traditionally accepted as factors driving this epidemic, these alone do not fully explain trends observed worldwide.[6] Therefore, in recent years, scientist have looked elsewhere for explanations and found that certain chemical and environmental factors, collectively called obesogens, can disrupt the endocrine system and affect weight gain.[6,92] Several pesticides have been found to be endocrine disrupting and have been thought of as putative obesogens.[22] There is, however, little evidence to cement the role of pesticides as obesogens, and more research is necessary to clarify this relationship.

The primary goal of this dissertation was to evaluate the influence of endocrine disrupting pesticides on body weight. The secondary goal was to determine whether agricultural work was a significant predictor of body burdens of endocrine-disrupting pesticides, and what dietary and non-dietary factors are predictive in a general population. These goals were accomplished via three specific aims. Aim 1 evaluated the impact of endocrine disrupting pesticides on BMI within an occupational cohort. Aim 2 sought to assess the exposure trend relationship between pesticide exposure and BMI outcome among farmers and commercial applicators. Aim 3 investigated whether being an agricultural worker was a predictor of biomarkers of endocrine-disrupting pesticides in the general population.

**Summary of Findings**

In Chapter 2 we discuss our study that investigated the impact of endocrine disrupting pesticides on BMIs in the range of overweight/obesity in an agricultural population. Using a longitudinal dataset, we found that pesticides in the estrogenic, anti-estrogenic, anti-androgenic, thyroid disrupting, and PXR agonist categories were positively associated with overweight/obesity. We found no significant associations with the pesticide in the "other" category, which has unspecified endocrine disrupting properties. We then delved further into associations and evaluated the impact of gender on these results. We found that estrogenic pesticides were inversely associated with high BMI for men, and positively associated with high BMI for women. In this study we only focused on establishing the existence of a relationship and to investigate gender effects.

Therefore, in Chapter 3 we investigated whether a relationship between increasing amounts of pesticides and increasing BMI existed, and whether the presence of one pesticide modified the association of another. To do this we utilized intensity-adjusted cumulative exposure scores, a measure of pesticide exposure calculated by AHS investigators that takes into consideration length and frequency of use, application methods, repair of application equipment, and use of personal protective equipment. This measure was not calculated for spouses of applicators; therefore, this sample consisted primarily of male participants. We found positive trends for anti-estrogenic, anti-androgenic, and thyroid disrupting pesticides. Interestingly, we saw the same inverse association as seen in Chapter 2, and an inverse trend for estrogenic pesticides, meaning that with increasing intensity-adjusted cumulative exposure scores, odds of overweight/obesity decreased. We found only one statistically significant

interaction between pesticides. However, some interesting patterns emerged within certain interactions. We found that in the presence of an above median score in the anti-androgen category, the protective effects of any category of estrogenic pesticide group score were reversed. Additionally, we found that a weak association between anti-estrogenic pesticides and BMI was strengthened in the presence of anti-androgenic pesticides.

Together, results from Chapters 2 and 3 provided compelling evidence that endocrine-disrupting pesticides may lead to overweight/obesity in an occupational population. Though this group of individuals is thought to have significantly increased exposures,[22] the general population is not immune from exposure to pesticides from a variety of sources.[101] Therefore, we wanted to investigate whether conclusions made in Chapters 2 and 3 could also apply to the general public. In Chapter 4, we sought to compare levels of pesticides in agricultural and non-agricultural workers, as well as describe whether a history of agricultural work was predictive of body burdens of endocrine disrupting pesticides using the general population NHANES dataset. In this study, we found that being an agricultural worker was not a significant predictor of pesticide levels. Our results also showed that increased age, race/ethnicity, and foreign birth were associated with increased levels of pesticides. Additionally, we found that consumption of vegetables were associated with higher levels of organophosphate pesticides.

## Limitations

Our results are subject to several limitations. Studies from Chapters 2 and 3 utilized the AHS dataset, which relied on self-report for the collection of the majority of data. However, as previously mentioned, questions on pesticide exposure and other

covariates showed good reliability between repeated assessments of the AHS instrument.[89] Second, the AHS dataset comes from a longitudinal study, and as such, is subject to a high attrition. The initial number of participants is large enough so that even with a large number of missing individuals, we still had a large study sample size. Lastly, in this dataset, intensity-adjusted cumulative exposure scores were not calculated for spouses, and women made up a low number of applicators. Thus, we could not investigate the effect gender on dose response.

The study design of the NHANES dataset is cross-sectional, which has its own set of inherent limitations. We are unable to determine causality since we cannot establish that our outcome preceded any exposure to any potential predictors. We also relied on self-reported covariates for the NHANES study. A major limitation of the NHANES study is that the definition of agricultural worker comes from the U.S. Census Bureau's Census Indexes of Industry and Occupations coding scheme, which groups forestry and fishing industries along with agricultural. We were unable to separate these individuals who are unlikely to have significant pesticide exposures. However, our sample consisted of 1.6% (weighted) agricultural workers, which is similar to the estimated 1.9% estimated in 2000.[126]

## Public Health Implications and Future Directions

Our results from the AHS studies show that exposure to pesticides that are anti-estrogenic, anti-androgenic, thyroid disruptors, and PXR agonists may be an important factor in the increasing obesity trends seen worldwide. Additionally, we found that three out of the five pesticide categories show a positive exposure trend with overweight/obesity. In a general population, we found that reporting working in agricultural as the longest work type was not a significant predictor of body burdens of

pesticide. Though we had a small number of individuals reporting a history of agricultural work, our results were weighted to represent the U.S. population. Therefore, while we cannot make any strong conclusions, we can provide some evidence that in a general population setting, agricultural work is not a significant factor in pesticide exposure, and that agricultural and non-agricultural workers did not have significantly different levels of pesticides currently in use. This provides evidence that we may be able to extend results found in Chapters 1 and 2 to non-agricultural populations.

Many of the pesticides included in this study have already been banned in the United States. Though we do not recommend a ban for those that have not yet been discontinued because of their importance both industry and public health, we do recommend that more caution be used to reduce exposure. This might be especially important for foreign countries, given our finding that country of birth are important predictors of pesticide body burdens. Pesticides are necessary in crop and livestock industry, as well in public health (e.g. malaria control). However, a reduction in use and perhaps development of safer pest control methods is crucial to alleviate any health risks they may pose.

Our research has provided many different ideas for future studies to build upon. We suggest that future studies investigate the obesogenic effects of other endocrine disruptors, not just pesticides. Additionally, there is still much to be learned regarding why we found the results in this study. Therefore, we recommend that future studies investigate the pathways in which pesticides affect weight. Similarly, there needs to be more evidence regarding the exact endocrine-disrupting effects of pesticides. Most of the evidence available comes from *in vitro* studies that may not be entirely suitable for

discerning multiple effects *in vivo*. We saw interesting patterns within interactions

between pesticides displaying different modes of action, though we did not find

statistically significant effects in our cohort. In this sense, studies should take one step

backwards and investigate how different hormones interact to affect weight. The likely

next step for our studies is to follow a younger group of individuals, to observe and

investigate weight gain trajectories associated with pesticide use.

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BIOGRAPHICAL SKETCH

Claudia Kusano Lissåker was born in São Paulo, Brazil in June of 1985. She moved with her family to Fort Lauderdale, Florida at the age of 10. She graduated from Fort Lauderdale High School in 2003, and began her studies at the University of Florida that same year. Claudia graduated with a Bachelor of Science in Telecommunications—Production in May of 2008. She was subsequently accepted into the University of Florida's Master of Public Health program with a concentration in epidemiology and graduated in May of 2010. During this time, Claudia worked as a Graduate Assistant in the Florida Office for Disability and Health and was a student-inductee into the Beta Upsilon chapter of the Delta Omega Honorary Society in Public Health. Claudia enrolled in the University of Florida's PhD in Epidemiology program, where she was a recipient of the University of Florida Graduate School fellowship. She received her PhD in the summer of 2015.