Pesticide Exposures and Body-Mass-Index (BMI) in a Cohort of Male Pesticide Applicators

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Dedication

The author wishes to dedicate this dissertation to all her friends who supported her throughout the DrPH program and the dissertation process, most importantly Mary.

From the Book of Psalms

51:6 ***Surely, thou desirest truth in the inner self, and thou makest me to understand hidden wisdom.***

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Abstract
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The rising trend in the number of obese and overweight individuals in the United States in recent years has led to much scientific hypothesizing to identify causes beyond the well-known impact of high calorie diet combined with lack of physical exercise. Animal and *in vitro* studies suggest that exposure to organochlorines, organophosphates, carbamates, PBBs, PCBs, plastics, heavy metals, and solvents may be associated with weight gain through disruption of hormones, neurotransmitters, and metabolic processes, and damage to body tissues, especially nerve and muscle tissue. Several pesticides are among the chemicals that have been hypothesized to induce metabolic changes associated with weight gain in animal studies. Human studies are limited. Some have shown that rural populations have higher mean weights for each age group compared to non-rural populations. Studies have examined the relationship between blood levels of pesticides or their metabolites in relation to current body mass index (BMI) and have found that subjects with higher BMIs had higher levels of these substances in their blood. No study could be identified that obtained BMI and pesticide exposure levels at one point in time and BMI at a later point in time to prospectively assess the effects of exposure on BMI.

Our research investigated the hypothesis that exposure to pesticides may be associated with higher BMI using epidemiologic data from the Agricultural Health Study (AHS). This large cohort study of almost 90,000 Iowa and North Carolina pesticide applicators

and their spouses was initiated in 1993 (Phase 1: baseline/enrollment) with a follow-up 5 years later (Phase 2). The population available for our analysis included the 9,076 male private applicators that completed Phase 1, Phase 2, and the diet history distributed during Phase 2. No energy input variable was available for baseline and no measure of energy expenditure was available for Phase 1 or Phase 2; thus, we used available data to indirectly estimate and control for these. The calculated cumulative intensity-weighted days of exposure to pesticide classes from the AHS database was our main independent variable. Data were available to assess the association between BMI and exposure to particular pesticide classes for three separate studies: cross-sectional analysis of exposure to pesticide classes and BMI at enrollment; prospective analysis of BMI at 5-year follow-up in relation to pesticide exposure at enrollment; and cross-sectional analysis of BMI and pesticide exposures at follow-up.

We found that triazine herbicides were significantly positively associated with BMI across stratified groups and in all three of our studies: enrollment cross-sectional, prospective, and follow-up cross-sectional. While we also saw positive associations between BMI and organochlorines and phenoxy herbicides, both of which were suggested by our literature review, these results were eliminated when we controlled for triazine herbicide exposure in our multiple and logistic regression analyses. Differences in results for the three studies are slight and may be influenced by differences in other predictors included in the models. Further investigation of triazine herbicides as a class and the individual agricultural pesticides included in this class, is warranted.

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CHAPTER 1: INTRODUCTION

1.1 Background

There has been a steady increase in the proportion of the U.S. population that is overweight and obese over the past several decades. This "obesity epidemic" transcends gender, age, socioeconomic status, and race/ethnicity, though prevalence is higher among minorities and the economically disadvantaged (Wang & Beydoun 2007). Current estimates based on the 2009-2010 National Health and Nutrition Examination Survey (NHANES) suggest that 37.5 % of U.S. adults are obese (Ogden et al. 2012). Current prevalence rates are the same for men and women. These age-adjusted prevalence rates indicate that in 2009-2010, over 78 million U.S. adults were obese. . Recent data point to a leveling off of increasing prevalence for adults based on the lack of a significant change in obesity prevalence between 2007-2008 to 2009-2010. Obesity in adults is defined as a BMI $\geq$ 30 (Ogden et al 2012).

The Centers for Disease Control and Prevention (CDC) utilized its annual Behavioral Risk Factor Surveillance System data from 1985 to 2010 to create alarmingly graphic representations illustrating the increase over time in obesity prevalence by state using changing colors on a U.S. map (CDC 2007). This illustration has become widely recognized by the public health community and is often used to inform the lay public about the need to take action to stop or reverse this trend (CDC 2007). Interestingly, global estimates developed by the World Health Organization (WHO) suggest a similar pattern in other westernized countries in the Middle East, Central and Eastern Europe,

as well as North America (James et al. 2001). Developing countries are also experiencing dramatic increases in the prevalence of obesity, though few of these countries have nationally representative longitudinal data to adequately assess trends (Caballero 2007).

Aside from the obvious aesthetic influence obesity has on the human form with resultant negative psychological impacts on self-esteem, obesity's greatest impact on human health stems from its association with a myriad of chronic diseases, including many with disabling effects and high mortality, as well as high costs of treatment. Some in the medical and public health community have lobbied for acceptance of obesity as a disease in and of itself, rather than define it as a symptom of various chronic diseases (Conway & Rene 2004).

While the impact of high calorie diet (energy intake) combined with low levels of physical activity (energy expenditure) on obesity trends cannot be denied, the steady rate of increase since 1980, estimated at 1 percentage point annually (Wang & Beydoun 2007), has led to the search for additional influencing factors. The obesity epidemic appears to have occurred in a relatively short amount of time among a large proportion of the population instilling curiosity among the scientific community to identify factors in addition to the high calorie-low exercise lifestyle. Researchers have hypothesized additional influencing factors using the results of ecologic studies and/or examinations of overlapping time trends data for various socio-demographic, economic, or environmental factors overlaid with prevalence of obesity and overweight. Additionally,

they have attempted to explain the social factors that influence changes in diet and physical activity patterns that subsequently influence weight. America's obesity problem has been linked to a complex set of interacting and often conflicting social, agricultural, industrial, and economic policies that favor increased food availability at low cost to consumers (Tillotson 2004). Urbanization and globalization of food production and marketing are suggested as the two most important factors influencing the rise in obesity prevalence worldwide (Caballero 2007).

Remarkably, Keith et al. (2006) suggest that the evidence linking diet and exercise with the increase in obesity is largely circumstantial, with the bulk of the studies relying on ecologic associations rather than studies based on individual-level data. They utilize sound reasoning and evidence to identify other potentially influencing factors for the obesity epidemic, including: reduced average amount of sleep; reduced average time spent in variable ambient temperatures with corresponding increases in time spent in thermoneutral zones; decreased smoking prevalence; increased use of pharmaceuticals associated with weight gain; changes in population distribution by ethnicity and age; increasing age for women at time of first birth; various prenatal and perinatal factors such as increased maternal obesity and gestational diabetes promoting these conditions in subsequent generations and increased incidence of low birth weight with consequent rapid catch-up growth leading to obesity; increased BMI associated with greater reproductive fitness thereby yielding selection for obesity predisposing genotypes; assortative mating (probability two individuals mate is positively related to their degree of phenotypic similarity) for adiposity with the presence of factors preventing extreme

thinness (i.e., floor effects accentuate the effects of assortative mating); and *increase in exposure to endocrine disruptors (industrially produced chemicals that can affect hormonal functions)* (Keith et al. 2006).

Baillie-Hamilton (2002) also hypothesized that toxic chemicals in the human environment may be responsible for the obesity epidemic. In her article in the *Journal of Alternative and Complementary Medicine,* she provides ecologic evidence suggesting that the levels of synthetic organic and inorganic chemicals in the environment have increased at the same time as rates of obesity and overweight in the population have increased. She points out that human exposure to these toxins occurs through contaminated air, food, and water, as well as through cutaneous absorption, and that many toxins can be found in human milk. In addition, she states that while very high exposure to toxic chemicals induces weight loss, weight gain can occur at lower, chronic exposure levels, without signs of obvious poisoning.

Baillie-Hamilton (2002) suggests many chemicals can induce weight gain including pesticides, especially organophosphates and carbamates. She mentions the exploitation of the weight-promoting properties of these chemicals in the development of drugs to fatten livestock and induce weight gain in the grossly underweight. She believes that current environmental exposure levels may have damaged the human body's weight control mechanisms and may interact with other factors to create overweight and obesity. She notes that, while less potent than natural hormones, these synthetic chemicals have endocrine-disrupting properties and are present in wildlife and in living

human tissues at concentrations many times higher than the natural hormones that they mimic (Baillie-Hamilton 2002). Her hypotheses are reasonable and have prompted reviewers of her work to suggest that while the evidence is limited, the signs, clues, and ideas have been around for a long time (Jobst 2002). Her article may not be relied on as a thorough scientific review of the evidence, but it provides the origin and the most compelling scientific articulation of a hypothesis that has attracted widespread interest and deserves further testing before it can be dismissed or taken seriously.

Other reviewers have also reached similar conclusions after examination of the limited evidence linking environmental exposures and weight gain (Walter & Heitmann 2007). The idea that chemical exposures could be causing hormonal imbalances that induce adipogenic effects in humans was also suggested in the early 1980s (Makarov et al 1981). However, Stebbing's review on potential growth from toxic exposures ascribes the idea to researchers from many years ago who found that low levels of potentially toxic agents can have stimulatory effects (Stebbing 1982). Indeed, the idea that substances which are toxic may be beneficial (stimulatory) in small amounts extends back to the writing of Paracelsus in the 16th Century.

Many of Stebbing's points are relevant to the study of the effects of toxic agents on weight gain. He points out that the frequency with which growth is caused by particular agents is reflected in the amount of effort given to their study. The beta-curve (Figure 1-1, Stebbing 2003) is the concentration-response curve most likely to be observed for chemicals that are associated with stimulatory effects at low levels (Stebbing 1982;

2003). This curve illustrates any adaptive response to toxic agents stimulated first on reaching a threshold of detection (point 1, Figure 1-1, Stebbing 2003). The response will reach a maximum (point 2) such that no increase in concentration will elicit any greater response. The descending arm of the beta curve is due to the inhibition of the adaptive response by higher concentrations as their effect becomes more toxic (point 3, Figure 1-1, Stebbing 2003) This is reflective of some of the animal studies investigating weight gain from low-dose pesticide exposures and weight loss at much higher doses. Stebbing concludes that the more concentrations of a toxicant used in an experiment, the more closely the data will resemble the beta-curve (Stebbing 1982). While not previously suggested by other researchers, this curve does illustrate our hypothesis that pesticides may cause an increase in BMI even though studies of the effects of high doses of pesticides in animal experiments and due to human accidents suggest they cause weight loss.

Indeed, the toxic effects of pesticides have been known about for many years and include weight loss in animal studies and when humans are accidentally exposed to very high levels (Hayes & Laws 1991). Pesticide poisoning causes many symptoms besides weight loss that are also symptoms of various other health conditions. Some examples include, but are not limited to, hypo- or hyper-thermia, chills, myalgia, thirst, anorexia, skin rashes, pallor, cyanosis, keratoses, jaundice, excessive loss or growth of hair, toxic eye effects, paresthesia, headache, mood disturbances, seizures, tremor, hearing loss, hypo- or hyper-tension, cardiac arrhythmias, pulmonary edema, dyspnea, nausea, diarrhea, kidney effects, low sperm count, hemolysis, and anemia. The fact that these

symptoms are associated with a wide array of health conditions often makes pesticide poisoning hard to diagnose (Reigart & Roberts 1999). With any type of chemical poisoning, the concern is with the acute effects related to exposure to high levels (Reigart & Roberts 1999). High dose exposure is represented in the beta curve (Figure 1-1) by the dramatic drop off (point 3 = onset of inhibitory effects such as weight loss) once a certain level of exposure is reached.

Our research deals with the chronic exposure effects of low levels of exposure to pesticides (i.e., the first part of the beta curve where a stimulatory effect is observed). This is an area of research that has not been investigated to any great extent as our literature review reveals (Chapter 2). Some reviewers suggest that the capacity to conform to the beta-curve requires the establishment of an end point specific lowest observable effect level (LOEL) and a no observable effect level (NOEL), with multiple doses within two orders of magnitude immediately below the NOEL (Calabrese & Baldwin 1998). Most toxicologic studies do not satisfy these design criteria (Calabrese & Baldwin 1998). A review of the literature on growth found that the majority of low-dose stimulations are 30 to 60% greater than controls, as shown in Figure 1-1. The distance between the maximum stimulatory response and the estimated NOEL is typically observed in the 3- to 6-fold range (Calabrese & Baldwin 1998).

There is relatively little research on the effects of toxins on the growth of organisms compared with that on lethality and this is logical given that lethality is the fundamental measure of adverse effects against which all other toxicologic tests are calibrated

(Stebbing 1982). The most likely reason for failing to observe a stimulatory effect at low exposure levels where such an effect exists is the experimental design (Stebbing 1982; Calabrese & Baldwin 1998). Another reason is the strong regulatory need for safety evaluation of environmental agents, which emphasizes the upper end of the dose-response continuum (Calabrese & Baldwin 1998). Concentrations of chemicals that are tested or evaluated must extend to levels below the range in which growth is inhibited in order to identify growth (Stebbing 1982).

In addition, intervals between concentrations must be less than an order of magnitude or the concentrations used might not span those producing stimulatory effects. Even when growth occurs, it may be dismissed as an aberrant result if the peak is due to a single data point (Stebbing 1982). In their review of over 4000 toxicologic studies, Calabrese & Baldwin (1998) found a direct relationship between the strength of the evidence supporting a stimulatory effect at low exposure levels and the number of doses studies evaluate. They estimate that 500,000 toxicologic studies were published in the 20th Century with only 2% of these including six or more doses; this suggests only 10,000 studies contain dose ranges adequate for evaluation of potential stimulatory effects at low levels, with only 10% of these having three or more doses below the NOEL. Assuming 90% of these 1000 studies have doses in the low-dose range within 1-2 orders of magnitude and close to the estimated NOEL, only 900 potentially relevant studies exist (Calabrese & Baldwin 1998).

Stebbing (1982) states that failure to observe a stimulatory effect at low exposure levels is not evidence that it would not occur if certain requirements of experimental design are satisfied. If growth occurs in a context where it is unexpected and unwanted, these results may not be published. In addition, toxicologists may be unwilling to publish results on a phenomenon that is not fully understood. He suggests that growth observed at low exposure levels is most likely the consequence of regulatory overcorrections by biosynthetic control mechanisms to low levels of inhibitory challenge resulting in growth that is greater than normal (Stebbing 1982). Many of Stebbing's points are reflective of our hypothesis that pesticide exposure will be associated with increased BMI, even though many studies based on high doses show that pesticide exposure reduces weight (Hayes & Laws 1991; Reigart & Roberts 1999).

A more recently published article provides a hypothesized rationale for specific mechanisms for the induction of weight gain from exposure to exogenous chemicals citing the fact that their hypothesis parallels models for the action of environmental estrogens (Grun & Blumberg 2006). Such models predict the existence of "obesogens", molecules that incorrectly regulate lipid metabolism and adipogenesis to promote obesity. They present evidence that suggests the existence of environmental endocrine disruptors (EDs) that mimic natural lipophilic hormones mediate their effects through various nuclear receptor transcription factors. However, they note that it is unclear whether the effects of EDs on promoting adipocytes are mediated solely through an estrogen receptor signaling pathway since estrogens have been shown to be antiadipogenic in some studies suggesting that increased obesity is associated with

inhibition, rather than activation, of estrogen signaling. The term “obesogens” is gradually being accepted by the scientific community as representing those toxic agents that may induce weight gain.

The bulk of Grun and Blumberg’s argument (2006) deals with mechanistic models of how organotins, including tributyltin, which has been used as a fungicide on food crops, could promote adipogenesis. They discuss the ability of organotins to alter transcriptional activity by functioning as agonist ligands to create disturbances in the body’s homeostatic hormonal controls that impact key adipogenic factors, fat depot size, and function. They note that the implications of these effects on long-term changes remain to be determined. The role of environmental chemicals in the etiology of obesity is poorly understood but Grun and Blumberg (2006) have suggested links between organotins and adipocyte differentiation that have opened a new area of research.

1.2 Statement of the Problem

The World Health Organization (WHO 2002) concluded that endocrine disruption is not considered a toxicological end point *per se* but a functional change that may lead to various adverse effects in humans and wildlife. Reviewers of the effects of endocrine-disrupting chemicals note that there are a variety of hypothesized mechanisms by which these chemicals may influence metabolism and, possibly, weight gain (Elobeid & Allison 2008). These mechanisms include direct binding to nuclear receptors such as the estrogen receptor; acting as nuclear receptor antagonists; functioning indirectly by

inhibiting aromatases such as the P450 family members CYP19 and CYP3A1 which convert testosterone to estradiol; activating expression of the P450 enzymes; altering neuronal synapse formation which could affect release of brain produced substances that bind to nuclear receptors and may affect energy regulation (Elobeid & Allison 2008). Table 1.1 lists all the AHS pesticides from Phase 1 that are known or likely to be endocrine disrupters (IEH 2005).

Jobst (2002) advocates bringing the idea that exposure to some chemicals could be responsible for the obesity epidemic into the forefront of scientific debate, if for no other reason than to stimulate research to refute the hypothesis of Baillie-Hamilton (2002). She notes that industry is likely to be wary of such research because the economy relies upon production and abundant use of toxic chemicals, including for intensive farming (Jobst 2002). Indeed, the use of pesticides has been increasing steadily since the post-WWII era (Padgitt et al 2000; Delaplane 2000; USDA 2003). Pounds of active ingredient per planted acre for each pesticide class have varied over the years (USDA 2003). Herbicide use peaked in 1982 at 1.682 pounds per planted acre; insecticides peaked in 1964 with 0.706 pounds per planted acre; fungicides peaked in 1995 at 0.208 pounds per planted acre; other pesticides peaked in 1997 at 0.452 pounds per planted acre; total pesticide usage peaked in 1994 at 2.419 pounds per planted acre (USDA 2003).

The proportion of cropland treated with pesticides varies by crop according to the Agricultural Resource Management Survey (ARMS) conducted annually (USDA 2011). The proportions treated by number of acres planted are as follows:

corn	=	97% of 81,740 acres in 2010;
soybeans	=	98% of 72,881 acres in 2006;
cotton	=	98% of 10,241 acres in 2007;
rice	=	95% of 2,838 acres in 2006;
spring wheat	=	97% of 13,165 acres in 2009;
winter wheat	=	63% of 36,908 acres in 2009;
durum wheat	=	99% of 2,227 acres in 2009;
sorghum	=	85% of 8,465 acres in 2003;
oats	=	33% of 3,114 acres in 2005;
peanuts	=	99% of 1,310 acres in 2004;
barley, feed	=	66% of 1,146 acres in 2003;
barley, malt	=	81% of 3,704 acres in 2003 (USDA 2011).

Over the years, the types of pesticides available have also increased with a wider variety of chemical structures and, therefore, potentially different mechanisms of action (Padgitt et al. 2000). Demand for pesticides will continue to rise into the future as farmers must increase yields on increasingly fewer farm acres – a consequence of a growing human population and increased urbanization (Delaplane 2000). It is interesting to note that among her final recommendations, Baillie-Hamilton (2002)

suggests studying the effects of the chemicals she identified as potential weight inducers on weight gain in specific subpopulations including farmers. Hence, the need for the present study has been identified by other researchers.

If the same relationship found in a few animal studies on low-dose exposure to pesticides causing weight gain exists for humans, it is likely to be more readily identified among population subgroups with a higher likelihood of exposure, such as pesticide applicators, rather than in the general population, though factors associated with the "healthy worker effect", or in this case, the "healthy farmer effect" may also be influencing variables.

1.3 Conceptual Framework

The conceptual framework for the conduct of this research is based on the existence of the perhaps coincidental correlation between increasing rates of obesity subsequent to increased use of manufactured chemicals, the results of animal studies that suggest chemical and other environmental exposures may induce weight gain, hypotheses on the effects of such substances on metabolic processes leading to weight change, and the need to provide human evidence for the existence or non-existence of weight gain associated with environmental exposures. Analyses are based on bivariable and multivariable statistics on body mass index (BMI) and pesticide class exposure variables. Investigations include whether past exposure leads to increased BMI, the existence of an exposure-response relationship, whether there are mediating factors in

the relationship between specific pesticide class exposures and BMI, and whether there are effect modifiers of the potential association between pesticide classes and BMI. The conceptual model that forms the basis for these proposed research questions stems from several hypotheses including ideas on how environmental estrogens may perturb adipogenic mechanisms and how such endocrine disrupters may induce weight gain (Grun & Blumberg 2006).

Some pesticides exhibit estrogenic or endocrine disruption characteristics. These pesticides are believed to affect lipid homeostasis and adipogenesis potentially leading to a predisposition to obesity and/or sensitization of exposed individuals to obesity and related metabolic disorders under the influence of the typical high-calorie, high-fat Western diet (Grun & Blumberg 2006). Additional hypothesized mechanisms as noted above include adaptation and alterations in patterns of gene expression (Calabrese & Baldwin 1998). Hypotheses on the impact of environmental exposures on weight gain are based on the results of animal assays showing increased weight due to changes in metabolism and other physiological processes induced by exposure to certain chemicals. Analyses of human data alone have so far failed to provide firm evidence of direct causal associations between measured low-levels of endocrine disruptors in human populations and adverse health outcomes (WHO 2002). Chapter 2 provides a review of both the animal and human evidence in support of weight-related variables and their association with exposure to environmental substances that are relevant to our research. A study such as ours has never been done before on a human population. This research provides information to help fill in a gap in current scientific knowledge

regarding the relationship between body weight and chronic low-level exposure to specific environmental substances – pesticides.

Figure 1-2 presents the median BMI by increasing age for males in the National Health and Nutrition Examination Survey III (NHANES III) cohort (1988-1994), a general population national survey (Roberts & Williamson 2002), along with a similar hypothesized curve for males in the AHS. For illustrative purposes, we assumed an increasing influence of pesticide exposures at various ages (i.e., hypothesized increases in BMI of 1-5% for the five age groups for which data were presented). This hypothetical curve represents AHS males exposed to pesticides over their entire lifetimes. Points in time are designated for 1994, when the bulk of the AHS enrollment began, 1999, when the 5-year follow-up (FUP) began, and 20 years prior to enrollment (1974). The mean age at AHS enrollment was approximately 46 years (Alavanja et al 1996), allowing for estimation of mean age at 5-year follow-up of 51 years and mean age 20 years prior to enrollment (26 years). It is important to note that the majority of the 52 pesticides for which information was collected in the enrollment questionnaire were all registered for use prior to 1970 (Hoppin et al. 2002). This means that at the time of enrollment when the average age of the cohort was 46 years, male applicators exposed throughout their lifetimes had already been exposed to most of the pesticides for more than 20 years.

1.4 Overview of AHS and Our Study Design

The Agricultural Health Study (AHS) is a large cohort study of almost 90,000 pesticide applicators and their spouses that was initiated in 1993 (Alavanja et al. 1996). Pesticide applicators were selected at the time of certification renewal in either Iowa or North Carolina. Detailed self-reports were obtained at baseline on pesticide usage, other agricultural exposures, lifestyle factors, and weight and height at the time of interview and at age 20. For some subjects with missing information on height and/or weight, this information was taken from state drivers' license records. During the Phase 2 follow-up, the cohort was recontacted and all information was updated, including self-reported weight. Our study's target population was the 50,641 AHS male private pesticide applicators aged 20 years or older from Iowa and North Carolina.

Our research evaluated BMI at enrollment with exposure at enrollment (enrollment cross-sectional analysis), BMI at follow-up with exposure at enrollment (prospective study), and BMI and exposures at follow-up (cross-sectional follow-up analysis) in the AHS male cohort of pesticide applicators. AHS has the most detailed exposure data on agricultural chemicals currently available. Pesticide applicators are much more highly exposed to pesticides than are persons in the general population. The AHS cohort includes pesticide applicators and their spouses. Since the majority of the applicators are male, since pesticide exposures are better defined and more intense for the applicators rather than the spouses, and since hormonal responses to pesticides are likely to be different for males and females, our study focused solely on male

applicators. Height and weight data were available to estimate BMI at enrollment and at five-year follow-up, and to estimate BMI at age 20. The association between exposure to specific pesticide classes and BMI was evaluated. We also examined prevalence of obesity, overweight, normal weight, and underweight as per definitions established by the World Health Organization (WHO) using ranges of BMI (WHO 2007).

Pesticide exposure was defined based on a previously developed "general" exposure algorithm that creates a subject-specific intensity of exposure score for individual pesticides (Dosemeci et al. 2002). The general algorithm is based on data from the enrollment questionnaire on mixing status, application method, equipment repair status, and personal protective equipment use, and was validated using biological monitoring results. No energy input variable was available for baseline and no measure of energy expenditure was available for Phase 1 or Phase 2; thus, we used available data to indirectly estimate and control for these. Self-reported diet and physical activity information were used to develop a method to control for total caloric intake and amount of physical activity (energy input and energy output) in all analyses. Our study design examines BMI at age 20 descriptively since no exposure information is available for that time period. For BMI at enrollment, and at 5-year follow-up, bivariable and multivariable analyses were conducted in relation to exposure intensity scores for each pesticide class at enrollment and at 5-year follow-up. All analyses were conducted including and excluding subjects who self-reported medical conditions at enrollment or follow-up that are generally associated with body weight such as cancer, diabetes, and thyroid disease. Additional stratified analyses by state were also conducted.

Prior analyses of the AHS data based on an average of 5 years of follow-up have found that compared to the general population in the two states included in the study, the cohort has experienced a low mortality rate overall and for specific causes, as well as a low cancer incidence rate overall (Alavanja et al 2005; Blair et al. 2005). Incidence of respiratory cancers was significantly lower than expected (Alavanja et al. 2005). Standardized mortality rates among the applicators for cancers of the lung, pancreas, bladder, prostate, esophagus, and buccal cavity and pharynx were significantly lower than expected with SMRs of 0.6 or lower (Blair et al 2005). SMRs were under 1.0 for all smoking-related diseases both for “ever” and for “never” smokers. Smoking prevalence in the cohort is significantly lower than the national average (Alavanja et al. 2005). The SMR for diabetes was significantly reduced at only 0.3 for applicators. However, the limited numbers in most mortality and cancer incidence categories makes these results unstable. The low smoking and alcohol use prevalence in the cohort combined with the low SMRs for smoking related diseases and the homogeneous nature of the cohort suggest there may not be a need to control for these factors in our study.

The strengths of the AHS study include its large size, the collection of exposure information prior to the development of disease, or in this case, prior to BMI estimation, the detailed information on pesticide use practices and procedures for specific chemicals, the availability of information on agricultural and non-agricultural exposures, the availability of detailed information on potential confounding factors, and completion of a 5-year follow-up. The use of the exposure algorithm to estimate

pesticide exposure scores takes into account individual differences in work practices including use of personal protective equipment; it is considered to be the best pesticide exposure data available anywhere. Sub-studies of small numbers of participants have directly measured exposures to pesticides and have validated the self-reports (Coble et al. 2005). Evaluations of the data to date have found very little collinearity among the pesticide exposures with most of the applicators and farmers having used a maximum of 15 pesticides in their lifetime.

1.5 Research Questions

This study sought to answer a number of questions:

- Are AHS male pesticide applicators who are more highly exposed to pesticide classes more likely to have a higher body mass index (BMI) than male pesticide applicators who are less highly exposed to these same pesticide classes?
- Is exposure to pesticide classes among the male AHS cohort associated with an increase in BMI years later?
- Is there data to examine exposure-response by level of pesticide class exposure in relation to BMI in the male AHS cohort?
- What are the identifiable confounders and effect modifiers in the relationship between pesticide exposure and BMI in the AHS cohort?
- Are there specific pesticide classes that are associated with current BMI or with BMI several years after exposure in the AHS cohort and how do these specific substances relate to current theories on chemicals that cause weight gain?

A priori hypotheses for those agents most likely to be associated with BMI have been delineated in Chapter 2 and are based on a thorough review of the animal and human evidence to date.

1.6 Significance of study

Our study is significant because the impact of pesticides on BMI has never been directly investigated for humans. The size and homogeneity of the AHS male pesticide applicator cohort suggests the likelihood of seeing an effect if one exists. This structured scientific inquiry investigated the hypothesis that occupational exposure to pesticides is associated with BMI. Our results may be used to spur additional research in this area. Our findings, in conjunction with any subsequent research, could affect pesticide policy and regulation, as well as worker protection practices in agriculture.

1.7 Validation of the AHS

Methodological studies of the AHS have provided evidence to suggest that the self-reported data collected via interview are reliable and valid (Coble et al. 2005; Lynch et al. 2005; Hoppin et al. 2002; Samanic et al. 2005; Dosemeci et al. 2002; Tarone et al. 1997; Coble et al. 2002; Blair et al. 2002). Previously published analyses of subjects who completed only the enrollment questionnaire versus those who completed the enrollment questionnaire along with the much longer take-home questionnaire (both part of Phase 1 data collection) found the characteristics of the two groups to be quite

similar indicating a lack of evidence for substantial selection bias based on completion of the take home questionnaire (Tarone et al. 1997). A reliability assessment based on repeat interviews with over 4,000 subjects one year after the initial enrollment interview revealed percentage agreement for pesticide use and application practices was very high, generally from 70% to over 90%, and did not vary by age, education, or farm size (Hoppin et al. 2002).

Quality of the self-reported pesticide use history was evaluated by comparing reported decade of first use and total years of use to the year the pesticide was first registered for use based on registration information from the U.S. Environmental Protection Agency (EPA) and other publicly available sources for the 52 pesticides included in the AHS interview (Hoppin et al. 2002). On average, only 1% of subjects overestimated total possible duration of use and, for decade of first use, 98% gave plausible responses. Generalizability of the AHS findings was evaluated by comparing AHS private applicators to the Census of Agriculture data for Iowa and North Carolina (Lynch et al. 2005). AHS farmers were found to represent the higher end of pesticide usage in both states mainly because they had larger farms. Higher usage translates to higher cumulative exposure with a greater potential influence on health effects, including BMI (if such a relationship exists).

Another methodological study of the AHS assessed the effects of potential confounding from non-pesticide agricultural exposures by examining associations between the use of pesticides and the frequency of performing various types of specific farming activities

that involved non-pesticide exposures. This research determined that the magnitude of bias due to confounding is likely to be minimal based on confounding risk ratios between 0.97 and 1.07 (Coble et al. 2002).

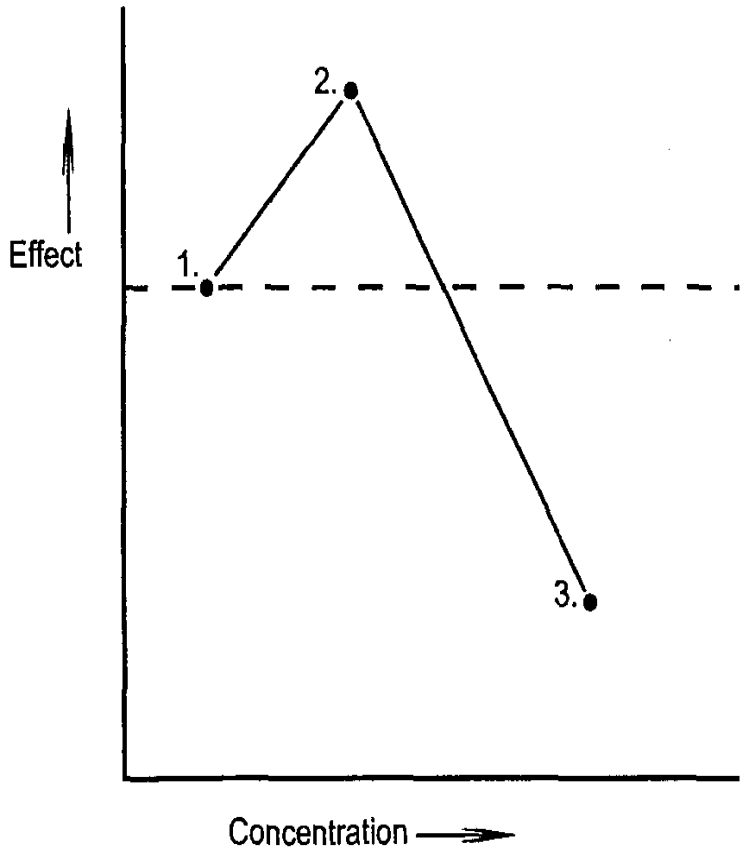
1.8 IRB and other regulatory issues

Approval to utilize the AHS data for the purposes outlined above was obtained from the AHS executive committee. The risks to human subjects from the proposed research were evaluated by the George Washington University (GWU) Medical Center Institutional Review Board (IRB). An exemption was obtained since this research study is a secondary analysis of already-collected data and NIH non-disclosure policy prohibits any outside researchers from accessing or viewing any identifying data. Bi-annual progress reports continue to be submitted to the AHS executive board in fulfillment of requirements for use of their data.

1.9 Conflicts of interest

No conflicts of interest existed for any of the researchers who worked on this project.

Figure 1-1: Typical dose-response beta-curve associated with substances that have a stimulatory effect at low doses (e.g., weight gain) and an inhibitory response at high doses (e.g., weight loss) (Stebbing 2003)



Hypothetical concentration–effect curve identifying the defining points (1 to 3) that characterize the β-curve. See text for further discussion.

Figure 1-2: Hypothetical Change in Median BMI in AHS Males Potentially Due to Agricultural Exposures Compared to Median BMI in NHANES III Males (NHANES data and graph extracted from Roberts & Williamson 2002)

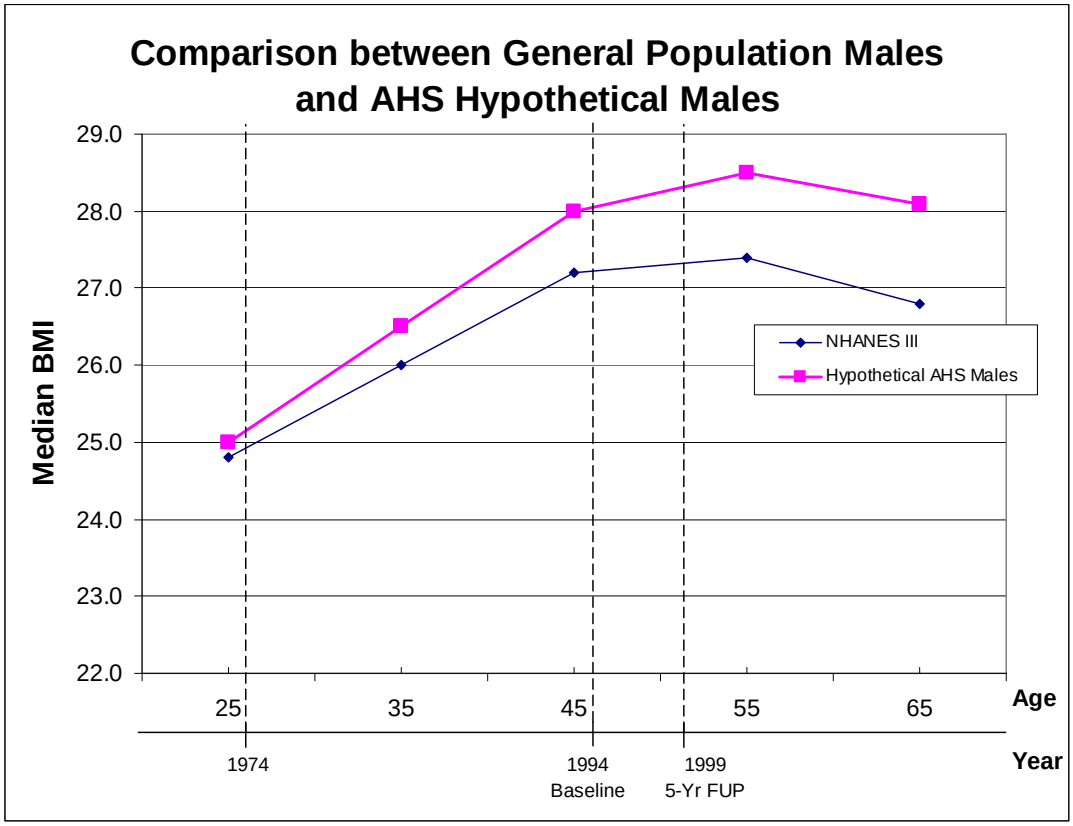


Table 1.1. Pesticides Likely to be Endocrine Disruptors Assessed in Phase 1 of Agricultural Health Study versus those not Identified as Endocrine Disruptors*

IDENTIFIED AS ENDOCRINE DISRUPTORS	**NO EVIDENCE FOR ENDOCRINE DISRUPTION**
HERBICIDES	**HERBICIDES**
Atrazine	Dicamba
Cyanazine	Metolachlor
Alachlor	EPTC
Glyphosate	Imazethapyr
Trifluralin	Chlorimuronethyl
2,4-D	Petroleum distillate
Metribuzin	Butylate
Paraquat	2.4.5-TP
Pendimethalin	Metsulfuran methyl
2,4,5-T	Chloramben
Bromoxynil	Bentazone
Fluazifop-butyl	Bicep (atrazine + metolachlor)
Linuron	Acifluorfen sodium
Simazine	Bronco (alachlor + isopropylamine salt of glyphosphate)
Oryzalin	Bullet (alachlor + atrazine)
INSECTICIDES	Canopy (metribuzin + chlorimuron ethyl)
Pyrethroid	Clomazone
Trichlorfon	Conquest (cyanazine + atrazine)
Carbofuran	Fluometuron
Chlorpyrifos	Napropamide
Permethrin	Diphenamid
Dichlorvos	EPTC
Lindane	Thifensulfuron methyl
Malathion	Diclofop-methyl
Parathion	Inorganic arsenic
Carbaryl	Marksman (dicamba + atrazine)
Diazinon	Isopropalin
Aldicarb	Organic arsenic
Aldrin	Sethoxydim
Chlordane	Propachlor
Dieldrin	Imazaquin
DDT	Ethalfluralin
Heptachlor	Squadron (pendimethalin + imazaquin)
Toxaphene	Sutazine (atrazine + butylate)
Dimethoate	Tridiphene
Tefluthrin	Vernolate

Dicofol	Norflurozon
Methomyl	**INSECTICIDES**
Methoxychlor	Terbufos
Acephate	Fonofos
Mevinphos	Coumaphos
Fenvalerate	Phorate
Endosulfan	Aastar (flucythrinate + phorate)
Fenthion	Trimethacarb
Tetrachlorvinphos	Dioxathion
FUMIGANTS	Bacillus thuringiensis
Carbon disulfide	Disulfoton
DBCP (dibromo-chloropropane)	Fenchlorphos
FUNGICIDES	Azinphos methyl
Benomyl	Phosmet
Maneb	Lead arsenate
Ziram	Methamidophos
Ferbam	Ethoprop
Vinclozolin	Rotenone
Thiram	Crufomate
Metiram	Trichlorfon
Iprodione	Oxanyl
Fenarimol	Nicotine
Etridiazole	Famphur
	Bufencarb
	Ivomec
	Ronnel
	Trichlorfon
	Phosmet
	Rotenoneapry
	Tackic
	FUMIGANTS
	Methyl bromide
	Aluminum phosphide
	Ethylene dibromide
	Dazomet
	Bin Spray
	Chloropicrin
	Calcium cyanide
	Dichloropropene
	Tetrafume
	Metam-sodium
	Methyl isothiocyanate
	FUNGICIDES
	Chlorothalonil
	Captan

	Metalazyl
	Triabendazole
	Propiconazole
	Triadimefon
	Triadimenol
	Captafol
	Copper ammonia carbonate
	Dodine
	Anilazine
	Triforine
	Cupric hydroxide
	Thiabendazole
	Dodemorph acetate
	Myclobutanil
	Folpet
	Piperalin
	Sulfur
	PCNB
	Thiocarabamates
	Triophanate-methyl
	Copper sulfate
	Triphenyltin acetate
	Carboxin

* Pesticide data collected at 5-year follow-up was collected via verbatim responses

CHAPTER 2 LITERATURE REVIEW

2.1 Introduction

This dissertation research will utilize the Agricultural Health Study (AHS) male cohort of private pesticide applicators who are farmers from Iowa and North Carolina to evaluate whether or not exposures to the AHS-defined pesticide functional and chemical classes -- commonly used pesticides in Iowa and North Carolina, are associated with BMI. Such exposures would result from contamination of the occupational or general environments. The pesticides of interest are, or were, in common use, currently or in the past, in production agriculture in these two states. Since current theories suggest that low-level, chronic exposure to certain chemicals, particularly organochlorines, may induce changes in metabolism that result in weight gain and eventually, overweight and obesity, this literature review will focus on studies that show a weight gain or an increase in a weight-related outcome in humans or animals following exposure to any of the pesticides in the AHS-defined functional or chemical classes. Only pesticides that are relevant to these exposures that can be assessed using data from the AHS database will be reviewed. The pesticides of interest include:

Functional classes

- **herbicides**
  - alachlor

- atrazine
- butylate
- chlorimuron ethyl
- cyanazine
- dicamba
- EPTC
- glyphosate
- imazethapyr
- metolachlor
- metribuzin
- paraquat
- pendimethalin
- petroleum oil / distillates
- trifluralin
- 2,4,5-T
- 2,4,5-TP (silvex)
- 2,4-D

- **Insecticides**
  - aldicarb
  - aldrin
  - carbaryl
  - carbofuran
  - chlordane

- chlorpyrifos
- coumaphos
- dichlorvos or DDVP
- DDT
- diazinon
- dieldrin
- fonofos
- heptachlor
- lindane
- malathion
- parathion (ethyl or methyl)
- permethrin or pyrethroid
- phorate
- toxaphene
- trichlorfon

- **fungicides**
  - benomyl
  - captan
  - chlorothalonil
  - maneb or mancozeb
  - metalaxyl
  - ziram
- **fumigants**

- aluminum phosphide
- carbon tetrachloride /carbon disulfide
- ethylene dibromide
- methyl bromide

Chemical classes

- **Organochlorine insecticides**
  - aldrin
  - chlordane
  - DDT
  - dieldrin
  - heptachlor
  - lindane
  - toxaphene
- **Organophosphate insecticides**
  - chlorpyrifos
  - coumaphos
  - diazinon
  - dichlorvos/DDVP
  - fonofos
  - malathion
  - parathion
  - phorate

- terbufos
- trichlorfon

- **Organothiophosphate insecticides**
  - chlorpyrifos
  - coumaphos
  - diazinon
  - fonofos
  - phorate
  - terbufos
- **Carbamates**
  - aldicarb
  - benomyl
  - carbofuran
  - carbaryl
- **Phenoxy herbicides (and contaminant TCDD--dioxins)**
  - 2,4-D
  - 2,4,5-T
  - 2,4,5-TP (silvex)
- **Triazine herbicides**
  - atrazine
  - cyanazine
  - metribuzin

Studies that examine increases in weight or weight-related variables in humans or animals due to changes in diet or specific dietary components, or the use of drugs or dietary supplements will be excluded, as will studies designed to specifically determine optimal environmental conditions and/or the effect of antimicrobial agents, dietary supplements, or drugs known or hypothesized *a priori* to induce weight gain. Studies that assess a weight-related outcome due to exposure to any of the above named pesticides or pesticide classes, including as contaminants in the diet or dietary supplements, or from occupational (indirect) exposure to drugs or dietary supplements, will be included.

2.2 Literature search methods

A number of online literature searches were performed using PubMed, ToxNet, and the Hazardous Substances Data Base (HSDB), available through the National Library of Medicine (NLM) website. Additional searches were performed using AGRICOLA, a database of agricultural literature available through the U.S. Department of Agriculture website, and CRISP, an online database available through the National Institutes of Health which lists biomedical research projects currently and previously funded by the U.S. Department of Health and Human Services (DHHS). A variety of keywords in various combinations were input. Each search included one or more outcome (dependent) variables and one or more exposure (independent) variables. BMI and "body mass index" were among the outcome variables included in the searches. It is defined as weight in kilograms divided by height in meters squared. Additional

keywords or phrases used for the outcome variable in the searches included: weight gain, body weight, overweight, obesity, growth, fattening, obese, significant weight gain, increase, gain, weight increase, body weight gain, body weight increase, metabolism, and weight change. These keywords were used in combination with one or more of the following keywords or phrases representing the exposure variable: environmental, occupational, chemical, industrial, synthetic, crops, pesticides, fertilizers, drugs, agents, additives, livestock, exposed, exposure, expose, test, challenge, assay, bioassay, exogenous, agrochemicals, agricultural chemicals, agricultural exposures, farm chemicals, farm exposures, ranch chemicals, ranch exposures, veterinary, domestic animals, farm, and agriculture.

The combined results of these searches yielded several thousand abstracts, including many duplicates and irrelevant papers, making it difficult to determine the exact number of distinct abstracts identified. All of the abstracts were reviewed to select the relevant papers to obtain in full. In addition to the exclusions already mentioned, additional exclusions included experimental animal studies of invertebrates, studies showing reductions in the amount or proportion of weight gain as a result of specific exposures, studies controlling for weight gain (BMI, body weight, etc.) while examining the impact of a certain exposure on some other outcome (diabetes, cancer, etc.), studies showing that exposure to one substance can reverse the weight loss effects of a prior substance, and studies discussing weight gain in animals as a result of substances given to the animals specifically to cause weight gain (drugs, fattening agents, new diet, growth hormones, etc.).

It was very difficult to identify animal studies showing weight gain from exposure to exogenous exposures. Most animal studies are designed to identify toxic effects, including weight loss, and therefore, tend to use very high doses due to the animals' short lifespans in comparison to humans. Occasionally, an exposed subgroup may show a weight increase at a particular dosage level. This increase may or may not be reported in the study's abstract. Since weight increase has not historically been a variable of interest in animal experiments, some effects may not have been reported or may not be identifiable using the online search strategies employed in this review due to absence of relevant information in the study abstracts or keywords. These difficulties were encountered by other reviewers as well (Baillie-Hamilton 2002).

Assuming that statistically significant increases are more likely to be reported in the abstract than non-significant increases, only animal studies reporting statistically significant increases will be included in this review, despite the fact that the identification of significant increases may be a function of the "multiple comparisons" phenomenon rather than a true weight increase induced by a particular exposure. Every effort was made to identify animal studies showing any statistically significant increase in a body weight-related variable in relation to exposure to any of the aforementioned pesticides and to include those findings in this review.

Among the thousands of abstracts reviewed were 644 human studies that examined a weight-related outcome in relation to levels of pesticides and other persistent organic

pollutants (POPs) in whole blood, serum, plasma, and tissue samples. Virtually all of these studies were cross-sectional and indicated that higher levels of these substances are found in people with higher weight or BMI. When we re-did the online search so as to specifically identify agricultural, farming, or rural populations among the 644 articles, only 6 articles were identified and only one of these was relevant to our study and is included in this review (Wolff et al. 1978).

For the remaining 638 studies, all of the abstracts were reviewed and articles were sampled to include in this review based on pesticides of interest to this study, the sample size, and the recency of publication in order to include a manageable number in this review.

Bearing in mind the inclusion and exclusion criteria discussed above, review of the abstracts from the various online searches yielded a list of several hundred papers of potential relevance, not including the studies of POPs in human samples. These articles were obtained in full. Additional papers were identified from the reference lists of the studies reviewed and from the National Toxicology Workshop working document (NTP 2011) and final report (Thayer et al 2012). All relevant papers were obtained and reviewed to determine if they met our inclusion and exclusion criteria. We only included published, peer-reviewed studies and excluded any studies that were unpublished.

Studies done in human populations that examine weight gain or a weight-related outcome in relation to an environmental exposure relevant to the AHS male cohort are basically of two types: evaluation of a weight-related outcome in a rural or agricultural population with no specific pesticide exposure information provided (exposure is the rural or agricultural environment itself); and, as noted above, studies examining a weight-related outcome in relation to levels of pesticides or their metabolites or contaminants in human blood, serum, plasma, or tissues. However, one recent case-control study that focused on Agent Orange (phenoxy herbicides) exposure (defined as yes/no) in prostate cancer patients provided enough data to calculate odds ratios (ORs) and 95% confidence limits (95% CL) for the association between BMI and exposure (Shah et al 2009).

In summary, study inclusion criteria for the above-named pesticides included:

- Examination of exogenous exposures, including contaminants in diet or dietary supplements and occupational (indirect) exposures;
- Studies of agricultural populations in general;
- Examination of a weight-related variable in relation to a relevant exposure;
- Exposure represents one of the AHS-defined functional or chemical classes or the pesticides included in these classes, and can, therefore, be assessed using available data in AHS database;
- For animal studies only, at least one statistically significant increase in a weight-related outcome was reported.

Exclusion criteria include studies examining:

- effects of pesticides that are not among the AHS-defined functional and chemical classes;
- effects of diet or specific dietary components;
- effects of drugs or dietary supplements;
- determination of the optimal environmental conditions to induce weight gain;
- invertebrate animals;
- exposures related to reductions in the amount or proportion of weight gain;
- control for weight gain (BMI, body weight, etc.) while examining the impact of a certain exposure on some other outcome (diabetes, cancer, etc.);
- how exposure to one substance can reverse the weight loss effects of a prior substance;
- exposure information that is only available for the *in utero* time period;
- weight gain in animals as a result of substances given to the animals specifically to cause weight gain (drugs, fattening agents, new diet, antimicrobials, growth hormones, etc.).

The final group of papers included in this literature review is summarized below and in the accompanying tables.

2.3 Results of literature search for animal studies

Table 2-1 provides detailed information on studies of weight gain or increases in weight-related variables in vertebrate animals associated with pesticide exposures. Each row summarizes one study. The studies are grouped by the exposure evaluated, which is listed in the second column next to the citation. Exposures include parathion, chlorpyrifos, diazinon, dieldrin, aldrin, DDT, lindane, chlordane, 2,4-D, and TCDD (dioxin - a contaminant of phenoxy herbicides). Additional columns list the animal species investigated, which include rats and mice. The fourth column provides the dosing methods and levels. These include subcutaneous injections; gavage; and *ad libitum* in feed, drinking water, or milk. Additional columns provide information on the study methods, response or results, and any additional relevant comments. All significant findings related to weight gain are in bold in the response column.

2.4 Summary of animal studies

The animal studies do not carry equal weight in terms of the relevance of their study methodologies and results to the hypothesis under study in the AHS adult male cohort. For example, the use of subcutaneous injections (Meggs and Brewer 2007; Roegge et al 2008; Lassiter et al 2008; Adigun et al 2010), catheter into stomach (Takahama et al 1972), and gavage (Lassiter & Brimijoin 2008) as dosing methods are far less relevant to humans than the use of peroral (Chadwick et al 1988) or dietary (Deichmann et al 1975, 1972; Nicolau 1983; Ball et al 1953; Lim et al 2009) exposures. Sometimes

significant findings in one gender were coupled with no significant effect in the other gender (Lassiter & Brimijoin 2008) or findings in one group were not duplicated in another similar group (Deichmann et al 1975; Cassidy et al 1994). Some studies found weight gain occurred from exposures occurring at specific developmental periods including while *in utero* (Lassiter & Brimijoin 2008) – time periods that cannot be directly assessed in the AHS. In several studies, weight gain was not significant until the animals had reached a particular age (Meggs & Brewer 2007; Lassiter & Brimijoin 2008), were of a particular age (Deichmann et al 1972), or were confronted with another environmental exposure such as high fat diet (Roegge et al 2008; Adigun et al 2010).

Interestingly, associations observed for chlorpyrifos (Meggs & Brewer 2007; Lassiter & Brimijoin 2008) and for parathion (Lassiter et al 2008), were not duplicated for diazinon (Roegge et al 2008), though all are organophosphate insecticides. DDT, dieldrin, and aldrin (Deichmann et al 1972, 1975; Ball et al 1953), all organochlorines, showed similar associations to the organophosphates parathion and chlorpyrifos. Research suggests organochlorines act by a very different mechanism than organophosphates. It may be that each is toxic and that the animal is, therefore, more lethargic and less active than an unexposed control but less affected by toxicity than an animal at higher dosage. Among the studies discussing the mechanism believed to have caused the weight gain in these animals, a number of different theories were purported including circulating metabolites increasing the deposition of total body lipids (Deichmann et al 1972); neonatal low dose exposure disrupting glucose and fat homeostasis (Lassiter et al 2008); impaired leptin production or enhanced clearance (Lassiter & Brimijoin 2008); growth

as a nonspecific response to stressor action of chemical administration (Ball et al 1953); changes in hormonal regulation of energy balance (Chadwick et al 1988); isomers of chlordane interacting with sex steroid receptors directly or altering the levels of sex steroids (Cassidy et al 1994); mild mitochondrial damage that mimics the characteristic of the insulin resistance state (Lim et al 2009).

A study by Sand et al. (2010) illustrates an important issue in any animal study and that is the different sensitivity levels of particular strains to chemical exposures. They examined two rat species: Hans-Wistar (H/W) and Long-Evans (L-E) with respect to 20 weeks exposure to TCDD. Non-exposed control groups were used but the data on controls are not reported. Female rats, 5 weeks old (10/dose) with mean weights of 70.1 or 81.7, respectively, were partially hepatectomized and initiated 24 hours later with a single ip dose of nitrosodiethylamine. After 5 weeks, TCDD was administered in corn oil by sc injection (2 ml/kg) once per week for 20 weeks; controls received only corn oil. A loading dose 5 times higher than the 19 treatment doses was given in order to rapidly achieve the kinetic steady state. Total doses were 0, 0.17, 1.7, 17 and 170 (H/W only) ug/kg bw. Daily doses were calculated which corresponded to calculated doses of 0, 1, 10, 100, and 1000 (H/W only) ng/kg bw/day. The maximum likelihood approach was used for model fitting. Differences in sensitivity to treatment were estimated by BMD ratios ($BMD_{H/W}$ / BMD_{L-E}). Matching liver concentrations were 76.9 ng/g dry weight for H/W rats and 49.3 for L-E rats. The difference in body weight gain (bw_{end} / bw_{start}) was used to estimate differences in sensitivity between the rat strains by calculating the ratio of the BMD and 90% confidence intervals. The result was $BMD_{H/W}$

/ $BMD_{L\text{-}E}$ = 9.4 (3.0; 30), which represents the difference in sensitivity between the strains. Log-likelihood models were calculated twice: 1. all parameters were strain specific (result=101.82); 2. relative range of response and Hill coefficient were common for both strains with remaining parameters being strain specific (result=101.36). The authors concluded that the use of parallel dose-response curves was supported by the data and the 2 strains differed statistically in their response to TCDD (Sand et al 2010). Therefore, selection of animal strain will influence results on changes in body weight due to different sensitivities between strains.

A review of the potential mechanisms that cause pesticide exposure to influence cellular metabolism of lipids, proteins, and carbohydrates, is illustrated in Figure 1 (Karami-Mohajeri & Abdollahi 2010). These authors extracted information from over 200 studies designed to investigate specific changes in the metabolic pathway caused by pesticide exposure to develop their hypothesized model of the complex interactions that occur at a cellular level to induce changes in metabolism. They concluded that organophosphates and carbamates impair the enzymatic pathways in the metabolism of carbohydrates, fats, and protein within cytoplasm, mitochondria, and proxisomes through inhibition of AChE or affecting target organs directly. Organochlorines affect lipid metabolism in adipose tissues and change glucose pathways in other cells. All pesticides induce cellular oxidative stress via their affects on mitochondrial function and therefore, disrupt neuronal and hormonal status (Karami-Mohajeri & Abdollahi 2010). They recognize that differences in the studies they reviewed in methods, exposure pattern, type of animal, and doses limit their conclusions but anticipate their

review will lead to more coherent strategies and studies to better understand the mechanism of toxicity of pesticides and ways to reduce their toxic effects.

Schug et al. (2011), in their recent review of the effects of endocrine disruptors (EDs) on disease, indicated that EDs are no longer thought to exert their actions solely through nuclear hormone receptors, but utilize much broader mechanisms than originally recognized. Studies have shown that EDs can also act through nonsteroid receptors, transcriptional coactivators, enzymatic pathways involved in metabolism, and direct effects on genes and their epigenetic impact. Similar to hormones, EDs can function at very low doses in a tissue specific manner, may exert non-traditional dose-responses due to complicated dynamics of hormone receptor occupancy and saturation, suggesting low doses may have a greater effect on a target tissue than higher doses. They stress that the age at exposure is very important. Exposure during development results in different effects than exposures during adulthood. Toxicity in adults requires higher concentrations and effects only last as long as the ED is present. Whereas, low dose exposure during development can result in disruptions that last long after the ED is gone from the body. This “fetal basis of adult disease” may relate to the development of obesity in later life. In addition, exposures during early postnatal development may have the same effect. Exposure to EDs may affect the exposed animal and also the offspring and subsequent generations. The mechanism of transmission involves non-genomic modifications of the germ line such as changes in DNA methylation and histone acetylation (Schug et al 2011; see Figure 2).

The authors note the importance of eating calorie-dense, nutrient-poor food combined with lack of exercise in causing obesity and diabetes in the young (Schug et al 2011). This rapid rise among the young suggests the influence of early life exposures to EDs may also play an important role. They conclude that the precise metabolic pathways targeted by most EDs are uncertain but the data linking EDs with obesity are strong and growing (Schug et al 2011).

2.5 Results of literature search for human studies

Tables 2-2, 2-3, and 2-4 present detailed information on the studies done in human populations that examine weight gain or a weight-related outcome in relation to an environmental exposure relevant to the AHS male cohort. Studies listed in Table 2-2 evaluated a weight-related outcome in a rural or agricultural population. In these studies, no specific pesticide exposure information is provided – the exposure is the rural or agricultural environment itself. Table 2-3 examines the studies identified that examined a weight-related outcome in relation to levels of pesticides or their metabolites or contaminants in human blood, serum, plasma, or tissues. Table 2-4 includes the human studies that do not fit into either of these categories.

Regarding the tables, the first column lists the citation followed by the exposures examined, alone or in combination. The third column lists the population examined, followed by a column summarizing the study design. The fifth and sixth columns explain how the exposure and the outcome were defined or assessed, followed by a seventh column summarizing the main findings, and finally, the last column includes

additional relevant comments. All significant findings related to weight gain or a weight-related variable are in bold in the results column.

The majority of the studies are cross-sectional in design and are, therefore, unable to examine weight gain as a result of exposure; they examine prevalence of weight gain, overweight, or obesity in relation to prevalence of exposure, with exposure defined using a single measurement at one point in time. Two studies utilized nested case-control designs within cohorts of residents of an agricultural area and residents of an area that underwent a chemical accident, respectively (Merchant et al 2002; Bacarelli et al 2005). One study was purely descriptive (Mokdad 2002). All studies are retrospective in terms of weight and/or exposure measures and do not provide the correct temporality to assess effects on a weight-related variable due to previous pesticide exposure. The study by Shah et al (2009) is the exception since it utilizes a case-control design with exposures designated as having occurred in the past in relation to BMI at the time of the study.

2.6 Summary of human studies

Several cross-sectional and one nested case-control study examined broad exposure definitions such as rural residence or occupation as a farmer in relation to BMI or another weight-related outcome. Among the findings are significantly higher prevalence of overweight among white men and women living in rural areas in the U.S. compared to middle-sized or urban areas (Sobal et al 1996); Finnish male and female farmers are

significantly more likely to be overweight than upper and middle non-manual workers (Rahkonen et al 1998); Finnish and Swedish female farmers are significantly more likely to be obese than upper and middle non-manual workers (Rahkonen et al 1998); average BMI was significantly higher for Swedish female farmers compared to other workers, but not for male farmers (Haglund 1987); young Dutch men living in rural areas have significantly higher BMIs than those living in other areas (Baecke et al 1982); rural residents of southern India are at increased risk of high BMI and this is affected by the agro-ecologic region in which they live (Sarkar et al 2012); Polish elderly men living in rural areas who are at risk of malnutrition have significantly lower BMIs than men with adequate nutrition (Wyka et al 2012); black, female, urban residents in the U.S. are more likely to have a favorable weight than those living in rural areas (Duelberg 1992); young men from rural areas in Denmark have a significantly higher prevalence of obesity than those from metropolitan areas (Sorenson and Price 1990); men and women living in rural areas in Czechoslovakia have high rates of severe obesity compared to the urban residents of Prague (Osancova & Hejda 1976); among students in India, urban dwellers are much more likely to show signs of obesity than those living in rural areas (Bandyopadhy & Chattopadhyay 1981); living in a city or small town is associated with significantly higher BMI among males and among females than living in rural areas in Benin, West Africa (Delisle et al 2012); based on the Behavioral Risk Factor Surveillance Survey (BRFSS) data with urban/rural defined by Metropolitan Statistical Area (MSA), rural residents had significantly higher BMIs than non-rural residents (Lutfiyya et al 2012); a study of non-pregnant adult women in

Mexico found that urban residents had significantly higher BMIs than rural residents (Colchero & Sosa-Rubi 2012).

However, oftentimes significant positive findings in one subgroup were offset by a lack of findings or a lack of significant findings in another subgroup. For example, contrary to the results for males, young Dutch women living in rural areas did not have significantly higher BMIs than those living in other areas (Baecke et al 1982) and Polish women in rural areas did not have significantly higher BMIs based on their nutritional status (Wyka et al 2012); contrary to the results for women, average BMI was not significantly higher for Swedish male farmers compared to other workers (Haglund 1987); Swedish male and female farmers were more likely to be overweight and Finnish and Swedish male farmers were more likely to be obese but the differences were not significant compared to other workers (Rahkonen et al 1998). The prevalence of obesity in US males > 59 years old is higher in rural areas but for men aged 17-35 years, half as many obese are found in rural areas compared to urban areas (Kohrs et al 1979).

In many studies, no significant positive association was found between rural residence or occupation and any weight-related outcome. There was no association between overweight and obesity and ever having farmed, having a farm residence, or having a rural residence in Iowa (Merchant et al 2002). No significant difference in mean BMI between urban and rural men from India was identified (Ramachandraiah et al 1994) and male college students from Eastern India who live in urban areas actually have a higher mean percent body fat and body weight than those from rural areas

(Bandyopadhyay & Chattopadhyay 1981). Exposure to farm environments was not related to being obese (Radon & Schulze 2006). There was no significant difference in Quetelet Index for male or female farmers versus townspeople from Iowa (Pomrehn et al 1982). Males in rural states had a greater risk of obesity than urban males but the difference was not statistically significant (Greenberg 1987). Several studies present descriptive data suggesting a relationship between rural residence or occupation and weight-related outcomes but the relevant findings were not evaluated using tests of statistical significance (Kohrs et al 1979; Bjelke 1971; Osancova & Hejda 1976; Rothacker & Blackburn 2000). Two studies present descriptive data only on rural residents or farmers without an unexposed comparison group (Pearson & Lewis 1998; Mokdad 2002). Some studies were limited by small sample sizes, either for the study as a whole (Ramachandrajah et al 1994; Delisle et al 2012; Wyka et al 2012), or for subgroups of interest (Sobal et al 1996).

The nested case-control study of BMI in relation to rural or farm residence or ever having worked as a farmer (Merchant et al. 2002), while utilizing a stronger study design than the correlational studies, found no relationship. This study is nested in a cross-sectional cohort whereby exposure and outcome were measured at the same time. The large cross-sectional study of Swedish and Finnish populations calculated odds ratios for overweight and obesity for farmers compared to other occupational groups and found increased risks with some significant relationships depending on country and gender (Rahkonen et al 1998). The U.S.-based study comparing farmers to non-farmers with respect to Quetelet index found no significant relationship for males or females

(Pomrehn et al 1982). The study of over 50 million participants in the U.S.-based BRFSS found that rural residents had significantly higher BMIs than non-rural residents (Lutfiyya et al 2012).

The study of rural residents from Wisconsin utilized a cross-sectional design to assess change in weight using presently measured height and weight and historical data from five years previous (Rothacker & Blackburn 2000). However, all subjects were rural residents so there is no "unexposed" comparison group and no information is available on the statistical significance of the increase in prevalence of overweight and obesity. The analysis of rural residence in relation to BMI in the NHANES sample of over 11,000 adults defined "rural" as residing in an area with population <10,000 with subjects classified as urban residents if they resided in an area with population >250,000 (Sobal et al 1996). Such broad distinctions are unlikely to provide meaningful results. Overall, the inconsistencies in these findings, coupled with the broad exposure classification, detract from the evidence linking rural residence or occupation with weight gain, overweight, or obesity. However, while studies utilizing such broad definitions of agricultural exposures (i.e., rural residence) may seem unimportant, researchers have pointed out that focusing on urban vs rural residence acknowledges the importance of place in social epidemiology and public health, especially with regard to health inequities and the need for targeted interventions (Lutfiyya et al 2012).

Pearson and Lewis (1998) discussed a mechanism for the high prevalence of obesity they observed among rural residents by describing rural populations as "slow adopters"

to positive health behaviors due to a high rate of poverty and low levels of education. This purely descriptive study also discussed rural families eating diets higher in calories and in fats with reduced energy expenditure due to the mechanization of modern farming. These factors may have contributed to the higher prevalence of obesity observed in rural residents. Overall the results suggest that the rural/farm lifestyle may be influencing activity patterns, which then influences weight gain.

The study by Shah et al (2009) provides some evidence for higher BMI when exposed to Agent Orange but all subjects had prostate cancer and no dose levels were available. Many cross-sectional studies compare levels of environmental or occupational contaminants in human blood, urine, or tissues with BMI and/or prevalence of overweight or obesity. These findings are almost always positive (i.e., higher levels associated with higher weight) but not always significant (Dirinck et al 2011). The studies listed in Table 2-3 provide a representative sample of these studies, with preference given to those with larger sample sizes or those examining a specific chemical rather than a broad class of chemicals.

One study utilized a nested case-control design in evaluating the occurrence of elevated TCDD levels (> 10 ppt vs. $\leq$ 10 ppt) among a sample of 321 subjects selected from the Seveso cohort (Baccarelli et al 2005). This study matched chloracne cases with non-chloracne controls from the cohort and examined TCDD levels in plasma taken in 1993-99 in relation to BMI recorded at the same time. TCDD level was found to increase with increasing BMI. Subjects with high TCDD levels were more likely to have a BMI of greater than 25 versus <21.7 (OR=2.8; 95% CI: 1.0-4.0, p=0.04). BMI between 21.7-

25 also showed an increased odds ratio, but it was not significant (OR=1.6; 95% CI: 0.6-4.0). Lee et al (2011) selected 90 diabetes-free controls randomly from each BMI category from a large prospective study with 20 years of follow-up. All measures were taken at baseline and at each follow-up exam. Organochlorines measured in stored serum samples collected at study year 2 were examined in relation to BMI at each exam through BMI at year 20. DDE and DDT levels predicted BMI at year 20 (Lee et al 2011). The remaining studies were all cross-sectional. Some of these studies were limited by relatively small sample sizes (Schildkraut et al 1999; Munoz-de-Toro et al 2006; Muscat et al 2003; Collins et al 2007; Dirinck et al 2011; Kanazawa et al 2012).

White race, BMI, and breastfeeding were significant positive predictors of DDE concentrations in plasma from women from North Carolina but there was no significant difference in mean DDE between women in the first versus the fourth quartile of waist-to-hip ratio or those with a 5-pound weight gain or loss (Schildkraut et al 1999). The lack of statistically significant findings for blacks in the race-specific analyses was most likely due to small numbers. DDE and total organochlorine pesticides (OCP) in blood showed significant positive correlations with BMI in women serving as controls in a New York-based breast cancer study (Stellman et al 2000). Argentinean women living in an agricultural area showed a significant positive correlation between organochlorine levels in adipose tissue and BMI (Munoz-de-Toro et al 2006). Organochlorine compounds in blood were significantly correlated with BMI in breast cancer cases from New York (Muscat et al 2003). Spanish women living in an agricultural area showed a significant positive correlation between DDE levels in adipose tissue and BMI but there

was no significant difference for lindane, endosulfan, endrin, or dieldrin (Cerrillo et al 2006). Some persistent organochlorines (POCs) identified in whole blood samples from 186 women showed significant findings for body weight and BMI but not others (Kanazawa et al 2012).

In their study of Ranch Hand veterans from the Vietnam War who sprayed Agent Orange, investigators divided subjects into background, low, and high exposure groups based on their current TCDD levels in blood. They found that Ranch Hand veterans in the high exposure group were younger and heavier than those in the background and low exposure groups but no information is provided on the statistical significance of these findings (Michalek et al 1999). BMI was significantly associated with levels of DDE in blood serum from a sample of over 2000 from the NHANES general population, but it showed no significant relationship with levels of oxychlordane or trans-nonachlor (Lee et al 2006). BMI was significantly related to about half of the dioxin analytes examined among chlorophenol production workers and among referents (Collins et al 2007).

Overall the findings on BMI or weight-related outcomes and levels of environmental contaminants in body fluids or tissues consistently show higher levels with higher weight. However, they are based on cross-sectional cohorts and, therefore, cannot distinguish temporality. The only nested case-control study did find a relationship between BMI and TCDD levels in blood plasma (Baccarelli et al. 2005), but it is based on a cross-sectional cohort whereby exposure and outcome were measured at the same

time. The one prospective study did find that DDE/DDT levels measured in study year 2 could predict BMI in study year 20 (Lee et al 2011). Several studies examined broad categories of contaminants (Stellman et al 2000; Munoz-de-Toro et al 2006; Muscat et al 2003; Cerillo et al 2006; Ronn et al 2011; Pelletier et al 2002) and found significant positive correlations. Other studies examined specific chemicals within these broad categories (Lee et al 2006; Collins et al 2007; Kanazawa et al 2012), and found significant positive correlations for some chemicals but not others.

Anderson (1985) indicated that since pesticides and related compounds favor bioaccumulation and are highly lipophilic, the primary accumulation site in humans is adipose tissue and this remains the preferred biologic index for estimating exposure. In our review, several studies based exposure level on adipose tissue samples and found significant associations between exposure level and BMI (Brauner et al 2012; Stellman et al 2000; Muscat et al 2003; Cerrillo et al 2006). Anderson recommends that when adipose tissue is obtained, blood should also be sampled to investigate the resulting partition ratio as an investigative tool. Partition ratios range from 160:1 to 284:1 for DDE/DDT. The only ratio known for lindane is 195:1. Adipose tissue sampling increases detectability, utilizes autopsy or surgical material, is useful for descriptive epidemiology, and provides a direct measurement of body depot. Disadvantages include the use of an invasive technique with risk, serial studies are difficult, time consuming for mass studies, and clinical interpretation is difficult. Adipose tissue levels tend to increase with advancing age. Studies have failed to show a dose response between health outcomes and tissue concentrations. Generally studies report a significant

correlation between adipose tissue concentration and serum. Adipose tissue sampling is most suitable when detection of low level exposure is important, in conjunction with serum analysis to evaluate suspected poisoning, and when autopsy or surgical tissue can be utilized for surveillance (Anderson 1985). Among the pesticides or their metabolites identifiable in human adipose tissue are aldrin, dieldrin, endrin, heptachlor, mirex, lindane, DDT, and dioxins – all relevant to our investigation of BMI and pesticide functional/chemical class exposure.

Several studies have shown that when obese individuals lose a large amount of weight, concentrations of POPs including organochlorines increase and, conversely, when people gain a large amount of weight, concentrations decrease (Lim et al 2011; Tremblay et al 2004; Pelletier et al 2003; Charlier et al 2002; Imbeault et al 2002a; Imbeault et al 2002b; Pelletier et al 2002; Imbeault et al 2001; Chevrier et al 2000; Tremblay & Doucet 2000). The authors indicate that weight loss releases toxins stored in adipose tissue into the blood stream so concentrations in blood are higher, and with weight gain, levels go down because of the increase in body lipids to retain the toxins.

Wolff et al (2005; 2007) explain that while a positive correlation between DDE and BMI seems correct, a negative correlation is dictated by the pharmacokinetics: during uptake or absorption of DDT the model predicts an inverse relationship because DDT concentrations in low BMI persons will be greater than those with high BMI. Since there is a greater lipid denominator in obese people, with comparable uptake, their concentrations should be lower than those of lean people. DDT in adipose tissue

decreases as BMI increases. Wolff et al's statements disagree with the majority of studies we have reviewed which have shown higher DDT/DDE levels in people with higher BMI. Wolff et al. explain this as BMI altering DDT pharmacokinetics during the elimination phase, after cessation of the substantial exposure which began around 1970 in North America, as levels in diet and the environment were reduced. The average clearance rate encompasses extremes that depend on total body fat depot but the half life is longer in obese versus lean people so obese people eliminate DDE more slowly. However, weight gain subsequent to exposure shortens the half-life by diluting the body burden yielding an inverse correlation between DDT and weight gain, but BMI lengthens elimination time and increases half-life. Therefore, weight change and time since peak exposure are key factors in pharmacokinetic variability of a single DDT biomarker. These estimates need to be adjusted for BMI and weight gain and other characteristics such as age and lactation. The use of one measurement for substances such as DDT, which accumulate in the body and change slowly, as a marker for long-term cumulative exposure in epidemiologic studies of chronic disease should be discouraged. Pharmacokinetic variability may cause misclassification when using a single measurement (Wolff et al 2007).

Other researchers have found that the magnitude of the negative slope for DDT versus BMI varies by year of birth; later birth cohorts (younger people) have smaller negative slopes in their first 10-20 years of exposure (Wolff et al 2007). Birth cohort can be a surrogate for onset of exposure. Duration of exposure equals period between birth date and the date of sample donation. During a long interval of exposure, weight gain will

differ for each birth cohort and this will alter correlations of DDT and BMI. There will be large variances and small correlations observed for DDT-BMI relationships in cross-sectional studies where birth dates vary and great heterogeneity exists in BMI and weight gain over time among cohorts. Cross-sectional studies with a single biomarker of exposure might be improved by including known and suspected pharmacokinetic factors and exposure information. Longitudinal data on both biomarkers and covariates would be ideal. BMI may be more important during the absorption phase and weight gain during the elimination phase of exposure. Measurements of DDT at more than one time point would be useful. These pharmacokinetic principles are identical for other exposure biomarkers with a significant body compartment or dilution effect. A more critical problem is when there is mutual confounding such as when BMI is related to both the DDT level and the outcome (Wolff et al 2007).

2.7 Synthesis of animal and human evidence

Few animal studies have examined weight gain due to exogenous exposures. Studies often utilize dosing methods such as injection or gavage that are not relevant to human exposures (Meggs and Brewer 2007; Roegge et al 2008; Lassiter & Brimijoin 2008; Adigun et al 2010). Studies with significant findings in one sub-group often show no effect in other sub-groups (Lassiter & Brimijoin 2008; Deichmann et al 1975; Cassidy et al 1994). Timing of exposure in terms of period of development may be related to whether or not an effect is observed (Lassiter & Brimijoin 2008; Meggs & Brewer 2007; Deichmann et al 1972). Among the studies with the strongest relevance to

humans based on dosing method and availability of detailed information to assess validity, exposures include dieldrin (Deichmann et al 1975), aldrin (Deichmann et al 1972; Ball et al 1953), lindane (Chadwick et al 1988), 2,4-D (Nicolau 1983), and atrazine (Lim et al 2009). As for mechanisms, a number of different theories were purported including circulating metabolites increasing the deposition of total body lipids (Deichmann et al 1972); neonatal low dose exposure disrupting glucose and fat homeostasis (Lassiter et al 2008); impaired leptin production or enhanced clearance (Lassiter & Brimijoin 2008); growth is nonspecific response to stressor action of chemical administration (Ball et al 1953); changes in hormonal regulation of energy balance (Chadwick et al 1988); isomers of chlordane are interacting with sex steroid receptors directly or altering the levels of sex steroids (Cassidy et al 1994); mild mitochondrial damage that mimics the characteristic of the insulin resistance state (Lim et al 2009).

Human studies are plagued by serious limitations including lack of formal statistical analyses (Kohrs et al 1979; Pearson & Lewis 1998; Osancov & Hejda 1976; Mokdad 2002), relatively small sample sizes (Ramachandrajah et al 1994; Schildkraut et al 1999; Munoz-de-Toro et al 2006; Muscat et al 2003; Collins et al 2007; Sobal et al 1996; Dirinck et al 2011; Pelletier et al 2002; Lee at al 2011; Kanazawa et al 2012), positive significant findings in one sub-group offset by lack of significant findings in other sub-groups with the same exposure classification (Kohrs et al 1979; Baecke et al 1982; Haglund 1987; Rahkonen et al 1998; Wyka et al 2012); and the majority of observational studies were limited by the inability to distinguish timing of exposure in

relation to effect due to reliance on cross-sectional cohorts, though there were a few non-cross-sectional studies (Shah et al 2009; Lee et al 2011; Merchant et al 2002).

Results are often inconsistent when available for the same or similar exposure measure. For example, having a rural residence and/or working on a farm has been significantly associated with weight measures in some studies (Sobal et al 1996; Rahkonen et al 1998), but not others (Merchant et al 2002; Bandyopadhyay & Chattopadhyay 1981; Radon & Schulze 2006; Pomrehn et al 1982; Wyka et al 2012; Delisle et al 2012; Lutfiyya et al 2012; Colchero & Sosa-Rubi 2012; Ramachandraiah et al 1994). A few studies that discussed mechanisms for the weight-related changes reported non-biological hypotheses such as rural populations are "slow adopters" to positive health behaviors due to a high rate of poverty and low levels of education (Pearson and Lewis 1998); greater migration of non-obese than obese to a metropolitan area (Sorenson & Price 1990); more favorable weight among urban residents is a result of higher education and income levels (Duelberg 1992; Colchero & Sosa-Rubi 2012); increased SES allows for overnutrition and obesity (Bandyopadhyay & Chattopadhyay 1981). A few studies suggest the obvious reasons: less physical activity and/or a high fat diet (Sarkar et al 2012; Wyka et al 2012). Others provided very complex mechanisms (Karami-Mohajeri & Abdollahi 2010; Schug et al 2011) or suggest environmental causes together with genetic predisposition (Sorenson & Price 1990). The one case-control study includes only prostate cancer patients limiting its generalizability (Shah et al 2009).

Human evidence indicates that environmental exposure to chemicals, including pesticides, can be confirmed by their presence in body fluids and tissues and exposures to these chemicals have been correlated with weight-related variables (Collins et al 2007; Baccarelli et al 2005; Cerrillo et al 2006; Muscat et al 2003; Munoz-de-Toro et al 2006; Stellman et al 2000; Schildkraut et al 1999; Lee et al 2006; Wolff et al 2005; Ronn et al 2011; Pelletier et al 2002; Brauner et al 2012; Lee et al 2011; Lee et al 2007; Elobeid et al 2010; Michalek et al 1999; Kanazawa et al 2012). Experimental data show significant relationships between certain exogenous exposures and weight gain (Lassiter & Brimijoin 2008; Meggs & Brewer 2008; Chadwick et al 1988; Cassidy et al 1994; Deichmann et al 1975 & 1972; Nicolau 1983). The exact mechanism for weight gain due to exposure to exogenous chemicals has not yet been determined. Current evidence suggests that such exposures induce a change in an enzyme or hormone that regulates metabolism, thereby affecting weight gain.

Evidence from animal and human studies may be linked for exposure to organochlorine pesticides since studies of rats and mice exposed to DDT, lindane, or chlordane, all organochlorine insecticides, showed increased weight gain among the exposed compared to the controls (Chadwick et al 1988; Cassidy et al 1994; Deichmann et al 1975 & 1972), and human studies have shown statistically significant correlations between BMI and levels of organochlorines in adipose tissue (Cerrillo et al 2006; Muscat et al 2003; Munoz-de-Toro et al 2006; Stellman et al 2000), blood plasma (Schildkraut et al 1999), or whole blood (Kanazawa et al 2012). A study of rats exposed to 2,4-D found increased body weight compared to controls (Nicolau 1983) and this is

consistent with human evidence showing correlations between BMI and TCDD (a contaminant of 2,4-D) in blood plasma (Baccarelli et al 2005; Collins et al 2007). As the aforementioned studies link evidence between animal and human studies, they represent the strongest evidence currently available on weight gain due to pesticide exposures.

2.8 Chapter Summary

A few animal studies examining weight gain in relation to pesticides or other environmental exposures have shown some significant findings for specific exposures in some animal species using various dosing methods with exposures occurring at different points in development, but results are inconsistent across genders and across similar dose levels. Timing of exposure in terms of development appears to be important. Body weight increase has not historically been a variable of interest in animal experiments with the major focus being on identifying toxic effects with its subsequent weight loss due to the use of extremely high doses. Various mechanisms have been theorized with the majority focused on changes in metabolism and/or hormones affecting metabolism.

Human studies have been mostly retrospective and cross-sectional in design making it difficult to delineate the temporal relationship between exposure and outcome. Additional limitations include lack of formal statistical analyses, small sample sizes, and positive findings in one subgroup offset by a lack of findings in another subgroup

with the same exposure classification. Levels of chemical contaminants in human blood, sera, plasma and adipose tissue appear to be correlated with increased BMI, and/or being overweight or obese, but again, temporality cannot be distinguished. Biological mechanisms for the effects of exposure to environmental or occupational exposures on weight-related outcomes in humans were rarely discussed. Animal evidence and human evidence are congruent for TCDD and organochlorine exposures in relation to weight-related outcomes. Based on the results of this literature review, exposures relevant to the AHS cohort likely to be associated with weight gain and/or overweight or obesity include TCDD and organochlorine pesticides generally.

2.9 Animal and Human Evidence in relation to AHS Exposures

Based on the results of the literature review summarized above and in Tables 2-1 through 2-4, agricultural exposures relevant to the AHS male cohort that may be associated with weight gain include DDT (organochlorine insecticide), organochlorine pesticides generally, TCDD, parathion, chlorpyrifos, lindane, 2,4-D, and chlordane. The most consistent evidence suggests BMI may be related to exposure to TCDD and organochlorine pesticides generally.

The remainder of this chapter will list the specific study hypotheses to be tested using the AHS male cohort. First, those hypotheses based on the most consistent evidence will be listed, followed by the hypotheses of interest based on more limited evidence. All of these hypotheses will be examined in relation to age, race, and state (Iowa or

North Carolina) to assess differences across sub-groups; and length of time spent living or working on a farm in relation to current age will be used to assess exposures during early stages of development. Bearing in mind the proposed analytical plan (multiple regression and ordinal logistic regression), the hypotheses to be tested include:

Phenoxy herbicides (based on exposure to 2,4-D or 2,4,5-T with subsequent exposure to the contaminant TCDD)

Ever exposure to phenoxy herbicides will be significantly correlated with current BMI and with BMI at 5-year follow-up.

Subjects with high BMI (at enrollment and/or follow-up) will be more likely to have ever had high exposure to phenoxy herbicides than subjects with average or low BMI.

Organochlorine pesticides (based on exposure to any organochlorine pesticide, as well as prior studies of lindane, chlordane, DDT)

Ever exposure to organochlorine pesticides will be significantly correlated with current BMI and with BMI at 5-year follow-up.

Subjects with high BMI (at enrollment, and/or follow-up) will be more likely to have ever had high exposure to organochlorine pesticides than subjects with average or low BMI.

Additional hypotheses of interest, though the scientific evidence is much weaker, include:

<u>Organophosphate insecticides (based on prior results for chlorpyrifos and parathion)</u>

Ever exposure to organophosphates will be significantly correlated with current BMI and with BMI at 5-year follow-up.

Subjects with high BMI (at enrollment and/or follow-up) will be more likely to have ever had high exposure to organophosphates than subjects with average or low BMI.

In addition, since we are investigating all pesticide functional and chemical classes regardless of whether or not prior research has associated them with a weight related outcome, we are thereby including exposures in which we do NOT expect a correlation to serve as "negative controls" (i.e., fumigants, fungicides, organothiophosphates, carbamates). If exposure to these non-hypothesized pesticides shows a correlation with BMI, it will be very helpful in deciphering how much weight to place on evidence of an association for those pesticide classes which we believe will show a significant association with BMI based on prior studies.

TABLE 2-1 ANIMAL STUDIES

REFERENCE	CHEMICAL / EXPOSURE	SPECIES	DOSE	METHOD	RESPONSE (including 95% CIs & p-values when available; significant effects BOLDED)	COMMENT
Lassiter et al 2008	parathion, an organophosphate insecticide	offspring of timed-pregnant Sprague - Dawley rats; 12 rats per treatment group for each sex	postnatal days (PND) 1-4 using doses of 0.1 or 0.2 mg/kg/day dissolved in dimethylsulfoxide and injected subcutaneously; controls received only vehicle; selected doses straddle threshold for cholinesterase inhibition and first toxic signs	12 hour light-dark cycle; food and water ad libitum; pups randomized and redistributed to dams at litter size of 10 (5 males, 5 females) on day after birth; randomization within treatment groups continued until weaning; dams were rotated among litters; offspring weaned PND21; beginning on PND105, half of adult rats maintained on standard diet and half given high fat diet for 7 weeks; blood samples taken during PN week 22; used multivariate ANOVA with log-transformed data; groups differing from control identified with Fisher's Protected Test	main treatment effect, $p<0.0001$ MALES: **low dose, increase in weight, $p<0.0002$**, & no change in food consumption; **high dose** similar to controls in weight & **food consumption reduced, $p<0.005$** FEMALES: weight deficit, $p<0.0009$, intensifying over time; **low dose increased body weight up to PND 80, $p<0.05$ with high food consumption, $p<0.002$**; high dose, normal food consumption; from 12 weeks on, significantly reduced weights, both dose groups, $p<0.0001$ HIGH FAT DIET: controls, weight increase, $p<0.0001$, & reduced food intake, $p<0.0001$ MALES: treated with parathion on high fat diet, no difference in weight gain; high dose group on high fat diet, less decrease in food consumption, $p<0.009$ FEMALES: **low dose group on high fat diet, enhanced weight gain, $p<0.0001$,** & could not assess food consumption; high dose group on high fat diet, reduced dietary effect on weight, $p<0.03$, with same food consumption pattern as controls	neonatal low-dose exposure disrupts glucose and fat homeostasis in a persistent and sex-selective manner; females more sensitive to negative metabolic and growth effects of parathion than males; females had greater interactions between exposure and high fat diet with low dose increasing weight gain caused by fat but high dose reduced it; results show lasting metabolic dysregulation due to neonatal exposure characterized in males by net anabolic response at low exposure and catabolic response at high exposures and females had greater sensitivity to catabolic effects; anabolic effects raise potential for gain by high fat diet; organophosphates are developmental neurotoxicants but can have metabolic effects; adverse events in fetal or neonatal life can lead to misprogramming of metabolism

Adigun et al 2010	Parathion, an organophosphate insecticide	Timed-pregnant Sprague-Dawley rats; pups given parathion; numbers of animals not given	Day after birth, all pups randomized & redistributed to dams with a litter size of 10: 5 male, 5 female. Parathion dissolved in dimethylsulfoxide injected subcutaneously in volume of 1 ml/kg once daily on postnatal days 1-4; control animals received vehicle only	Studies conducted on 1 male & 1 female from each litter with 6 animals for each neonatal treatment group. At 15 weeks of age, half switched to high-fat diet providing 58% total calories as fat; remaining rats continued on standard diet (13.5% total calories as fat).	**Global ANOVA** identified significant main effects of diet, sex, and tissue; **diet x sex interaction for each tissue/peripheral measure including body weight ($p<0.04$). Body weight showed main significant effect of diet in males ($p<0.0003$) & females ($p<0.0001$) with sex difference reflecting greater effect of high fat diet in females.**	
Meggs & Brewer 2007	chlorpyrifos, an organophosphate insecticide	female Long-Evans rats, about 6 months of age; 10 exposed and 10 control rats	10 rats, subcutaneous injections of 5 mg/kg/day for 4 months (final concentration of 4.0 mg/ml chlorpyrifos); 10 control rats, injected with equal volume of vehicle (dimethyl sulfoxide – DMSO, diluted with saline) for 4 months	euthanized at 4 months; body parts weighed; preadipocytes placed in cell culture and stimulated to differentiate using 1) mixture of methylisobutlylxantine, dexamethasone, and insulin; 2) 10 ul of DMSO, or 3) increasing concentrations of chlorpyrifos (0.002, 0.004, 0.006, or 0.008 mg/ml) dissolved in 10 ul of DMSO.	**Exposed rats significantly heavier than controls by 2 months: 335.7 g vs 318.6 g ($p=0.03$). Difference increased at 3 months: 350.1 vs 322.3 ($p=0.006$); and 4 months: 374.4 vs 340.2 ($p=0.006$).** For cell culture study, up to 0.008 ug/ml chlorpyrifos or 10 ul DMSO associated with normal cell growth with evident fat accumulation but no growth acceleration or increased cell number.	No acute toxic effects after dosing were observed. Small sample size but large enough to detect statistically significant results. Specific mechanism for weight gain not elucidated.

Lassiter & Brimijoin 2008	chlorpyrifos, an organophosphoro-thionate pesticide	Experiment 1: progeny of time-pregnant, primiparous, Long-Evans rats; litters culled to 4 male and 4 female pups; number of dams not given; total of 9-10 males and 9-10 females Experiment 2: same type and number of rats but only males used	Experiment 1: dams gavaged from gestation day (GD) 7 through postnatal day (PND) 21 with corn oil vehicle, 1 mL/kg, or chlorpyrifos at 1, 2.5, or 4 mg/kg Experiment 2: same doses	dams maintained on relatively fat-rich diet; litters not randomized, cross-fostered, or dosed directly; 2 female and 2 male rats euthanized on PND 22; remaining 4 rats per litter weighed weekly until euthanasia PND 95-101; quantified serum leptin and brain RNA	body weights as % of control, progeny PND 95-101: 1.0 mg/kg 104.8% 2.5 mg/kg 107.5% 4.0 mg/kg 100.7% Weight (g), Exposed vs control, PND 100: **males: 536 g vs 491 g ($p<0.05$)** females: 310 g vs 306 g; BMI (weight/length squared), males: 0.689 vs 0.670 BMI, females: 0.542 vs 0.537 For 2.5 mg/kg/day group at pre-weaning - no affect on body weight of pups; **at post-weaning - increased body weight of males becoming statistically significant on PND 51 ($p<0.05$) and forward;** maximum increase of 10.5% above control at PND 72; no affect on females. Experiment 2: dose-response results showed inverted u-shaped curve; no statistically significant treatment effect; by end of experiment, exposure to 2.5 mg/kg showed same relative weight as first experiment; **pooled all values at 2.5 mg/kg (n=21) and still found significant difference from pooled controls (n=20).**	no changes in maternal weight gain or offspring viability; no toxic affects evident; exposure induced increase in weight and volume and decrease in specific gravity suggest increased adiposity; Mean leptin levels did not increase significantly in exposed males; slope of linear regression: body weight for exposed was half as steep as control suggests impaired leptin production or enhanced clearance. No affects on brain weight or brain RNA levels in pups or adults. Second study failed to fully replicate first but weight and age of dams at breeding differed with first study group weighing more than second (284 g vs 235 g).

Roegge et al 2008	diazinon, an organophosphorus pesticide	35 Sprague-Dawley time-pregnant rats and male and female pups (N=10-12 of each sex per treatment group)	diazinon (DZN) subcutaneous daily injections on postnatal days (PND) 1-4: 0, 0.5, or 2 mg/kg/day; DZN dissolved in dimethylsulfoxide; controls received vehicle only	developmental study also examining interaction between diet and weight gain; on day of birth all pups randomized and redistributed to dams to achieve litter of 12 (6 male, 6 female); pups weaned on PND 21; one male and one female from each litter randomly selected for emotional tests; high fat diet introduced to subset of animals on PND 105: half of rats in each condition put on an ad libitum diet of high fat food and other half maintained ad libitum on regular food; high fat diet given for 5 weeks, then regular diet for 2 weeks; data analyzed using ANOVA	no significant effect of neonatal DZN treatment on body weight gain; **high fat diet increased weight gain in rats given low dose DZN (DZN x diet x time, p<.05);** no effect in controls or high dose group; on regular diet, low dose group tended to have lower weights but not significantly lower but high fat diet reversed this so body weights equaled or exceeded the other groups, but not significantly; no differential effect by sex mean weight (g), week 21, males (M) and females (F): M, control, regular diet = 670 M, control, high fat diet = 710 M, 0.5 mg/kg, regular diet = 620 M, 0.5 mg/kg, high fat diet = 725 M, 2 mg/kg, regular diet = 700 M, 2 mg/kg, high fat diet = 705 F, control, regular diet = 325 F, control, high fat diet = 350 F, 0.5 mg/kg, regular diet = 300 F, 0.5 mg/kg, high fat diet = 340 F, 2 mg/kg, regular diet = 315 F, 2 mg/kg, high fat diet = 340	other results showed neonatal exposure at doses below threshold for cholinesterase inhibition have lasting effect on emotional responses, with preferential effects on males; DZN has different effects than chlorpyrifos reflecting different mechanisms

Deichmann et al 1975	dieldrin or p,p'-DDT - insecticides	Swiss-Webster mice	7-generation study (P - F6); P = parent generation - divided into 3 groups of 10 females and 6 males each and fed dietary supplements to age 260 days: Group 1 = aldrin 5 ppm Group 2 = aldrin 10 ppm Group 3 = DDT 100 ppm Group 4 = controls, 20 females and 12 males, fed regular diet	concentration and total retention of pesticides in abdominal fat and total carcass and total body lipid content determined for 7 generations; all first litters were discarded; when second litters reached weaning age, parents sacrificed to analyze tissues; 0.2 g of abdominal fat removed from each mouse at sacrifice for analysis; control pesticide-free diet fed to all F4 generation experimental mice from weaning to age 260 days; experimental diets resumed with weanlings of F5 generation	**higher weight gain in some groups in some generations** for treated groups, especially F5 & F6 generations but unknown as to significance of each group; text states: "while there were **some statistically significant differences (p<.05) in the body weights** of some of the groups.... there was no significant pattern"; **all groups showed significant (p<.05) increase in total body retention and concentration of dieldrin or total DDT in total carcass of F1, F2, and F3 generations** generally directly related to increases in total body lipids compared with P generation; concentrations in total body lipids & in abdominal fat were essentially the same; pesticides absorbed by F4 generation while *in utero* and via lactation found at sacrifice to have been completely excreted; F5 generation showed repetition of general findings in P and F1 generations	as generations progressed from F3 to F6, conception frequently delayed for 3-5 weeks; results indicate pesticide retention and total body lipids closely related and high body lipid content favors higher retention rate
Deichmann et al 1972	DDT, aldrin, or a combination - - pesticides	groups of 12 male and 12 female weanling, adult (4-6 months), and old (18-20 months) Osborne-Mendel strain rats (to take account of age and sex related differences in metabolism rate of chemicals)	4 weeks diet supplemented with 50 ppm DDT, 7.5 ppm aldrin, or a combination of 50 ppm DDT plus 7.5 ppm aldrin; similar groups fed control diet	after 4 weeks on treatment diets, 6 males and 6 females per group sacrificed; remaining rats starved for 6 days with free access to water, then sacrificed; tissues analyzed; data examined with Student's t-test	**DDT and/or aldrin supplement: significant increase in growth rate of male & female weanling rats vs rats fed control diet;** group, male(M) / female(F), weight (g) after 4 weeks: control, M, 147; control, F, 131 **DDT 50 ppm, M, 188 (p<.05)** DDT 50 ppm, F, 150 **DDT + aldrin, M, 222 (p<.05)** **DDT + aldrin, F, 165 (p<.05)** **aldrin 7.5 ppm, M, 244 (p<.05)** **aldrin 7.5 ppm, F, 184 (p<.05)** data strongly suggest circulating DDT & metabolites, particularly aldrin or dieldrin, responsible for increased deposition of total body lipids & increased body weight; aldrin + DDT showed less marked effects	concentration of pesticide in blood & in abdominal or subcutaneous body fat not directly related to total body pesticide load; therefore, relating human tissue pesticide concentrations directly to total body load misleading especially in persons suffering from disease; hepatic microsomal enzyme activity stimulated in rats but starved animals showed further augmented degree of stimulation in spite of reduced total body pesticide load due to starvation

Ball et al 1953	Aldrin, an organochlorine insecticide	Sprague-Dawley rats	3 groups of 25 female rats with statistically comparable weights fed from age 30 days a series of aldrin preparations at 5, 10, and 20 ppm in feed; 4th group acted as control.	Exposed for 6 weeks – no difference in growth from controls. From 6th week, aldrin supplied in form of wettable powder containing 2.5% aldrin for 20 weeks. Withdrawal effects assessed from 26-38th week of feeding. Resumption effects assessed from 38th weeks with wettable powder containing 20% aldrin. From 55th week aldrin levels doubled.	**20 ppm group reached maximum 24% higher than controls (<0.01). 10 ppm group significantly increased growth (p<0.01). At withdrawal, 20 ppm group decreased weight for 6 weeks but failed to drop to level of controls; final weight difference of 9% (p<0.01).** 10 ppm group had initial weight loss but gained between 6-12 weeks of withdrawal; rats on 5 ppm gained throughout study at same rate as controls. At resumption, 20 ppm group gained abnormally until end of experiment. 40 ppm group was 14% higher at week 64 than controls but did not differ in length.	Growth effect associated with increased food consumption which by 64 weeks reached 180% of controls for 10 ppm group and 270% for 20 ppm group. Constituents of different trade formulations other than aldrin responsible for growth effects. Growth effect is nonspecific response to stressor action of chemical administered.
Takahama et al 1972	Endrin, lindane, DDT	20 healthy adult rabbits (but only 3 rabbits per treatment group and one control rabbit)	0.1 mg/kg Endrin 6.25 mg/kg Lindane 50 mg/kg DDT; given twice a week via Nelaton catheter inserted into stomach	Body weighed once per week. Weight reported at 5, 12, 18, and 24 weeks.	No statistical analyses of differences between groups. We used PROC GLM (general linearized model) to analyze difference in weight among groups due to unbalanced numbers in each group eliminating ability to use PROC ANOVA. Overall model containing variables group (pesticide), subject (rabbit), week, and **weight highly significant (p<0.0001). Type 1 Sum of Squares group variable highly significant (p<0.0001). Compared to reference rabbit, significant differences identified in weight based on positive parameter estimates (higher weight) for DDT (p=0.0007, endrin (p<0.0001), and lindane (p=0.0186)**	

Chadwick et al. 1988	lindane or DDT, insecticides; 2,4-D, a herbicide	Study 1: 24 Fischer 344 weanling, female rats Study 2: 44 female pups from timed pregnant Fischer 344 rats	Study 1: daily peroral doses of vehicle or 0.069 mmol/kg lindane, p,p'-DDT, or 2,4-D for 189 days; dosed at volume = 0.1% body weight Study 2: starting at age 21 days, gavaged daily with 0, 5, 10, 20, or 40 mg/kg lindane for 15 weeks; n = 6, 6, 8, 12, or 12 rats respectively	Study 1: free access to food and water; 12-hour light/dark cycle. Study 2: food and water same as above; obesity measure used was Lee index: [body weight (g)]$^{0.33}$ x 10^4 / naso-anal length (mm)	Study 1: DDT & 2,4-D = no significant effect on body weight; **lindane significantly increased body weight after 84 days treatment (183.5 vs 166.9 g; p not given)**; weight increased with treatment time; at 112 days treatment, lindane increased body weights 20% more than controls (207.4 vs 174.3 g; p not given). Study 2: **20 mg/kg lindane significantly increased body weight vs controls after 77 days (189.3 vs 172.5, p<0.05);** for 40 mg/kg group, gain increased only at 13 weeks treatment (numbers not given) and approached significance; 7/12 dosed at 40 mg/kg died; by week 12, all treated rats had significantly higher Lee index than controls; appears to be dose-response but no info on test for trend. Lee Index by dose at 14 weeks (experiment end): 0 mg/kg 301.9 5 mg/kg 306.1 10 mg/kg 305.8 20 mg/kg 319.4 40 mg/kg 319.0	lindane may interfere with female rat reproductive function by acting as antiestrogen due to change in tissue's ability to recognize endogenous estrogen or change in availability of endogenous estrogen; changes in body weight may be due to altered ovarian hormone secretion and/or impaired response to circulating estrogen levels; changes in hormonal regulation of energy balance could account for obesity of treated rats; if lindane interferes with appetite-suppressing and exercise-stimulating activity of estradiol, more energy available for weight gain would go to body fat and less would be available for other tissues.
Nicolau 1983	2,4-D (2,4-dichlorophenoxyacetic acid) -- a herbicide	120 random-bred male Wistar rats	60 rats received daily 100 ppm 2,4-D mixed with regular diet over 90 days	housed under natural lighting conditions; on day of study, subgroups of 5 animals receiving 2,4-D and 5 controls were weighed and sacrificed at 6-hour intervals for a total of 12 timepoints; concentrations of RNA, DNA, and protein determined in tissues from adrenals, thyroid, and testes; single cosinor analyses performed for each of 3 24-hour spans and for entire time span of 3 days; differences in rhythm parameters statistically evaluated	**at sacrifice, body weight of exposed rats = 273 and controls = 255, p<0.01**	alterations in circadian rhythm in thyroid and adrenal, and to a much lesser degree, testis, were observed

Cassidy et al. 1994	chlordane, a cyclodiene insecticide used for termite control and in agriculture during 1950-1970s; it is classified as an organochlorine in AHS	time-pregnant Sprague-Dawley dams and offspring; litters culled to 10 offspring; the number of dams and pups used is unclear	Dosed daily using peanut oil; dosing method is unclear; control and 3 dose groups: 100, 500, and 5000 ng/g chlordane; dams dosed from gestation day 4 to lactation day 21; pups dosed from postnatal day 22 to 80.	Dams and offspring housed together until pups aged 22 days; litter sizes culled to 10 offspring (5 of each sex) by postnatal day (PND) 4; from PND 22-80, pups housed separately; 12-hr light/dark cycles; food and water ad libitum	Dose-related increases in body weight observed in female offspring dosed with 100, 500, and 5000 ng/g chlordane with increases in body weight of 8, 11, and 4%, respectively. **Only female offspring in 500 ng/g group significantly different from controls (<0.05) with 5 litters tested.** Male offspring had only modest weight gains. Nonsignificant weight gain also observed in dams during gestation and lactation. Effect was both dose and sex dependent.	Low dose group (100 ng/g) generated levels in dam and offspring representative of serum levels found in U.S. at 99th percentile (i.e., only 1% of U.S. values higher); suggest isomers of technical chlordane are interacting with sex steroid receptors directly or altering the levels of sex steroids
Lim et al. 2009	Atrazine, a triazine herbicide	48 male Sprague-Dawley rats	Dosed for 5 months with vehicle or 30 or 300 ug/kg/day in drinking water; regular diet for one group; second group fed regular diet for 3 months followed by high fat diet for 2 months	Initial weights equivalent for controls & exposed groups. Dose calculated by multiplying atrazine concentrations by volume of water consumed; diet and water consumptions measured 2 times/month; movement evaluated using spontaneous motor activity analyzer for 4 hours/month	Used t-tests, ANOVA, correlations. **Atrazine increased body weight 5.5% compared to controls & high fat diet group increased by 9.8% (p<0.05)**—not related to water/food intake or physical activity level. **Exposed had more visceral fat than controls (p<.05);** exposed with high fat diet enhanced visceral fat accumulation; regular diet group showed lipid accumulation in muscle and liver. Measured energy expenditure using indirect calorimetry – energy expenditure of exposed rats on regular diet reduced in dose dependent manner.	Previous studies using dose levels 10-100 fold higher caused weight loss. Acute exposure is toxic. Chronic, low-dose exposure might lead to mild mitochondrial damage that mimics the characteristic of the insulin-resistance state, and hence, leads to weight gain.

TABLE 2-2 HUMAN STUDIES, GENERAL RURAL ENVIRONMENT

REFERENCE	CHEMICAL / EXPOSURE	POPULATION	STUDY DESIGN	EXPOSURE ASSESSMENT	OUTCOME ASSESSMENT	RESULTS (with 95% CIs or p-values when available; significant effects BOLDED)	COMMENTS
Merchant et al 2002	agricultural & rural environmental exposures	1000 households in Keokuk County, Iowa (entirely rural): 34% farm, 20% rural nonfarm, & 46% town households.	nested case-control in large cross-sectional cohort; age-adjusted ORs & 95% CIs computed using multiple logistic regression.	Baseline in-person interviews with household members. Obtained complete job history for adults. Separate head-of-household interview by industrial hygienist with environmental measurements taken using direct reading instruments.	Baseline in-person interviews with household members from 1994-1998; round 2 of data collection began 1999.	Overweight & obese OR (95%CI) ever farmed 0.97 (0.71-1.32) farm residence 0.88 (0.66-1.17) rural residence 1.18 (0.83-1.69) Obese OR (95% CI) ever farmed 1.03 (0.77-1.39) farm residence 0.90 (0.77-1.39) rural residence 1.18 (0.86-1.61)	Of 2496 eligible households, 67.1% agreed to participate; enrollment stopped when 1000 goal was reached. Power = 80% to detect assumed differences; alpha of 0.01 to accommodate multiple comparisons. Extent of farm exposures for entire cohort diminished chance to detect differences for health outcomes related to farming.

Kohrs et al 1979	rural residence	multistage stratified cluster sample of 718 subjects after exclusions; statewide sample = 7458 persons; drawn from all individuals residing in housing units in Missouri according to 1970 U.S. Census; sampling assured representative sample of persons below poverty level, from 5 age classes, from all state regions	cross-sectional; used data from U.S. Health Examination Survey tables as standards to determine % of average weight for age and height. Descriptive information only.	Rural defined as living in the country or any community with population <2500; all others were classified as urban.	Weight and height were measured. Normal weight = 90-109% of average; weight >10% above average = overweight; weight >10% below average = underweight; weight <80% of average=thin; weight >=120% of average=obese; weight >159% of average=super obese. BMI >27 also = obese.	Prevalence of obesity, men & women: aged > 16 years old: rural greater than urban when weight >=120% of average = obese; using BMI definition showed similar trends for women but not men. Prevalence of obesity, men: > 59 years old: increased from about 14% in metro areas to about 36% in rural low income areas. aged 17-35 years: half as many obese from rural & low income rural as from metro areas. aged 36-59 years: 1/4 as many obese from metro areas and rural areas as from low income rural area. Prevalence of obesity, women: aged 10-59 years: 20-25% from rural area obese aged > 59 years: almost twice as many from low income rural area obese vs women 36-59 in same area; about twice as many from low income rural areas vs other 2 areas in this age group.	Comparison group: U.S. data from NCHS collected 1960-62 using nationwide probability sample. No tests of statistical significance were performed.

Sarkar et al 2012	Rural residents of southern India state of Karnataka which has experienced 6 decades of agricultural practice; current use of pesticides is widespread	Data collection May-August 2009 including survey in 6 villages; used in depth personal interviews among 240 household key informants. 10 households randomly selected from 4 landholding categories – 40 households from each village = 240 households from 6 villages; 9.5% of all households in the area.	Cross-sectional study used GLM analysis based on BMI applied to estimate RR & 95% CI for low BMI (<20) & high BMI (>25) based on gender & agricultural practices.	Samples of rice (n=15) & straw (n=1) collected to assess pesticide levels. Used various pesticides especially organo-phosphates. Most widely used: buprofezin, endosulfan, phorate, monocrotophos, imidacloprid with application rates for latter 2 71% & 73% higher than recommended. Rice principal crop in recent years replacing coarse cereal grains. 8 rice samples from head area showed detectable insecticides & fungicide. 7 rice samples & 1 straw sample showed levels of endosulfan & mercury. Water samples showed high levels of fluorides & nitrates. However, for RR analyses, BMI examined in relation to area of state & land ownership.	BMI measured at time of personal interview for each adult household member.	BMI levels varied for different areas. Overall, females (F) with low BMI = 102 & high BMI = 51 vs males (M): (82 & 44 respectively). RRs of low & high BMI presented by agro-ecological area. **Low BMI in dryland vs head end** **RR, M, 2.05 (1.33,3.17)** **RR, F, 1.59 (1.12, 2.24)** **High BMI head end vs dryland** **RR, M, 2.38 (1.10, 5.11)** **RR, F, 2.50 (1.29, 4.86)** **Low BMI dryland vs ancient areas** **RR, M, 1.95 (1.28, 2.99)** **RR, F, 1.70 (1.19, 2.44)** **Low BMI females landless vs small landowners** **RR=1.89 (1.21, 2.95)** **Low BMI landless vs small landholders** **RR=1.54 (1.07, 2.23)** **High BMI small landholders vs landless** **RR=3.00 (1.16, 7.73)** **High BMI large landholders vs medium** **RR=2.10 (1.08, 4.07)**	Prevalence of overweight (BMI > 25) most evident in large landholding households; women had greater risk of extremes of undernutrition and overweight. Results demonstrate land ownership is significant factor in BMI. Principal causes of high BMI: less physical work, high fat intake. Four major visible impacts: occupational hazards, vector borne diseases, changing nutritional status, inequity in development. Modern agricultural practices have changed ecology & disease pattern in this area of India. Indian government now banned use of endosulfan. Study utilized limited information from health facilities supplemented by perspectives of sampled population, health & agriculture officials, & outcomes of ecological surveys. Under nutrition in early life may be contributing to increased obesity in the area.

Sobal et al 1996	rural residence	Second National Health and Nutrition examination survey (NHANES II) -- probability sample of U.S. residents conducted 1976-80; of 16,510 in full sample, 11,578 adults aged 17-74 years included here; current sample included 10% blacks	cross-sectional; analyses stratified by sex and race	rural-urban residence coded by NCHS using 1970 census data: rural = <10000 population; middle-sized= 10000-249,999; urban=250,000+.	BMI based on measured heights and weights. Overweight=BMI >=27.8 for males or 27.3 for females. Extreme overweight=BMI> =31.2 for males or 32.4 for females.	**White women: rural significantly higher than urban for relative weight, overweight, & extreme overweight (p<.001) and than middle for relative weight (p<.001) & overweight (p<.05) but no longer significant after controlling for mediating variables except for overweight in rural vs urban (p<.01).** Black women: no significant rural-urban differences probably due to small sample size since coefficients sometimes > white women. **White men: rural significantly higher relative weight & overweight than middle or urban (all p<0.05) & for extreme overweight vs middle only (p<.05); controlling for other variables diminished all effects except for overweight in rural vs urban (p<.05).** **Black men: no significant urban-rural differences; rural significantly higher than middle on relative weight & extreme overweight (p<.001)**; effect diminished only for relative weight after controlling for mediators.	Small sample size for rural blacks limited statistical power. Variables controlled for: age, age-squared, education, family income, employment, marital status, parity for females, energy expenditure, energy intake, smoking.

Ramachandrai -ah et al. 1994	rural residence	Men from India; 190 urban men from Tirupati and Chittoor and 190 men from 10 rural villages within 40 km of these 2 urban centers selected through electoral registers; age range = 40-76 years; excluded smokers, alcoholics, and those with relevant past illnesses	cross-sectional; compared 130 urban men vs 115 rural men based on interview data and blood tests	urban/rural determination based on residence	not clear if BMI from measurements or self-reports	Mean BMI not significantly different between the groups: rural = 20.97 +/- 0.19; urban = 21.37 +/- 0.16.	All men performed heavy labor; participation rates: urban = 76%; rural = 48%
Wyka et al 2012	Rural residence	174 elderly women & 64 elderly men (over age 60 years) living in rural areas in Olesinica, Poland. National registration used to select 1010 women & 762 men over age 60 years. Response rate was 17% for women & 8% for men.	Cross-sectional; used mini nutritional assessment questionnaire (MNA) to classify subjects as at risk of malnutrition (score = 17-23.5) or adequate nutrition (score >24) & malnutrition (score <17). Personal interviews, plus blood collection (cholesterol & triglycerides), & anthropometric measures. Statistics: chi-square, t-test, Mann-Whitney rank sum test.	No description of how rural residence identified or defined.	Anthropometric measures taken using standardized methods to calculate BMI.	Most women had high BMI (mean=28.8**). Men at risk of malnutrition had statistically significantly lower BMI (26.0 vs 28.7, p<0.04) than men with adequate nutrition.** Women at risk of malnutrition had lower BMI than those with adequate nutrition but not significant (27.9 vs 29.0, p<0.29). Mean BMI in men = 28.3 indicating excess weight.	Despite low energy value of diet for subjects with adequate nutrition, anthropometric parameters paradoxically showed presence of excessive fatty tissue. Low energy value of diet associated with incorrect energy structure (too much energy from fat). Place of residence urban/rural may affect nutrition & health of elderly with rural elderly having poorer nutrition & lifestyle.

Bjelke 1971	agricultural & forestry municipalities versus others	respondents to mail survey from systematic sample drawn from general population of Norway in 1964; 8638 men & 10331 women; response rates were 79% and 82%, respectively;	cross-sectional; sampling frame: all men born between 1893-1927; all women born between 1893-1932; sampled more from south west and from sparsely populated northern most region; sampled twice as many born before 1912 as after	used region of residence at time of 1960 census, which for most coincided with current region of residence; agricultural/forestry area corresponds to characteristic occupational pattern among economically active population; also used urban/rural classification based on 1960 residence	index weight divided by square of height, sometimes called Davenport index, here referred to as bulk index, chosen as measure of weight adjusted for height using self-reported information	bulk index lower in densely populated areas than in sparsely populated areas; no difference between areas characterized by agriculture and forestry and the fishing areas; variation with municipality type more pronounced in females than males; in each sex, those born in rural areas had higher bulk index than those born in urban areas, irrespective of urbanization of current place; persons living in rural areas had higher bulk index than those living in urban areas irrespective of urbanization of birthplace; associations somewhat stronger for males for birthplace; in females association with current place was strongest; variation of bulk index by broad occupational groups reflects associations based on population density and urban/rural; no mention of statistical significance of any results	bulk index correlates well with relative adiposity as estimated from body density measurements; bulk index varied more according to characteristics of current place like population density rather than geographical location; variation in bulk index by occupational group did not reveal any consistent association with social class

Haglund 1987	resident in farming municipality; also examines physical activity at work and at leisure	sample of 7986 persons from county of Skarsborg, Sweden, consisting of 16 municipalities; 3993 men and 3993 women aged 25-75 years; selected using stratified random sampling; participation rates: 70% men, 71% women	cross-sectional; in 1977, teams visited 16 municipalities; questionnaires distributed 2 weeks in advance & reviewed prior to physicals; data age standardized using indirect method; Fisher's permutation test and stepwise multiple linear regression used to analyze data; also used forced regression analysis	socioeconomic and occupational data from questionnaires; coded occupation using Nordic system; physical activity at work and leisure measured with one multiple choice question;	health exam included height, weight measurements; calculated ponderal index, BMI, and Quetelet's index	municipalities with high BMI are dominated by farming; **workers and farmers have a significantly higher mean BMI for women; BMI significantly correlated to physical effort on the job and during leisure**; greater weight is correlated with higher physical effort on the job but the opposite applies to physical effort during leisure; significantly lower weight in higher SES groups for men and women; **over weight in men observed mainly in occupations engaged in heavy physical work;** mean BMI for male farmers = 25.8 & **female farmers = 26.0 ($p<.05$);** for all workers, males = 25.3, females = 24.6; regression using **BMI as dependent variable shows significantly related to physical activity at work for women ($p<.05$) and to physical activity during leisure for men ($p<.05$) and women ($p<.0001$)**	used higher level of significance ($p<0.005$) to allow for mass significance problem; analysis of non-participants indicated higher morbidity & mortality; findings may be explained by differences in SES distribution between municipalities: those with higher proportion of farmers have higher mean BMI; geographical variations probably contingent on differences in economic & business structure & other SES preconditions; analysis of differences in weight index between SES groups & occupations shows differences within respective groups is often greater than differences between groups; greater differences between SES groups for extremely overweight more pronounced for women versus men; hard labor positively correlated with overweight (may reflect greater muscle mass but more likely cultural and SES factors since physical activity during leisure negatively correlated with weight)

Baecke et al 1982	rural versus urban residents	3936 young adults in municipality of Ede in The Netherlands representing 33% of all invited; 3 age groups: 19-21, 24-26, 29-31 years in 1980	cross-sectional; questionnaire completed at home and visit to a mobile research unit; pregnant women excluded; nonparticipants study of 472 subjects (response rate 92%) to obtain education, height, weight, reasons for not responding; statistical analyses utilized t-tests, ANOVA, correlation coefficients, multiple regression	questionnaire collected info on sociodemographic, behavioral and medical factors, including urbanization; Ede consists of 3 districts in an urban area and 4 villages in a rural area; urban districts have > 10000 inhabitants; rural districts have < 3000 inhabitants	body weight and height measured to nearest 0.1 kg and 0.1 cm; BMI calculated; correlation between BMI and % body fat was 0.81 in males and 0.79 in females indicating BMI suitable index of relative weight in this population	mean BMI of males in rural area higher than males in urban area; **males in rural area had BMI 0.6 kg/m^2 higher than urban males ($p<.05$);** no relationship between urbanization and BMI for females; 1765 males: 7.1% rural; 2092 females: 7.3% rural	age and socioeconomic status were most important socio-demographic determinants of BMI; no differences between participants and nonparticipants with respect to age and BMI, but nonparticipants had lower education levels; most important reason for low response rate was invitation by mail; nonparticipants study showed no bias introduced to BMI (i.e., non-response was not differential by BMI);; adjusting for level of education may have reduced any urban-rural differences; only a small proportion of the variation in BMI explained by socio-demographic variables

Greenberg 1987	Rural vs urban residents	CDC 1981 Behavioral Risk Factor Survey; individual surveys obtained from New Mexico, New York, Pennsylvania, Wyoming; 1,164 surveys for 18-34 year old population	Cross-sectional;	phone numbers used to place each subject into county group: 75% urban, 50-74.9% urban, and rural	Collected info on smoking, obesity, hypertension and sedentary lifestyles in survey	Examined only whites due to Insufficient numbers of non-whites; used parametric statistics. Urban/rural differences in 4 risk factors were small; when different, indicated better practices in urban areas. Rural population reported less risk than urban for sedentary lifestyle – rural advantage from jobs requiring physical activity. Rural states have greater prevalence of obese than urban states, especially among elderly (i.e., 37% & 42% respectively, of elderly males and females in North Carolina were reported to be obese vs 19% and 21% in California. Males 18-34 years old in rural states had greater risk than urban males for all 4 behavioral risk factors; females for 3 of 4. None were statistically significant. **Having < 8th grade education = 2 times as likely to be obese as those with some college (21% vs 11%, p<.05).**	
Duelberg 1992	rural versus urban residents	19,027 female respondents: 16.2% black, 83.8% white; participated in 1985 National Health Interview Study (NHI): sampled civilian, non-institutionalized U. S. residents; blacks over-sampled; response rate 96% for supplement on health promotion & disease prevention (1 adult per household)	cross-sectional; races other than black & white excluded; conducted series of multiple regressions using progressive adjustment	urban/rural residence determined according to person's place of residence: metropolitan area = urban; others are non-metropolitan areas; other independent variables were race, education, income, marital status	all dependent variables based on self reports; high score = positive health behavior; variables included exercise, favorable weight, and non-smoking; degree of overweight measured by Quetelet index (ratio of weight to height squared)	**bivariate correlation between urban/rural and favorable weight = 0.038, p<0.001;** urban residents are more likely to have favorable weight than rural residents; black women less likely to have favorable weight; **controlling for education and income reduced the effect of urban residence but it was still significant and positive;** interaction of race and urban residence is not significant so effect of race on favorable weight not conditioned by urban residence; both urban and rural black women less likely than whites to be normal weight	Quetelet index most adequate weight-to-height measure: least correlated with height & highly correlated with skinfold measures; therefore, good indicator of total body fat. Large part of positive effect of urban residence on favorable weight due to higher education & income levels in urban areas; black women less likely to exercise, be normal weight, & be non-smokers than whites; black women less likely to exercise or be normal weight even at same level of education & income as whites; urban residence had no effect on exercise & negative effect on non-smoking

Osancova & Hejda 1976	rural versus urban residents	12,000 men and women from an urban area (Prague) and two rural areas (Eastern and Western Bohemia) in the former Czechoslovakia	cross-sectional; nationwide survey conducted to study blood pressure	not stated	heights and body weights were recorded	obesity prevalence more frequent in women than men; % of desirable weight by sex and region: Men -104 105-14 115-24 125+ Prague 48.6 30.5 16.7 4.2 E. Bohemia 45.2 23.5 16.3 15.0 W. Bohemia 44.6 26.8 17.9 10.7 Women -104 105-14 115-24 125+ Prague 52.6 17.9 11.6 17.9 E. Bohemia 28.3 19.3 13.0 39.4 W. Bohemia 25.7 20.8 13.4 40.1 Rural areas ratio of severely obese men much higher than in Prague and rural women with low or normal body weight is half that of Prague and ratio of severe obesity is more than twice that of Prague.	no information on statistical significance of findings
Bandyopadhy-ay & Chattopadhy-ay 1981	rural versus urban residents	381 male college students residing in several districts of Eastern India; aged 16-24.5 years	cross-sectional; subjects randomly selected from healthy students	urban-rural classification based on residence using guidelines of Indian Census of 1971; urban = places with municipality, corporation, cantonment, or designated town area or minimum population of 5000 or at least 75% of male workers nonagricultural or population density of at least 400/sq km; all other places are rural	anthropometric measurements of skinfold thickness at several body sites; thicknesses of biceps, triceps, subscapular, suprailiac, and abdominal skinfolds measured with caliper on right side of body; measures used to estimate body density, body fat, and lean body weight; body surface area determined	**mean percent body fat significantly higher in urban group of students (12.07) vs rural (11.04) (p<.001); mean body height (p<.05), weight (p<.01), fat body weight (p<.001), lean body weight (p<.05), & surface area (p<.05) significantly higher in urban group; mean thickness of biceps (p<.001), triceps (p<.001) & subscapular (p<.01) skinfolds significantly greater in urban group;** 10.3% of urban group & 2.1% of rural group could be classed as obese & overnourished based on > 12 mm for triceps fold, > 8 mm for suprailiac fold, & >15 mm for subscapular folds	authors note measurements of fatness in both groups showed significant positive skew but large sample avoided use of nonparametric tests; per capita income of rural population significantly lower than urban throughout India; increase in prosperity positively correlated with overnutrition and obesity; compared to other studies in other countries, college students of Eastern India are leaner than youths of North America, Europe, Japan, and Northern India;

Sorenson & Price 1990	rural versus urban residents based on population density of region of residence	38,132 Danish young men; 10% of all those undergoing mandatory draft board exam; birth cohorts 1939-58 for Greater Copenhagen and western provincial region and cohorts 1947-58 for 2 other provincial areas	cross-sectional; used records of draft board in Denmark - obligatory for all males past age 18 years; about 5% unfit for military service but obesity not grounds for exemption; sample of 10% of population of draftees drawn in 1985 by selecting those born on 3 days of each month in 1939-58 with complete height and weight data; analyzed with linear and multiple logistic regression	1968 regional population densities: Region 1 = 806 per km^2 Region 2 = 88 per km^2 Region 3 = 123 per km^2 Region 7 = 58 per km^2 Compared metropolitan region 1 to other 3 provincial regions.	at draft board exam, height and weight measured and BMI calculated; systematically recorded since 1957 in region 1 (greater Copenhagen) and region 3, but only since 1964 in region 2 and region 7. Quartiles used to describe distribution of BMI by years and regions; obesity = BMI > 30.	no significant interactions between variables region and year born; no difference between regions 1 and 3 (p=0.46) and trend over time did not depend on region; increase in prevalence of obesity was significant (p=0.03); for analysis of all 4 regions, prevalence of obesity also rose but much less than earlier period; distinct increase in region 2 not seen in other regions; **overall increase in obesity prevalence significant, as was the difference between metropolitan and provincial regions (rural higher), but no significant interaction between the two (p values not given)**	authors conclude the secular increase in obesity prevalence without similar trends in median BMI suggests existence of environmental causes of obesity to which population increasingly exposed together with genetic predisposition; greater increase in obesity prevalence in provincial region closest to Copenhagen may be due to greater migration of non-obese than obese to metropolitan area
Delisle et al 2012	Rural vs urban residents	541 male and female residents aged 25-60 years selected randomly from a city, small town, and rural area in Benin; response rate = 86.9%; must have lived in area <u>> 6 months;</u> <u>residents with diabetes, hypertension or CHD excluded</u>	Cross-sectional; used chi-square, t-tests, ANOVA, multiple linear regression	Personal interviews for age, sex, education, smoking, alcohol; urbanization level determined by criteria of the Benin government; diet assessed with 2-3 non-consecutive 24 hour dietary recall questionnaires; 2-3 24-hour physical activity recall questionnaires;	Weight, height, and waist circumference (WC) measured using standard methods	**For males, living in city or small town significantly associated with higher BMI and WC than rural residents. For women, only BMI significantly higher in urban and small town residents versus rural residents.** **All mean BMI measurements significantly different from each other across locations ($p<0.05$):** Men Urban = 22.8 Small town = 22.5 Rural = 21.2 Women Urban = 27.5 Small town = 26.0 Rural = 23.7	First study done in West Africa examining these factors. Authors note limitations of cross-sectional design, use of subjects from one area of Benin, measured diet with 2-3 24-hour recalls, no information on early part of life which may influence current obesity levels.

Lutfiyya et al 2012	Rural vs urban residents	Subjects selected from participants in 2009 Behavioral Risk Factor Surveillance Survey (BRFSS); analyzed 52,259,789 U.S. adults who consumed ≥ 5 servings of fruits & vegetables per day; of these, 8,983,840 lived in rural areas. Missing data excluded.	Cross-sectional; bivariate analyses & logistic regression & descriptive comparisons between rural & urban residents based on diet, gender, race, marital status, household income, physical activity, BMI, personal physician, routine medical exams, self definition of health status, deferred medical care due to cost.	Rural defined using Metropolitan Statistical Area (MSA) identifications included in BRFSS	BMI determined by self-reported response to questionnaire on weight and height. BMI categorized as < 25 (not overweight or obese); >25 to <30 (overweight), >30 (obese)	**Bivariate significant results include rural had higher BMI than non-rural. All percent differences had p<0.05.** **% rural, BMI < 25 = 32.8 vs 37.2% non-rural** **% rural, BMI 25-<30 = 36.6 vs 36.2% non-rural** **% rural, BMI ≥ 30 = 30.6 vs 26.6% non-rural** **Logistic regression results based solely on rural residents found they were more likely to have BMI < 25 or BMI 25 to <30. Adjusted odds ratios:** **BMI <25 1.126 (1.124,1.129)** **BMI 25-<30 1.066 (1.064, 1.068)** **BMI ≥ 30 = referent group**	Focusing on urban vs rural residence acknowledges importance of place in social epidemiology & public health, especially with regard to health inequities & need for targeted interventions. Study limitations: telephone survey so those without a phone not included; questionnaire used closed-ended questions; answers all self-reported creating possibility of recall bias, only included English or Spanish speakers, no standard U.S. definition for rural. Study strength: large sample size.

Colchero & Sosa-Rubi 2012	Rural vs urban residence	Sample of 12,302 non-pregnant adult women over age 20 years.	Data from cross-sectional survey conducted in 2006 of 48,308 households. Used two stage least squares regression to control for endogeneity of income stratified by urban/rural; secondly explored determinants of weight using latent class models. Household income weighted to reflect household composition. Used Lincom test to compare urban/rural samples in bivariate analyses.	Urban – rural assessment not described.	BMI used as standard measure of weight, and as dependent variable. BMI measured by trained personnel following international standards.	Latent class model shows positive marginally significant association between income & BMI only in overall sample**. For bivariate analysis of BMI by urban/rural, mean for urban group (28.63) significantly higher than rural (27.6), p<0.05. Two stage least squares regression models showed living in urban area associated with higher BMI vs rural area. Positive, non-significant association between income & BMI in overall & urban samples but significant positive relationship in rural sample. OLS naïve regression & Independent Variable (IV) regression both found urban residence to be significantly associated with higher BMI (p<0.01). An increase of household income of 1000 pesos associated with 1.2 increase in BMI in rural areas.**	Limitations from omitted variables: weight may be correlated with lower wages if there is discrimination in labor market so women who weigh more are penalized with lower wages or if women of low weight have low productivity resulting in lower earnings or if there is self-selection into less paid occupations among women of very high or very low weights. Women with more educated parents and/or more self-control or higher self-esteem may be less likely to be obese & earn more income. Authors note limitations of cross-sectional design.

Radon & Schulze 2006	farm versus non-farm	rural adults aged 18-45 years living in 4 towns in northwestern Germany; all 10,252 inhabitants invited to complete mail questionnaire (67% response); only 7080 randomly selected for additional participation in medical exam; 4052 eligible for medical exam; 2745 participated (64%)	cross-sectional; final analysis excluded those with regular contact to farm animals after age 3, pregnant or breast feeding, underweight, or missing data on BMI, farm contact during childhood, or one of confounders; 1861 included in final analysis; used chi-square and ANOVA in analyses	determined from response to validated mail questionnaire, along with info on confounders (age, sex, recent or childhood ETS exposure, active smoking, education level, parental history of allergies, number of siblings, currently living on a farm)	weight and height measured at medical exam and BMI calculated (objective measures)	divided into 4 groups: --nonobese (BMI < 30), nonfarm (no farm contact during first 3 years of life) = reference group; --obese (BMI = 30+) nonfarm; --nonobese farm (lived on a farm or regularly visited animal houses during first 3 years of life); --obese farm; recent exposure to farm environments did not differ between obese & nonobese farm subjects; BMI not different between farm & nonfarm subjects; inclusion of subjects starting exposure after age 3 did not change results (data not shown); limited number of obese subjects so power not sufficient to prove statistically significant interaction between farm contact & obesity but its presence suggested by data	study focused on investigating allergy and obesity: results indicated the protective effect of early childhood farm contact on allergic sensitization might be diminished by adult obesity; due to time and finances, only 69% invited to medical exam (selected a priori) but only 67% of these completed questionnaire; among 433 nonrespondents, more likely to be male, born outside West Germany, more likely to have lived on a farm during first 3 years of life; selection bias could have occurred if over-representation of nonfarm subjects were dependent on BMI and outcome; possibility of recall bias (i.e., living on farm in early childhood from self reports); also possible selective migration out of study area occurred

Pomrehn et al 1982	farmers versus townspeople	targeted all adults aged 25-59 years living in Cedar County, Iowa on July 1, 1973 = 5594 eligibles; 3918 screened for a participation rate of 70%; random sample of 605 invited for second visit; 91% returned = 549	cross-sectional; risk factor data gathered using questionnaire and physical exam; 2nd visit underwent fasting plasma samples, 24 hour diet history, and exercise ECG; used student's t-test, chi-square, and multivariate analyses	visit 2 participants classified by occupation and place of residence; townspeople all live in communities with populations less than 3000	height, weight, BP, and triceps skinfold measured; plasma blood samples obtained	192 farmers and 353 nonfarmers participated in visit 2; no significant differences in Quetelet Index for male or female farmers versus townspeople; no significant differences in blood pressure; farm people reported engaging in regular exercise more frequently; farmers less likely to smoke; farmers consumed more total calories and less alcohol; plasma lipids all measured higher in farm men and women	authors state results indicate differences in smoking and physical fitness and less frequent alcohol use contribute to lower mortality seen among farmers in Iowa; higher dietary intake related to higher exercise level
Rahkonen et al. 1998	farmers	5306 Swedish men and women aged 18-75 years; plus 8650 Finnish men and women aged 20-74 years	cross-sectional; in-person interviews in 1991 in Sweden and 1994 in Finland; logistic regression used to calculate age and social class differences in overweight and obesity	In-person interviews; analyses on social class restricted to those currently employed and aged 20-74 years; social class measured by occupation: upper and intermediate nonmanual, lower nonmanual, skilled manual, unskilled manual, self-employed, and farmers	in-person interviews; BMI used to evaluate overweight and obesity based on WHO definitions.	OR (95% CI) for overweight in farmers; upper/middle non-manual=reference: **Finnish males: 1.57 (1.11-2.21)** **Finnish females: 1.94 (1.23-3.07)** Swedish males: 1.14 (0.53-2.45) Swedish females: 2.17 (0.73-6.44) OR (95% CI) for obesity in farmers; upper/middle non-manual=reference: Finnish males: 1.37 (0.82-2.28) **Finnish females: 2.65 (1.55-4.55)** Swedish males: 2.14 (0.46-9.83) **Swedish females: 6.45 (1.26-33.15)**	Finland response rate = 73%; Sweden response rate = 79%.

Rothacker & Blackburn 2000	rural residents	white adults aged 20-74 years in Marinette County, Wisconsin; 613 women and 352 men	cross-sectional; estimating prevalence of overweight and obesity and weight changes over 5 year period; collected age, weight, and height data from volunteers in 1997 and similar data collected retrospectively using medical records or self-reports for 1992	no unexposed control group; entire population were rural residents	weights obtained from medical and employment records and from self-reports in 1992 (retrospective); BMI calculated from measured weights and heights in 1997; 1992 weights obtained from actual measurements for 61% of sample; overweight=BMI 25-29.9; obesity = BMI 30+	**Weight gain over 5 yrs, mean in kg: women=7.3 (p<.001); men=7.9 (p<.001)** **Youngest age group (20-30 years) had greatest 5-year increase in kg: women=11 (p<.001); men=12.1(p<.001)** Increase in prevalence of overweight & obesity, 1992-1997: women: 11% & 13%; men: 2% & 16% Increase in incidence of overweight & obesity, 1992-1997: overweight women: 48% had no change; 44% became obese. obese women: 93% had no change overweight & obese women: 4% reduced weight normal weight men: 77% became overweight or obese obese men: 94% had no change overweight men: 41% became obese overweight or obese men: 3% reduced weight **BMI increased by 2 or more in all age groups, both genders** **age-adjusted mean BMI changes: women=0.55; men=0.52**	no significant differences between documented and measured 1992 weights; no interaction of BMI with age or sex

Pearson & Lewis 1998	resident in rural county	Otsego county, NY -- central NY State; mainly rural with 76% of inhabitants living in places with <2500 people; officially designated as part of Appalachia; most common occupations: agriculture and small businesses; 17,147 households enumerated (86.6% response rate) with 44,406 persons enumerated (25,614 adults aged 17-64 years)	cross-sectional; examined prevalence rates of various chronic diseases, use of preventive services, and health behaviors; descriptive data only	mail questionnaire-based survey conducted in 1989	mail questionnaire-based survey conducted in 1989; obesity defined as BMI >27.8 for men and >27.3 for women	Prevalence of obesity in sample=20% & by education: 17-29 year olds, males & females: < 8 yrs 23% 19% 9-11 yrs 13% 23% high school 15% 14% some college 13% 12% college grad 12% 8% 30-49 year olds, males & females: < 8 yrs 29% 38% 9-11 yrs 37% 38% high school 30% 29% some college 30% 28% college grad 22% 16% 50-64 year olds, males & females: < 8 yrs 37% 48% 9-11 yrs 30% 41% high school 32% 33% some college 29% 29% college grad 24% 23%	Persons who had not completed high school & lived in town had high rates of obesity but those living in rural areas who had not completed high school had still higher rates regardless of educational attainment (data not shown). Rural population seen as "slow adopters" to positive health behaviors due to high rate of poverty & low rate of educational attainment. Rural families believed to consume high-fat, high-calorie diet, previously counterbalanced by equally high caloric expenditure from farming physical labor but mechanization of farming has reduced levels of caloric expenditure leading to obesity, which is strongest in persons of low education level.

Mokdad 2002	farmers	514 male farmers engaged in field activities from both private and government sectors and the 4 date-palm regions	descriptive; provides anthropometric data; measurements always on right hand side of subjects to nearest millimeter; all measurements taken in morning; compared descriptively with results from other studies of farmers	all subjects were farmers	anthropometric data obtained on 36 body dimensions including ideal body weight (IBW), body fat percentage, and body surface area (BSA); used standard anthropometer, sliding calipers, squeeze dynamometer, skinfold calipers, weighing scare to take measurements	mean age = 36 (range 15-75), coefficient of variation (CV%) = 30.8; mean weight = 64, (range 49-97), CV = 17.0% mean stature = 1726 (range 1449-1909), CV = 4.0% Quetelet Index = 21 (range 19-27), CV = 9.6% %body fat = 13 (range 8-30), CV = 50.0% means by age: < 26, 26-45, > 45 Quetelet index: 19.9, 22.9, 21.7 %body fat: 12, 14, 12 Algerians observed to be lighter and shorter than farmers from developed countries and taller and heavier than farmers from developing countries; ratio of BSA to body mass = 0.027; previous study was 0.028; India = 0.031; Canada = 0.024	7 million farmers in Algeria (23% of 1998 population); study sample = 0.008% of whole population -- not large enough to decrease CV; Quetelet index increased from 21.19% in 1966 (stature=167 cm, weight=59.1 kg) to 21.48% (stature=172.6 cm, weight = 64 kg) in 1998 - attributed to nutritional and health variables; ratio of BSA to body mass confirms Bergmann's rule of body size variety inc increasing with decreasing mean temperature of habitat; small ratio in cold/moderate area; high ratio in hot and warm areas; small size helps dissipate heat; large body size helps maintain body heat

TABLE 2-3 HUMAN STUDIES, PESTICIDES IN BLOOD, PLASMA, SERUM OR TISSUE IN RELATION TO WEIGHT OUTCOME

REFERENCE	CHEMICAL / EXPOSURE	POPULATION	STUDY DESIGN	EXPOSURE ASSESSMENT	OUTCOME ASSESSMENT	RESULTS (with 95% CIs or p-values when available; significant results in BOLD)	COMMENTS
Schildkraut et al 1999 **Comment by Wolff & Anderson 1999**	DDE, a metabolite of DDT -- an organochlorine pesticide now banned from use	99 North Carolina women residing in a 24-county region enrolled as controls in a breast cancer study; 42 black & 57 white women selected on basis of waist hip ratio (WHR) (1st or 4th quartile)	cross-sectional; Chi-Square tests comparing frequencies; t-tests for mean DDE levels within groups of covariates; multiple linear regression models using analysis of covariance to examine relationship between ln(DDE) plasma levels & 1st or 4th quartile of WHR	blood sample collection for plasma analysis of 20 organochlorine pesticides via gas chromatography; only DDE detectable in most subjects	body measurements used to calculate BMI and WHR (used as measure of upper body obesity)	Total lipids not correlated with DDE. For all women combined: no significant difference in mean DDE in 1st vs 4th WHR quartile; **race, BMI, & breastfeeding were significant positive predictors of DDE.** DDE consistently higher among heavier women, women who breastfed, & women reporting farming or living in a rural area as a child. In multiple regression, no relationship found between DDE and 4th vs 1st quartile of WHR or > 5 pound weight gain or loss. Race-specific analyses yielded similar results. **BMI & breastfeeding significantly associated with DDE for whites;** DDE not associated with any variables for blacks.	Results suggest BMI may affect circulating levels of contaminants but do not suggest central obesity modifies plasma levels of DDE. DDE more accurate when measured in adipose tissue. Limited sample size. Wolff & Anderson 1999 comments: using pharmacokinetic model, only 15 years or 1-2 half-lives after exposure ends can positive association with obesity be seen for OCs. Patterns in this paper related to pharmacokinetics of DDE; present DDE levels represent long period of elimination exceeding intake. Association between BMI & DDE differ in reports since 1970 which may be attributable to timing and duration of OC exposure relative to measurement time. Hence, current OC measurements may require statistical approach based on timing & duration of OC exposure & BMI.

Wolff et al 1978	DDE and PBB in serum (DDE is a metabolite of DDT, an organochlorine insecticide)	524 Michigan dairy farm residents and consumers of their products; 55 chemical workers; 56 Wisconsin farm residents (controls); for weight-related analysis, only farm residents and consumers evaluated.	Cross-sectional	Serum levels of PBB and DDE obtained in 1976; DDE is a DDT-derived environmental contaminant.	Assumes females have higher residue in tissues due to higher body fat. For farm families, adult male-female pairs of similar age examined for PBB and DDE levels in serum. Results also examined by quarantined versus non-quarantined farmers and consumers with quarantined assumed to have had greater exposure.	Observed serum PBB and DDE follow trend for male-female pairs such that <20 of 108 pairs PBB in males < females yet males weigh more than females. FQ=farmers, farms quarantined; FNQ=farmers, farms not quarantined; CQ=consumers, farms quarantined; CNQ=consumers, farms not quarantined. For DDE, males had higher DDE than females for 42/67 FQ, 22/29 FNQ, 5/6 CQ, 6/6 CNQ; females had higher DDE than males for 25/67 FQ, 7/29 FNQ, 1/7 CQ, zero CNQ. Males weigh more than females and generally showed higher levels of DDE than females. No tests for statistical significance conducted.	Authors concluded: Differences not explicable by greater male obesity; both PBB and DDE are higher for males but other factors should be considered: male consumption of more contaminated meat or more direct contact with PBB.

Wolff et al 2005	DDE and PCB (DDE will be the focus of our review due to its relevancy to AHS)	1,508 breast cancer cases and 1556 controls from the Long Island Breast Cancer Study; due to high assay cost, random subset of population (646 cases, 429 controls) selected for organochlorine analyses of blood samples; final analyses conducted on 1008 women: 1007 with DDE and 998 with PCB values	Cross-sectional; Spearman correlations, Kruskal-Wallis test, Mantel Haenszel chi square test used for analysis; main predictors included age, body size, breastfeeding history, BMI at each decade of life, change in BMI over 20 and over 30 years	Blood samples taken; DDE and PCB levels obtained; DDE levels log transformed to achieve normality; modeled levels corrected for total plasma lipids and non-corrected models also examined; pharmacokinetic models generated using diet exposure data & compared to actual plasma levels	In-person interview including questions on weight by decade of adult life starting with age 20 with self administered food frequency questionnaires	**DDE**: positive correlation with current BMI; increased with age, black race, breastfed, longer residence on Long Island, less education, & parity; parous women who lactated had lower levels; BMI at every decade & 20 & 30 year gains increased; age adjustment reduced correlation from 0.2 to 0.15. DDE: Leanest vs obese had lower levels across age strata; DDE: at age < 70 years women with least weight gain had lower level vs women with BMI increase. "…these different directions of the BMI effect on DDE… have proven misleading because body size and weight change required simultaneous consideration" **Older age strongest predictor of DDE; strong inverse association between lactation & DDE; reduced in adjusted model with BMI gain. Final model: higher levels of DDE associated with higher BMI, lower BMI gain & no lactation – all p <.0001.**	DDT/DDE levels in data sets from 1950-2000 show downward trend--corresponds to elimination rates; dietary intake of DDT show similar trend. Obese have lower DDE concentration during uptake given an equivalent absorbed amount; obesity & higher body fat means longer DDE half-life during time of absorption – DDE lower in heavier than lean people until crossover (i.e., DDE inversely correlated with BMI until crossover of elimination curves for lean & obese persons after exposure declines); after crossover, BMI positively associated with DDE: explains cross-sectional data showing heavier people with higher DDE levels than lean years after exposure. Weight gain over time leads to lower DDE levels and weight loss leads to higher levels; timing of crossover depends on how high levels are when exposure stops and elimination exceeds intake. Data represent model consistent with high BMI to lengthen elimination, weight gain to reduce levels, & pharmacokinetic model.

Dirinck E et al 2011	POPs including DDE, which will be the focus of our review	98 obese men & women retrospectively selected from patients visiting weight management clinic between 1998-2007 – all 18 years old or older with BMI ≥ 30. Controls: 46 men & women matched by age and sex recruited from hospital staff; BMI range 18-25	Cross-sectional; analyzed using Mann-Whitney U test & Spearman rank correlation & results presented as median with minimal and maximal values	Venous blood samples obtained for analysis of POPs; lipid normalized concentrations were calculated	Anthropometric measurements taken; body composition determined by bioimpedance analysis; CT scan measured amount of total abdominal adipose tissue, visceral abdominal adipose tissue, and subcutaneous abdominal adipose tissue. Median BMI was 40 for obese and 23 for controls.	Abdominal adiposity more prevalent among obese. Median DDE concentration was 209 for obese & 205 for controls, p=0.97, r=-0.002. Spearman rank correlation for BMI vs DDE = 0.013, ns; for waist circumference, 0.033 for males and 0.039 for females; for % fat mass, 0.067 for males and 0.113 for females.	Lack of relationship between DDE & weight measures may be due to young population used. Limitations: no data on other obesogenic factors; study design cross-sectional; data collected over 10 years (1998-2007) so POPs burden may have dropped; no info on occupational exposures; no fat samples collected; none of groups displayed normal distribution of POP levels so use of nonparametric tests necessary.
Ronn et al 2011	POPs, including DDE, which will be the focus of our review	Randomly chosen from Swedish population register; 50% participation rate; 890 evaluated by DXA	Cross-sectional; completed questionnaire on lifestyle habits; all POPs entered as independent variables with gender in multiple regression model	Measured in stored plasma samples collected at baseline; 21 POPs measured; POPs all had skewed distribution so ln-transformed; lipid standardized; related to indices of fat mass by DXA for regression; POP levels divided into quintiles to evaluate non-linear relationship with fat mass.	2 years after baseline, fat mass & lean mass in body estimated by DXA; precision error was 1.5% for total fat mass & 1% for total lean mass.	Highest median concentration was 325 ng/g lipid for DDE. **Regression analyses showed DDE positively related to total fat mass (p values 0.029 to 0.0001 depending on number of adjustment variables).** Regression coefficient = 1.90 (1.29, 2.51). Gender interaction = 0.888. **ORs for DDE:** Q1=referent Q2= 2.2 (0.3, 4.0) **Q3 = 3.3 (2.1, 5.7)** **Q4 =3.9 (2.1, 5.7)** **Q5 = 5.0 (3.1, 6.8)** **P values from 0.026 to 0.0001 depending on number of adjustment variables**	Major strength: applied direct measure of fat mass using DXA screening which allowed for adjustment of lean mass. Limitations: many statistical tests performed increasing chance of false positive; cross-sectional design; age of subjects could influence results – endocrine function declines with age but positive relationship between age & plasma concentration of POPs; probably not exposed during fetal period or childhood.

Pelletier et al 2002	Organochlorine pesticides (DDT, DDE, mirex aldrin, chlordane, oxychlordane and other organochlorine substances not relevant to AHS cohort	3 groups: 8 male endurance athletes aged 48-65 years, 26 male lean sedentary subjects aged 40-58, 17 male obese subjects aged 36-55 years	Cross-sectional	Examined total plasma concentrations of 17 chlorinated substances adjusted for age and for differences in total plasma lipids	Body density measured by hydrostatic weighing technique; percent body fat multiplied by body weight to obtain body fat mass and fat free mass calculated as difference between body weight and body fat mass. 3 groups pooled to assess relationship between BMI & fat mass.	BMI athletes = 23.7 +/- 2.8; lean sedentary = 24.4 +/- 1.4; obese = 34.3 +/- 2.7; **obese significantly higher BMI than each of the other groups ($p<0.0001$). Athletes had significantly lower concentration of DDT compared to lean sedentary group. Obese had significantly higher levels than lean sedentary for 5 compounds including DDE and oxychlordane. Compared to athletes, obese had significantly higher levels of 7 compounds including DDE and DDT. Total OC concentration significantly higher for obese than lean sedentary or athletic groups. 9 compounds significantly correlated with BMI and fat mass. Total OC concentration adjusted for age positively correlated to BMI ($r=0.41$, $p=0.007$) and to fat mass ($r=0.43$, $p=0.004$).**	Obese subjects significantly younger than other 2 groups. Large adipose tissue compartment in obese subjects is associated with increased levels of circulating organochlorines. Limitations: missing data on diet; small number of subjects in each group; blood sampling done in different years for each group.
Brauner et al 2012	Organochlorine pesticides: cis-nonachlor, trans-nonachlor, oxychlordane, DDT, DDE, HCH, dieldrin, HCB; will focus only on those relevant to AHS	245 randomly selected subjects used as controls in a nested case-control study based on a prospective cohort of 57,053 subjects enrolled 1993-1997	Cross-sectional; questionnaire on diet, health status, lifestyle; used generalized linear models for analysis; each pesticide transformed by natural logarithm; LOD not considered; performed univariate regression and multiple regression	Adipose tissue biopsy from buttock of each subject. Mean lipid adjusted concentration: oxychlordane = 24 for males & 27 for females; DDT = 27 for males & 25 for females; DDE= 869 for males & 834 for females; dieldrin= 22 for males & 24 for females	Baseline BMI means: males = 27; females = 26. Not stated how BMI was measured.	**BMI positively associated with total chlordane residues, % difference** (mean concentration according to each given increment in determinant variable) **& 95% CI = 1.99 (0.10, 3.92); p=0.04**, r-squared = 0.26. DDT % difference = 2.15 (-0.29, 4.65), p=0.09, r squared=0.24. DDE % difference = 2.43 (-0.15, 5.08), p=0.07, r squared =0.19. **Dieldrin % difference = 3.34 (0.97, 5.76), p=0.01**, r-squared = 0.22.	Strength: concentrations determined in adipose tissue. Performed 135 tests expecting 7 significant by chance but found 26 significant results. Limitation: relatively high proportion of samples with concentrations below LOD. Diet at enrollment may not reflect previous diet which may have been relevant for body burdens of pesticides leading to low r-squared; another explanation for low r-squared are predictors not accounted for. Participants in study are older Danish people with median age of 56 years.

Lee et al 2011	Organochlorine pesticides and PCBs (the latter exposure not evaluated – not relevant to AHS data)	90 controls selected from a large prospective study on heart disease (CARDIA) and remained diabetes free over the course of 20 years of follow-up; selected at random from each BMI category (<20, 20-24, 25-29, 30-39, 40+); stratified random sample. . Male 31.1%, 51.1% white.	Prospective – can low dose organochlorine exposures predict future adiposity; all measures taken at baseline and each follow-up exam; analyses adjusted for triglycerides, total cholesterol, baseline values; analyses done across quartiles of individual compounds	9 Organochlorines measured in stored serum samples collect at study year 2 (1987-1988)	BMI measured at each exam; mean BMI of study subjects at baseline = 29.1; analyzed BMI at year 20 (2005-2006)	Many significant compounds exhibited inverse U shaped dose response**. DDE most consistent significant year 2 predictor of high BMI at year 20 but mean BMI decreased in 4th quartile. DDT also predicted BMI at year 20 but dose response close to linear increase (trend p=0.05).**	Limitations: potential false positive associations; small sample size,, small blood volumes so could not measure dioxins or furans. Strength: long term follow-up with baseline measures of all outcome variables.
Lee et al 2007	POPs, including organochlorines, which will be the focus of our review	721 non-diabetic participants aged 20+ years selected from 1999-2002 NHANES	Cross-sectional; for each POP, participants with serum concentration below LOD used as reference group	19 POPs in 5 subclasses selected because detectable in at least 60% of participants; measured in serum from blood samples collected during physical exam	Waist circumference measured as part of NHANES in home physical exam	OC pesticides were weakly and positively associated with BMI. **Adjusted ORs & 95% CI for OC pesticides in quartiles compared to waist circumference > 102 cm in men or > 88 cm in women:** 25th-50th = 1.2 (0.8, 1.9) 50th-75th = 1.7 (1.0, 2.9) **$\geq$ 75th = 2.4 (1.3, 4.6)** **p for trend = <0.01** ORs for oxychlordane: < 25th = 1.4 (0.9, 2.4) 25th-50th = 1.5 (0.9, 2.5) 50th-75th = 1.4 (0.8, 2.6) $\geq$ 75th = 1.2 (0.6, 2.4) P for trend = 0.57 ORs for DDE: 25th-50th = 1.0 (0.6, 1.5) 50th-75th = 1.2 (0.7, 2.0) $\geq$ 75th = 1.2 (0.7,, 2.0) p for trend = 0.40	Limitations: cross-sectional design but adjustment for changes in body weight did not change the results. Misclassification bias due to participants whose POPs would have been detectable with higher sample volume. Multiple comparisons.

Stellman et al 2000	organochlorine pesticides and polychlorinated biphenyls (PCBs); data for latter exposure not relevant to AHS so not presented here	323 hospital controls (250 benign breast disease and 73 non-breast surgery) from a study that also included 232 breast cancer cases; few women employed in occupations or industries with exposure to pesticides or PCBs	cross-sectional for control data	7 pesticide residues & 14 PCB congeners assayed followed by gas chromatography. In-person interviews, blood samples, & adipose tissue samples from surgery were obtained.	BMI appears to be from interview data but it is not clear in the paper	**Spearman correlations were significant ($p<0.01$) and positive for correlations between DDE and total OCP and each of these factors: age, BMI,** and number of full term pregnancies; between total PCB and age and number of full term pregnancies; significant negative correlations were identified between DDE and total OCP and each of these factors: education and age at first full term pregnancy; and between total PCB and education, age at first full term pregnancy, and number of months of breastfeeding.	Age & BMI were strongly correlated with organochlorine levels; relationship with age reflects older members of population who lived more years during the era before DDT & PCBs were banned. 95% participation rate.
Kanazawa et al 2012	Persistent organochlorine pesticides (POCs)	186 pregnant women aged 17 to 47 years from the 514 women participating in the Hokkaido Health Study from 2002-2005; 186 samples evaluated out of total of 374	Cross-sectional; demographics from medical records & self-administered questionnaires Used Jonchkeere-Terpstra test to determine trends in concentrations if detection rate >50% and to assess relationship to demographics	Whole blood samples quantified for 29 POCs using standard laboratory methods; compared within 3 calendar year groups: 2002, 2003, 2004-5	Pre-pregnancy body weight and BMI obtained from medical records and divided into tertiles. Median BMI = 20.6; Mean BMI = 21.4	21/29 pesticides identified in blood including some never used in Japan; log transformed concentrations significantly correlated with each other; most declined inversely to past conceptions ($p<0.05$); maternal age positively associated with 10 pesticides**; maternal pre-pregnancy body weight positively associated with concentrations of DDT, DDE, dieldrin, HCH, Parlar 26, Parlar 50 ($p<0.05$); associations with BMI more strongly related to body burden of POCs vs body weight; BMI associations significant & positive for dieldrin, Parlar 26, Parlar 50; significant & negative for mirex.**	Difficult to compare with other studies since most use levels in sera or plasma. Most levels decreased from year-to-year.

Elobeid et al 2010	POPs selected present in >80% of NHANES population: ln(Hpcdd), ln(Ocdd), ln (oxychlordane), ln(trans-nonachlor), ln(DDT); TCDD; only DDT & TCDD relevant to AHS	2464 NHANES particpants with valid BMI scores (1140 males, 1324 females) and 2448 particiants with valid waist circumference (WC) scores (1133 males, 1315 females); all aged from 6 to 40+ years	Cross-sectional; nationally representative of US non-institutional-ized population; surveyed 1999-2000 & 2001-2002	POPs assessed in participants on a 1/3 serum samples; determined levels of PCDDs, PCDFs, PCBs, OCs adjusted for serum total cholesterol & triglycerides; numeric levels transformed by taking natural log (ln) of each in order to improve linearity & analyzed in transformed form	BMI & WC determined using anthropometric measurements via standardized protocols	**Additive Regression Model ln(DDT)** **Beta (p-value)** **BMI = 0.48 (p=0.0353)** WC = 0.81 (p=0.1283) Every unit increase in ln DDT associated with BMI increase of 0.106 **Gender Interaction Model** **BMI = 1.08 (p=0.0020)** **WC = 2.73 (p=0.0010)** Male BMI decreased by 0.05 & female BMI increases by 0.344 for every unit increase in ln DDT; male WC decreased by 0.12 cm but female WC increased by 0.111 cm for every unit increase in ln DDT	POPs with high affinity for Ahr (Aryl hydrocarbon receptor) in NHANES such as TCDD did not show significant associations with BMI or WC.
Munoz-de-Toro et al. 2006	Organo-chlorine compounds (OCCs) and polychlor-inated biphenyls (PCBs); the latter exposure not relevant to AHS cohort	76 women from Santa Fe, Argentina area, who underwent excision biopsy of a breast lesion or had plastic surgery; 42% reported having resided in a rural area for different periods of time	cross-sectional examination of organochlorine residues in mammary fat tissue (adipose tissue) in relation to other factors; Spearman's correlation coefficients calculated	Women from Northeastern Argentina, near the Parana River ("littoral" area) -- an area characterized by intensive farming and agricultural activities and industrial development. Telephone interviews were conducted. None of the subjects were occupationally exposed to organochlorines and none were ever involved in rural work.	BMI appears to be from interview data but it is not clear in the paper	**Bioaccumulation was evidenced by the significant positive association between organochlorine levels and BMI (p=0.0003)** and patient's age (p=0.0002). Mean BMI=25.2. Total organochlorines: geometric mean=906.6; arithmetic mean=1435.7; range=20.0-5861.	no comparison group; diet found to be relevant source of exposure; levels similar in subjects who underwent plastic surgery and those treated for benign and malignant disease so all subjects combined for analysis

Muscat et al. 2003	Organo-chlorine compounds (OCCs) and polychlor-inated biphenyls (PCBs); the latter exposure not relevant to AHS cohort	224 women with nonmetastatic breast cancer enrolled in a New York-based case-control study; diagnosed between 1994-1996; data analysis based on 224 women because 8 of 232 had advanced disease	Cross-sectional for examination of BMI and OCC/PCB concentrations. Women followed 5 years to determine recurrence. Subjects mailed survey to determine postdiagnosis changes. Correlation coefficients calculated.	OC and PCB concentrations determined for 232 randomly selected cases from adipose surgical specimens taken prior to any chemotherapy. Women interviewed prior to surgery.	BMI appears to be from interview data but it is not clear in the paper	**Concentration of pesticides and PCBs was correlated with baseline age and BMI** but not cancer stage. **For BMI, r=0.35 (p<0.01) for total pesticide concentrations** and 0.14 (p=0.04) for total PCB concentrations. Pearson correlation between BMI at diagnosis and at follow-up was 0.90; for those with recurrence, it was 0.92 (n=23); p values not given.	30 women (13.4%) were diagnosed with a breast cancer recurrence. Pesticide levels not related to recurrence. >95% participation in original sample of 359 cases with 86% providing surgical samples. Only 185 completed post-diagnostic mail questionnaire.
Cerrillo et al. 2006	Organo-chlorine pesticides: DDE (metabolite of DDT), lindane, endosulfan, endrin, dieldrin	458 women undergoing surgery for various diseases, aged 33-75 years, living in Southern Spain (area of intensive greenhouse agriculture and high pesticide use)	cross-sectional analysis of adipose tissue levels of pesticides in relation to various factors assessed by in-person interview	Pesticide residues measured in adipose tissue samples.	BMI examined but unclear if from measurements or self-reports.	**Significantly higher DDE levels (p<.01) found for BMI >=30 vs BMI <30.** No significant difference based on BMI for lindane, endosulfan, endrin, or dieldrin.	Participation rate was 74.8%.
Baccarelli et al. 2005	TCDD (2,3,7,8-tetrachloro-dibenzo-p-dioxin) or simply dioxin -- a contaminant of the herbicides 2,4-D and 2,4,5-T	101 chloracne cases and 211 controls from Seveso, Italy cohort -- site of an industrial accident in 1976 that exposed the population to TCDD	matched nested case-control in cross-sectional sample; 101 controls matched (age, sex, & zone of residence at time of accident); remaining controls randomly chosen if non-chloracne.	plasma samples taken to determine TCDD levels in 1993-1998	Interviewers administered questionnaire from 1993-1998 on demographics, lifestyle, diet, residential history, occupations; BMI measured at interview. ORs calculated using unconditional logistic regression	**Occurrence of elevated TCDD levels (> 10 ppt vs ≤ 10 ppt) increased with increasing BMI, p for trend = 0.04.** BMI OR 95% CI p-value <21.7 1.0 --- 21.7-25 1.6 0.6-4.0 0.29 **>25 2.8 1.0-4.0 0.04**	20 years after Seveso accident, dioxin levels still elevated in exposed individuals, especially for those with higher BMI. Plasma dioxin strongly associated with chloracne.

Michalek et al 1999	TCDD (2,3,7,8-tetrachloro-dibenzo-p-dioxin) or simply dioxin -- a contaminant of the herbicides 2,4-D and 2,4,5-T	male Air Force veterans exposed to Agent Orange during Vietnam War; veterans of Operation Ranch Hand - unit spraying in Vietnam from 1962-71; other air force veterans serving in southeast Asia during the same period but not involved with spraying were comparisons; analyses based on 871 Ranch Hands and 1121 comparisons (1992 total)	cross-sectional; data collected over 10 years of follow-up from ongoing Air Force Health Study; comparison group matched for age, race, & military occupation; statistics include: analysis of covariance, partial correlations, & linear models; exclusions: taking hormones, prostate, testicular, or genital cancer, testicular surgery, history of diabetes prior to tour, no dioxin measurement or non-quantifiable result	estimated initial dioxin dose at end of tour of duty in Vietnam in Ranch Hands having current dioxin levels above background; Ranch Hand veterans assigned to exposure group based on current dioxin level assayed in 1987 or 1992: 376 background (<=10 ppt), 247 low (>10 ppt), or 248 high (>10 ppt); exposures in Vietnam occurred from 24-35 years ago; comparison veterans had current dioxin <= 10 ppt	defined percent body fat as PBF = 1.26 x BMI - 13.305; BMI measured at time of blood draw for dioxin	Ranch Hand veterans in high dioxin category younger and heavier than those in low and background categories (no info on statistical significance): mean % body fat by group: comparison = 21.7; background = 20.1; low = 22.4; high = 23.4	study designed to examine associations between exposure and insulin, glucose, and SHBG levels; info on body fat, weight descriptive only
Lee et al 2006	persistent organic pollutants (POPs): oxychlor-dane, DDE, PCB 153, HpCDD, OCDD, and trans-nonachlor; only oxychlor-dane & DDE relevant to AHS population (organo-chlorine pesticide)	2016 adult participants in NHANES 1999-2002; national sample of noninstitution-alized civilian population;	cross-sectional; POPs selected if detectable in 80% of participants; data from 2 NHANES combined	serum concentrations of POPs: hexachlorobiphenyl, heptachlorodibenzo-p-dixoin, octachlorodibenzo-p-dioxin, oxychlordane, DDT, & trans-nonachlor; adjusted for total cholesterol & triglycerides; 1/3 of participants from specific years selected to provide serum samples for measuring specific POPs.	home interview with physical exam; substituted median values of nonsubjects for missing BMI, waist circumference or poverty income ratio in 291 subjects; exclusion of these 291 did not change any conclusions	**significant positive associations (all p <.01) of BMI with HpCDD, OCDD, or DDE; BMI inversely associated with PCB 153; strong correlations in serum concentrations among all POPs**	study focused on diabetes: prevalence strongly positively associated with lipid adjusted serum concentration of all 6 POPs; cannot make causal inferences on cross-sectional data; >90% of POPs come from animal foods in general population without occupational or accidental exposure; U.S. serum concentrations of POPs in general population have been decreasing over several decades; could not examine TCDD because so few had detectable levels

Collins et al. 2007	2,3,7,8-substituted dioxins and furans from chlorophenol exposure	62 chemical manufacturing workers with chlorophenol exposures and 36 workers without chlorophenol exposures from Dow chemical plant in Midland, Michigan who worked during chlorophenol production between 1937-1980	cross-sectional; multiple linear regression used to examine relationship between current serum dioxin and furan levels and current BMI, age, change in weight in last 6 months, and smoking status; for production workers, also examined previous exposure estimates and days since last exposed	Serum samples collected to assess exposure; all results lipid adjusted. Used industrial hygiene data, product impurity data, job descriptions, and descriptions of processes and changes to determine relative exposure levels.	weight and height measured to calculate BMI; information on smoking and recent weight change obtained from questionnaires	**Among exposed, BMI significantly related to 7/18 analytes (p values):** **2378TCDD (0.02)** **12378P5CDD (0.03)** **123478H6CDD (0.003)** **123789H6CDD (<0.0001)** **1234678H7CDD (<0.0001)** **OCDD (0.008)** **TEQ (0.008)** **Among referents, BMI significantly related to 9/18 analytes (p values):** **2378TCDD (0.002)** **12378P5CDD (0.007)** **123678H6CDD (0.02)** **123789H6CDD (0.02)** **1234678H7CDD (0.04)** **OCDD (0.02)** **123478H6CDF (0.02)** **123678H6CDF (0.03)** **TEQ (0.005)** Referents showed negative significant associations for BMI and OCDF. Regression model showed age and BMI were predictors in assessing background levels of TCDD and a few other dioxins and furans.	Exposed had higher levels of each dioxin congener than referents; not stated if differences were statistically significant. Furan levels were similar for exposed and referents. Results similar whether using actual serum levels or log transformed levels.

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TABLE 2-4 HUMAN STUDIES, PESTICIDE EXPOSURE AND BMI

REFERENCE	CHEMICAL / EXPOSURE	POPULATION	STUDY DESIGN	EXPOSURE ASSESSMENT	OUTCOME ASSESSMENT	RESULTS (with 95% CIs or p-values when available; significant results in BOLD)	COMMENTS
Shah et al 2009	Agent Orange (2,4-D; 2,4,5-T; TCDD)	1495 male veterans who had undergone radical prostatectomy (RP); BMI data available for 1307 men (87%)	Case-control study of Agent Orange	Abstracted from medical records for men receiving RP from 1988-2007 at 4 VA medical centers; exposed vs non-exposed only; 206 exposed (14%).	BMI calculated based on height and weight in medical records	Men exposed to Agent Orange more likely to have higher BMI (p=0.001) based on Chi-Square analysis. ORs & 95% CIs with 25-29.9 as referent: BMI <25, 0.86 (0.56, 1.30) BMI 30-34.9, 1.02 (0.66, 1.58) BMI ≥ 35, 2.28 (1.38, 3.76)	Results based on population of men who all have prostate cancer. DISTRIBUTION: BMI unexposed exposed <25 302 (27%) 42 (22) 25-29.9 494 (44) 80 (42) 30-34.9 235 (21) 39 (20) ≥35 84 (8) 31 (16)

Figure 2.1: Hypothesized influence of pesticides on cellular metabolism (Karami-Mohajeri & Abdollahi 2010)

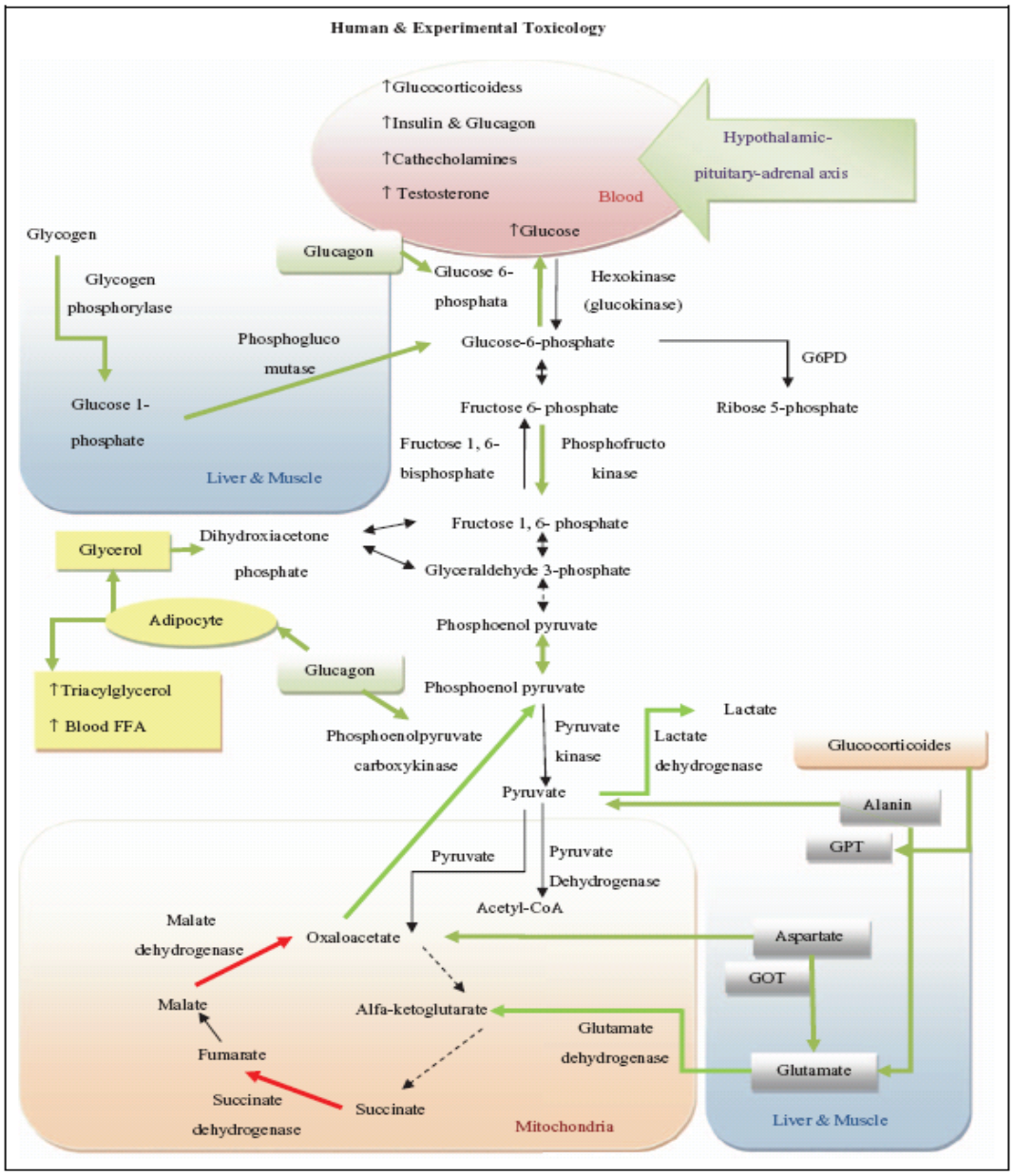


Figure 3. A model of metabolic impairment of carbohydrates, fats, and proteins caused by organophosphate (OP), organochlorine (OC), and carbamate (CB). Positive effects are shown by green arrows and negative effect by red.

Figure 2.2: Effect of Endocrine Disruptors (EDs) on epigenetic programming (Schug et al 2011)

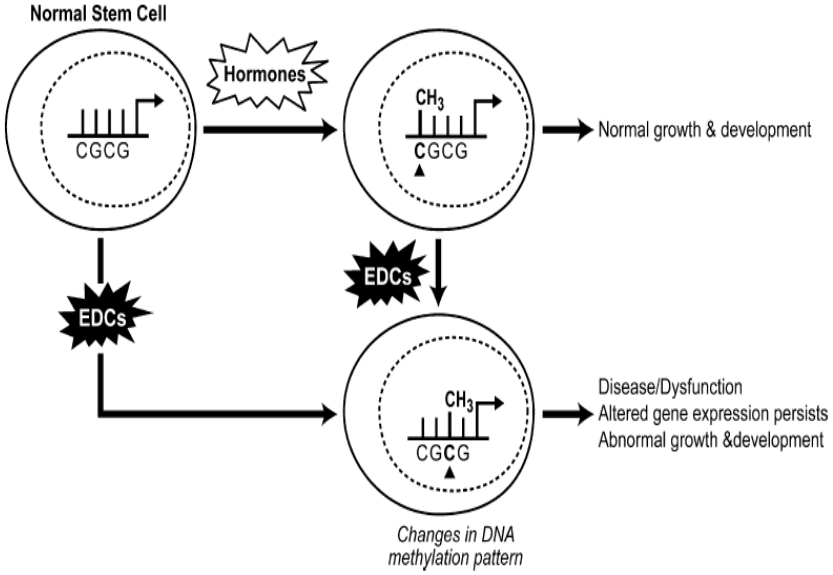


Fig. 4. Model depicting how EDCs can alter methylation patterns and normal epigenetic programming in cells. Alterations in the epigenetic status of somatic cells can lead to disease in developing tissues, whereas changes in the epigenetic programming in stem cells can lead to multi- and transgeneration effects in the offspring.

CHAPTER 3: DESIGN AND METHODS

3.1 Background on the Agricultural Health Study (AHS)

This dissertation research utilizes data from the AHS – a large, ongoing prospective cohort study of 57,310 pesticide applicators: 52,394 private applicators (farmers) and 4,916 commercial applicators. In addition, 32,347 applicators' spouses were included in the study. The AHS is conducted by the National Cancer Institute (NCI) in collaboration with the National Institute of Environmental Health Sciences (NIEHS), the U.S. Environmental Protection Agency (EPA), and the National Institute for Occupational Safety and Health (NIOSH). Battelle/Survey Research Associates was the original contractor coordinating the data collection, which is now being coordinated by Westat.

AHS objectives include identifying and quantifying cancer risks associated with occupational exposure to pesticides and other agricultural chemicals. This includes evaluating disease risks among spouses and children arising from occupational contact with pesticides and agricultural chemicals, as well as the applicators themselves; assessing current and past agricultural exposures; and examining the relationship between agricultural exposures, biomarkers of exposure, biologic effect, and genetic susceptibility in relation to cancer. Additional objectives include evaluating disease risks among spouses and children from non-occupational contact such as spray drift, laundering work clothes, or consuming contaminated food or water; and identifying and

quantifying other disease risks associated with lifestyle factors. In addition to the prospective cohort study, AHS also involves nested cross-sectional biological marker studies, nested case-control studies, and exposure assessments (Alavanja et al. 1996).

AHS methods have previously been described in detail (Alavanja et al. 1996), with updates as results are reported (Bonner et al. 2007; Montgomery et al 2008). Briefly, certified, restricted-use pesticide applicators and their spouses were enrolled over a 4-year period from December 1993 through December 1997 in North Carolina and Iowa. There were many reasons Iowa and North Carolina were selected to participate in the study, among them the fact that Iowa represents typical mid-western farm exposures and North Carolina represents farm exposures in the agricultural southeast. Applicators were identified when they sought a certification from the state Cooperative Extension Services or Departments of Agriculture. Certified applicators include farmers (private applicators) and commercial applicators employed by pest control companies or businesses that use pesticides on their premises. Applicators were approached at the certification facilities where they underwent required training and/or testing in the safe handling of pesticides in order to be certified and were asked to complete the enrollment questionnaire. In Iowa, commercial and private applicators attended some of the same sessions; whereas, in North Carolina they attended separate sessions. Therefore, only private applicators (farmers) were enrolled in North Carolina. Applicators who completed the enrollment questionnaire were given a take-home questionnaire to complete, along with take-home questionnaires for their spouses to complete. The

response rate for the enrollment questionnaire was about 77% and the response rate for the take-home questionnaires was about 50% (Alavanja et al. 1996).

The majority of the enrolled applicators are farmers (91%) with the remaining 9% being commercial pesticide applicators (Alavanja et al. 1996). Approximately 2.6% of the private applicators are women, 4.1% of the commercial applicators are female, and about 2.2% of all applicators are minorities. Commercial applicators are the more diverse group with respect to exposure with almost half applying pesticides to crops; more than a third applying pesticides to lawns and gardens; over 10% applying pesticides to homes; and less than 5% engaged in forestry applications. Commercial applicators tend to be younger, with fewer years of experience than farmers, but they tend to apply pesticides more frequently than farmers.

The enrollment questionnaire obtained detailed information on the use of 22 specific pesticides, including the decade of first use, the number of years used, and the number of days per year each pesticide was personally mixed or applied (Montgomery et al 2008; Bonner et al. 2007; Alavanja et al. 1996). An additional 28 pesticides were included in the enrollment questionnaire, but with only limited exposure questions. General information on application methods and use of personal protective equipment (PPE) was also obtained. Additional questions evaluated smoking, alcohol use, fruit and vegetable consumption, non-farm occupational exposures, and other agricultural exposures and lifestyle factors. Participants who completed the enrollment questionnaire were also asked to complete a take-home questionnaire that included all

the detailed questions on the 28 additional pesticides, including the decade of first use, the number of years used, and the number of days per year each pesticide was personally mixed or applied. The take-home questionnaire also obtained current weight and height and weight at age 20. This allowed for calculation of body mass index (BMI), which utilizes the formula: weight in kilograms divided by the square of the height in meters (kg/m^2).

Information was obtained on use of broad pesticide classes that were mixed or applied while using protective equipment during two time periods: 10 years ago and over the past 12 months (Dosemeci et al. 2002). Completion of the enrollment and take-home questionnaires represents baseline data for the cohort. This data collection effort is referred to as Phase 1. The selection of pesticides to be included in the questionnaires was based on their importance in Iowa and North Carolina agriculture and/or because of human or animal data suggesting possible adverse health effects associated with their use (Dosemeci et al 2002). The AHS was designed to capture chemical-specific intensity- and duration-related pesticide exposure information. Exposure algorithms (general and detailed) were developed by Dosemeci et al. (2002) based on both professional judgment and existing literature. The enrollment questionnaire contains more limited information on pesticide exposures so it forms the basis for the general exposure algorithm while the take-home questionnaire, with its more detailed exposure information, is used for the detailed exposure algorithm (Dosemeci et al. 2002). Coble et al. (2011) modified the weights of some protective factors and some application factors and this research utilizes his updated exposure classification; the basic

algorithms did not change. These algorithms provide the most detailed method for quantifying pesticide exposure from questionnaire data that currently exists anywhere.

Vital status for the AHS cohort has been ascertained through 2008 using the National Death Index (NDI) and state death registries (Montgomery et al 2008; Bonner et al. 2007; Alavanja et al. 1996). Incident cancers were identified through state tumor registries. Participants who moved out of Iowa or North Carolina were censored at the year in which they moved.

During Phase 2 of the AHS, information was updated using telephone and mail questionnaires approximately five years following enrollment. Phase 2 for private applicators was conducted from 1999 to 2003, while for commercial applicators, Phase 2 was conducted from 2003 to 2007. Phase 2 obtained additional information on exposure variables, along with current self-reported weight and height, and a detailed dietary questionnaire. Buccal cell collection was also performed on some participants for genetic analyses.

3.2 Study Population

The proposed study focuses on the 50,641 male private pesticide applicators aged 20 or more years at enrollment (see Table 3.1). The rationale for focusing on the males stems from the fact that the majority of the applicators are male (97%), the majority of the spouses are female, and pesticide exposures are better defined and more intense for

applicators rather than spouses. It is also possible that metabolic effects stemming from pesticide exposure are likely to be different for males and females. Only one-third of spouses of farmers used pesticides. The average farmer applicator used pesticides for 16 years for a median of 20 days per year (Alavanja et al. 1996). However, spouses of farmers used pesticides for an average of 13 years at a median frequency of 12 days per year (Alavanja et al. 1996). If agricultural exposures are related to weight gain, it logically follows that such a relationship is more likely to be identified among the male applicators due to their more intense and frequent exposures compared to the female spouses. After the various exclusions, there were too few commercial applicators to allow for rigorous analyses of these data. The sample available for each analysis utilizes only those applicators who provided weight and height data for calculating BMI at Phase 1 and/or Phase 2, as BMI is the outcome variable of interest. Individual analyses varied because of high non-response rates and/or missing values for some key variables.

Male pesticide applicators were excluded from secondary analyses if they self-reported, either at enrollment or at follow-up, any diseases or conditions associated with weight gain or loss. These conditions include any type of cancer (except non-melanoma skin cancer), diabetes, or thyroid disease. In addition, subjects self-reporting medical conditions such as ALS, emphysema, kidney failure, lupus, multiple sclerosis, scleroderma, sarcoidosis, colitis, respiratory diseases, cardiovascular disease, and Crohn's Disease were also excluded, though these numbers were very small. Inclusion of these individuals could bias the results since weight gain is the outcome variable of interest in relation to several independent exposure variables. At enrollment, subjects

self-reported whether or not they were told by a doctor they had these conditions. Similar responses were obtained at 5-year follow-up. Medical exclusions (any positive – yes—responses for the aforementioned conditions) were utilized as a stratifying variable in all bivariable and multivariable analyses, along with state of residence (Iowa or North Carolina).

Use of stratified analyses allowed for the potential evaluation of the impact of these conditions as confounders or effect modifiers. This may identify some impact of disease processes in general. There are not enough subjects in any one disease category to accurately assess the impact of including versus excluding subjects with a particular disease or condition.

Table 3.1 illustrates the available sample sizes for private applicators. The demographic data file supplied by NCI is considered to be the "gold standard" in terms of identifying participant status (applicator or spouse), age, enrollment date, gender, and a number of other factors. The Phase 1 data file originally contained 52,395 private applicators. This was reduced to 50,641 when merged with the demographic file, excluding females, and excluding males under age 20 years. The Phase 2 data file contained 33,457 applicators illustrating the fewer numbers of applicators who participated in the second phase. Merging with the demographic file, deleting the females, and deleting males under age 20 left 32,384 subjects in the Phase 2 file. However, Phase 2 also included a self-administered diet history completed by 17,626 male applicators, which was reduced to 16,557 after merging with the demographic file and deleting duplicates. Merging the

Phase 2 data file with the diet history file left a total of 16,558 male private applicators in Phase 2 with a diet history.

Merging the demographic file, Phase 1 file, and Phase 2 originally created a sample size of 32,384 private applicators. Merging this file with the diet history file and the nutrient file (calculated from the diet histories) yielded 15,976. Subjects who were missing a BMI variable at enrollment and a BMI variable at follow-up were deleted leaving a final sample size of 9076 (see Table 3.1).

3.3 Dependent Variables

The dependent variable is body mass index (BMI). The World Health Organization (WHO) defines BMI as a simple index of weight-for-height that is commonly used to classify underweight, overweight, and obesity in adults (WHO 2007). It is calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2). For example, an adult who weighs 70 kg and whose height is 1.75 m will have a BMI of 22.9: BMI = 70 (kg) / $(1.752\ m)^2$ = 22.9. Self-reported height, current weight, and weight at age 20 were obtained in the take-home enrollment questionnaire and were used for calculation of current BMI and BMI at age 20. In some cases, missing information on height and weight was supplied from driver's license records. Height and weight were also self-reported in the 5-year follow-up questionnaire and were used for calculation of follow-up BMI.

We decided to calculate BMI ourselves rather than use the BMI variable in the data files. Subjects with a height of 3 feet were all changed to 5 feet (n = 4) to account for probable data entry errors. Subjects with differences in height between enrollment and follow-up greater than 2 inches (n = 371) were coded to missing height. Since BMI is based on metric measurements and the weight and height measurements in the AHS are based on the English system, each BMI was calculated as the weight at that time period, divided by height squared, times 703 (CDC 2011). This formula corrected for using the English measurement instead of the metric.

BMI2 (at enrollment) and BMI3 (at follow-up) are the main outcome variables for this study. While the sample size of 9,076 may appear small compared to the original cohort size, it is still large enough to be able to detect an effect size (change in BMI) between 21 and 68% for the relationship between BMI and exposure to particular pesticide functional classes or chemical classes based on statistical power of greater than 80% and an alpha of 0.01 (see Table 3.2; Lenth 2011). The average change in BMI per year in the U.S. general population is about 0.115 kg/m^2 based on a number of studies and this was used along with the sample sizes of exposed and unexposed to each pesticide class to determine the effect size we have the ability to detect (Truong & Sturm 2005; Newby et al 2003; Baum & Ruhm 2007; Ostbye et al 2011).

Table 3.3 presents the BMI classifications developed by WHO representing underweight, normal weight, overweight, and obese. While BMI values are age-independent and the same for both sexes, they may not correspond to the same degree

of fatness in different populations due, in part, to different body proportions. Studies of BMI in different ethnic groups present increasing evidence that the associations between BMI, percentage of body fat, and body fat distribution differ across populations. Therefore, the interpretation of BMI gradings in relation to risk may differ for different populations (WHO 2007). WHO recommends that countries should use all cut-off categories (i.e., 18.5, 23, 25, 27.5, 30, 32.5 kg/m^2, and in many populations, 35, 37.5, and 40 kg/m^2) for reporting purposes (see Table 3.3) to facilitate international comparisons.

Variability across exposure groupings and outcomes is needed to see any effect. A review of Table 3.2 indicates that the proportion of the cohort exposed to each pesticide class at baseline (18% to 96%) varies greatly suggesting there is much variability in the exposure variables. Preliminary data received from AHS staff on mean and median BMI among AHS applicators suggested there was enough variability in BMI across age groups and also indicated that BMI among male applicators was usually greater than in the general U.S. population for all age groups under age 60 (Hou 2007; Table 3.4).

3.4 Independent Variables

Several independent variables were examined, including intensity-weighted cumulative pesticide exposure as defined below. Exposures from non-agricultural jobs that can be assessed with the available AHS data were also evaluated. The take-home questionnaire includes data on exposure to non-agricultural occupational exposures

(solvents, gasoline, asbestos, x-rays, grain dust, wood dust, cotton dust, mineral or mining dust, silica/sand dust, engine exhaust, lead solder, welding fumes, electroplating fumes, lead, mercury, cadmium, other metals, pneumatic drills) and these were evaluated as potential confounders.

Additional variables assessed for potential confounding influences are age, race/ethnicity, diet, physical activity, smoking, alcohol use, education, and number of years lived/worked on a farm. Age was based on date of birth and race/ethnicity was based on self-report in the enrollment questionnaire. Mediating factors such as geographic location (based on state: NC or IA), and presence or absence of weight-related health conditions were utilized as stratifying variables.

3.4.1 Pesticide Exposure Classification

Pesticide applicators are potentially exposed to pesticides while transporting, mixing, loading, or applying these substances (Dosemeci et al. 2002). Additional exposures may occur when cleaning or repairing application equipment or when entering treated areas. There are many factors that affect the level of exposure including the type of activity (i.e., applying, mixing, loading, harvesting, etc.), the method of application (air blast, backpack, aerial spray, hand spray, or ground boom), pesticide formulation (spray, aerosol, or dust), application rate (weight of active ingredient per acre), use of personal protective equipment (PPE) (i.e., gloves, respirators, face shields, boots, overalls, etc.),

personal habits and cleanliness (i.e., changing into clean clothes, bathing or showering, or washing hands after using pesticides), and frequency of healthcare visits.

Dosemeci et al. (2002) developed an exposure algorithm using AHS questionnaire responses from the enrollment and take-home questionnaires, respectively, combined with pesticide monitoring data from the published literature. Information from the Pesticide Handlers Exposure Database, developed mainly by EPA and based on over 100 studies submitted by companies wishing to register a specific pesticide, was used to make relative comparisons between different application methods and various types of PPE to refine the scoring system. The EPA pilot AHS pesticide monitoring surveys conducted at six AHS farms were also reviewed.

The enrollment questionnaire includes time-related exposure information including duration (number of exposed years) and frequency (average annual number of days used); intensity-related questions include frequency of mixing pesticides, method of application, repair of equipment, and use of PPE. The take-home questionnaire distributed at enrollment obtained additional information beyond time- and intensity-related questions including pesticide handling, use of enclosed mixing systems, type of tractor used, procedures used to clean equipment, personal cleanliness, practice of changing clothes after a spill, and frequency of replacing gloves. The pesticides included in the enrollment and take-home questionnaires are listed in Table 3.5. Together the exposure information from the enrollment and take home questionnaires constitutes the baseline pesticide exposure data for the AHS male cohort.

Pesticide exposure data from the 5-year follow-up questionnaire was collected verbatim (i.e., subjects were asked open-ended questions over the telephone about the types of pesticides they used). The previous pesticides used by the individual applicators were preloaded into the computerized interviews.

Dosemeci et al. (2002) used the questionnaire responses to develop chemical-specific exposure scenarios with quantitative intensity levels for each scenario calculated using an algorithm that was based on data from the enrollment questionnaire on mixing status, application method, equipment repair status, and PPE use; it was validated using biological monitoring results. Details on the algorithm definitions and scores can be found in Table 3.6. The exposure algorithm employs an additive model for mixing, application, repair, and washing activities because each contributes independently to overall exposure (Dosemeci et al 2002). However, a multiplicative model was used for PPE and other potential protective factors such as using an enclosed mixing system, tractors with enclosed cabs and/or use of charcoal filters, replacing old gloves, hygiene, and changing clothes after a spill, because they depend on the basic exposure determinants.

Each variable in the algorithm was weighted based on monitoring data from over 100 published articles supplying numerous measurements of exposure levels in relation to mixing, application, or work practices in agriculture. More than half of the articles provided extensive monitoring data on dermal, inhalation, and/or internal exposures.

The weights were generated by comparing the results of various monitoring data between individual exposure variables and within a selected variable. The ratio between exposure levels of mixing and application was found to be dependent on method of application. PPE intensity scores were based on articles providing data on exposure to parts of the body when using particular PPE and the proportion of the particular body part that can be protected using PPE was included in the overall body exposure. Lifetime cumulative exposure scores were calculated by combining exposure intensity scores for each scenario with chemical specific information on duration and frequency from the questionnaires such that cumulative exposure for a particular pesticide was calculated as: intensity level times duration times frequency.

Our version of the AHS data included the updated algorithm scores based on the new weighting factors developed from two biomonitoring studies (Coble et al. 2011). The weighting factors used in the original algorithm converted categorical responses to specific questions from the baseline questionnaire for each applicator into a relative exposure intensity. The results of the two biomonitoring studies allowed for adjustments to the intensity levels based on post-pesticide-application urine levels extracted from measures of 2,4-D and chlorpyrifos for a subgroup of AHS participants (Thomas et al. 2010) and urine levels from a second study used to evaluate relative exposure levels associated with airblast versus hand spray pesticide application methods (Hines et al. 2007).

Dosemeci et al. (2002) found that based on the enrollment questionnaire data, 4% of applicators did not personally mix pesticides and 3% did not personally apply pesticides. The most commonly used herbicides and insecticides were identical for the enrollment cohort and the sub-cohort completing the take-home questionnaire. Figure 3.1 shows the prevalence of these 6 pesticides in both questionnaires. Lynch et al. (2005) compared farmers in the AHS from Iowa and North Carolina to respective data on farmers from the Censuses of Agriculture for Iowa and North Carolina. They found that the AHS farmers from Iowa and North Carolina were younger, worked on larger farms, and more frequently applied pesticides when compared with the farmers surveyed in the Censuses of Agriculture for Iowa and North Carolina, respectively.

Our analyses examined pesticide functional and chemical classes as defined in the AHS Phase 1 Data User's Manual (Legum et al 2003).

Functional classes included:

- Herbicides
- Insecticides
- Fungicides
- Fumigants

Chemical classes included:

- Organochlorine insecticides
- Organophosphate insecticides

- Organothiophosphate insecticides
- Carbamates
- Phenoxy herbicides
- Triazine herbicides

Since there were many missing values for the questions on "did you ever use PESTICIDE?", we included these missing values as zero scores in our analyses based on the assumption that subjects went through the questionnaire and marked all the pesticides they ever used but left the questions on pesticides they never used blank. In this way, we had a larger sample for analysis of each pesticide class. We also determined the "true zero" exposures to various pesticides based on the applicators who answered every question as "yes" or "no", with "no" representing non-exposure. We conducted our multivariable analyses using the two groups: those who had missing values for various pesticides coded as zero exposure, along with those who actually coded each response as "yes" or "no" (non-exposed) combined; the second analysis group only included the subjects who actually answered the pesticide usage question as "no" – we refer to this group as the "true zero" group since it does not assume that missing values indicate no exposure.

3.4.2 Race/Ethnicity

The AHS cohort is 97% white indicating ethnic or racial variation is minimal. Nevertheless, race/ethnicity (nominal variable) was among the independent variables investigated for inclusion in the regression models. Questions were included in the

enrollment questionnaire on race (white, black, American Indian/Alaska Native, Asian or Pacific Islander, and Other). A separate question determined if the subject was of Hispanic origin. Race/ethnicity was evaluated as a potential confounder through correlation analyses with the outcome (BMI) and the exposure. Variables not correlated with exposure and outcome were not evaluated as confounders in the models.

3.4.3 Age

Age at enrollment was calculated using birth date subtracted from enrollment date, which yielded the number of days between these two dates. Division by 365.25 determined age in years. Similarly, age at follow-up was calculated by subtracting birth date from date of follow-up interview and dividing by 365.25. All subjects under age 20 at enrollment were deleted from the analyses. Care was taken to assure the correct age variable (at enrollment or at follow-up) based on time period of outcome variable was included in the regression models. Controlling for age controls for the increase in weight that normally occurs as people age due to the muscle mass decrease and fat mass increase.

3.4.4. ENERGY INPUT AND OUTPUT

Limitations of the study of BMI and exposure to pesticides in the AHS male cohort include difficulties in fully controlling for the influences of diet and exercise due to lack of adequate dietary and physical activity information at baseline and follow-up. A

justifiable method for controlling for energy input and energy expenditure was developed using the limited dietary and physical activity data available.

3.4.4.1 ENERGY INPUT

The diet history questionnaire (DHQ) dataset completed by the applicators during Phase 2 of the study was evaluated using the information from NCI's website devoted to the DHQ: http://riskfactor.cancer.gov/DHQ. The Diet*Calc software available on the website was downloaded and used to generate nutrient estimates using the DHQ food frequency data and the nutrient content for each food by sex and serving size. Measures of total energy input at one point in time (i.e., total calories in kcal), as well as a total measure of fat intake at one point in time were also provided. The DHQ file contains a total of 35,011 subjects, males and females. In some cases the gender given in the DHQ did not match the gender given in the applicator files. However, the DHQ file included the variables COMPL for "who completed the questionnaire" (responses: applicator, spouse, or someone else) and the variable REFER for "to whom do the responses refer" (responses: myself, spouse, or both) and SEX for "what is your sex" (responses: male or female) to assist us in correctly assigning each DHQ to its corresponding subject.

Of the 35,011 records in the original DHQ file, only 0.7% of the records were problematic. The gender in the DHQ was checked against the gender in the demographic file, which NCI had informed us was the best source of gender information. Each of these cases was examined to determine if the responses were for a

male applicator, a female applicator, a male spouse, or a female spouse, or could not be deciphered. Those that could not be deciphered were deleted (n = 571). The DHQ entries for male private applicators were merged with the Phase 1 and Phase 2 datasets and then with the DHQ files for which nutrient values were available. This yielded a dataset containing 9,076 private applicators—our study sample (see Table 3.1).

a. Correlations between fruit and vegetable consumption variables in DHQ and Phase 1 questionnaire

Since we did not have dietary history data from Phase 1 (enrollment), we needed to develop a variable we could use to control for energy input for the analyses based on the enrollment data alone. In order to be able to use total energy input per day at Phase 2 (which was calculated from the dietary histories completed at follow-up) to predict energy input per day at Phase 1, it was necessary to examine any similar food questions asked during the two phases to see if a relationship existed between what the applicators ate during Phase 1 and what they ate during Phase 2. There were some similar food items in the Phase 1 questionnaire administered at the certification facility and the DHQ self-administered at 5-year follow-up. The Phase 1 questionnaire focused on two categories of food eaten during the past 12 months. One question asked "About how many servings of vegetables did you usually eat, not counting salad or potatoes?" It also included a question on "About how many servings of fruit did you usually eat, not counting juices?" The DHQ included two summary questions on usual intake of vegetables and fruits. Respondents were asked "over the past 12 months, how many servings of vegetables (not including salad or potatoes) did you eat per week or per

day?" A second question on fruits inquired "Over the past 12 months, how many servings of fruit (not including juices) did you eat per week or per day?" Response categories were very similar but some re-coding was needed to assure they matched as precisely as possible. The categories were similar enough to continuous variables to justify the use of correlation analyses. Responses were as follows:

DHQ	**Enrollment Questionnaire**
< 1/week	< 1/week
1-2/week	1-2/week
3-4/week	3-4/week
5-6/week	5-6/week
1/day	1/day
2/day	1½/day
3/day	2/day
4/day	3/day
5 or more/day	4 or more/day

The correlation between overall vegetable and fruit consumption in Phase 1 versus Phase 2 was evaluated. Correlation coefficients examining Phase 1 and Phase 2 data were all highly significant ($p<0.0001$) for both vegetable intake and fruit intake. The correlation coefficient for vegetables was 0.46 and for fruit it was 0.52. Mean consumption (servings) of both vegetables (3.6/week versus 3.5/week) and fruits (2.7/week versus 2.6/week) in Phase 1 and Phase 2 revealed that Phase 2 values were

only slightly lower. These results suggest there was minimal change in diet as measured by fruit and vegetable consumption between Phase 1 and Phase 2.

b. Correlations & mean consumption (servings): meat variables, Phase 1 vs Phase 2

Several questions on meat consumption (hamburger, chicken, beef, pork, bacon) were utilized in the Phase 1 questionnaire and in the cooking practices module of the Phase 2 questionnaire. In the take-home applicator questionnaire done at baseline, respondents were asked "how often have you eaten the following meats [hamburger or cheeseburger; beef-steaks; chicken; pork-chops or ham steak; bacon or breakfast sausage]". In the cooking practices module during Phase 2, the questions were phrased "During the past year, how often did you eat MEAT [hamburgers or cheeseburgers; beef steaks; pork chops; bacon; and chicken including nuggets]". Response categories were as follows:

Phase 1 – take home	**Phase 2 – cooking practices**
Never or < 1/month	Do not eat
1/month	1-6/year
2-3/month	7-11/year
1/week	1/month
2/week	2-3/month
3-4/week	1/week
5-6/week	2/week

1/day	3-4/week
2 or more/day	5-6/week
	1/day
	2 or more/day

Re-coding was performed to make the response categories as similar as possible. The categories were similar enough to continuous variables to justify the use of correlation analyses. Correlations and frequencies were calculated to assess the within person stability of the data for each meat category between Phase 1 and Phase 2 – a period of approximately 5 years. All correlation coefficients were highly significant ($p<0.0001$). Correlations ranged from 0.48 for beef and chicken to 0.77 for pork chops or ham steaks. When examining mean consumption (servings) of the different meat categories among the applicators in Phase 1 and Phase 2, a slight upward trend was seen during the 5-year follow-up time period such that Phase 2 averages were slightly higher: for hamburger (enrollment = 4.4/week versus follow-up = 4.6/week); with a more pronounced increase for pork chops and ham (enrollment=3.5/month versus follow-up = 5.2/week) and bacon and sausage (enrollment = 3.4/month versus follow-up = 5.7/week). Mean consumption (servings) of chicken did not change over the 5-year time period from Phase 1 to Phase 2 and consumption of beef was lower in Phase 2 (3.2/month) than Phase 1 (3.5/month). While these differences in some categories of meat intake from Phase 1 to Phase 2 are noteworthy, as discussed above, all estimates for frequency of consumption for the two time periods were significantly correlated indicating that Phase 2 data may be used to predict Phase 1 data.

c. Meat, Vegetable, and Fruit Correlations with Total Daily Food Energy and Related Regression Models

A meat summary variable was developed by summing the consumption of hamburgers, sausage, beef, pork, bacon, and chicken from Phase 2. Similarly, a meat summary variable for Phase 1 was developed by summing the consumption of hamburgers, chicken, beef, pork, and bacon/sausage. Correlational analyses were performed to determine if baseline data on servings of vegetables and fruits per week or per day and meat intake per month, week, or day were correlated with total caloric intake per day based on the calculated nutrient values for the DHQ administered at Phase 2 (Table 3.7). The correlation coefficients between total daily food energy at Phase 2 and meat, vegetable, and fruit consumption at Phase 1 were all highly significant ($p<0.0001$) (see Table 3.7). However, the coefficients were 0.29, 0.10, and 0.06, respectively, and these very low correlations limit our ability to precisely predict Phase 1 energy input using Phase 2 energy input.

In addition, regression models were run using total food energy in kcal/day at Phase 2 as the dependent variable and Phase 1 consumption of meat, fruit, and vegetables as the independent variables to determine the prediction equation we used to represent total caloric intake at enrollment (Table 3.7). To summarize, we used the Phase 1 variables to predict total caloric intake/day at baseline, which was used to control for diet in regression models examining pesticide exposure and BMI at Phase 1. Subjects with a total food energy of less than 600 kilocalories/day based on the Phase 2 DHQ filled out

at 5-year follow-up were excluded from the analyses (n = 72) as this low food consumption is incompatible with life. The overall regression model for the relationship between total daily food energy at Phase 2 (dependent variable) and meat, vegetable, and fruit consumption at Phase 1 (independent variables) among all private applicators was highly significant ($p<0.0001$). In terms of the contributions to the model of each independent variable, the intercept, vegetable consumption, and meat summary parameter estimates were all highly significant ($p<0.0001$) but the parameter estimate for fruits did not get included in the final model because it contributed very little to the overall model. The R-square for the model was 0.08, which is relatively low. Based on these results, we used the following equation to calculate food energy in our regression models examining pesticide exposure and BMI among all private applicators in our sample at Phase 1: food energy (total kcal/day) = 889.0 + [34.3* (vegetable consumption at Phase 1)] + [65.6* (meat summary at Phase 1)]. For Phase 2 analyses, the total kcal/day provided by subjects' responses to the DHQ was used as the energy input variable. The formula was re-calculated for each subgroup evaluated in the stratified analyses, as well as the "true zero" group, so as to accurately characterize total kcal/day for each subject in each subgroup.

3.4.4.2 ENERGY OUTPUT

The enrollment and take home questionnaires were not designed to measure expenditure of calories, nor was the follow-up questionnaire. Data were collected at baseline on hours of leisure time physical activity in summer and in winter but this is unlikely to be

the major caloric expenditure for farmers given the highly physical nature of their occupation. Since hours spent doing various occupational activities were generally unavailable, calculation of the metabolic equivalents (MET) – the standardized method for calculating energy expended, is not possible. Because MET cannot be calculated by these data, a substitute method for controlling for energy output was identified.

a. Development of the energy output variable for Phase 1 data (at enrollment)

The Phase 1 questionnaire included two items useful in estimating energy output at enrollment: 1) hours per day of heavy lifting; and 2) hours per week of leisure time strenuous exercise. With regard to heavy lifting, subjects were asked "during a typical day in the growing season, about how many hours per day do you spend lifting or carrying heavy objects?" This question was asked for two time periods: currently (at enrollment) and 10 years ago (10 years prior to enrollment). Hours per day of heavy lifting during the growing season at the time of enrollment was significantly correlated with hours per day of heavy lifting 10 years ago (0.78, $p<0.0001$). Mean hours per day of heavy lifting decreased from the period 10 years ago to the present time (at enrollment). The difference was 0.4 hours between the two time periods (3.2 hours versus 2.8 hours). It is important to note that the heavy lifting variable was examined in detail by Racine et al. (2012) and found to be positively associated with the majority of farm activities indicating it may be a viable variable to use to control for physical activity in AHS studies.

The Phase 1 data also included the question "On average, how many hours per week do you spend doing strenuous exercise during your leisure time in the summer?" The same question was also asked for the winter time period. Similar questions were not asked during Phase 2. Hours per week of exercise was significantly (0.86; $p<0.0001$) correlated for the two time periods. Average hours of exercise were slightly higher in summer (1.9 hours) versus winter (1.6 hours). The amount of reduction from summer to winter was 0.3 hours.

The baseline data on hours per day of heavy lifting at enrollment was combined with the weekly leisure time physical activity hours (in summer and in winter) collected at enrollment to develop a single variable that represented physical activity to control for energy expenditure in all models based on Phase 1 data. Table 3.8 provides the frequencies and percents for applicators' responses to the relevant questions with the calculations we performed illustrated. While the response values for the two questions are identical, the lifting question deals with hours per day and the leisure exercise question deals with hours per week. This was taken into consideration in calculating each applicator's physical activity based on combining the answers to these two questions. In addition, since hours of heavy lifting was asked for the "growing season", we assumed half the number of days in a year represented this period. However, recent communications indicated that the growing season may actually be seven months per year (Alavanja 2012). Therefore, there may be some misclassification in our calculated hours of heavy lifting for growing season. For leisure activity, we assumed 6 months of

summer and 6 months of winter for the calculation and adjusted for hours per week versus per day. Heavy lifting and leisure time exercise were combined to create the overall hours per year of physical activity, which was divided by 365.25 to give hours per day of physical activity at enrollment. This variable was used for models based on Phase 1 data only to control for energy output. This is shown in detail in Table 3.9.

b. Development of the energy output variable for Phase 2, the 5-year follow-up

The Phase 2 questionnaire did not include questions on hours of heavy lifting or hours of exercise. However, using the difference between hours of heavy lifting at enrollment and hours of heavy lifting 10 years ago, we were able to determine the percent change per year in hours of heavy lifting by dividing the difference by 10. We calculated the number of years between Phase 1 and Phase 2 for each subject. In order to estimate hours of heavy lifting at Phase 2 for each subject, we multiplied the percent change per year times the hours of heavy lifting at enrollment for the number of years between Phase 1 and Phase 2. Our calculation process followed a depreciation model with the value for each year calculated based on the number of hours from the year before multiplied by the percent reduction in the value from the year before. However, this involved an assumption that the reduction was the same for each of 10 years and this may not be precise. Our calculations assume a linear depreciation model, which may not accurately represent the true change in hours of heavy lifting over time. The variable we calculated using our linear depreciation model was used in the regression

models to control for energy output for the Phase 2 follow-up data. It was found to be significantly correlated with the hours of heavy lifting at enrollment (0.58, $p<0.0001$) and the hours of heavy lifting 10 years prior to enrollment (0.30, $p<0.0001$). However, as noted, it is based on a linear depreciation model, which may not reflect the actual reduction in hours of heavy lifting over time.

3.4.5 Additional Variables Evaluated for Inclusion in the Models

Several categorical variables were evaluated to assess whether or not they were associated with BMI and with exposure. This required creating categorical outcome and exposure variables, which we based on our univariable results for the 25^{th}, 50^{th}, and 75^{th} percentile as dividing points for our categories. Categorical variables evaluated include whether or not the subject lived half his life on a farm before age 18 as determined by his response in the enrollment questionnaire. This variable taken at enrollment only was evaluated in frequency calculations with corresponding chi-squares for the categories for BMI at enrollment, BMI at follow-up, exposure at enrollment, and exposure at follow-up. For models examining BMI at enrollment, whether or not the subject had ever had a high exposure incident through enrollment was evaluated through chi-square analysis with the categorical variables created for enrollment BMI and exposure at enrollment; a similar analysis was conducted for whether or not the subject had ever had a high exposure incident through follow-up since the variable was included in the follow-up questionnaire.

Questions were asked at enrollment regarding whether or not the subject was ever exposed to a list of hazardous substances during non-farm employment. Any "yes" response included the subject in a variable representing non-farm occupational exposures. This variable was assessed to determine if it was associated with BMI and exposure for two time periods: at enrollment and at follow-up. Frequency distributions and chi-square testing determined whether or not there was an association.

We used education as a crude measure of socioeconomic status (SES) in our occupational cohort. In terms of education, approximately 44% of the private applicators are high school graduates (Mahajan 2007). Schooling beyond high school was accomplished by 36% of private applicators (Mahajan 2007). Subjects were asked to report the highest level of education they completed in the enrollment questionnaire and this categorical variable was also assessed using chi-square analyses to determine if it was significantly associated with BMI and with exposure at enrollment and at follow-up.

Several continuous variables were also assessed using correlation analyses to determine if they were correlated with BMI and exposure at enrollment. These include the number of cigarettes smoked per day at enrollment, the number of alcoholic drinks consumed per month at enrollment, and the total number of years the subject worked in farming. Follow-up data were available to assess whether or not BMI at follow-up and exposure at follow-up were correlated with number of alcoholic drinks per month at follow-up and total number of years of farming through follow-up. Smoking status at follow-up

was evaluated as a categorical variable since the updated data in phase 2 included smoking information as a categorical variable. Chi square analyses were used to determine if it was associated with BMI at follow-up and exposure at follow-up.

The relationship between smoking and BMI is complex; smokers are generally lighter than non-smokers but chronic, heavy smokers tend to be heavier than light or non-smokers (Chiolero et al. 2008). Similarly, moderate alcohol use is associated with weight gain, while chronic, heavy use is associated with weight loss (Suter 2005). Blair et al. (2005) found an SMR for lung cancer of 0.4 among private applicators in the AHS cohort and SMRs for other smoking and alcohol-related cancers were all less than 1.0. Similarly, the SIR for lung cancer was 0.47 for private applicators (Alavanja et al. 2005). These low SIRs are believed to be due to the low prevalence of smoking and high prevalence of physical activity in the cohort. Nevertheless, smoking and alcohol use were investigated as potential confounders.

After the primary analyses were completed, they were repeated to exclude the subjects with potentially confounding medical conditions and to examine differences by state to determine the relationship between BMI and pesticide exposure for each of these subgroups. In addition, the analyses were repeated using the "true zero" exposure group.

3.5 Study Aims

The ultimate goal of this research was to determine if occupational exposures to pesticide functional and chemical classes are associated with BMI in the AHS male cohort and, if so, which exposures. There is a legitimate scientific rationale on which to base this investigation, as per the literature review and evaluation in Chapter 2. To achieve the overall goal, the following specific research questions were investigated, building on the more descriptive questions posed in Chapter 1:

- Do pesticide exposures (based on pesticide classes defined by chemical structure or function) at enrollment predict BMI at enrollment?
- Do pesticide exposures at enrollment predict BMI at the end of 5-year follow-up?
- Do pesticide exposures at 5-year follow-up predict BMI at follow-up?

These questions were evaluated using a number of statistical methods. Frequency and proportion differences among sub-groups were examined, along with univariable measures. Graphs showing BMI in relation to the independent variables were assessed. Bivariable analyses included calculation of correlation coefficients. Multiple regression was used to determine the unit change in BMI associated with a unit change in exposure to a specific pesticide class. It was also used to help develop the energy input variable for the baseline time period. The assumptions for using multiple regression were evaluated for this dataset to assure our data were viable for use with regression

modeling. An exposure-response effect was evaluated using analyses based on general linearized models and the least squares means with graphic illustrations of the relationship between increasing least squares means and increasing exposure categories.

As noted above, examining the correlation between specific potentially confounding variables with BMI and with exposure allowed us to determine if such factors were in fact potential confounders. For pesticide classes found to be associated with BMI, we evaluated if controlling for other influencing pesticide classes impacted the relationship. As noted above, we evaluated whether or not the AHS data could be used to develop variables for controlling for the effects of diet and exercise on the relationship between exposure to pesticides and BMI. We also conducted analyses using only potentially confounding variables found to exert a 10% or more change on the outcome variable in the models.

Based on the literature review summarized in Chapter 2, pesticide exposures likely to influence BMI in the AHS cohort include TCDD (based on exposure to phenoxy herbicides such as 2,4-D or 2,4,5-T) and organochlorine pesticides. Despite literature pointing to the aforementioned exposures as potential weight-inducers, all pesticide classes in the AHS database were evaluated for their impact on BMI.

3.6 Research Design

This investigation assessed the relationship between pesticide exposures and BMI. Both retrospective and prospective analyses were conducted using cumulative intensity-weighted exposure days for each pesticide class as the exposure variable and BMI as the outcome variable. These analyses are referred to as enrollment cross-sectional (retrospective: utilizes data on exposure and outcome at enrollment); prospective (utilized data on exposure at enrollment and outcome at 5-year follow-up); and follow-up cross-sectional (retrospective: utilized data from the 5-year follow-up for both exposure and outcome). Therefore, some analyses represent a cross-sectional look at the data at two points in the lifespan of the subjects, at enrollment and at the end of 5-year follow-up. In order to evaluate the longitudinal impact of specific exposures and to take the prospective nature of the AHS into consideration, with its ability to supply the correct temporality between exposure and disease, additional analyses were conducted to determine the impact of exposure at enrollment on BMI assessed at the end of 5-year follow-up. The models were adjusted for the identified confounders (i.e., age, race, etc.). The incremental increase in BMI associated with a particular incremental increase in pesticide exposure intensity was determined. The significance level was set at <0.01 to control for multiple comparisons in all our analyses. SAS software (SAS Institute 2001) was used to analyze the data (Cody & Smith 2006).

Multiple regression analyses examined BMI as a continuous variable in relation to cumulative intensity-weighted exposure to pesticide classes at enrollment and at 5-year

follow-up. BMI was available for three time periods (at age 20, at enrollment, and at the end of 5-year follow-up); however, exposure data are only available for enrollment and for 5-year follow-up so this study focused on the two BMI measures for which exposure data were available. Only descriptive statistics are provided for BMI at age 20. Exposure quantiles for exposure at enrollment and at follow-up were used to investigate if there was an exposure-response. Attempts were made to determine if an exposure-response existed for the exposures under study by distributing exposed subjects into quantiles representing the 25%, 50%, and 75% categories based on the univariable analyses of cumulative exposure intensity for pesticide classes.

Basic models were developed on the basis of a set of covariates and confounders of potential interest that were found to correlate with both exposure and outcome. The tables presenting the correlation results between potential covariates and exposure and outcome are not shown but will be made available upon request. The effects of time on the outcome variable, BMI, were controlled by adjusting for age when it was found to be correlated with both the exposure and outcome variables. If age was not correlated with both exposure and outcome, it was left out of the model and we assumed that time (age) was not an important factor in the relationship between BMI and the particular pesticide class exposure. Rejection of the null hypothesis implies that there is some effect beyond what would be expected by chance in the response between pesticide exposure class and BMI measurement.

Indicator variables that were investigated as stratifying variables include geographic location (state: NC or IA) and presence or absence of weight-related health conditions. Additionally, the assumption that missing values for the pesticide usage questions represents zero exposures was evaluated by using a second analysis group that included only subjects who answered the pesticide usage question as "no" (the true zero group). An investigation was conducted to identify potential confounders based on their correlation with the outcome and the exposure. The multiple regression models included the independent variables cumulative exposure intensity and other identified confounders. Parameter estimate for the pesticide class exposure variable, its standard error, and its *p-value* were presented for change in BMI due to cumulative exposure calculated in the models. Information was also provided on the significance of the initial model including pesticide exposure and all relevant confounders.

All analyses took temporality into account. For example, if an exposure was newly identified at the end of 5-year follow-up, it was not examined in relation to BMI at enrollment. Exposures newly identified at the end of 5-year follow-up were only examined in relation to BMI at the end of 5-year follow-up (cross-sectional follow-up). Exposures identified at enrollment were examined in relation to BMI at enrollment (cross-sectional enrollment) and BMI at the end of 5-year follow-up (prospective). Pesticide functional and chemical classes were calculated based on the exposure to the pesticides in each class at each time period. The particular pesticides that make up the functional and chemical classes were previously defined by AHS (Legum et al. 2003). Note that the follow-up data are cumulative and include the exposure data from Phase 1

as well as Phase 2. Variables such as race, ethnicity, and educational level, which are unlikely to change over the course of 5 years, were used in the models examining enrollment and follow-up BMI.

The strength of the impact of each potential confounder included in the regression analyses was evaluated. Classical epidemiologic methods have relied on the identification of potential confounders depending on whether the variable may cause disease (condition) or a surrogate measure of a cause in unexposed people; be correlated with exposure in the study population with a different distribution among the exposed and unexposed groups; and not be an intermediate step in the causal pathway between the exposure and the disease or condition (McNamee 2003). Researchers generally selected confounders based on *a priori* knowledge and/or by evaluating the statistical relationships between the potential confounder and the outcome variable, and the exposure variable. We have utilized the latter method in our selection of confounders and the final covariates included in all regression models were identified through backward elimination (BE). Detailed tables showing the statistical results for our selection of confounders are not shown but are available upon request.

We also examined effect modification by stratifying our results by state (both states, Iowa, North Carolina) and by presence or absence of weight-related health conditions in these three stratified groups. However, both selection of confounders and variables used to examine effect modification have limitations: validity of results depends on distributional assumptions, which, sometimes may be violated; both analyses are limited

by the size and quality of the data set (Greenland 1989). We also evaluated our assumption that missing values for pesticide usage questions indicate zero exposure by conducting additional analyses using only "true zero" scores (subjects who answered the pesticide usage question as "no").

Recent publications have identified additional selection methods and/or criteria for confounders (McNamee 2005; Viallefont et al 2001; Dominici et al 2008; Budtz-Jørgensen 2007; Weng et al 2009; Schrier & Platt 2008; Maldonado & Greenland 1993; Tong & Lu 2001; Tong & Lu 2001; Hogan & Lancaster 2004; Cole & Hernan 2008). Maldonado & Greenland (1993) evaluated the change in estimate, significance test of the covariate, significance test of the difference, equivalence test of the difference, and weighted averages of adjusted and unadjusted estimates strategies for covariate selection. They found that the change-in-estimate and equivalence-test-of-the-difference strategies performed best when the cut-point for deciding whether crude and adjusted estimates differed by an important amount was set to a low value (10%). The significance test strategies performed best when the alpha level was set to a much higher than the conventional level (0.20) (Maldonado & Greenland 1993).

Walter & Tiemeier (2009) assessed the frequency of use of several confounder selection procedures in published studies including change in the effect estimate, stepwise selection, shrinkage, and penalized regression. After screening the methods sections of 300 articles published in 2008 in 4 prominent epidemiologic journals, they identified 28% using prior knowledge to select covariates and 20% used stepwise selection with

or without univariate pre-screening. They found that stepwise selection methods remain the predominant method for variable selection. Shrinkage methods were not used in any of the studies. They stated that prior knowledge from the scientific literature is formally seen as the most important rationale for including or excluding covariates but it is not always available. Stepwise regression was often combined with pre-specified change in estimate (15%) generally focused on at least a 10% change in risk measure. Together, stepwise regression and change in estimate procedures made up 34% of all methods used. Nine studies used other methods: principal components, propensity scores, all variables in the regression, causal diagrams, and deletion/substitution/addition algorithm. For the 4 journals evaluated, 25-39% of the studies in these journals did not describe their confounder selection procedures (Walter & Tiemeier 2009).

Based on current thinking, we re-evaluated each non-pesticide covariate by calculating the percent difference inclusion of the covariate in the model caused between the unadjusted model and the adjusted model. Any covariates causing a 10% or greater difference in the unadjusted parameter estimate for pesticide class exposure were identified and the adjusted regression analyses were re-run using just these covariates.

The association between exposure to each pesticide class and BMI was also examined by plotting the least squares mean BMI by increasing exposure categories. This was done for each of the time periods analyzed (cross-sectional enrollment, prospective, cross-sectional follow-up). Multicollinearity was assessed by observing the apparent

contribution of an independent variable to estimation of the dependent variable as other independent variables were added to and removed from the regression models.

Since there are no pre-existing data available to estimate the latency associated with agricultural and non-agricultural occupational exposures and the development of overweight or obesity in humans, BMI at 5-year follow-up was investigated in relationship to exposure through 5-year follow-up and for exposure only through enrollment in the hopes of identifying any lag period between exposure and increase in BMI. The main independent variable of interest was "cumulative intensity weighted exposure days" to each pesticide class, bearing in mind temporal logic when possible. Exposure-response relationships were evaluated using PROC GLM in SAS, including calculation of the least squares means with exposure-response trends evaluated using graphical illustrations. Exposure-response was also examined in our ordinal logistic regression analyses.

3.7 Descriptive Analyses

Prior to conducting inferential analyses, descriptive statistics were examined. The numbers and proportions (and means with standard deviations, where applicable) of male private applicators overall and in North Carolina (NC) and Iowa (IA) were categorized by:

Independent Variables

Demographic Factors

- Age by 10-year frequency groupings and univariable statistics
- Race/ethnicity
- Number of years worked in agriculture
- Number of years lived on a farm
- Highest level of schooling completed
- Marital status
- Size of farm where currently living/working
- Geographic location
- Behavioral and Medical Factors
- Physical activity variables at enrollment
- Summary diet variables at enrollment and 5-year follow-up
- Smoking at enrollment and 5-year follow-up
- Alcohol use at enrollment and at 5-year follow-up
- Presence or absence of weight-related health conditions at enrollment and 5-year follow-up
- Completion of take-home questionnaire
- Completion of 5-year follow-up questionnaire
- Completion of Phase 1 and Phase 2

Dependent Variables (Weight-related)

- Weight at enrollment by 20-pound groupings
- Height at enrollment by 3-inch groupings
- Weight at age 20 by 20-pound groupings (collected at enrollment)
- BMI at age 20 by WHO groupings

- Weight at 5-year follow-up by 20 pound groupings
- Height at 5-year follow-up by 3-inch groupings
- BMI at enrollment by WHO groupings (i.e., underweight, normal weight, etc.)
- BMI at 5-year follow-up by WHO groupings
- Obesity and overweight at each of 3 time periods: age 20, at enrollment, at 5-year follow-up
- Consistently obese and overweight across 3 time periods
- BMI univariables for age 20, enrollment, and 5-year follow-up

Trends in prevalence of obesity and overweight over time were examined by looking at prevalence in the male cohort based on data for age 20, data at enrollment, and data obtained at 5-year follow-up. Age and BMI were evaluated as univariables with standard statistics such as mean, median, mode, range, and 25th, 50th, and 75th percentiles.

3.8 Primary Inferential Analyses

3.8.1 Bivariable Analyses

BMI was evaluated as a continuous variable in a number of correlation analyses. These bivariable analyses were used to interpret the results of multivariable analyses (i.e., if the bivariable results are different from the multivariable results, there is likely to be

confounding). The following calculations were performed twice: including missing values for pesticide usage as zero scores and excluding missing values from the zero scores (true zero group):

- Correlation coefficients for BMI at 5-year follow-up versus cumulative intensity-weighted exposure for pesticide classes as determined by exposure at enrollment and at 5-year follow-up.
- Correlation coefficients for BMI at enrollment versus cumulative intensity-weighted exposure for pesticide classes as determined by exposure at enrollment.
- Correlation coefficients for BMI calculated using self-reported height and weight at enrollment versus BMI calculated using self-reported height and weight at the end of 5-year follow-up.

The numbers and proportions of subjects exposed to each pesticide class were calculated and reviewed. Univariable statistics were presented for each pesticide class with missing value scores for pesticide usage questions excluded and included. The nature of how the pesticide exposure questions were asked suggests that a zero or missing score indicates that the subject did not use that pesticide or pesticide class. Therefore, for individual pesticides, missing scores were set to zero. However, we conducted the analyses including and excluding missing values as zero scores for all pesticide classes. This allowed for assessment of whether or not the inclusion of missing

values as zero scores for pesticide classes would mitigate any relationship between pesticide exposures and BMI.

3.8.2. MULTIVARIABLE ANALYSES

3.8.2.1 Analyses to determine incremental increase in BMI associated with incremental increase in exposure

Prior to running the regression models, residual analysis was conducted at each time period (cross-sectional enrollment, prospective, cross-sectional follow-up) in relation to each corresponding cumulative intensity-weighted pesticide exposure controlling for age to assure our data matched the assumptions for regression analysis. We defined a confounder as an independent variable that is statistically significantly related to the dependent variable (BMI) and to the independent variable of interest (pesticide exposure) in our main adjusted regression analyses. These analyses were re-run including only those covariates found to cause a 10% or greater change in the effect size. Each independent variable considered to be a potential confounder was examined in relation to BMI and to exposure at phase 1 (enrollment) and at phase 2 (follow-up). Once the potential confounders were identified through correlation or chi-square analyses to identify those factors associated with both the exposure and the outcome, multiple regression models were run for each pesticide class using cumulative intensity weighted exposure days as the variable of interest. Exposure at enrollment was included in the model with BMI at enrollment as the dependent variable (enrollment cross-

sectional analyses) and in a separate model with BMI at 5-year follow-up as the dependent variable (prospective analyses). Additional models examined exposure at 5-year follow-up in relation to BMI at 5-year follow-up (follow-up cross-sectional analyses).

3.8.2.2 Analyses to evaluate exposure-response

Generalized linear models (PROC GLM in SAS) postulate that a linear relationship exists between the independent variables and the expected value of the dependent variable. The observed value of the dependent variable (response) is the sum of its expected value and an error term. PROC GLM is advantageous because it allows the inclusion of exposure as a class variable, it includes a means procedure, there is no need for dummy variables, it does not require that interaction terms be written in advance, and categorical variables with more than two levels can go directly into the model. PROC GLM produces Type I sum of squares which show the effect of each model predictor sequentially and Type III sum of squares that show the effect of each model predictor controlling for all other effects.

PROC GLM in SAS was used to evaluate each pesticide class using the categorical exposure variable in comparison to the dependent variable, BMI, and including all other relevant independent variables (potential confounders). Levels for each exposure category were determined from the quartiles calculated using PROC UNIVARIATE. The Least Squares Mean option was used to calculate the predicted mean BMI for each

pesticide class category. The significance of any exposure-response trend was evaluated by examining the graphs of Least Squares Means by increasing exposure level.

3.8.2.3 Analyses to calculate odds ratios and 99% confidence limits

We used ordinal logistic regression (OLR) analyses to calculate odds ratios and 99% confidence intervals to evaluate exposure-response. OLR models cumulative probability over the lower ordered values when the dependent variable is ordered from low to high (Snedker et al 2002). In our analyses, weight level increases from the lower ordered values to the higher ordered values. OLR simultaneously estimates multiple equations. The number of equations it estimates is equivalent to the number of categories in the dependent variable minus one. To perform these analyses, the outcome variable (BMI) was categorized based on the WHO classifications of underweight, normal weight, overweight, and obese. Therefore the number of equations estimated is three:

	Pooled categories	compared to	**Pooled categories**
Equation 1	underweight		obese, overwt, normal wt
Equation 2	underwt, normal wt		overweight, obese
Equation 3	underwt, normal wt, overwt		obese

Each equation models the odds of being in the set of categories on the left versus the set of categories on the right based on each increase in pesticide class exposure level (i.e.,

from level one to level 2, from level 2 to level 3, and from level 3 to level 4). OLR provides only one set of coefficients for each independent variable. There is an assumption of parallel regression such that the coefficients for the variables in the equations would not vary significantly if they were estimated separately. A Chi-square test was performed for each analysis of exposure level to a particular pesticide class versus weight category to assess whether or not the assumption of parallel regression was accepted or rejected. If the assumption was accepted, we reverted to using a binary dependent variable and a regular logistic regression analysis (i.e., obese and overweight versus normal and underweight). If the assumption was rejected based on the results of the Chi-square test, we continued with the OLR analysis.

In the OLR analyses, a positive coefficient indicates an increased chance that a subject with a higher exposure will be observed in a higher BMI category with an odds ratio greater than one. A negative coefficient indicates the chances that a subject with a higher exposure will be observed in a lower BMI category with the odds ratio being less than one (Snedker et al 2002; UCLA accessed 2012).

We calculated the odds that increasing exposure categories (one through 4) for a particular pesticide class are associated with increasing BMI category (underweight, normal weight, overweight, obese) with the odds being cumulated over the lower ordered values. For example, we would say that for a one unit (category) increase in exposure (i.e., going from exposure level one to exposure level two), we would expect an increase in the ordered log odds (odds ratio) of being in a higher weight level versus

a lower weight level (i.e., odds ratio greater than one) given that all the other variables in the model are held constant. In summary, what is being modeled is the probability of being in a higher weight category versus a lower weight category depending on going from a lower level of exposure to the next highest level of exposure (Snedker et al 2002; UCLA accessed 2012).

Each pesticide class was divided into levels based on the 25th, 50th, and 75th percentile scores calculated during the univariable analyses. This categorical variable was compared with the BMI categorical variable created by grouping BMI according to the WHO levels for underweight, normal weight, overweight, and obese. We used a 99% confidence interval in keeping with our decision to use 0.01 as the level to determine statistical significance for all analyses. This more conservative level also controlled for multiple comparisons. The ordinal logistic regression procedure calculates a Wald Chi-square to evaluate if there is a significant positive or negative relationship between the dependent variable (BMI group) and the main independent variable (exposure group). We used this variable to assess whether or not a significant positive or negative relationship existed between BMI group and exposure group.

3.9 Additional Analyses to Evaluate Positive Findings

Each analysis as originally conceptualized was to evaluate one pesticide class at a time. However, when we found consistently significant findings for triazine herbicides and BMI across all studies and stratified groups, we re-analyzed the multivariable results for

all other pesticide classes to further understand the confounding impact of triazine herbicides on any other positive results identified. To assure that any change caused by including triazine herbicides in the models was not by chance or over-control of exposure variables, we also examined multiple regression results including each pesticide class as a covariate in the analysis of all other pesticide classes. Care was taken not to include multiple related pesticide classes (that is, classes that include some of the same substances) in the same model.

3.10 Summary

The relationship between BMI and exposure to agricultural chemicals was evaluated using the AHS male cohort of 50,641 subjects aged 20 years or more. Data to calculate exposure intensity and BMI at two time periods were available, along with data to assess and control for confounding. Male applicators were the focus of this research due to the better defined and more consistent and intense exposures. The sample available for analysis was 9,062 after relevant exclusions for missing data. The sample used in each analysis differed depending on whether or not data were available for the variables in the models. However, statistical power calculations indicate a power of 80% with alpha set at 0.01 to detect changes in BMI between 21% and 68%. Subjects with medical conditions self-reported at baseline or follow-up that are associated with weight gain or loss were excluded in stratified analyses. Additional stratified analyses examined subjects from Iowa and from North Carolina separately. The ultimate goal was to determine which, if any, exposures to classes of pesticides are related to BMI in

the AHS cohort. A number of analyses were conducted to evaluate potential associations between pesticide classes and BMI. Data are available on exposure to broad classes of pesticides at enrollment and at 5-year follow-up. Data are available to calculate BMI at age 20, at enrollment, and at 5-year follow-up. This wealth of data allows for 3 types of analyses: cross-sectional using BMI and exposure at enrollment; prospective using BMI at 5-year follow-up and exposure at enrollment; and cross-sectional using BMI and exposure at 5-year follow-up.

Descriptive and analytical analyses were performed. Correlation coefficients, regression coefficients, and least squares means were calculated, along with odds ratios and 99% confidence intervals. Correlation coefficients were calculated to examine the association between BMI and exposure intensity using cumulative intensity weighted exposure for pesticide classes. Multiple regression was used to determine the increase in BMI in relation to the increase in pesticide class exposure intensity score calculated using algorithms that have been validated. Both unadjusted and adjusted multiple regression analyses were conducted and consistently significant findings for a particular pesticide class were further evaluated using that class as a covariate in the analyses of other pesticide classes. We also conducted the regression analyses including only those confounders found to cause a 10% or greater change in the unadjusted parameter estimate. Factors such as the impact of geographic location (NC or IA) and presence or absence of weight-related health conditions were also evaluated in stratified analyses in order to identify potential effect modification. In addition, we evaluated our assumption that missing values for the questions on ever use of a particular pesticide were

equivalent to zero exposure by conducting analyses using only "true zeros" – that is, the respondent answered "no" to the question on ever use of a particular pesticide.

Prospective analyses evaluated the impact of exposures of interest as identified at the point in time when baseline data collection began in relation to BMI at the end of 5-year follow-up. A justifiable method for controlling for energy input and energy expenditure was developed using the limited dietary and physical activity data. The main limitations to the study are the lack of clear variables to use to control for these important factors. The study is also limited by the lack of childhood exposures, the large amount of missing information, and the reliance on self-reported data to calculate BMI. Study strengths include the best available questionnaire-based pesticide exposure data, large sample size, as well as the ability to prospectively assess the impact of prior pesticide exposures on BMI.

TABLES FOR CHAPTER 3

Table 3.1. Development of Sample for Analysis

FILE NAME	Private Applicators	
demographicfile	**Entire Cohort**	**89658**
	Delete Commercial Applicators	**-4916**
		84742
	Delete Females	**-33488**
		51254
	Delete Males Under Age 20	**-394**
		50860
	Delete Subjects Remaining Identified as Spouses	**-219**
Maleprivapps	**TOTAL MALE PRIVATE APPLICATORS UNDER AGE 20 IN DEMOGRAPHIC FILE**	**50641**
p1privapps + maleprivapps	**Merge Phase 1 data file with Male Demographic File**	**52395**
	Delete missing age	**-3**
	Delete Females	**-1358**
	Delete Males Under Age 20	**-393**
demop1apps	**Total Merged Phase 1 with Demographic File**	**50641**
p2privapps + maleprivapps	**Merge Phase 2 data file with Male Demographic File**	**51714**
	Delete females	**-921**
	Delete males under age 20	**-152**
Demop2apps		**50641**
p1only	**Delete Males in Phase 1 Only**	**-18257**
P1p2demoapps	**Merge Phase 2 with Demographic File (in both files)**	**32384**
p1p2demo	**Merge Phase 1, Phase2, and demographic files**	**50641**
Totalprivapps	**merge Phase 1, Phase2, and demographic files (in all 3)**	**32384**
Diethistquest	**diet history questionnaire**	**35011**
	delete if unknown to who responses refer	**-571**
	subtotal	**34440**
	unknown who completed questionnaire	**-44**

	subtotal	**34396**
	delete spouse diet histories	**-8151**
	subtotal	**26245**
	delete female applicators and additional spouse questionnaires	**-7697**
	subtotal	**18548**
	delete commercial applicators	**-1613**
	subtotal	**16935**
	delete duplicate IDs	**-377**
Dietupdate	**subtotal**	**16558**
demodietupdate	**merge in demographic file**	**82972**
	delete if not in demo and diet files	**-65346**
	in both demo and diet files	**17626**
	delete duplicates	**-1069**
Demodiet	**male private applicators in demographic and diet files**	**16557**
Dietplusfinal	**nutrient data available**	**17165**
	delete duplicates	**-3**
Dietnutrients	**final nutrient file**	**17162**
Demodiet	**merge nutrient file with male private applicators in demo and diet files**	**16557**
	delete if not in both demo and diet files	**-491**
dietplusnutrients	**demo-diet-nutrient file**	**16066**
dietplusnutrients	**merge demo-diet-nutrient file with male private applicators**	**16066**
totalprivapps	**merge male private applicators with demo-diet-nutrient file**	**32384**
	delete applicators not in diet file	**-16318**
	delete applicators not in both files	**-90**
Demodietfile	**male private applicators in Phase 1 and Phase 2 with diet & nutrients**	**15976**
finaldemodietfile	**delete if missing BMI2 and BMI3 [or BMI2<13 or BMI3<13 (n=2)]**	**-6900**
finaldemodietfile	**FINAL ANALYSIS FILE**	**9076**

TABLE 3.2

FREQUENCY OF EXPOSURE TO PESTICIDE FUNCTIONAL AND CHEMICAL CLASSES WITH STATISTICAL POWER CALCULATIONS*

	ALL PRIVATE APPLICATORS				IOWA PRIVATE APPLICATORS				NORTH CAROLINA PRIVATE APPLICATORS			
	Number	Percent	% chg detected / % power**		Number	Percent	% chg detected / % power**		Number	Percent	% chg detected / % power**
animal insecticides				animal insecticides				animal insecticides			
unexposed	885	9.8	37.57%	unexposed	555	8.2	48.35%	unexposed	330	14.2	67.30%
exposed	8191	90.3	80.08%	exposed	6199	91.8	80.13%	exposed	1992	85.8	80.14%
crop insecticides				crop insecticides				crop insecticides			
unexposed	801	8.8	39.57%	unexposed	492	7.3	51.48%	unexposed	309	13.3	69.48%
exposed	8275	91.2	80.25%	exposed	6262	92.7	80.10%	exposed	2013	86.7	80.04%
fumigants				fumigants				fumigants			
unexposed	7371	81.2	26.78%	unexposed	6113	90.5	42.09%	unexposed	1258	54.2	43.57%
exposed	1705	18.8	80.11%	exposed	641	9.5	80.14%	exposed	1064	45.8	80.05%
fungicides				fungicides				fungicides			
unexposed	6390	70.4	31.57%	unexposed	5439	80.5	30.87%	unexposed	951	41.0	44.70%
exposed	2686	29.6	80.06%	exposed	1315	19.5	80.18%	exposed	1371	59.0	80.10%
herbicides				herbicides				herbicides			
unexposed	285	3.1	68.87%	unexposed	121	1.8	113.83%	unexposed	164	7.1	97.30%
exposed	8791	96.9	80.09%	exposed	6633	98.2	80.43%	exposed	2158	92.9	80.04%
organochlorines				organochlorines				organochlorines			
unexposed	4674	51.5	21.04%	unexposed	3546	52.5	24.52%	unexposed	1128	48.6	43.65%
exposed	4402	48.5	80.36%	exposed	3208	47.5	80.07%	exposed	1194	51.4	80.13%
organophosphates				organophosphates				organophosphates			
unexposed	1189	13.1	32.61%	unexposed	652	9.7	44.52%	unexposed	537	23.1	53.91%
exposed	7887	86.9	80.26%	exposed	6102	90.4	80.14%	exposed	1785	76.9	80.24%

Organothio-phosphates				Organothio-phosphates				Organothio-phosphates			
unexposed	2138	23.6	25.30%	unexposed	1275	18.9	32.43%	unexposed	863	37.2	45.74%
exposed	6938	76.4	80.22%	exposed	5479	81.1	80.10%	exposed	1459	62.8	80.18%
carbamates				carbamates				carbamates			
unexposed	3870	42.6	21.30%	unexposed	3335	49.4	24.52%	unexposed	535	23.0	53.91%
exposed	5206	57.4	80.13%	exposed	3419	50.6	80.04%	exposed	1787	77.0	80.10%
phenoxy herbicides				phenoxy herbicides				phenoxy herbicides			
unexposed	1506	16.6	29.22%	unexposed	698	10.3	43.04%	unexposed	808	34.8	46.52%
exposed	7570	83.4	80.09%	exposed	6056	89.7	80.19%	exposed	1514	65.2	80.10%
triazine herbicides				triazine herbicides				triazine herbicides			
unexposed	1729	19.1	27.57%	unexposed	695	10.3	43.13%	unexposed	1034	44.5	44.00%
exposed	7347	81.0	80.23%	exposed	6059	89.7	80.18%	exposed	1288	55.5	80.00%
all pesticide classes				all pesticide classes				all pesticide classes			
unexposed	183	27.4	103.48%	unexposed	73	30.4	185.65%	unexposed	110	25.7	139.13%
exposed	485	72.6	80.01%	exposed	167	69.6	80.31%	exposed	318	74.3	80.08%

ALL PRIVATE APPLICATORS, MEDICAL EXCLUSIONS				IOWA PRIVATE APPLICATORS, MEDICAL EXCLUSIONS				NORTH CAROLINA PRIVATE APPLICATORS, MEDICAL EXCLUSIONS			
	Number	Percent	% chg detected / % power**		Number	Percent	% chg detected / % power**		Number	Percent	% chg detected / % power**
animal insecticides				animal insecticides				animal insecticides			
unexposed	619	9.7	45.83%	unexposed	413	8.5	57.39%	unexposed	206	13.7	52.67%
exposed	5748	90.3	80.08%	exposed	4449	91.5	80.35%	exposed	1299	86.3	88.35%
crop insecticides				crop insecticides				crop insecticides			
unexposed	563	8.8	48.09%	unexposed	373	7.7	60.43%	unexposed	190	12.6	92.09%
exposed	5804	91.2	80.05%	exposed	4489	92.3	80.14%	exposed	1315	87.4	80.09%

fumigants				fumigants				fumigants			
unexposed	5269	82.8	33.39%	unexposed	4455	91.6	53.13%	unexposed	814	54.1	55.57%
exposed	1098	17.3	80.11%	exposed	407	8.4	80.01%	exposed	691	45.9	80.41%
fungicides				fungicides				fungicides			
unexposed	4580	71.3	28.00%	unexposed	3965	81.6	37.48%	unexposed	615	40.9	56.96%
exposed	1787	28.1	80.11%	exposed	897	18.5	80.08%	exposed	890	59.1	80.12%
herbicides				herbicides				herbicides			
unexposed	180	2.8	90.09%	unexposed	85	1.8	140.43%	unexposed	95	6.3	134.87%
exposed	6187	97.2	80.16%	exposed	4777	98.3	80.17%	exposed	1410	93.7	80.31%
organochlorines				organochlorines				organochlorines			
unexposed	3579	56.2	25.48%	unexposed	2798	57.6	29.48%	unexposed	781	51.9	55.30%
exposed	2788	43.8	80.25%	exposed	2064	42.5	80.20%	exposed	724	48.1	80.06%
organophosphates				organophosphates				organophosphates			
unexposed	837	13.2	39.48%	unexposed	492	10.1	52.26%	unexposed	345	22.9	53.27%
exposed	5530	86.9	80.05%	exposed	4370	89.9	80.05%	exposed	1160	77.1	80.44%
Organothio-phosphates				Organothio-phosphates				Organothio-phosphates			
unexposed	1510	23.7	30.52%	unexposed	957	19.7	38.17%	unexposed	553	36.7	58.43%
exposed	4857	76.3	80.14%	exposed	3905	80.3	80.17%	exposed	952	63.3	80.11%
carbamates				carbamates				carbamates			
unexposed	2872	45.1	25.48%	unexposed	2539	52.2	29.22%	unexposed	333	22.1	70.26%
exposed	3495	54.9	80.03%	exposed	2323	47.8	80.14%	exposed	1172	77.9	80.06%
phenoxy herbicides				phenoxy herbicides				phenoxy herbicides			
unexposed	1081	17.0	35.13%	unexposed	539	11.1	50.00%	unexposed	542	36.0	58.70%
exposed	5286	83.0	80.22%	exposed	4323	88.9	80.24%	exposed	963	64.0	80.01%

triazine herbicides				triazine herbicides				triazine herbicides			
unexposed	1170	18.4	33.91%	unexposed	504	10.4	51.74%	unexposed	666	44.3	56.09%
exposed	5197	81.6	80.17%	exposed	4358	89.6	80.27%	exposed	839	55.8	80.07%
all pesticide classes				all pesticide classes				all pesticide classes			
unexposed	111	28.2	140.78%	unexposed	51	35.4	240.87%	unexposed	60	24.1	199.39%
exposed	282	71.8	80.00%	exposed	93	64.6	80.22%	exposed	189	75.9	80.02%

* mean change per year in BMI = 0.115 for general population (Truong & Sturm 2005; Newby et al 2003; Baum & Ruhm 2007; Ostbye et al 2011)

**assumes alpha=0.01 using Russ Lenth's online Power Calculator: http://www.stat.uiowa.edu/~rlenth/Power/

Table 3.3: The International Classification of adult underweight, overweight and obesity according to BMI

Classification	BMI(kg/m²)	
	Principal cut-off points	Additional cut-off points
Underweight	**<18.50**	**<18.50**
Severe thinness	<16.00	<16.00
Moderate thinness	16.00 - 16.99	16.00 - 16.99
Mild thinness	17.00 - 18.49	17.00 - 18.49
Normal range	**18.50 - 24.99**	**18.50 - 22.99**
		23.00 - 24.99
Overweight	**≥25.00**	**≥25.00**
Pre-obese	25.00 - 29.99	25.00 - 27.49
		27.50 - 29.99
Obese	**≥30.00**	**≥30.00**
Obese class I	30.00 - 34-99	30.00 - 32.49
		32.50 - 34.99
Obese class II	35.00 - 39.99	35.00 - 37.49
		37.50 - 39.99
Obese class III	≥40.00	≥40.00

Source: WHO 2007.

Table 3.4: Mean and Median BMI distribution by age group among AHS applicators (1993-1997) and the overall U.S. population 1988-1994 (NHANES III) (Flegal and Troiano. Int J Obes (2000) 24,807-818). (Hou 2007)

	AHS			U.S. Population		
Age (Years)	N	Mean	Median	N	Mean	Median
20-29	2,625	26.53499	25.968	1,639	25.2	24.3
30-39	8,634	27.34276	26.831	1,470	26.5	25.7
40-49	10,622	28.00471	27.37	1,222	27.3	26.3
50-59	8,535	27.9849	27.436	858	27.8	27.2
60-69	5,763	27.32805	26.972	1,179	27.4	27.1
70-74	1,237	26.62224	26.444	548	26.8	26.1

Table 3.5. Pesticides Included in Enrollment versus Take-Home Questionnaires*

	ENROLLMENT QUESTIONNAIRE					TAKE-HOME QUESTIONNAIRE				
	Ever mixed or applied	Number of years used	Decade first used	Used 10 years ago	Used in past 12 months	Ever mixed or applied	Number of years used	Decade first used	Used 10 years ago	Used in past 12 months
PESTICIDES	X	X			X	X			X	
HERBICIDES	X				X	X			X	
Atrazine	X	X	X							
Dicamba	X	X	X							
Cyanazine	X	X	X							
Metolachlor	X	X	X							
EPTC	X	X	X							
Alachlor	X	X	X							
Imazethapyr	X	X	X							
Glyphosate	X	X	X							
Trifluralin	X	X	X							
2,4-D	X	X	X							
Chlorimuronethyl	X					X	X	X		
Metribuzin	X					X	X	X		
Paraquat	X					X	X	X		
Petroleum distillate	X					X	X	X		
Pendimethalin	X					X	X	X		
Butylate	X					X	X	X		
2.4.5-TP	X					X	X	X		
2,4,5-T	X					X	X	X		
Metsulfuran methyl						X				
Chloramben						X				
Bentazone						X				
Bicep (atrazine + metolachlor)						X				
Acifluorfen sodium						X				
Bronco (alachlor + isopropylamine salt of glyphosphate)						X				
Bullet (alachlor + atrazine)						X				
Bromoxynil						X				
Canopy (metribuzin + chlorimuron ethyl)						X				
Clomazone						X				
Conquest (cyanazine + atrazine)						X				
Fluometuron						X				
Napropamide						X				
Diphenamid						X				
EPTC						X				
Fluazifop-butyl						X				
Thifensulfuron methyl						X				
Diclofop-methyl						X				
Inorganic Arsenic						X				
Linuron						X				
Marksman (dicamba + atrazine)						X				
Isopropalin						X				
Simazine						X				
Organic Arsenic						X				
Sethoxydim						X				
Propachlor						X				
Imazaquin						X				
Ethalfluralin						X				
Squadron (pendimethalin + imazaquin)						X				

Oryzalin						X				
Sutazine (atrazine + butylate)						X				
Tridiphene						X				
Vernolate						X				
Norflurozon						X				
INSECTICIDES	X				X	X			X	X
Pyrethroid	X	X	X							
Terbufos	X	X	X							
Fonofos	X	X	X							
Trichlorfon	X	X	X							
Carbofuran	X	X	X							
Chlorpyrifos	X	X	X							
Coumaphos	X	X	X							
Permethrin	X	X	X							
Dichlorvos	X	X	X							
Lindane	X					X	X	X		
Malathion	X					X	X	X		
Parathion	X					X	X	X		
Carbaryl	X					X	X	X		
Diazinon	X					X	X	X		
Aldicarb	X					X	X	X		
Phorate	X					X	X	X		
Aldrin	X					X	X	X		
Chlordane	X					X	X	X		
Dieldrin	X					X	X	X		
DDT	X					X	X	X		
Heptachlor	X					X	X	X		
Toxaphene	X					X	X	X		
Aastar (flucythrinate + phorate)						X				
Trimethacarb						X				
Dimethoate						X				
Dioxathion						X				
Bacillus thuringiensis						X				
Disulfoton						X				
Fenchlorphos						X				
Tefluthrin						X				
Azinphos methyl						X				
Phosmet						X				
Dicofol						X				
Methomyl						X				
Lead Arsenate						X				
Methoxychlor						X				
Methamidophos						X				
Ethoprop						X				
Rotenone						X				
Acephate						X				
Mevinphos						X				
Fenvalerate						X				
Crufomate						X				
Endosulfan						X				
Trichlorfon						X				
Oxanyl						X				
Fenthion						X				
Nicotine						X				
Famphur						X				
Bufencarb						X				
Ivomec						X				
Ronnel						X				
Methomyl						X				
Trichlorfon						X				
Phosmet						X				
Tetrachlorvinphos						X				
Rotenoneapry						X				
Tackic						X				

FUMIGANTS	X				X	X			X	X
Methyl bromide	X	X	X							
Aluminum phosphide	X					X	X	X		
Carbon disulfide	X					X	X	X		
Ethylene dibromide	X					X	X	X		
Dazomet						X				
Bin Spray						X				
Chloropicrin						X				
Calcium cyanide						X				
DBCP (dibromo-chloropropane)						X				
Dichloropropene						X				
Tetrafume						X				
Metam-sodium						X				
Methyl isothiocyanate						X				
FUNGICIDES	X				X	X			X	X
Chlorothalonil	X	X	X							
Captan	X	X	X							
Benomyl	X					X	X	X		
Maneb	X					X	X	X		
Metalazyl	X					X	X	X		
Ziram	X					X	X	X		
Triabendazole						X				
Propiconazole						X				
Triadimefon						X				
Triadimenol						X				
Captafol						X				
Ferbam						X				
Copper ammonia carbonate						X				
Vinclozolin						X				
Dodine						X				
Anilazine						X				
Thiram						X				
Triforine						X				
Cupric hydroxide						X				
Thiabendazole						X				
Dodemorph acetate						X				
Myclobutanil						X				
Folpet						X				
Piperalin						X				
Metiram						X				
Iprodione						X				
Fenarimol						X				
Sulfur						X				
PCNB						X				
Thiocarabamates						X				
Triophanate-methyl						X				
Copper sulfate						X				
Triphenyltin acetate						X				
Etridiazole						X				
Carboxin						X				

* Pesticide data collected at 5-year follow-up was collected via verbatim responses

Table 3.6. Exposure Algorithm with Definitions and Scores

General algorithm: Intensity Level = (Mix + Appl + Repair) * PPE	
Definitions for Algorithms with Scores:	
Mix (mixing status):	**SCORE**
if [Mix] = Never	0
if [Mix] = <50% of time mixed	3
if [Mix] = 50%+ of time mixed	9
Appl (Application methods identified for 5 different groups of pesticide separately):	**SCORE**
if [Appl] = Does not apply	0
For herbicides	
if [Appl] = Aerial-aircraft	1
if [Appl] = Distribute tablets	1
if [Appl] = In furrow/banded	2
if [Appl] = Boom on tractor	3
if [Appl] = Backpack	8
if [Appl] = Hand spray	9
For crop insecticides	**SCORE**
if [Appl] = Aerial-aircraft	1
if [Appl] = Seed treatment	1
if [Appl] = Distribute tablets	1
if [Appl] = In furrow/banded	2
if [Appl] = Boom on tractor	3
if [Appl] = Backpack	8
if [Appl] = Hand spray	9
if [Appl] = Air blast	9
if [Appl] = Mist blower / fogger	9
For fungicides	**SCORE**
if [Appl] = Seed treatment	1
if [Appl] = Distribute tablets	1
if [Appl] = In furrow/banded	2
if [Appl] = Boom on tractor	3
if [Appl] = Backpack	8
if [Appl] = Hand spray	9
if [Appl] = Air blast	9
if [Appl] = Mist blower/fogger	9
Application methods for livestock insecticides (with Scores):	**SCORE**
if [Appl] = Ear tags	1
if [Appl] = Inject animal	2

if [Appl] = Dip animal	5
if [Appl] = Spray animal	6
if [Appl] = Pour on animal	7
if [Appl] = Powder duster	9
Application methods for fumigants (with Scores):	**SCORE**
if [Appl] = Gas canister	2
if [Appl] = Row fumigation	4
if [Appl] = Pour fumigant	9
Status of repairing equipment:	**SCORE**
if [Repair] = Does not repair	0
if [Repair] = Repair	2
PPE (Personal Protective Equipment use):	
4 groups of PPE categories identified considering combinations of PPE used:	
PPE-0 (0% PROTECTION):	
[PPE] = never used PPE	
PPE-1 (20% PROTECTION) one or more indicated PPE:	
[PPE] = Face shields or goggles	
[PPE] = Fabric/leather gloves	
[PPE] = Other protective clothing, such as boots	
PPE-2 (30% PROTECTION) one or more indicated PPE:	
[PPE] = Cartridge respirator or gas mask	
[PPE] = Disposable outer clothing	
PPE-3 (40% PROTECTION):	
[PPE] = Chemically resistant rubber gloves	
PPE or combinations of PPE use:	**SCORE**
PPE-0	1.0
PPE-1	0.8
PPE-2	0.7
PPE-3	0.6
PPE-1 & PPE-2	0.5
PPE-1 & PPE-3	0.4
PPE-2 & PPE-3	0.3
PPE-1 & PPE-2 & PPE-3	0.1

TABLE 3.7
ALL PRIVATE APPLICATORS WHO COMPLETED PHASE 1, PHASE2, AND THE DIET HISTORY

CORRELATIONS COEFFICIENTS FOR TOTAL DAILY FOOD ENERGY IN KILOCALORIES AT PHASE 2 VERSUS SERVINGS OF ALL MEATS SUMMED (PHASE 1) AND FRUIT AND VEGETABLE SUMMARIES (PHASE 1), ELIMINATING ZEROS

UNIVARIABLE STATISTICS FOR FOOD VARIABLES

REGRESSION MODEL FOR PHASE 2 KCAL = PHASE 1 FOOD SUMMARIES

	Meats at Phase 1 vs Total Food Energy at Phase 2	**Vegetables at Phase 1 vs Total Food Energy at Phase 2**	**Fruits at Phase 1 vs Total Food Energy at Phase 2**
Correlation coefficient	**0.29 (p<0.0001)**	**0.10 (p<0.0001)**	**0.06 (p<0.0001)**

UNIVARIABLE STATISTICS FOR MEATS, VEGETABLES, AND FRUITS FROM PHASE 1 AND TOTAL KCAL FROM PHASE 2

	MEAT Summary, Phase 1	**VEGETABLE Summary, Phase 1**	**FRUIT Summary, Phase 1**	**TOTAL KCAL, Phase 2**
Number of Subjects available for use	8682	8510	8623	9004 subjects
Mean food intake	18.9 = 4+ servings/day	3.6 = < once/day but > 6/week servings	2.7 = 3-6 servings/week	2280.0 kcal/day
Standard Deviation for mean food intake	4.3	1.5	1.6	1034.0 kcal/day
Median food intake	19.0 = 4+ servings/day	4.0 = once/day	2.0 = 3-4 servings/week	2105.0 kcal/day
Minimum value for food intake	5.0 = 2 servings/week	0.0 = none	0.0 = none	600.8 kcal/day
Maximum value for food intake	45.0 = 10 servings/day	7.0 = 4+ servings/day	7.0 = 4+ servings/day	31609.0 kcal/day

REGRESSION MODEL: TOTAL FOOD ENERGY/DAY AT PHASE 2 (DEPENDENT VARIABLE) = COMBINATION OF MEAT SUMMARY, FRUIT SUMMARY, AND VEGETABLE SUMMARY OF SERVINGS PER MONTH/WEEK/DAY (INDEPENDENT VARIABLES ALL FROM PHASE 1)

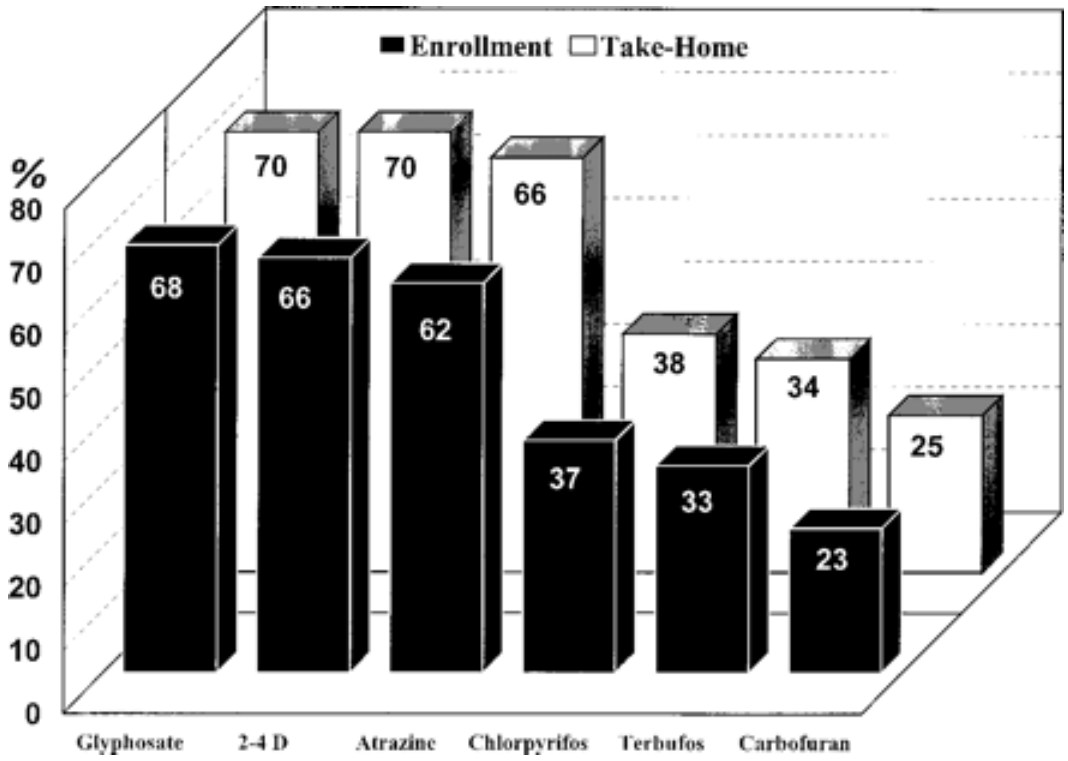
Full Model: # subjects, Anova F value, p-value, R-square	**Variable**	**Parameter Estimate**	**Standard Error**	**F value (p-value)**
n = 8190 F = 237.5 p<0.0001 R-sq=0.08	Intercept	889.0	55.4	**257.3 (p<0.0001)**
	Vegetables --- Phase 1	34.3	8.4	**16.6 (p<0.0001)**
	Meat Summary -- Phase 1	65.6	2.6	**654.9 (p<0.0001)**
	Fruits - Phase 1	13.5	8.1	2.8 (p=0.0971)
Calculation of total food energy at enrollment based on regression model	total kcal = 889.0 + 34.3(phase 1 vegetable intake) + 65.6(phase 1 meat intake)			

TABLE 3.8: DAILY HOURS OF PHYSICAL ACTIVITY AT ENROLLMENT

	PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, AND DIET HISTORY		
		mean=357.7	s.d.=400.8
	Hours	Frequency	Percent
Number of hours per day of heavy lifting over the past year multiplied by half the number of days in a year to represent the growing season	0	425	4.8
	0.5*182.6=91.3	3368	38.2
	1.5*182.6=273.9	2901	32.9
	4*182.6=730.4	1498	17.0
	8*182.6=1460.8	500	5.7
	10.5*182.6=1917.3	117	1.3
PLUS		mean=73.0	s.d.=88.6
	Hours	Frequency	Percent
Number of hours per week of exercise divided by 7 for number of hours per day then multiplied by the number of days in half a year to represent the summer	0	2288	25.5
	0.5 / 7 * 182.6=13.0	1843	20.5
	1.5 / 7 * 182.6=39.1	1552	17.3
	4 / 7 * 182.6=104.3	1615	18.0
	8 / 7 * 182.6=208.7	887	9.9
	10.5 / 7 * 182.6=273.9	792	8.8
PLUS		mean=53.7	s.d.=73.6
	Hours	Frequency	Percent
Number of hours per week of exercise divided by 7 for number of hours per day then multiplied by the number of days in half a year to represent the winter	0	2386	26.8
	0.5 / 7 * 182.6=13.0	2349	26.4
	1.5 / 7 * 182.6=39.1	1809	20.3
	4 / 7 * 182.6=104.3	1380	15.5
	8 / 7 * 182.6=208.7	587	6.6
	10.5 / 7 * 182.6=273.9	403	4.5
SUM		mean=482.1	s.d.=444.8
Total number of hours of physical activity in one year (hours of lifting + hours of exercise in summer + hours of exercise in winter)	Hours	Frequency	Percent
	0	117	1.4
	< 300	4221	48.8
	300 - < 600	1850	21.3
	600 - < 900	1374	15.8
	900 - < 1200	359	4.1
	1200 - < 1500	335	4.0
	1500 - < 1800	186	2.1
	1800 - < 2100	164	1.9
	> 2100	49	0.6
TOTAL DIVIDED BY 365.25 TO REPRESENT NUMBER OF DAYS IN 1 YEAR = CALCULATION OF TOTAL DAILY HOURS OF PHYSICAL ACTIVITY AT ENROLLMENT		mean=1.3	s.d.=1.2
	Hours	Frequency	Percent
	0	117	1.4
	>0 - < 1.0	4865	56.2
	1.0 - < 3.0	2845	32.9
	3.0 - < 5.0	615	7.1
	5.0 - < 6.0	170	2.0
	6.0 +	43	0.5

FIGURES FOR CHAPTER 3

Figure 3.1: Prevalence of most commonly used herbicides and insecticides by questionnaire type (from Dosemeci et al 2002)



CHAPTER 4

RESULTS

To assess the links between pesticide exposure and BMI, we used descriptive and multivariable analyses. We developed methods to control for energy input (diet) and energy output (physical activity) using the available AHS data. Because there are differences between the questionnaires for Phase 1 and Phase 2 related to diet and physical activity, modifications and adjustments were needed to calculate the potential confounding in the AHS data. Development of surrogate measures to control for energy input and energy output is essential due to the strong relationships among energy input, energy output, and body weight. A number of additional potential confounders were evaluated including age, race, Hispanic ethnicity, smoking, alcohol use, number of years lived on a farm, non-farm occupational exposure, whether or not the subject had lived at least half of his life on a farm, whether or not the subject had ever had a high exposure incident, and education level.

We also assessed exposure-response based on increasing categories of exposure and their association with BMI category. Three analysis periods were considered: a cross-sectional analysis using exposure and BMI at enrollment; a prospective analysis using exposure at enrollment and BMI at five-year follow-up; and a cross-sectional analysis using exposure and BMI at five-year follow-up. Stratified analyses were conducted using state of residence (Iowa or North Carolina) and exclusion or inclusion of subjects

with a weight-related health condition as stratifying variables. We used $p<0.01$ to identify significant results rather than the more traditional 0.05 in order to be more conservative in evaluating our results due to the number of multiple comparisons. All tables for Chapter 4 are included in the Appendix to this document. Stratified results are presented in the supplemental tables to this chapter (available upon request) and will only be discussed if a noteworthy finding was identified.

4.1. DESCRIPTIVE ANALYSES

4.1.1. Population numbers

Table 4.1 presents the final sample sizes used in the overall analysis and the stratified analyses by state and by presence or absence of a weight-related health condition. Table 3.1 in the Methods Chapter provides detail on the subjects who were excluded to create our analysis file. Excluded are: female applicators (n=1358), commercial applicators (n=4916), males under 20 years of age (n=393), private applicators who did not participate in both Phase 1 and Phase 2 of the study (n=18257), applicators who do not have at least one relevant BMI measure (i.e., at enrollment or at 5-year follow-up; n=6900), and applicators who did not complete the diet history with nutrients calculated (n=494). Since all subjects in our sample have at least one BMI measure, height is present for all subjects, along with at least one weight response.

Note that for this study, BMI1=BMI at age 20; BMI2=BMI at enrollment; BMI3=BMI at 5-year follow-up. BMI1 and BMI2 were collected at enrollment and BMI3 was collected at follow-up. Descriptive data will be provided for BMI1 but only BMI2 and BMI3 are analyzed in relation to exposure since we have no exposure data from the subject's childhood and adolescence that could potentially influence BMI1. However, we have exposure information available for the enrollment and 5-year follow-up periods.

4.1.2. Demographics for full sample and study sample

Table 4.2 presents the frequency and percent for various demographic factors among the total private applicator sample of 50,641 men aged 20 or older who completed only Phase 1 or both phases, and our analysis sample of 9,076 men aged 20 or older who completed both Phase 1 and Phase 2, as well as the diet history with calculated nutrients. The distributions for race, Hispanic origin, education, and marital status were very similar between the full sample of private applicators and our study sample. Mean age varies by almost three years between the two groups with our sample being slightly older and we know that weight increases with age. Our sample has more subjects with weight-related health conditions than the larger sample. Inclusion and exclusion of subjects with weight-related health conditions is a stratifying variable in this study. However, it is difficult to assess if this creates the potential for bias because some excluded health conditions are associated with reduced weight, while others are associated with increased weight.

Our sample includes a larger proportion of applicators from Iowa with fewer from North Carolina (NC) than the full sample and this may have reduced our statistical power in the NC analyses making it difficult to identify associations between pesticides and BMI. Based on these results our analysis sample does differ from the relevant cohort of applicators on some key variables. However, all frequencies of weight-related variables between the full sample of 50,641 male applicators and our study sample of 9,076 were very similar (Table 4.3) and BMI is our only dependent variable of interest.. Since there is little difference between the samples regarding the frequencies of the outcome or dependent variable (BMI), selection bias should not be a problem in our study.

4.1.3. BMI at age 20, at enrollment, and at follow-up

Mean BMI and range for the three time periods for which BMI is available were evaluated for all applicators completing Phase 1 and 2 included in our study sample (see Table 4.4). For all private applicators combined, the mean BMI at age 20 was 23.5 with a range of 13.0-43.3; at enrollment the mean was 27.4 with a range of 13.1-56.3; at follow-up the mean was 27.9 with a range of 15.4-57.2. The increase in mean BMI with age is as expected.

BMI1 (age 20), BMI2 (enrollment), and BMI3 (follow-up) were highly correlated with each other ($p<0.0001$). Correlation coefficients ranged from 0.53 (BMI1 vs BMI3) to 0.90 (BMI2 vs BMI3) for our study sample.

4.1.4 Univariable statistics on pesticide classes at Phase 1 and at Phase 2

AHS data were employed to calculate intensity-weighted cumulative exposure days for each pesticide class, using the specific exposure algorithm described in Chapter 3. We first set all missing values for the cumulative intensity-weighted days of exposure to specific pesticides making up each class to zero. Most of the questions on use of specific pesticides allowed the applicators to select "yes" or "no"; however, applicators may have just selected the "yes" response for the pesticides they used and skipped the questions on pesticides they did not use. This was our justification for setting missing values for questions on use of specific pesticides to zero. Also, as noted in Chapter 3, information from many questions was used to calculate the cumulative intensity-weighted days. It is likely that most applicators with a missing value for cumulative intensity-weighted days of exposure did not use that particular pesticide. To assess whether or not this procedure led to different results then when only subjects with true non-zero scores were included (i.e., answered "no" to question on use of particular pesticide), our initial analyses examined the data including subjects assigned zero scores due to missing values and examining only the subjects with "true" zero scores (answered 'no' to pesticide use question). Therefore, we examined the relationships between BMI and cumulative exposure days using samples that included all subjects

with zero scores from missing values for the pesticide classes under study and again excluding individuals with zero scores due to missing values. Any differences in the results for these two samples were evaluated. Since all of our subjects were recruited when they went to obtain or renew their license to apply agricultural pesticides, all subjects are exposed to one or more pesticides. Therefore, we do not have a group of applicators non-exposed to all pesticides for comparison with our exposed group.

Univariable statistics on numbers of exposed and non-exposed applicators by pesticide class are presented in Table 4.5. For the exposed subjects, Table 4.5 also presents the mean (sd), range, and 25%, 50% and 75% quantiles for intensity-weighted cumulative exposure days. These statistics are presented for cumulative exposure at enrollment and cumulative exposure at 5-year follow-up and when including or excluding subjects with zero scores due to missing values. For the functional pesticide classes during Phase 1 with subjects with zero scores from missing values deleted, the means varied from 541.2 intensity-weighted cumulative exposure days for fumigants to 1668.5 intensity-weighted cumulative exposure days for herbicides; the corresponding numbers for Phase 2 were 540.8 and 1727.1, respectively. The mean exposure for fumigants decreased slightly when examining Phase 1 and Phase 2 variables but the range and 25^{th}, 50^{th}, and 75^{th} percentiles remained the same. For fungicides, the mean and range decreased from Phase 1 to Phase 2 but each of the percentiles increased. When including subjects with zero scores from missing values, the means ranged from 101.1 for fumigants to 1569.1 for herbicides.

Table 4.5 also provides univariable statistics for subjects exposed to each pesticide chemical class at enrollment and follow-up and shows the number of exposed and non-exposed subjects when including and excluding missing values as zero scores. When excluding subjects with zero scores from missing values, mean pesticide intensity-weighted cumulative exposure days ranged from 705 for organochlorines to 2862 for phenoxy herbicides. The mean intensity-weighted cumulative exposure days for each of the chemical classes using follow-up data ranged from 701 (organochlorines) to 2902 (phenoxy herbicides). Mean organochlorine use was reduced only slightly from enrollment to follow-up for our study sample (705 versus 701). The finding of small reductions in usage of these pesticide classes in the 5-year period between the phases for some exposures may have occurred due to restricting our sample to 9,076 men. When including subjects with missing values as zero scores, this discrepancy did not occur: enrollment means ranged from 338.5 for organochlorines to 2287 for phenoxy herbicides; follow-up means ranged from 340 for organochlorines to 2420 for phenoxy herbicides.

By excluding missing values from zero scores, the non-exposed group was reduced by from 9% to 26% depending on the particular pesticide class. Eliminating a large number from the analyses may have reduced the statistical power to identify an effect. However, from a positive perspective, we are more certain that our exposed and unexposed groups are correctly defined. In order to assure adequate power for our stratified analyses, these analyses were only performed when including missing values as zero scores.

4.2. DEVELOPMENT OF ENERGY INPUT AND OUTPUT VARIABLES

4.2.1. Energy Input

As discussed in detail in Chapter 3, Methods, an approach to control for total caloric intake (energy intake) in all analyses utilized self-reported diet information collected at baseline and at follow-up. A detailed dietary history questionnaire (DHQ) was collected at the end of 5-year follow-up, but not at baseline, so correlational analyses determined whether or not baseline data on servings of vegetables and fruits per week and/or an overall meat intake variable correlated with similar variables and total caloric intake in the DHQ. Such correlations provided support for the assumption that these baseline variables could be used to develop a method to control for diet in regression models. Since only 40% of the cohort completed the DHQ collected at the end of 5-year follow-up, all analyses are conducted on the portion of the cohort for which all data were available. Models investigating BMI and exposure at follow-up utilized the actual total caloric intake variable calculated using the DHQ.

A dietary variable was developed using regression modeling that represented total food energy in relation to intake of fruits, vegetables, and meat at enrollment. This variable was used to control for diet in the models based on BMI at enrollment. It was possible to develop a variable that predicted past overall caloric intake modeled from intake of meats and fruits and vegetables at enrollment versus at the end of 5-year follow-up. This is discussed in detail in Chapter 3.

4.2.2. Energy Output

Data are not available on number of hours per day spent performing particular occupational tasks to calculate the metabolic equivalent (MET) needed to sum the total energy expenditure across tasks to control for physical activity from occupation. Patterns of physical activity at enrollment were based on questions on hours per day of heavy lifting at baseline and 10 years prior, and hours per week of strenuous leisure exercise in winter and in summer. These responses were examined to assess the variability and homogeneity across subgroups of the AHS cohort by age and state. As there was little variability across sub-groups, the baseline variables on hours per day of heavy lifting and hours per week of strenuous leisure exercise were used to develop a variable to represent amount of physical activity to be used to control for energy expenditure in all models dealing with exposure at enrollment.

We examined mean hours per day of heavy lifting at enrollment and 10 years prior, as well as the correlation between these two values. Similarly, we examined mean hours per week of exercise in summer and winter and the correlation between these two values. We used number of hours per day of heavy lifting during the past growing season (6 months), and number of hours per week of exercise in summer and in winter, to develop a total number of hours of physical activity per year, which was then divided by 365 to determine daily hours of physical activity. For 5-year follow-up, the rate of decline in hours of heavy lifting from 10 years ago until enrollment was used in a depreciation model to calculate hours of heavy lifting at follow-up. This is described in detail in Chapter 3 and its corresponding tables.

We compared our crude measures of physical activity at enrollment with our crude measurements at 5-year follow-up. As there were differences in physical activity between the two time periods, it was necessary to evaluate physical activity as an independent variable in the models for each time period. Each of these variables was evaluated for their correlation with the corresponding exposure variables and outcome variables based on representative time period in order to assess whether or not they should be included in our statistical models. Only variables related to both outcome and each specific pesticide class exposure were included in the model examining that specific exposure.

4.3. BIVARIABLE ANALYSES

4.3.1. Correlations between exposure to pesticide functional and chemical classes and BMI using data from Phase 1 and Phase 2

Table 4.6 presents the correlations between each pesticide class and BMI both excluding and including the individuals with missing values from zero scores for pesticide class exposure. Pesticide class exposures at enrollment (Phase 1) were compared with BMI2 (cross-sectional analysis) and BMI3 (prospective analysis). Pesticide class exposures at follow-up (Phase 2) were compared with BMI3 only (cross-sectional analysis) since exposures at follow-up are not relevant to BMI2 (at enrollment). The strongest age-adjusted correlations between BMI and pesticides were

observed for herbicides. Correlations for herbicides at enrollment and BMI2 (cross-sectional), and herbicides at enrollment and BMI3 (prospective) were identical (0.09 $p<0.0001$). When subjects with zero scores from missing values were included, the herbicide correlation coefficient dropped to 0.08 ($p<0.0001$). The unit of measurement for each of these correlation coefficients is change in BMI ($kg/(meter)^2$) per intensity-weighted exposure day. Among the functional classes at Phase 2, only herbicide exposure is significantly correlated with BMI3 (0.07, $p<0.0001$).

When examining the results of our stratified analyses, we observed that triazine herbicides were consistently significantly correlated with BMI in every sub-group. Therefore, we created an additional pesticide class that included only non-triazine herbicides. However, deleting the triazine herbicides from the overall herbicide class made little difference in the overall results. For BMI2, insecticide exposure at enrollment was also significantly correlated with BMI regardless of inclusion/exclusion of subjects with zero scores from missing values..

Table 4.6 also presents correlations between BMI at follow-up and intensity-weighted cumulative exposure days to pesticide chemical classes using the Phase 1 and Phase 2 data. For Phase 1, all but one class (carbamates) was found to be significantly correlated with BMI2 and BMI3 when subjects with zero scores from missing values were excluded. This group included “non-exposed” individuals (those who answered “no” to exposure to a particular pesticide and those with missing values for the question) and the results indicated that both organochlorines and carbamates were not correlated with

BMI. All correlation coefficients were small ($\leq$ 0.1); however the largest correlation coefficients were seen for phenoxy and triazine herbicides. All of the same chemical classes found to be significantly correlated with BMI3 in Phase 1 were significantly correlated with BMI3 in Phase 2 with almost exactly the same coefficient values. For both functional and chemical classes, inclusion or exclusion of subjects with zero scores from missing values did not change the overall results very much except that the correlation coefficients were generally smaller when including subjects with zero scores from missing values than when subjects with missing values for exposure to a pesticide class were excluded from the non-exposed.

4.3.2 SUMMARY

Overall, these unadjusted results provide evidence of a positive association between BMI and exposure to several pesticide functional and chemical classes. The cross-sectional analysis of enrollment BMI yielded similar results as the prospective analyses of follow-up BMI when examining exposure to functional and chemical classes at Phase 1. The cross-sectional analyses of BMI at follow-up using exposure at Phase 2 yielded similar results to the prospective analyses of BMI at follow-up using Phase 1 data. Inclusion or exclusion of subjects with zero scores from missing values made little difference in the results. The most consistent finding when examining all our stratified sub-groups was the significant correlation between triazine herbicides and BMI. Our literature review and study hypotheses identified organochlorines and phenoxy herbicides as substances likely to cause weight gain. Many of the correlations discussed

above support these findings. However, all correlation coefficients, even when found to be statistically significant, were small – in the range of -0.02 to 0.09, indicating that if there is an effect of pesticide exposure on BMI it is likely to be modest.

4.4. RESIDUAL CHECK OF THE DATA

Since simple and multiple regression analyses are our main analytic methods, residual analysis was necessary to assess whether or not any of the assumptions that pertain to regression analysis were being violated. In regression analysis, it is assumed that the residuals adhere to 1) normality; 2) homoscedasticity; and 3) linearity. Each residual represents the difference between the observed value and the predicted value based on the regression model. An evaluation of the residuals was performed for the dependent variables BMI2 (at enrollment) and BMI3 (at follow-up) based on the independent variables cumulative exposure intensity-weighted days to each pesticide class at enrollment for BMI2 and exposure to each pesticide class at follow-up for BMI3 including zero scores for missing values. Increasing the size of our sample by including missing values increased our ability to assess the relationships among the residuals. Age at the corresponding time period was also included in the model. The histogram, boxplot, normal probability plot, and plot of the residuals versus the predicted values obtained from the residual analyses for each pesticide class at Phase 1 and Phase 2 are presented in the supplemental tables to this chapter that can be made available upon request.

For example, the regression model BMI2 = insecticide intensity-weighted cumulative exposure days at enrollment and age at enrollment resulted in a histogram illustrating a normal distribution skewed slightly to the right. This finding, combined with the normal probability plot showing a backward S-shape rather than a straight line, suggest that the data are not completely normal. However, with a population size of over 9,000 subjects, normality may be assumed regardless of the shape of the histogram and probability plot. The plot of the residuals versus the predicted values clearly shows linearity in that the shape of the data points plotted tend to look like a horizontal line. In addition, the plot illustrates homoscedasticity in that the variances appear equal – there is a similar spread of the residuals (data points) across the range.

The regression model with the dependent variable BMI2 at enrollment and the independent variables intensity-weighted cumulative exposure days to insecticides at enrollment and age at enrollment satisfies the necessary assumptions for regression analysis. All other residual analyses were similar: the histograms showed either a normal probability skewed to the right or a leptokurtic normal distribution, the normal probability plots suggested a backward S-shape, and the plots of the residuals versus the predicted values all illustrated linearity and homoscedasticity. The normal distribution criterion is fulfilled by the size of the sample. Therefore, regression modeling is a viable method to analyze these data. Since there is adherence to the three criteria for regression (normality, homoscedasticity, and linearity), these data do not require log transformation or any other type of transformation prior to regression modeling.

4.5. UNADJUSTED ANALYSES

Table 4.7a presents the regression analysis results when comparing BMI at enrollment with each of the Phase 1 cumulative pesticide class exposure categories without controlling for any other variables (cross-sectional analysis). Two tables are presented representing the unadjusted analyses excluding and including subjects with zero scores from missing values for pesticides. F value, *p*-value, and R^2 are presented for the initial model, which are identical to those that are associated with the parameter estimate for the pesticide class because pesticide class was the only independent variable in the model. The *p*-values indicate only herbicides, herbicides less triazines, organochlorines, organothiophosphates, phenoxy herbicides, and triazine herbicides are significant when excluding missing values from zero scores. However, the R^2 values for these models were all very small (from 0.0010 to 0.0050).indicating the model predictor explained only a small amount of the variance in the model.

When subjects with missing values from zero scores were included, the only difference in the statistically significant findings was that phenoxy herbicides were no longer significant. . The percent differences in the change in the parameter estimate when excluding versus including missing values as zero scores were highly variable. R^2 values generally went down very slightly or stayed the same when subjects with zero scores from missing values were included. Nevertheless, the results in terms of statistically significant findings were very similar.

When BMI at follow-up was used as the outcome variable in a regression analysis using pesticide functional or chemical class at Phase 1 as the only independent variable (prospective analysis; Table 4.7b), the statistically significant findings when excluding missing values from zero scores were identical to the cross-sectional enrollment results (Table 4.7a). R-squared values remained small (<0.0045). When missing values were included in the zero scores, there were slightly fewer significant findings: organochlorines and phenoxy herbicides were no longer significant. R^2 values when including missing values as zero scores were also very small (<0.0044) indicating only a small amount of the variance can be explained by the model. Again, percent differences in parameter estimates were highly variable and did not exhibit any particular pattern.

Similar results in terms of significant exposure parameter estimates were obtained when BMI at follow-up was used as the dependent variable with pesticide functional and chemical class at follow-up as the independent variable (Table 4.7c; cross-sectional follow-up analysis) regardless of whether or not zero exposures were included. The only difference between when missing values were excluded or included as zero scores was a significant finding for organochlorines when excluding missing values versus a significant finding for organothiophosphates when including missing values as zero scores. Again, R^2 for these models were all very small (<0.0043) indicating that only a small amount of the variance for each of these respective models can be explained by the model predictor (cumulative pesticide class exposure).

A column showing the percent change in the parameter estimate for the association between BMI and pesticide class exposure is included for each of the three unadjusted analyses (cross-sectional enrollment, prospective, and cross-sectional follow-up. No pattern can be observed when looking across the three types of analyses. The range of changes in parameter estimates was very broad: -306% for carbamates (cross-sectional follow-up) to 33% for carbamates (cross-sectional enrollment). Despite the large changes that occurred in some parameter estimates, identification of statistically significant findings was very similar for the three analyses regardless of whether or not missing values were included as zero scores.

4.6. DETERMINATION OF CONFOUNDERS

A number of potential confounders were identified and discussed in Chapter 3, Methods. We defined a variable as a confounder if it was statistically significantly associated with both the outcome and the exposure. We also considered the amount of change in the unadjusted parameter estimate for pesticide exposure and BMI when including and excluding the identified confounders (this is discussed further in Section 4.9). The size of our sample suggests that statistical significance would be achieved if there was a relationship between any potential confounder and the outcome and exposure variables. Since we have outcome and exposure data through enrollment (Phase 1) and we also have outcome and exposure data through follow-up (Phase 2),

confounders were investigated for the two time periods using available data on the potential confounders at the particular phase of interest.

We used the Phase 1 exposure data distributed into quartiles as per the 25th, 50th, and 75th percentiles listed in Table 4.5, the categorical potential confounders (lived half life on a farm since age 18 as determined at enrollment (yes/no), ever had a high exposure incident through enrollment (yes/no), ever had non-farm occupational exposures through enrollment (yes/no), Hispanic origin as determined at enrollment (yes/no), race as determined at enrollment, and educational level as determined at enrollment), and the two outcome variables (BMI2 and BMI3) distributed into quartiles as per the 25th, 50th, and 75th percentiles presented in Table 4.4 to determine if either the outcome or the exposure was statistically significantly associated with any of the potential confounders based on chi-square results. The only potential categorical confounder associated with an outcome variable was the significant positive relationship between BMI at enrollment and educational level. Educational level was also significantly related to each of the exposure categories. None of the potential categorical confounders investigated was significantly related to BMI at follow-up. Therefore, education was controlled for in all models examining BMI at enrollment and each pesticide exposure class at Phase 1 but it was not controlled for in analyses of BMI at follow-up and exposure at enrollment.

Next we examined the outcomes (BMI2 and BMI3) and the pesticide exposure variables at Phase 1 to determine if any of them were correlated with the potential confounders

that are continuous variables: age at enrollment, physical activity per day at enrollment, number of cigarettes smoked per day at enrollment, number of alcoholic drinks consumed per month at enrollment, total years worked in farming at enrollment, and energy input (kcal per day) at enrollment. For BMI at enrollment, significant positive correlations were found with number of cigarettes per day, total years worked in farming, and daily energy input. For BMI at follow-up, a significant correlation was found with age at enrollment along with the same relationships that were found for BMI2 and the continuous potential confounders: number of cigarettes per day, total years worked in farming, and daily energy input. Neither BMI2 nor BMI3 was correlated with physical activity at enrollment. Various pesticide classes were correlated with all or some of these variables. Only the potential confounders correlated with both the particular outcome (BMI2 or BMI3) and the particular pesticide class exposure level at Phase 1 were controlled for in the regression models based on Phase 1 exposure data.

BMI3 (follow-up) was evaluated in relationship to the Phase 2 data on exposure and categorical potential confounders. There was a highly significant relationship between smoking status at follow-up and BMI3, along with most of the exposure variables.. Smoking status was used as an independent variable in the models examining Phase 2 data when it also correlated with the particular pesticide class.

An examination of the continuous variables that are potential confounders based on Phase 2 exposure data was conducted for BMI3 and all pesticide categories. Any relationships identified between BMI3 and potential confounders do not imply that

these relationships also apply to BMI2 since BMI2 is unrelated to Phase 2 exposures. Age at follow-up was correlated with BMI3 and was correlated with the majority of the intensity-weighted cumulative exposure days variables.. Therefore, age at follow-up was used as an independent variable in the models examining BMI3 and the relevant exposures. Physical activity at follow-up was not correlated with BMI3. Food energy based on total kilocalories calculated using the diet history questionnaire administered at Phase 2 was highly correlated with BMI3 (follow-up) and with most of the exposure variables. Therefore, energy input was controlled for in the models examining BMI3 and exposure at Phase 2 to the relevant pesticide classes.

When identifying confounders for analyses including subjects with zero pesticide scores from missing values in the analyses, again education was the only categorical variable related to BMI2 and to all the pesticide classes. None of the categorical variables were related to BMI3. For the Phase 1 continuous variables, BMI2 was related to cigarettes smoked per day, number of years worked in farming, and energy input. BMI3 was also related to these same variables and also showed a relationship with age at enrollment. The selection of confounders used in the Phase 1 models was based on whether or not any of the confounders related to BMI were also related to the particular pesticide class.

For Phase 2 data, again smoking status was the only categorical variable related to BMI3. Continuous variables related to BMI3 were age at follow-up and energy input at follow-up. If any pesticide class was related to any of the variables associated with BMI3, then the potential confounder was included in the model.

We did not adjust for exposure to all the other classes of pesticide exposure when examining a particular pesticide class. Such an adjustment would greatly limit the sample size for analysis of the particular pesticide class making any results extremely unstable.

4.7. MULTIPLE REGRESSION RESULTS FOR THE ADJUSTED MODELS

4.7.1 CUMULATIVE PESTICIDE FUNCTIONAL OR CHEMICAL CLASS EXPOSURE INTENSITY-WEIGHTED DAYS IN RELATION TO BMI

Two sets of backward elimination multiple regression analyses were run relating Phase 1 pesticide exposure data (at enrollment) to BMI data at enrollment – including and excluding subjects with zero pesticide exposure scores from missing values. We controlled for the following potential confounders: cigarettes smoked/day at enrollment, educational level at enrollment, kcal/day (energy input) at enrollment, and total years of farming through enrollment whenever the confounder was significantly related to BMI2 and to exposure to the particular pesticide class at enrollment. The parameter estimate for pesticide class exposure was always presented even when it was not significant and not a variable in the final model – the most parsimonious model. A column in Tables 4.8a, 4.8b, and 4.8c indicates at what point the exposure parameter estimate was eliminated from the model. Note that columns 2, 3, and 4 in these tables

provide the results for the initial model with all the covariates included prior to the backward elimination procedure. This same information is provided in all of the tables for multiple regression analyses and in almost every case, the initial model was significant indicating the exposure variable with all the covariates included explained the parameter estimate for the dependent variable, BMI.

Table 4.8a examines BMI2 in relation to exposure at enrollment; this is a cross-sectional analysis. Parameter estimates represent the change in BMI (kg/m^2) per intensity-weighted cumulative days of exposure. When missing values were excluded from zero scores, significant parameter estimates were obtained for herbicides ($p=0.0031$) and for triazine herbicides ($p=<0.0001$). Most parameter estimates were positive suggesting exposure to any of these pesticide classes was associated with increased BMI (though not always significantly), while fungicides and organophosphates had negative but not significant parameter estimates. R-squared values were small for the initial models (0.02 to 0.03) and were almost identical for the reduced models that included the pesticide exposure variable. This indicates that the models only explain about 2-3% of the variance in BMI. The remainder of the variance may be explained by uncontrolled confounding or limitations in calculation of our exposure scores and/or our values for the covariates we included in our models.

When the analyses were repeated including the individuals with zero exposure scores from missing values, again, herbicides and triazine herbicides were significantly related to BMI. The only other difference from the analysis excluding zeros scores from

missing values was that there were more negative parameter estimates, though none were significant (fumigants, fungicides, organophosphates, and carbamates).. Again, percent change in parameter estimates when excluding or including missing values in zero scores were highly variable and did not exhibit a particular pattern. The percent change in parameter estimates whether or not missing values were included or excluded from zero scores varied from -4970% to 94%. However, including or excluding zero scores from missing values for the pesticide classes did not change the statistically significant results.

Table 4.8b represents a prospective analysis as exposure at enrollment is related to BMI3 (follow-up). Most of the parameter estimates were positive and two were negative (fungicides and carbamates) suggesting an inverse relationship between the latter two pesticide classes and BMI at follow-up. Significant positive parameter estimates were identified for the functional class herbicides ($p=0.0004$) and for the chemical classes organochlorines ($p=0.0027$) and triazine herbicides ($p<0.0001$). Confounders controlled for included age at enrollment, number of cigarettes smoked per day at enrollment, energy input at enrollment, and total years farming at enrollment, depending on the specific relationships between these confounders and each pesticide class. The results were almost the same when subjects with zero scores from missing values were included except that a significant positive parameter estimate for herbicides less triazines was observed ($p=0.0038$). The percent change in parameter estimates when excluding versus including missing values as zero scores varied from -75% to 75% and did not exhibit a particular pattern.

Table 4.8c represents a cross-sectional analysis as exposure at follow-up is related to BMI at follow-up. When excluding missing values from zero scores, significant parameter estimates were obtained for herbicides ($p=0.0002$), herbicides less triazines ($p=0.0006$), organochlorines ($p=0.0006$), phenoxy herbicides ($p=0.0031$), and triazine herbicides ($p<0.0001$). Additional independent variables in the models, if they were correlated with the particular pesticide class under examination, were age at follow-up, smoking status at follow-up, and energy input at follow-up. Most parameter estimates were positive, with only fungicides illustrating a non-significant inverse relationship with BMI at follow-up. Results were similar when subjects with zero scores from missing values were included except that phenoxy herbicides was no longer significant. Percent differences in parameter estimates when excluding or including missing values in zero scores varied from -7% to 48% with no particular pattern observed. When examining the percent changes in parameter estimates across the three studies, no consistent pattern was observed.

In summary, the positive significant findings for organochlorines in the prospective and Phase 2 cross-sectional analyses are supportive of the hypotheses under consideration, which suggests that these pesticides might be positively associated with weight gain. We found a significant result for phenoxy herbicides in the cross-sectional follow-up analysis and this supports findings suggested by our literature review. We also found significant associations for herbicides and triazine herbicides for all three analyses (two cross-sectional and one prospective).

4.8. COMMENTS ON STRATIFIED AND OTHER ANALYSES

When examining the effects of stratifying by state and/or presence or absence of a weight-related health condition, the number of significant findings was reduced for the North Carolina subgroups, probably because the sample sizes were decreased reducing the statistical power for identifying effects. The positive association between triazine herbicide exposure and BMI was the only consistent association noted across all stratified sub-groups and for all three study groups. The association between herbicides and BMI was consistent for all groups and time periods except for North Carolina applicators.

Organochlorines and organothiophosphates are somewhat consistently associated with BMI in the regression analyses for the Iowa applicators, regardless of including or excluding subjects with weight-related health conditions, across the three study groups. Fungicides and insecticides show a negative relationship with BMI at low level exposures for the Iowa subgroup in some of the analyses. There were a number of negative parameter estimates in all the regression analyses but none were significant.

When we recalculated the full-sample analyses for herbicides deleting all individuals with reported triazine herbicide exposures from the herbicide category, herbicide exposure remained significantly associated with BMI across the three study groups.

These results suggest that the significant relationship identified for herbicides and BMI is not caused by the existence of triazine herbicides in the overall herbicide category.

4.9. EVALUATION OF STRENGTH OF CONFOUNDERS

We used a second method to identify confounders that assessed the impact of the confounder on changing the parameter estimate for pesticide exposure and BMI at least 10% (tables posted in the Supplemental Tables to Chapter 4; available upon request). For each outcome (BMI2 or BMI3) and each study type (cross-sectional enrollment, prospective, and cross-sectional follow-up), different confounders were included in our re-calculated models based on whether the variable caused a >10% percent change in the unadjusted parameter estimate when zero scores for missing values were included. We performed regression analyses using only confounders that caused a >10% change in the unadjusted parameter estimates for pesticide class versus BMI in the models. Differences between the regression models including only the covariates associated with a >10% change in the pesticide class parameter estimate versus the unadjusted parameter estimate did not cause significant changes in the multiple regression results. Based on these results, we decided that using only those confounders causing a >10% change in the unadjusted parameter estimates did not change the overall results enough to warrant using only these confounders in all models from now on. Therefore, results of all further analyses include all covariates significantly related to the exposure and the outcome based on correlation coefficients or chi-square values regardless of the percent change in unadjusted parameter estimates for pesticide class and BMI.

4.10 CONTROLLING FOR EXPOSURE TO OTHER PESTICIDE CLASSES

The consistent significant positive findings for triazine herbicides and BMI, including in the stratified analyses, prompted us to control for triazine herbicides in the adjusted regression analyses. Table 4.9 shows the percent change in the original parameter estimate caused by adjusting for triazines, along with the new parameter estimate and its corresponding *p*-value. These adjustments were made both in the analyses that included subjects with zero scores from missing values and when excluding missing values from zero scores. These analyses were performed for all three studies: cross-sectional enrollment, prospective, and cross-sectional follow-up. Any parameter estimates for a particular pesticide class and BMI that were statistically significant were no longer significant after adjusting for triazines.

In order to assess whether multiple pesticide class exposures might also be confounders, we also adjusted for each unrelated pesticide class in the analyses of other pesticide classes. This was done to assure that any changes related to including triazine exposure as a covariate in the regression models for all other pesticide classes was specific to triazines, we also used each pesticide class as a covariate in the regression models for all other pesticide classes (tables included in supplemental tables to Chapter 4). When the triazine analyses included each of the other pesticide classes, all updated parameter estimates remained statistically significant. Our results suggest that controlling for other pesticide classes did not change the overall results as the categories identified as having

significant parameter estimates for exposure and BMI (mainly herbicides and triazines) remained statistically significant after adjusting for the other pesticide classes.

In summary, adjusting for other pesticide classes did not generally affect the parameter estimates for triazine herbicides. The herbicide class estimates were also not greatly affected by controlling for other pesticide classes in the cross-sectional enrollment, prospective, or cross-sectional follow-up analyses. However, it is important to remember that the category "herbicides" includes all triazine herbicides; therefore, adjustment for triazines was not possible. Considering that when we remove triazines from the herbicide category and then adjust for triazines, no significant effect is noted for herbicides and the parameter estimates change dramatically, it is safe to say that triazines are driving the significant findings for herbicides and the triazine pesticide class is the only pesticide class with consistently significant findings and with large changes in parameter estimates for other pesticide classes when adjusting for triazines. Therefore, we decided to conduct all remaining analyses with and without adjustment for triazines.

Adjusting for triazine exposure in all the stratified analyses virtually eliminated any significant findings for other pesticides (supplemental tables to Chapter 4 available upon request). Triazine exposure was a significant factor in the final models for all pesticide classes ($p<0.01$). For the Iowa stratified groups (including and excluding applicators with weight-related health conditions), there were no significant findings for any pesticide class and triazine exposure was included in all final models as a

significant factor ($p<0.01$). For the North Carolina group, there were no significant findings for any pesticide class after adjusting for triazine exposure. Triazine exposure was included in almost all of the final models as a significant factor ($p<0.01$). Results were similar for the North Carolina group excluding those with a weight related health condition. Therefore, we decided not to continue with any further stratified analyses as controlling for triazines would likely eliminate any significant findings identified.

4.11. EXAMINATION OF EXPOSURE-RESPONSE TRENDS

In order to investigate exposure-response relationships, we used generalized linear models with calculations of least squares means for 5 categories of cumulative pesticide exposure with the highest exposure level being the referent category. Intensity-weighted cumulative exposure days were converted into categorical variables for each of the pesticide classes using the quartiles formerly identified in our examination of the univariable statistics for each pesticide class. R^2 values for the overall models including pesticide class as a categorical variable with relevant confounders were all very low (1% to 3%) indicating that the models only explained a small amount of the variance in the dataset.

Table 4.10a shows the estimates for BMI at enrollment compared to exposure at enrollment including missing values as zero scores. Insecticides were identified as a significant factor in the overall model based on Type 1 Sum of Squares (SS) only. Herbicides were identified as a significant factor in the overall model based on Type 1

and Type 3 sum of squares (SS). In addition, parameter estimates for each pesticide level in the model were significant for levels one, two, and three. Least Squares Means (LSM) were significant for the first and second level of exposure. Similar results were obtained for the pesticide class “herbicides less triazines” suggesting there are other herbicides besides triazines that can affect BMI. Statistically significant results were observed for organophosphates in the full model based on Type 1 SUM OF SQUARES and exposure level one had a significant parameter estimate in the model. Similar results were obtained for organothiophosphates except that the significant pesticide exposure level was the second level rather than the first.

Phenoxy herbicide exposure levels were a significant factor in the overall model based on Type 1 and Type 3 SUM OF SQUARES. All exposure levels for phenoxy herbicides had significant parameter estimates and the LSM for the second level of exposure was also significant. Triazine herbicides showed the strongest findings with exposure being a significant factor in the overall model based on Type 1 and Type 3 SUM OF SQUARES, plus all exposure levels and all least squares means were statistically significant. Controlling for triazines eliminated some of the significant findings for the class of herbicides without triazines and eliminated the exposure-level specific significant findings for organophosphates, organothiophosphates, and phenoxy herbicides.

We repeated all analyses excluding missing values from zero scores but there was little affect on any of the findings. Least squares means were either identical or varied by 0.1-

0.2. Therefore, we only show our findings when including missing values in zero scores.

Figure 4-1 illustrates BMI least squares means by exposure categories including missing values as zero scores for each of the linear models based on outcome and exposure at enrollment, both with and without adjustment for triazines, and thus provides a visual assessment of dose-response. Based on these figures, none of the graphs illustrate a true dose response because adjusted BMI is higher for the non-exposed group compared to subjects classified at the first level of exposure. Phenoxy herbicides and triazine herbicides show a monotonic increase in BMI as the cumulative exposure category increases and the zero categories has a smaller drop than in the graphs for the other classes. These results were virtually unchanged when controlling for triazines.

Table 4.10b represents the results obtained for the prospective analyses: BMI3 (BMI at follow-up) with Phase 1 exposure (at enrollment) including missing values as zero scores. Results were similar to those for the enrollment cross-sectional analyses. R^2 for these models ranged from 1% to 3% indicating the models explained only a small amount of the variance in the data. Pesticide exposure was found to be a significant variable in the overall model based on Type 1 and/or Type 3 SUM OF SQUARES for insecticides, herbicides, herbicides less triazines, organochlorines, organophosphates, organothiophosphates, phenoxy herbicides, and triazine herbicides. Significant parameter estimates were identified for Phase 1 pesticide cumulative exposure

distributed into categories for levels 2 and 3 for herbicides and herbicides less triazines; level 2 of exposure for insecticides, organochlorines, and organophosphates; level 3 of exposure for organothiophosphates; and for all levels of phenoxy herbicides and 3 levels for triazines. Significant *p*-values for specific least squares means were only observed for the first and second levels of exposure for herbicides and herbicides less triazines; the second level of phenoxy herbicides, and the zero and second levels for triazines.

Controlling for triazines eliminated some of the significant findings for herbicides less triazines and the exposure level specific findings for organochlorines, organophosphates, organothiophosphates, and phenoxy herbicides. Visually scanning the least squares means and examining the plots in Figure 4-2 for each pesticide class revealed that controlling for triazines had no apparent effect on the graphs. There were no precise exposure-response relationships when the least square means for zero exposure were included. Phenoxy herbicides exhibited the least drop from zero level to the first level of exposure, with a dose response apparent after that point. Triazines herbicides illustrated a slight decrease from zero exposure to level 2 of exposure and an increasing response from that point on. Insecticides, fumigants, fungicides, and carbamates showed no exposure response. These results were unchanged by controlling for triazine exposure in the models.

Table 4.10c presents the follow-up cross-sectional analyses. These findings used the Phase 2 exposure data (at follow-up) and BMI3 (at follow-up) and were very similar to

the prospective results. R^2 ranged from 0.5 % to 2% indicating the models only explained a small amount of the variance in the dataset. The particular pesticide classes found to be significant predictors in the overall models were insecticides, herbicides, herbicides less triazines, organochlorines, organophosphates, organothiophosphates, phenoxy herbicides, and triazine herbicides based on results for Type 1 and/or Type 3 Sum of Squares. The parameter estimates for the Phase 2 categorical cumulative pesticide exposure variables were significant for level 1 insecticides, levels 1 and 2 herbicides, herbicides less triazines, and organophosphates; level 1 organochlorines, levels 2 and 3 organothiophosphates, and all 4 levels for phenoxy herbicides and triazine herbicides. The specific exposure levels significantly associated with its least squares mean were level 1 for insecticides, levels 1 and 2 for herbicides and herbicides less triazines, level 1 organochlorines, level 2 organothiophosphates, levels zero, 1, and 2 for phenoxy herbicides, and all Least squares means for triazine herbicides. Controlling for triazines eliminated some of the significant findings for insecticides, and the exposure level specific significant findings for herbicides less triazines, organochlorines, organophosphates, organothiophosphates, and phenoxy herbicides.

When examining Figure 4-3, which illustrates Least squares means by exposure level, it is apparent that aside from fumigants and fungicides, all of these analyses exhibited a high least squares mean for zero exposure with a decrease to level 1 and then an increasing outcome with increasing exposure from level 1 to level 4. Only phenoxy herbicides showed a true exposure response trend with increasing BMI at follow-up. The drop off from zero level to the first level of exposure was slight for triazines and a

dose-response was observed after that point. Again, controlling for triazines did not change these results, which are similar to what was observed with Phase 1 exposure data versus BMI at enrollment and at follow-up (Tables 4.10a and 4.10b; Figures 4-1 and 4-2). The similarities in the results for the three types of analyses (enrollment cross-sectional, prospective, follow-up cross-sectional) including missing values as zero scores lend credibility to the findings. We conducted all these analyses excluding missing values from zero scores but there was little difference in the findings (data not shown). Again, the exposure with the most consistent positive exposure-response relationship was phenoxy herbicides.

4.12 ORDINAL LOGISTIC REGRESSION EXAMINING EXPOSURE-RESPONSE

Table 4.11a presents the adjusted odds ratios relating exposure quantiles developed in our univariable analyses to four categories of BMI (underweight, normal weight, overweight, and obese) as defined by the WHO (2007; see Table 3.3). Zero exposure was used as the referent category for all calculations and included missing values set to zero. These analyses were performed using the ordinal logistic regression procedure in SAS, which allows for multiple categories for the exposure and outcome variables provided the score test for the proportional odds assumption is not rejected. In situations where the score test for the proportional odds assumption was rejected, then the outcome variable was collapsed into binary categories (underweight + normal weight versus overweight + obese). The table indicates which analyses are based on a binary

outcome variable. The table is shown twice, with the second set of results controlling for triazine exposure. Both unadjusted and adjusted ORs were calculated but we have presented the adjusted analyses in Table 4.11a. Unadjusted results are available upon request. The adjusted ORs take the relevant potential confounders previously identified for that outcome and exposure variable into account.

In ordinal logistic regression, each odds ratio (OR) represents the odds of an individual in that particular exposure category having a higher BMI compared to an individual from any of the lower ordered categories. For example, regarding the significant finding for the highest level of triazine herbicide exposure in the cross-sectional enrollment analyses, the odds of an individual with intensity weighted triazine herbicide exposure days > 1956 having a higher BMI than an individual exposed to triazine herbicides at any of the lower ordered exposure categories is 1.263.

The adjusted cross-sectional enrollment analyses also revealed a significant increase in BMI for the highest level of phenoxy herbicide exposure . (The third level of exposure for both triazine and phenoxy herbicides was also modestly, but not significantly, elevated.) Also identified were significantly reduced ORs for the first level of insecticides, herbicides, and herbicides less triazines exposures. In general, analyses for all pesticide groups except triazine herbicides and phenoxy herbicides showed ORs less than one or close to one for most levels of exposure. In reviewing the “Wald Chi Square” from the ordinal logistic regression analyses, a statistic that indicates whether or not a particular variable in the model had a significant effect on the dependent

variable, herbicides, herbicides less triazines, phenoxy herbicides, and triazine herbicides are all significantly related to BMI. The second half of Table 4.11a included triazine exposure as a covariate in the models. Differences from the original table that are apparent when controlling for triazines are highlighted in red. This table shows that the first level of insecticide exposure was no longer significantly reduced compared to the zero categories but the third and fourth levels of insecticide exposure were now significantly negatively related to BMI. Other differences included the elimination of a significant negative finding for the first level of herbicide exposure; the lack of a relationship between phenoxy herbicides and BMI based on the Wald Chi-Square results and the fourth level of phenoxy herbicide exposure was no longer significant.

For the prospective analyses, the zero category of exposure (lowest ordered value) was significantly more likely to have a higher BMI than the high ordered levels of insecticides and fungicides. Most ORs were less than one or close to one for most levels of exposure. The highest level of phenoxy herbicide exposure (OR = 1.23), and the highest level of triazine herbicide (OR = 1.33) exposure had significantly higher odds of having a higher BMI than the lower ordered exposure levels. Based on the Wald Chi-Square results, insecticides, herbicides, herbicides less triazines, organothiophosphates, phenoxy herbicides, and triazine herbicides are all significantly related to BMI. Differences in the results when adjusting for triazines include significantly reduced ORs for all insecticide categories but no exposure-response is apparent. The highest level of carbamate exposure was significantly negatively associated with having a higher BMI than any of the lower ordered categories. The significant positive effect previously

identified for the highest level of phenoxy herbicide exposure was no longer significant but the ORs for increasing levels of phenoxy herbicide exposure remained suggestive of a dose-response. The Wald Chi Square results were no longer significant for herbicides less triazines, organothiophosphates, and phenoxy herbicides.

For the cross-sectional analyses of follow-up data, the lowest level of fungicide exposure was significantly more likely to be associated with a lower BMI than the lower ordered zero category of exposure. Significantly higher odds (OR = 1.42) of the highest (fourth) level of herbicide exposure and the highest level of herbicide exposure less triazines (OR=1.35) had significantly higher BMIs than the lower ordered categories. This was also shown for the highest level of organothiophosphates (OR = 1.37). The third and fourth (highest) levels of phenoxy herbicide exposure (ORs 1.20 and 1.35, respectively) and triazine herbicide exposure (ORs 1.20 and 1.48, respectively) were associated with significantly higher odds of having a higher BMI than the lower ordered exposure categories. For the follow-up cross-sectional study group, significant findings based on the Wald Chi Square were identified for the association between pesticide class and BMI for fungicides, herbicides, herbicides less triazines, organophosphates, organothiophosphates, phenoxy herbicides, and triazine herbicides. Differences when controlling for triazines indicate that all categories of insecticide exposure are significantly negatively related to BMI when compared to the lower ordered BMI categories but no exposure response exists. Level one of fumigant exposure was also significantly negatively related to BMI compared to the lower ordered value (zero). The highest level of carbamate exposure was significantly

negatively related to BMI compared to the lower ordered categories of BMI. The highest level of exposure for herbicides less triazines and the highest level of organothiophosphates were no longer significantly positively related to BMI compared to the lower ordered levels of BMI and the third and fourth levels of phenoxy herbicide exposure also were no longer significantly positively related to BMI compared to the lower ordered levels of BMI. The Wald Chi Square for herbicides less triazines, organophosphates, organothiophosphates, and phenoxy herbicides were also no longer significant indicating no relationship exists between these pesticide classes and BMI

Table 4.11b presents the logistic regression results based on missing values excluded from the zero scores. There are slight differences in the levels of particular pesticide classes that showed significant negative odds ratios. Positive significant odds ratios were obtained for the highest levels of phenoxy herbicide and triazine herbicide exposure in all three analyses. The cross-sectional follow-up analysis also included a positive significant odds ratio for the highest level of organothiophosphate exposure. Changes that occurred when adding triazine exposure as a covariate in the models are shown in red in the second half of the table. Again, there were some differences in particular levels of pesticide classes showing significant negative odds ratios. The significant Wald Chi Square observed in all three analyses for herbicides less triazines and phenoxy herbicides were no longer significant after controlling for triazines. The same was true for organothiophosphates in the prospective and cross-sectional follow-up analyses and carbamates in the cross-sectional follow-up analysis. Almost all odds ratios were more or less 1.0. The positive significant findings for the highest level of

phenoxy herbicide exposure remained for the cross-sectional enrollment analysis but was eliminated for the other two analyses.

Overall, controlling for triazines eliminated any significant positive associations between having a higher BMI than that observed for any of the lower ordered BMI categories. This was also observed for phenoxy herbicides, herbicides less triazines, and organothiophosphates. Controlling for triazines consistently increased the number of significantly negative ORs for levels of insecticide, fumigant, and carbamate exposure indicating higher exposure was associated with a lower BMI than the BMI observed for any of the lower ordered levels across the three studies when missing values were included in the zero scores. The results when excluding missing values from zero scores were more scattered with particular levels of insecticides and organophosphates no longer showing significant negative odds ratios while other levels of insecticides and the highest level of carbamates showed significant negative odds ratios. Adjusting for triazines eliminated most of the significant Wald Chi Square results and eliminated the significant positive odds ratios for the highest level of organothiophosphates in the cross-sectional follow-up analyses and the highest levels of phenoxy herbicides in the prospective and cross-sectional follow-up analyses.

4.13. CHAPTER SUMMARY

While our initial results based on multiple and logistic regression suggested significant positive effects between BMI and exposure to herbicides and triazine herbicides at

enrollment (cross-sectional analysis); herbicides, organochlorines, and triazine herbicides at enrollment and BMI at follow-up (prospective analysis); and herbicides, organochlorines, organothiophosphates, and triazine herbicides at follow-up with BMI at follow-up (follow-up cross-sectional analysis), adjusting for triazines virtually eliminated any significant findings for other pesticide classes. This suggests that triazines are the main pesticide class of interest in the relationship between increase in BMI and pesticide exposure.

When triazine exposure was used as an additional covariate in the general linearized models (GLM) for each pesticide class where least squares means BMIs were calculated in order to evaluate exposure-response, they had a minimal effect on the results. This is evident in the almost non-discernable differences in the graphs of BMI least squares means by each pesticide class exposure level when including and not including adjustment for triazines. Only one true exposure response was identified for phenoxy herbicides in the cross-sectional follow-up analyses.

Our study sample included the 9,076 private applicators that completed Phase 1, Phase 2, and the diet history distributed during Phase 2. Frequency distributions comparing the original cohort of 50,626 male applicators aged 20 or more years to our reduced study sample, did show differences between the groups on several important demographic variables (age, presence of a weight-related health condition, and state of residence). However, since the distribution patterns of weight-related variables showed minimal differences between the full sample and our sample, and a weight-related variable

(BMI) is our outcome or dependent variable, it is unlikely that selection bias is operating in this study.

We developed surrogate measures for energy input and output at enrollment and for energy output at follow-up. We used the diet history data and some dietary information from Phase 1 to perform a regression analysis to create a formula to calculate energy input at Phase 1. Using data in Phase 1 on hours per day of heavy lifting and hours per week of leisure exercise, we created a surrogate variable for energy output for Phase 1. The surrogate variable for energy output at phase 2 took the change over 10 years (from enrollment to 10 years prior to enrollment) in amount of heavy lifting into account.

Bivariable analyses using Spearman correlation coefficients provided evidence to suggest a positive effect on BMI from exposure to pesticide functional and chemical classes, though all correlation coefficients were small (-0.02-0.09). A residual check of the data suggested it met the criteria for multiple regression analysis. Potential confounders were identified by a significant correlation or chi-square value when compared with both the outcome variable and a specific exposure variable. The same confounders were used in the stratified analyses as were identified for the corresponding full sample to avoid the possibility that the reduction in statistical power resulting from stratifying the sample by state and presence or absence of weight-related health conditions would affect the identification of appropriate confounders.

The regression models were re-run with the subjects with zero scores from missing values using only those confounders causing a >10% change in the unadjusted parameter estimate for the relationship between exposure and BMI. These results did not suggest significant differences compared to when all related confounders were included in the models. Therefore, we retained all originally identified potential confounders in all remaining analyses.

Approximately 180 separate analyses were conducted with our study sample not counting the stratified analyses, and omitting the 10% confounders analyses, and the analyses adjusting for triazines. While some might suggest that a Bonferroni adjustment is needed based on the number of analyses, we used alpha=0.01 as our criteria for statistical significance rather than the usual alpha=0.05. Use of the Bonferroni adjustment would require that alpha be reduced at a minimum to 0.05 divided by 180 (i.e., 0.00028 or less). This would control for any possibility of a false positive but would increase the probability of a false negative. Since our research is really the first study to investigate weight gain in people chronically exposed to low-levels of pesticides, placing such a strong restriction on identifying any significant findings might divert future investigations designed specifically to evaluate this hypothesis with better data on outcomes and potential confounders. Interestingly, if we accept the significance level associated with the Bonferroni adjustment, we can still identify a few significant findings in our pesticide adjusted multiple regression analyses.

Ordinal logistic regression analyses using zero exposure as the referent group yielded ORs that supported the consistent relationships identified for herbicides and triazine herbicides. We found that the parameter estimates in the regression analyses also showed significant findings for herbicides whether or not triazines were included in the category. Similar findings were identified whether or not subjects with zero scores from missing values were included in the regression analyses.

The lack of exposure-response trends for almost all our least squares mean analyses regardless of whether or not we controlled for triazines may indicate that our data may not be linear. In summary, we have identified increases in BMI associated with exposure to specific classes of pesticides in our regression analyses, including some suggested by our literature review. However, controlling for triazines eliminated positive significant findings in the adjusted multiple regression analyses and the ordinal logistic regression analyses for all pesticide classes. We have provided enough evidence to warrant further investigation of the pesticide class triazine herbicides and its effects on weight.

CHAPTER 5: DISCUSSION AND CONCLUSIONS

5.1 Summary of Findings and Comparability with Literature Review

Based on the literature review summarized in Chapter 2, agricultural exposures likely to influence BMI in the AHS cohort include TCDD (based on exposure to phenoxy herbicides such as 2,4-D or 2,4,5-T) and organochlorine pesticides. While we did find some positive significant findings that support the results of our literature review (i.e., some significant findings for organochlorines and phenoxy herbicides), these findings were virtually eliminated when controlling for triazine herbicides.

Findings for our multiple regression models when stratifying by state of residence and presence or absence of weight-related health conditions indicated there were differences across these subgroups and across studies (enrollment cross-sectional, prospective, and follow-up cross-sectional). Besides the effect modification caused by the medical and residence variables, these findings also suggest the possibility that each pesticide class acts differently with BMI with some showing short-term effects and others showing long-term effects. However, controlling for triazine exposure eliminated virtually all significant findings identified. Interestingly, triazine herbicides are among the pesticides believed to be endocrine disruptors (see Table 1.1).

The association between triazine herbicide exposure and BMI was the only consistent association noted across all stratified groups and for all three study groups (cross-

sectional enrollment, prospective, cross-sectional follow-up) using multiple regression, GLM, and ordinal logistic regression. The significant results for the association between triazine herbicide exposure and BMI are the most consistent finding emerging from this analysis. As our study provides the first data to prospectively evaluate the relationship between BMI and pesticide exposure, we must be cautious in discussing our key findings with respect to a direct relationship between exposure to triazine herbicides and BMI.

Initially, deleting triazines from the overall herbicide category indicated a significant association with BMI suggesting herbicides besides triazines might be related to BMI. However, when we controlled for triazine exposure in the regression analyses of herbicides less triazines, any significant findings were eliminated. Therefore, any significant results for the overall herbicide category are being driven by the effects of triazines.

While we can suppose that some of these significant positive associations are due to chance because of the large number of separate analyses that were conducted, we tried to minimize Type I error by using <0.01 as our *p*-value for identifying significant results. To have conducted a Bonferroni adjustment would have made our significance level <0.0002. While this would have eliminated the identification of any false positives, the chances of a false negative would have increased dramatically. As our research is the first to examine increase in BMI in relation to pesticide exposure, we felt that use of the more conservative <0.01 criteria for significance would give us some

confidence in reducing false positives, it would still leave the door open for us to identify any associations that might exist.

We graphed the least squares means for each pesticide class (Figures 4-1, 4-2, 4-3) to visualize their exposure-response relationships with BMI. It is difficult to understand why, in most instances, the zero exposure level should be associated with a higher BMI than the majority of the exposed categories and why a dose-response trend was seen only after excluding the zero category. We tried to evaluate this by graphing the overall mean BMI for each pesticide exposure level but found similar results (presented in supplemental tables). We also re-did these analyses using only the "true" zero scores (i.e., subject answered "no" to ever use of pesticide) and did not include missing values as zero scores. However, the results were almost identical. Including the zero level, the only exposure-response identified was for phenoxy herbicides in the cross-sectional follow-up analysis. In one instance, the drop from the zero level exposure to the first level of exposure was very small so a potential exposure-response trend can be seen for triazine herbicides in the prospective analysis. Controlling for triazine herbicide exposure had little effect on these graphs. In conclusion, we can only deduce that subjects in the zero exposure group, including the "true" zero group, are not like those who are exposed for reasons that are not analyzable within this data set..

At the start of our study, we had hypothesized that our data might conform to the beta-curve (Figure1-1). However, most of our graphs of least squares means (LSMs) by exposure level indicate a more traditional direct dose-response shape, although the zero

exposure levels tend to be associated with higher BMIs than some of the exposed levels creating graphs that show a slight U- shape or V-shape, rather than the inverted J-shaped beta curve. Graphs of phenoxy herbicide and triazine herbicide LSMs versus exposure level are suggestive of a direct dose-response with a possible threshold. The levels of exposure in our study are likely too low such that the data could never show the peak stimulation (weight gain) followed by the inhibition (weight loss) as exposures increase that are characteristic of the beta-curve. Our exposure data is based on intensity-weighted cumulative exposure days that do not correspond directly to pesticide levels known to cause poisoning including weight loss. Exposure levels causing poisoning differ greatly across pesticides and are also influenced by exposure route and personal characteristics (Hayes & Laws 1991).

When weight loss begins, those exposed would be experiencing many other health symptoms. The Agricultural Health Study (AHS) data includes information on subjects' responses to questions about symptoms associated with high levels of pesticide exposure, but as noted in Chapter 1, these symptoms can also be associated with a wide variety of medical conditions (Hayes & Laws 1991). Without medical confirmation, we cannot know if any of our subjects is currently being exposed at a level that could induce the inhibitory response (weight loss). We evaluated the data on whether or not the subject had ever had a high exposure incident as a potential confounder but it was not correlated with the outcome or the exposure so was never used as a covariate in any of our models. Most likely our data conform to the very first part of the beta curve where exposure directly increases stimulatory response (increase in BMI); therefore,

this part of the beta curve can be evaluated with linear models, such as we utilized. Linear models are the most simplistic and parsimonious models. Since we are engaged in a new line of research, and there are no prior human studies available to evaluate dose-response, it is reasonable to utilize linear models as our initial analysis models. If we were to have included analyses examining other dose response shapes, this would affect the alpha level we could use to determine statistical significance. Hence, it is wise that we began with the simplest type of modeling.

We also initially hypothesized that our study subjects would show higher median BMIs by increasing age than the general population (see Figure 1-2). In comparing the midpoints of each age group from 20-29 to 60-69 years of age with the results of NHANES, our actual data show that for BMI at enrollment, pesticide applicators had higher median BMIs than the general population until approximately age 50 (see Figure 5-1). From that point onward, BMIs for pesticide applicators and males in NHANES were approximately equivalent. Our graph using BMI at follow-up compared to BMI in the general population males by age group shows consistently higher BMIs for pesticide applicators across all age groups. In general, male pesticide applicators tend to have higher BMIs than general population males, at least through middle-age. However, there are other factors to consider since all subjects are farmers and farmers are generally healthier than the general population, as noted by previous AHS studies showing lower levels of cardiovascular disease and cancer, and reduced rates of smoking and alcohol use. Health status may effect selection into farming as an occupation and obese farmers may be more likely to enter other professions. Farmers

may consume larger amounts of food than the general populations because of the physical nature of their jobs. Yet we have found a potential link between exposure to triazine herbicides and BMI despite the more healthy characteristics of farmers compared to the general population.

5.2 Study Limitations

This study's main limitations stem from difficulties in fully controlling for the influences of diet and exercise due to lack of adequate dietary and physical activity information at baseline. These are the two most important factors that must be controlled in any study examining an exposure's influence on BMI. Therefore, we developed crude measures of physical activity and diet at enrollment and a crude measure of physical activity at follow-up. The development of new variables for diet and physical activity was based on many assumptions which may or may not be accurate. Our inability to fully control for these factors detracts from the validity of our findings.

5.2.1 Limitations related to energy output variable

The enrollment and take home questionnaires (baseline data) were not designed to measure expenditure or intake of calories. Data were collected at baseline on hours of leisure time physical activity in summer and in winter but this is unlikely to be the major caloric expenditure for farmers given the highly physical nature of their

occupation. Data are not available on the number of hours per day subjects spent performing particular occupational tasks making it impossible to calculate the metabolic equivalent (MET) associated with each task for summing across tasks to more precisely control for the overall energy expenditure associated with physical activity from occupation.

Patterns of physical activity based mainly on enrollment interview questions on hours per day of heavy lifting and hours per week of strenuous leisure exercise were used to create substitute variables that rely on assumptions regarding the generally physically demanding work experience of this homogeneous cohort. This variable was assessed to be used to control for energy output in the cross-sectional enrollment and the prospective analyses. The heavy lifting question specified "during the growing season" which we interpreted as 6 months, but this may in fact be incorrect as many who work in agriculture consider the growing season to be 7 months. This may have contributed to misclassification for this variable as our calculated energy outputs may be less than what might be expected with a 7-month growing season. If energy output levels were actually higher than what we calculated, this may have influenced our findings in some way.

Using the available data on hours of heavy lifting during the growing season 10 years prior to enrollment, we were able to estimate hours of heavy lifting at follow-up using the percent change per year in hours of heavy lifting and a linear depreciation model assuming that the change per year was constant. This may not reflect the actual

reduction in hours of heavy lifting over time. This variable was assessed for use in the regression models to control for energy output for the Phase 2 follow-up data. It was significantly correlated with hours of heavy lifting at enrollment and hours of heavy lifting 10 years prior to enrollment. It is also based on a growing season of 6 months rather than 7 months so there is a strong indication that misclassification may have occurred, though it is difficult to predict if this misclassification biased our results towards the null or towards a greater effect. Interestingly, when we assessed our physical activity variables as potential confounders by correlating them with the outcome measure and with the exposure measures, there were no significant correlations. Therefore, physical activity was not used in any of our models as a potential confounder...

A recently published paper utilized AHS data from Phase 1 on farmers and spouses to assess the relationship between the responses to the question on heavy lifting and other farm activities (Racine et al. 2012). This cross-sectional study based on data from the enrollment period found that adjusted odds ratios (ORs) (demographic, health status, health behavior, and farm activities, BMI, state of residency, and history of chronic disease) were all non-significant for the relationship between heavy lifting and other farm activities. The authors estimated that the MET for heavy carrying and lifting is 8, which indicates vigorous physical activity.

The results of Racine et al (2012) lend support to our decision not to use size of farm to represent physical activity in our models since after adjusting for potential confounders,

they found that planting ≥ 200 acres was associated with a significantly reduced OR for heavy lifting and acres planted. This indicates that heavy lifting ≥ 1 hour/day and planting 200+ acres were less likely to be associated with each other compared to the referent group of 1-199 acres. Also, it is difficult to determine if larger farms were more mechanized with less physical activity or with more physical activity due to their size. Racine et al. (2012) concluded that they could not validate whether or not heavy lifting was an adequate measure of occupational physical activity in farmers since few objective measures exist for comparison. However, the heavy lifting variable was positively associated with the majority of farm activities indicating it may be a viable variable to control for physical activity in AHS studies (Racine et al. 2012), though it was not a significant factor in our study.

A study done in Finland examined the physical strenuousness of work and BMI at 5-year intervals from 1972-2002 using self-reported data and defined a physically demanding occupation as involving lifting and carrying heavy objects (Bockerman et al. 2008). Changes in the physical strenuousness of work explained 7% at most of increased BMI for Finnish males over the past 30 years. BMI was 2.4% lower when a male's occupation was physically demanding compared with sedentary work (reference group). Contrarily, Lakdawalla & Philipson (2006) used data from the U.S. National Longitudinal Survey of Youth to investigate physical activity at work and weight in the U.S. and were able to estimate that a man who spends 18 years in the most physically demanding occupation is about 25 pounds (14%) lighter than his peer in the least demanding occupation but a male spending 18 years in the most strength demanding

occupation is about 28 pounds (15%) heavier than his counterpart in the least demanding job. A survey conducted in a rural county of New York on physical activity evaluated the mean number of times per week participants were involved in “sweat activity” (Eaton et al. 1994). In general, a high level of physical activity was found in this rural county. Farmers had an increased amount of sweat activity compared to all other occupations and 46.4% of male farmers were involved 4 or more times per week in sweat related activity (Eaton et al. 1994).

Mayer et al. (1991) assessed the consistency of three methods of measuring work and leisure activity (a 7-day physical activity recall, self-assessed rank, and vigorous activity score) in the rural (17% farmers) biethnic population of San Luis Valley, Colorado. Among 503 adults (49% white, 51% Hispanic) physical activity at work rather than leisure time largely determined total energy expenditure as measured using the 7-day recall questionnaire. In comparison to the self-assessment and vigorous activity score, subjects indicated they expended more energy at work than with the 7-day recall. Men also self-reported and had higher vigorous scores for their leisure activity; these levels were higher than with the 7-day recall. Differences between the assessment instruments among gender, ethnic, and occupational groups may be related to differences in patterns of physical activity. Internal consistency of physical activity assessment was determined using a rural population and the results suggest a need to adapt or design assessment instruments more specific to the particular population (Mayer et al. 1991).

Another possibility we considered to develop an energy output variable was to utilize data from another study done on a similar population that provides data on energy intake and energy expenditure and adapt these variables to the current study. Bazzarre et al. (1992) conducted a cross-sectional analysis of 195 North Carolina farmers and their wives to measure cardiovascular disease risk factors including diet and exercise. Their paper provides data on energy intake and energy expenditure separately for males and females, and by three broad age groups, that could potentially be adapted to the current study. However, we felt that using data from the AHS itself to develop the energy output variable would yield a more valid variable.

As noted, our energy output variable was never used in any of our models because it was not found to be correlated with both the outcome and the exposure measures – the method by which we selected variables to assess as confounders in our models. This leads to a few potential conclusions: 1) our study sample was very homogeneous in terms of energy expenditure to the point that our energy output factor showed no variability across subjects and so was not found to be correlated with both outcome and any pesticide exposures; 2) the variable we developed based on the data we had available to us did not adequately assess energy output so it was not related to outcome and exposure; 3) our physical activity variable was heavily misclassified reducing or masking any real risk.

We found very high correlation coefficients (> 0.7, $p<0.0001$) for heavy lifting at enrollment compared with heavy lifting 10 years prior to enrollment. Similarly, amount

of leisure time exercise in summer and winter at enrollment were highly correlated (> 0.8, $p<0.0001$). When examining the frequency distributions for the full sample and the stratified samples in terms of our calculated physical activity variable for Phase 1, distributions were very similar with a slight tendency for North Carolina applicators to have higher levels of physical activity. Our correlation coefficients for our calculated physical activity variables for Phase 1 and Phase 2 were significantly correlated (≥ 0.5, $p<0.0001$). This lends support to the idea that energy expenditure based on responses to questions on heavy lifting and leisure time exercise was stable over time.

Figure 5-2 shows the histograms for our calculated variables, energy output at enrollment and at follow-up. The distributions extend well beyond the normal distribution line for the lower values where they tend to cluster. However, physical activity in hours per day at enrollment ranges from zero to 13.5. This wide range might suggest heterogeneity across the sample, but the majority of the sample falls into the zero to 7 hours per day categories so, in fact, the sample appears to be homogeneous with respect to our physical activity variable. The histogram for Phase 2 physical activity shows clusters of subjects with hours of physical activity per day at zero, 0.4, 1.6, 4.0, 8.0, and a small cluster at 10.4. While the range from zero to 10.4 is quite wide, the majority of the sample falls into the zero to 4.0 hours per day range. We have provided some support for the idea that our sample is homogeneous for our energy output variables. However, it is still possible that our energy output variables did not assess energy output very well and/or that there is a great deal of misclassification in our energy output variables; such misclassification should be non-differential.

We agree with Racine et al.'s suggestions that detailed daily diary information about farming activities with subjects wearing measurement devices to validate their responses would have provided a better physical activity variable. However, the expense of such a data collection would not be feasible for the more than 90,000 subjects in the AHS. Therefore, a detailed survey among a subset of subjects to assess bouts, intensity, and frequency of physical activity would be more feasible.

5.2.2 Limitations due to energy input variable

With regard to dietary influences, a detailed dietary history was collected at 5-year follow-up but not at baseline. Nutrient levels calculated from the dietary history questionnaires administered at follow-up were only available for a portion of the male private applicators who completed Phase 1 and Phase 2 and were aged 20 or older (n = 9076); no nutrient levels were available for the commercial applicators or the spouses. Since energy input is such an important variable to control for in any analysis of BMI, our sample size was reduced to include only those male private applicators with these values available. Correlational analyses determined that baseline servings of vegetables and fruits per week and overall meat intake were all highly significantly correlated with similar summary measures in the diet questionnaire conducted at Phase 2; the correlation coefficients were all moderate (0.44 to 0.77). The baseline servings of vegetables, fruits, and meats were highly correlated with total caloric intake based on the nutrient calculations for the 5-year follow-up dietary history questionnaire but these

correlation coefficients were even smaller (0.06 to 0.29). This detracts from our ability to correctly estimate Phase 1 energy input based on Phase 2 data.

Nevertheless, we utilized available AHS data to develop a variable to predict past overall caloric intake modeled from intake of meats, fruits, and vegetables at enrollment and total caloric intake at 5-year follow-up using multiple regression. Our energy input variable was based on the model parameter estimates which we used as a formula for calculating energy input during Phase 1. Our variable was found to be significantly correlated with the outcome variable (BMI) and exposure to specific pesticide classes for many of our analyses. In some instances, it was the only potentially confounding variable used in the model because none of the other potential confounders were related to BMI and the particular pesticide class under analysis.

Figure 5-3 presents the histograms for the two energy input variables – at enrollment and at follow-up. Energy input at enrollment illustrates a normal distribution with a peak at 2200-2300 kilocalories per day and a range from 1300 to 3200 indicating a more even spread for the enrollment energy input variable. The histogram for energy input at follow-up, which is based on actual self-reports from the diet history questionnaire, is represented by a normal distribution curve heavily skewed to the left (lower values), with a peak at about 1900 kilocalories per day and a range from 100 to 7300 kilocalories per day. The majority of subjects fall in the 900 to 4100 kilocalories per day range. This suggests greater variability for the energy input variable at Phase 2. Energy use at enrollment is calculated from energy input at follow-up and is used in the

cross-sectional enrollment and prospective analyses; energy input at follow-up is used in the cross-sectional follow-up analysis. Our calculated variable suggests less variability across subjects than the actual reported values from Phase 2. Nevertheless, both variables were found to be correlated with the outcome variables and with some of the exposure variables for specific pesticide classes so they were included in those regression models.

A study done in Denmark estimated the degree of misreporting of energy input in relation to self-reported BMI among 309 men (Nielsen et al. 2009). The results indicated 35% underreported and 7% over-reported their energy input and obese men were more likely to underreport than those who were not obese. Underreporting of energy input was significantly related to current and previous BMI (Nielsen et al. 2009). If such underreporting did occur in our study, it would bias the results towards the null.

As mentioned for energy output, we did consider the possibility of utilizing energy intake data from another study done on a similar population, and adapting these variables to the current study. The paper by Bazzarre et al. (1992) on measured cardiovascular disease risk factors in North Carolina farmers included diet. Their paper provides data on energy intake separately for males and females, and by three broad age groups. However, as with energy output, we felt that using data from the AHS itself to develop the energy input variable would yield a more valid variable.

Despite the somewhat questionable validity of our energy input and energy output variables, and the potential for energy input to be influenced by BMI (Nielsen et al. 2009), we utilized the best available data in the AHS to control for these two factors in our analyses. The fact that energy input was used in many of our models since it was correlated with both BMI and pesticide exposure, while energy output was not, possibly suggests homogeneity of the energy output variable at both baseline and follow-up as discussed above. Of course, the potential for heavy non-differential misclassification for both our energy input and energy output variables exists but this would have biased risk estimates for BMI towards the null.

5.2.3 Limitations related to lack of exposure data for entire lifespan

No information on childhood exposures is available so exposure to specific agricultural chemicals over the lifespan is unknown and the use of BMI at age 20 as more than a descriptive variable is not possible. While researchers have provided limited evidence to suggest exposure at a particular developmental stage – particularly intrauterine and childhood exposures – affects the development of overweight and obesity at later ages (Hu 2008a), we cannot assess this due to lack of information on intrauterine and childhood exposures. Our regression models did control for age and for the number of years lived or worked on a farm.

5.2.4 Limitations due to self reports of weight, height, and medical conditions

Additional limitations stem from the use of self-reports of weight and height to calculate BMI rather than anthropometric measures. While anthropometrically measured height and weight, with precise measurement of body fat level using medical devices designed specifically for this purpose are the ideal, the majority of health studies still rely on BMI based on self-reported weight and height. Also, anthropometric measures may be difficult and/or costly to obtain. Such is the case with the AHS data. For some subjects missing information on height and weight at enrollment, data were supplemented with information from drivers' licenses. Self-reports were also utilized for the medical exclusion criteria since medical records were unavailable for confirmation.

Several studies on height/weight validity that are based on large samples with comparisons of actual measurements and self-reports have found that self-reports are reasonably reliable and valid measures for some subgroups of the population (Goodman et al. 2000; Gunnell et al. 2000; Rimm et al. 1990; Gillum & Sempos 2005; Ezzati et al. 2006; Kuczmarski et al. 2001). Some studies have found that compared to actual anthropometric measurements, respondents tend to under-report weight, especially at the higher weight levels (Black et al. 1998; Flood et al. 2000; Gunnell et al. 2000; Nawaz et al. 2001; Gillum & Sempos 2005; Stewart et al. 1987; Elgar & Stewart 2008; Villanueva 2001; Ezzati et al. 2006; Rowland 1989). However, underreporting is

generally less among males (Elgar & Stewart 2008; Villanueva 2001; Ezzati et al. 2006; Rowland 1989) and greater among older age groups ≥ 70 years (Kuczmarski et al. 2001; Rowland 1989). Less than 10% of our study subjects fell into these older age groups at enrollment.

Another study found a high degree of reproducibility of self-reports of past body weight at ages 25 and 40 among 120 middle-aged subjects; a difference of +/- 3 kg was observed for 75.8% of subjects for weight at age 25 and 81.7% of subjects for weight at age 40 indicating self-reports of body weight have high reliability (Klipstein-Grobusch, et al. 1998). The results were not influenced by age, sex, current BMI, or education level (Klipstein-Grobusch, et al. 1998). In conclusion, if under-reporting occurred in the AHS, it is likely to create a bias towards the null in terms of the research questions under study.

5.2.5 Limitations due to other occupational exposures

There is also the potential for confounding by non-pesticide agricultural exposures and non-farm occupational exposures. A methodological study of AHS data assessing the effects of potential confounding from non-pesticide exposures on associations between pesticide usage and various outcomes determined the magnitude of bias due to confounding based on confounding risk ratios (CRR) – the ratio of the unadjusted relative risk to the relative risk adjusted for the confounding factor (Coble et al. 2002). Prevalence of various farm activities that involve non-pesticide exposures reported by

farmers from Iowa and North Carolina show that the percentages vary from a low of 6% (NC) and 2% (IA) for “work in poultry confinement areas” to a high of 49% (NC) for “repair pesticide application equipment” and 86% (IA) for “handle stored grain” (see Table 5-1).

In terms of having non-farm jobs, 74% of North Carolina and 59% of Iowa farmers reported working in jobs off the farm. The prevalence of exposure to particular substances from non-farm jobs for the AHS farmers was highest for non-farm exposure to engine exhaust (20% in NC; 21% in IA) (see Table 5-2). CRRs calculated based on the proportion of farmers reporting various activities among those who mixed or applied pesticides 10 or more days per year versus those who mixed or applied pesticides less than 10 days per year varied from a low of 0.97 for milking cows to a high of 1.07 for repairing engines, grinding metal, welding, or driving trucks. Thus, the effects of bias from confounding due to non-pesticide exposures are likely to be minimal (Coble et al. 2002).

We attempted to control for exposure to non-farm occupational exposures using a very crude variable we developed to determine if exposure to any of the non-farm hazardous substances listed in the questionnaire were correlated with both the outcome and pesticide class exposure variables. Our non-farm exposure variable was based on whether or not the subject had checked off any one of a group of non-farm exposures listed in the baseline questionnaire. A checked response to any of these occupational exposures indicated the subject had non-farm occupational exposure; if none of the

exposures were checked, the subject was assumed to have no non-farm occupational exposures. We did not find this variable to be significantly correlated with both outcome and any pesticide class, so non-farm exposure was not included in the models as a potential confounder. However, this very simplistic method of controlling for non-farm occupational exposures may have led to heavy non-differential misclassification biasing our results towards the null.

All adjusted regression analyses including subjects with zero scores for exposure to specific pesticide classes were re-run controlling for intensity-weighted cumulative exposure to each of the other pesticide classes.. Only adjustment for triazine herbicides affected the results. For all three study groups (cross-sectional enrollment, prospective, cross-sectional follow-up), there were no significant regression parameter estimates for pesticide exposure and BMI when controlling for triazines. In addition, controlling for exposure to triazine herbicides changed the sign of the parameter estimate from positive to negative for many pesticide classes in all three studies (enrollment cross-sectional, prospective, follow-up cross-sectional). This suggests that the relationship between triazine herbicide exposure and BMI is dominant, and detracts from our findings for all other pesticide classes.

5.2.6 Limitations due to low response rates and missing data

One source of bias stems from the large amount of missing information – as high as 50-60% for some items. The low response rate (50%) for the take home questionnaire

distributed to the applicators at baseline suggests there may be problems with the quality and generalizability of studies based on these data. However, when farmer applicators who completed the take-home questionnaire were compared to those who did not complete the take-home questionnaire, all differences were small and etiologically insignificant suggesting any bias resulting from using data from the take-home questionnaire should be minimal (Tarone et al. 1997).

To evaluate potential selection bias for the sub-cohort completing both the enrollment and take-home questionnaires, a comparison was made between pesticide-specific mean exposure intensity scores for 2,4-D and chlorpyrifos for the cohort completing just the enrollment questionnaire versus the sub-cohort completing both questionnaires (Dosemeci et al 2002). The enrollment cohort showed slightly more application days for each pesticide than the sub-cohort but both populations showed a similar intensity of exposure and a similar distribution of exposure levels by demographic variables including state, gender, age, and applicator type. This suggests that the sub-cohort that completed the take-home questionnaire is representative of the entire cohort on exposure. In addition, the mean exposure levels in the sub-cohort were compared using the exposure algorithm. There was high concordance between the scores obtained with the algorithm in terms of percent agreement and correlations suggesting the results of the general algorithm can be used to evaluate health risks.

In order to be able to include an energy input variable in our models, our study was limited to those private applicators who completed Phase 1 and Phase 2 with the diet

history. It was further limited by the need to use those dietary history questionnaires for which nutrient intakes had been calculated and the need to include subjects with at least one BMI measure (enrollment and/or follow-up). This reduced our sample size from 50,641 male private applicators aged 20 years or older to 9,076 men: 18% of the total sample. No diet history questionnaires with nutrient values were available for the commercial applicators so we could not analyze them in our study.

We compared our study sample to the full private applicator sample on demographic and weight-related variables. There was little difference between the groups with regard to weight-related variables; thus the study subjects did not appear to be differentially included on the outcome variable and selection bias is unlikely to be an issue in our analyses. However, there were important differences based on demographic factors such as age (mean 3 years younger), state of residence (larger proportion from Iowa), and weight-related health conditions (fewer health problems).

5.2.7 Limitations due to exposures assessed through self-reports

While biological monitoring data would be useful, this data collection method was not employed for AHS as the size of the cohort made it not feasible due to factors such as cost and logistical constraints. In addition, biological monitoring is not useful to assess past exposure to pesticides with short half-lives. Pesticide exposure was also not validated through sales receipts data. However, the validity of the general algorithm that was used to create an exposure score for each pesticide for each subject was

evaluated by comparing algorithm scores with biological monitoring data from a previously conducted study of pesticide applicators applying the herbicides MCPA or 2,4-D known as the Pesticide Exposure Assessment Study (PEAS) (Coble et al. 2005).

PEAS employed a detailed questionnaire similar to the AHS questionnaire and involved collection of urine samples from 126 pesticide applicators prior to application and for 2 days following application to identify factors affecting exposure under actual field conditions. No attempt was made to modify or control application methods or exposures. Algorithm scores were calculated based on the study's questionnaire data and compared with the post application urine concentrations to evaluate the relationship between exposure intensity scores and urine concentrations of pesticides (Coble et al. 2005).

Scores were grouped into low, medium, and high ranges. It is important to note that variability in pesticide exposure levels can be due to a variety of factors including, but not limited to, the application method, personal hygiene, and the use of personal protective equipment (PPE). Since almost all of the PEAS applicators used the same application method, the influence of this factor could not be evaluated. Similarly, almost all of the applicators mixed herbicides prior to application so the mixing variable contributed little to score variability, which was found to be mainly due to differences in use of PPE (Coble et al 2005).

The concentrations of pesticides detected in post-application urine samples were found to vary considerably reflecting variability in exposures received during application along with variability in absorption, metabolism, distribution, and excretion rates (Coble et al 2005). Other factors that could have influenced the absorbed dose include differences in breathing rates, physical exertion, temperature, humidity, pesticide formulation, and dermal factors such as skin surface area, exposed skin locations, skin damage, and presence of other chemicals on the skin. However, AHS algorithm scores are supposed to represent exposure intensity rather than absorbed dose. Despite the study's limitations, statistically significant trends in the geometric mean of the urine concentrations across algorithm exposure categories (low, medium, high) were observed for applicators using 2,4-D and MCPA indicating that the algorithm is robust. The algorithm scores we used in our study were updated after calculation of the original scores to take account of the results of two exposure monitoring studies. The authors concluded that the algorithm scores provided a reasonably valid measure of exposure intensity (Coble et al 2005). The AHS first follow-up was 5 years after enrollment with additional follow-ups planned for the future. Each follow-up will obtain data on pesticide use. This will allow researchers to assess how well the algorithm works over time with continued biomonitoring of small samples for validation.

Exposure assessment is also complicated by the fact that use of various pesticides often occurs simultaneously and because pesticides registered for specific uses have changed over time (Samanic et al. 2005). Factor analysis was used to characterize the underlying patterns of self-reported exposure to the 50 different pesticides (enrollment

plus take home questionnaires) examined among the AHS cohort. Separate analyses were performed for farmers, commercial applicators, and spouses of farmers. Three factors were identified for farmer applicators: 1. Iowa agriculture and herbicide use; 2. North Carolina agriculture and use of insecticides, fumigants, and fungicides; and 3. older age and use of chlorinated pesticides (Samanic et al. 2005).

Because farmers provided more detailed information on their activities that could be compared to pesticide use, additional analyses were conducted to determine if type of crops or livestock, application methods, protective equipment worn, and lifestyle characteristics would cluster with pesticide use. However, results were similar to those seen when pesticides only were included in the analyses. A fourth factor was identified that included other vegetables, spraying of animals, use of ear tags, and injecting animals. Exposures were not found to correlate with the lifestyle characteristics investigated for farmers (smoking, race, education, fruit consumption, vegetable consumption, and alcohol consumption) (Samanic et al. 2005).

While it was not possible to directly validate each respondent's use of pesticides, or the underreporting of use, an analysis was undertaken to determine the plausibility of applicators' responses (Hoppin et al. 2002). Quality of the self-reported pesticide use history was evaluated by comparing reported decade of first use and total years of use to the year the pesticide was first registered for use based on registration information from the U.S. Environmental Protection Agency (EPA) and other publicly available sources for the 52 pesticides included in the AHS Phase 1 interviews (Hoppin et al. 2002). The

majority of the more than 57,000 certified applicators in the AHS cohort provided plausible responses for both decade of first use and total duration of use.

On average, less than 5% of applicators overestimated total possible duration of use (Hoppin et al 2002). For decade of first use, 98% of applicators gave plausible responses with overestimates no higher than 7% for an individual pesticide. However, more applicators did not report decade of first use than duration of use with an average of 6% missing decade of first use information for an individual pesticide (Hoppin et al. 2002). Based on these findings we felt it was unnecessary to assess our study sample for plausibility in terms of their pesticide exposure responses, including decade of first use, total years of use, and comparability to dates of availability of the pesticide. In addition, our study investigated pesticide classes, not individual pesticides, which would likely dilute any effects from providing the wrong dates of use for a specific pesticide.

Generalizability of the AHS self-reports was evaluated by comparing AHS data on private applicators to data from the 1992 and 1997 Censuses of Agriculture for Iowa and North Carolina (Lynch et al. 2005). AHS Iowa farmers were younger, lived or worked on larger farms, more frequently applied pesticides, and were more likely to raise cattle and swine and grow corn, soybeans, hay, and oats compared to farmers included in the Iowa Censuses of Agriculture. AHS North Carolina farmers were younger, lived or worked on larger farms, more frequently reported growing crops common to the State, and used pesticides more frequently compared to farmers included

in the North Carolina Censuses of Agriculture. The authors concluded that AHS farmers represent the higher end of pesticide usage in both states mainly because they have larger farms (Lynch et al. 2005). However, higher usage translates to higher cumulative exposure with a greater potential influence on health effects, including weight gain (if such a relationship exists).

Several sub-studies of small numbers of AHS participants have directly measured exposures to pesticides and have validated the self-reports (Thomas 2010). Evaluations of the data to date have found very little collinearity among the pesticide exposures with most of the applicators and farmers having used a maximum of 15 pesticides in their lifetime (Alavanja 2006). Recently Blair et al. (2011) assessed the impact of exposure misclassification in the AHS on relative risks using the range of correlation coefficients observed between measured post-application urinary levels of 2,4-D and a chlorpyrifos metabolite and exposure estimates based on an algorithm from 83 pesticide applications. They found that the algorithm scores were more closely related to measured urinary levels than individual exposure determinants. Their estimates of potential bias in relative risks based on the correlations indicate non-differential misclassification of exposure using the algorithm would bias estimates towards the null, but less than individual exposure determinants.

Studying pesticide classes rather than individual pesticides increased our statistical power to identify an effect if one exists for the sample of over 9,000 applicators included in our study. However, examining pesticide classes rather than individual

pesticides could dilute any association between BMI and a specific pesticide that might differ from the overall association between BMI and that pesticide's class. Any bias would be towards the null. Since specific pesticide classes were identified as having an effect on BMI, particularly triazine herbicides, our results set the stage for detailed examination of each pesticide in these classes to identify the effect of individual pesticide exposures on BMI. However, such analyses will be affected by the proportion of applicators using a particular pesticide included in the class than the others (i.e., atrazine is used more frequently than the other triazine pesticides).

5.2.8 Random Error

There is always the potential for random error in any research study. However, we were able to determine that at 80% statistical power, with alpha = 0.01, our study has the ability to detect a change in BMI from 21-69% due to exposure to particular pesticide classes. For all statistical analyses conducted we used 0.01 to determine statistical significance in order to be more conservative in evaluating associations in this new line of research and also to account for multiple comparisons.

5.3 Study Strengths

The known strengths of the AHS include the existence of the best questionnaire-based pesticide exposure data available anywhere in the world and the large sample size**.** Another strength of this investigation is the fact that both retrospective and prospective

analyses of the relationship between BMI and pesticide exposures can be evaluated. Approximately 1700 deaths occurred among male applicators in the cohort (about 3% of the cohort) between enrollment and 5-year follow-up (Blair et al 2005). The small proportion of deaths suggests that the prospective analyses were not performed on a select group of “survivors”.

5.3.1 Study Design

Since the AHS utilizes a prospective design, periodic assessments of recent exposures are obtained, which should improve recall and reduce nondifferential misclassification (Alavanja et al. 1996). Prospective analyses examine BMI at 5-year follow-up in relation to exposure at enrollment. This assures correct temporality between exposure and BMI and should eliminate differential recall by subsequent BMI status. Using the AHS data, current pesticide exposure can be used to predict BMI in the future for those pesticide classes for which we have found consistent results. This has never been evaluated in any study before.

The AHS includes data to calculate BMI at two points in time (enrollment and 5-year follow-up) to be compared, respectively, to exposure to broad pesticide classes at two points in time (enrollment and 5-year follow-up). These two cross-sectional analyses provided the opportunity for further analyses of the BMI and pesticide exposure relationship, however reverse causation cannot be ruled out. Furthermore, if individuals of varying BMI recalled pesticide exposures differentially, this could bias the cross-

sectional analyses. However, it is unlikely that this may have occurred because the research on BMI and pesticide exposures is not widely known. The issue of temporality has been a problem in all previous human research studies on this topic. When exposure and outcome are assessed at the same time there is always some potential for bias. That is why the prospective analyses in our study are of greater importance.

As discussed under "Limitations", several studies have assessed the validity, reliability, and generalizability of the data with results indicating that the AHS data can provide clear insight into the exposure-outcome relationships evaluated and these relationships are unlikely to be greatly effected by bias (Coble et al. 2005; Lynch et al. 2005; Hoppin et al. 2002; Samanic et al. 2005; Dosemeci et al. 2002; Tarone et al. 1997; Coble et al. 2002; Blair et al. 2002). Indeed, Blair et al. (2002) found very high agreement in responses in their evaluation of over 4,000 AHS applicators who completed either a subset of, or the complete enrollment questionnaire, one year after initial enrollment. Agreement on ever mixed or applied any pesticide was 95% and reliability of reporting use for specific chemicals was quite consistent (79-88%). Agreement was also high for smoking and diseases (both >90%).

In addition to our overall study sample of 9,076, we also did analyses on sub-samples of this group stratified by state of residence (Iowa or North Carolina) and by presence or absence of weight-related health conditions. These stratified analyses allowed us to determine if state of residence or presence of weight-related health conditions were confounders or effect modifiers in the relationship between BMI and exposure to

specific pesticide classes. As noted above, our study sample was large enough to detect an effect size (change in BMI) between 21 and 68% for the relationship between BMI and exposure to particular pesticide functional classes or chemical classes based on statistical power of greater than 80% and an alpha of 0.01. We verified that the data adhered to the criteria for regression analysis: normality, homscedasticity, and linearity. Therefore, transformation of the data was not necessary.

5.3.2 Exposure Variable

Variability across exposure groupings and outcomes is needed to see any effect. The proportion of the cohort exposed to each pesticide class at baseline (18% to 96%) varies greatly. There was also enough variability in BMI across age groups.

The AHS exposure data include many variables that could be used to assess exposure including number of exposed years, number of days per year mix/load/apply pesticides, average annual number of days used, rate of application, number of acres to which pesticides are applied, exposure score, numbers of farm animals, whether or not the applicator ever applied each particular pesticide, whether or not the particular pesticide was ever used on the farm, lifetime days used each pesticide, intensity score, total days used per year, intensity days, lifetime intensity days, intensity adjusted cumulative exposure score, decade first used, whether or not pesticide used last year, total cumulative days over lifetime mixed/applied pesticide, apply score, mix score, PPE score, and repair score.

The score variables were developed by Dosemeci et al. 2002 and were used to create an exposure algorithm. Each score variable utilized professional judgment and existing literature, along with the questions on exposure from the AHS questionnaires to develop an exposure score for mixing, applying, and repairing pesticide equipment. These individual scores were used to develop the following formula: Intensity Level = (Mix + Apply + Repair) * PPE (see Table 3-6). We selected intensity weighted cumulative exposure as the best overall exposure variable for all analyses since it utilized information on intensity, duration, type of usage, and use of PPE. Our investigation was based on the general exposure algorithm with the update (revised scores) constructed by Coble et al. (2011) that are subsequent to additional exposure monitoring studies and validation efforts and so make minimal changes to improve the weighting factors without changing the exposure algorithm itself. We used the definitions developed by the AHS to define pesticide functional and chemical classes (Legum et al. 2003).

5.3.3 Confounders

In addition to energy input and energy output, we selected a number of AHS variables to assess as potential confounders based on their known relationship to weight gain, obesity, or health conditions in general, or their potential to influence exposure. These variables included age, race, Hispanic ethnicity, education (a potential surrogate for socioeconomic status), smoking, alcohol use, number of years lived on a farm, non-farm occupational exposure, having lived half their lifetime on a farm, and having had a

pesticide high-exposure incident. In some cases, new data on the confounder were obtained at Phase 2 (age, smoking, alcohol use, number of years lived on a farm). When updated data were available on a particular confounder, these data were used in the follow-up cross-sectional analyses; otherwise, the same value for the confounder obtained in Phase 1 was used in the follow-up cross-sectional analyses. In this way, we were able to control for changes in some of the confounding variables over time.

In individuals with the same BMI, the relative compositions of their fat mass versus lean body mass depend on age, sex, and ethnicity (Hu 2008b). Aging is associated with a substantial loss in lean body mass and with some increase in fat mass; therefore, age is an important variable to consider in any study of BMI. Since our entire study sample is male, we did not have to adjust for gender differences in BMI. Our study population is 97% white so it is unlikely our results were influenced by race and ethnicity. We did evaluate these two factors in terms of the strength of their chi-square values in relation to increasing categories of BMI and in general, found no relationship due to the very small numbers of non-Caucasians in our study. Those analyses where both BMI and exposure to a particular pesticide class were significantly related to age included age in the models using the most temporally appropriate age variable..

The accumulated evidence suggests an inverse association between socioeconomic status (SES) and obesity (Bennett et al. 2008). We used education as a measure of socioeconomic status (SES), a variable frequently used for this purpose (Bennett et al. 2008). Educational categories were found to be statistically significantly related to BMI

categories in our study sample so those models utilizing pesticide classes that were also found to be significantly related to education controlled for this factor. Those farmers with higher educational levels tended to have lower BMIs than farmers with low educational levels and this is as expected (Bennett et al. 2008).

As far as the exposure-oriented categorical confounders such as lived half life on a farm since age 18, ever had a high exposure incident, and ever had non-farm occupational exposures, they were controlled for infrequently in the models as there were not many instances where both BMI and a particular pesticide class were both significantly related to these variables.

Many studies have examined the relationship between alcohol intake and body weight but almost all are cross-sectional, show an inverse relationship for women, and show very inconsistent results for men (Hu 2008c). The prospective studies have not shown that alcohol consumption is an important predictor of weight gain. However, the general consensus among researchers is that heavy alcohol consumption may increase weight but light to moderate consumption has no effect on weight gain (Hu 2008c). Since we had data on alcohol use during Phase 1 and for Phase 2 for the AHS cohort, we included it among the potential confounders we considered. The Phase 1 variable was used for the cross-sectional analyses of enrollment data and for the prospective analyses. The Phase 2 variable was used for the cross-sectional analyses of the follow-up data. There were only a few instances where alcohol use was correlated with both BMI and

exposure to a particular pesticide class -- alcohol use was used as a predictor in these models.

Smoking was also among the confounders we considered. Adult smokers generally weigh less than non-smokers and are less likely to be overweight or obese (Audrain-McGovern & Benowitz 2011). Smoking cessation is associated with weight gain. The mechanisms by which smoking causes weight loss are poorly understood. It has been suggested that nicotine reduces body weight by raising the resting metabolic rate while blunting the expected increase in food intake in response to the increase in metabolic rate (Audrain-McGovern & Benowitz 2011). Data were available in Phase 1 and in Phase 2 on smoking. We utilized smoking as a confounder in all models where BMI and the particular pesticide class under consideration were significantly correlated, being careful to select the appropriate smoking variable based on temporality.

In order to assess the strength of our selected confounders, we identified the percent change inclusion each of the particular confounders had on the unadjusted parameter estimate for each pesticide class exposure and BMI. Then we re-ran the regression models using only the confounders causing a 10% or greater change in the unadjusted estimates. We found very little difference in our results suggesting that our choice of confounders based on their statistically significant relationship to the outcome and the exposure provided accurate identifications. This gave us confidence to conduct all further analyses using the confounders we originally identified rather than only those

causing a 10% or greater change in the unadjusted parameter estimates for BMI and pesticide class exposure.

We utilized all potential confounders for the potential association between BMI and pesticide exposure for which data were available in the AHS dataset. For the stratified analyses, we utilized the same covariates in the models as were used for the corresponding full analyses, therefore the results can be compared between the overall study population and the subgroups. Stratification on state and medical exclusions identified few significant findings for North Carolina, which may be a result of the small sample size for North Carolina (n=2322) leading to a reduction in statistical power to identify any effects. There may have been some effect modification in the Iowa subgroup because of differences in findings compared to the overall study group and when medical exclusions were taken. While possible effect modification by state of residence and/or by inclusion/exclusion of specific medical conditions was evident in our regression results, controlling for triazine eliminated any significant findings and any differences across sub-groups. Therefore, we did not continue to conduct stratified analyses for least squares means or ordinal logistic regression. For analyses of triazines as a pesticide class, all subgroups maintained similar estimates as the overall group and all were significant. In fact, the only significant finding for the North Carolina group was for triazine exposure.

5.3.4 Internal Comparison Group

Studies of occupational cohorts that make comparisons between these subjects and the general population on various health-related outcomes are always subject to the "healthy worker effect". Our sample included only men who were private pesticide applicators. All comparisons utilized AHS cohort members who were still exposed to other factors associated with the agricultural environment. Our study did not include a group of non-agricultural individuals to use as a comparison group. Therefore, our results are not influenced by the "healthy worker effect". This effect has been seen in other occupational studies when members of the occupational cohort under study are compared to a general population sample. The occupational cohort tends to show better outcomes than the general population because all subjects in the occupational cohort were healthy enough to maintain jobs. This could be a particularly large problem when studying an agricultural cohort such as the AHS as these subjects are healthy enough to perform a physically demanding type of work, indicating they are likely to be healthier than the general population and to have lower BMIs. Different results may have been obtained if we had used a non-farmer, non-agricultural control group. Our stratified analyses that excluded subjects with weight-related health conditions still utilized men who were healthy enough to work. This stratification variable did not yield results that were very different than when all subjects were included in the analyses.

The non-exposed group utilized in our GLM and ordinal logistic regression analyses are only non-exposed with respect to the particular pesticide class being analyzed. All

subjects in this study are exposed to agricultural chemicals by virtue of their occupation. Therefore, our non-exposed group in each pesticide class analysis is contaminated with subjects who may have been exposed to other agricultural chemicals that affect BMI. All univariable and bivariable analyses were conducted excluding and including subjects with missing values used within the zero score (non-exposed) group for the particular pesticide class. Therefore, we were able to identify when different results were obtained due to the inclusion or exclusion of a non-exposed group that includes missing values as non-exposed and a "true" non-exposed group that only includes subjects who distinctly answered "no" to use of the particular pesticide under consideration. Remarkably, there were few differences in the significant findings for each pesticide class by including or excluding subjects with missing values as zero scores for a particular pesticide included within a particular pesticide class.

5.4 Biological Mechanisms

It is important to consider how the results on triazine herbicides (atrazine, cyanazine, metribuzin) relate to current hypotheses about how chemicals may increase body weight in humans. We located one article that discussed atrazine in relation to weight gain in rats treated for 5 months with low concentrations (Lim et al. 2009). One group of rats was fed a regular diet and the other was fed a high-fat diet. Atrazine increased body weight without causing a change in food intake or physical activity level. A high-fat diet further increased these findings. The authors concluded that mitochondria in skeletal muscle and liver of atrazine treated rats was swollen with disrupted cristae.

Atrazine blocked the activities of oxidative phosphorylation complexes I and III, resulting in decreased oxygen consumption, while it suppressed the insulin-mediated phosphorylation of Akt. This suggests that long-term exposure to atrazine might cause insulin resistance and obesity (Lim et al. 2009).

Lim et al. (2009) also discuss the CDC and USGS maps that have often been used to suggest there may be an association between atrazine and obesity since high obesity counties are also high atrazine usage counties (see Figure 5-4). Examining maps of obesity and atrazine usage in the United States provides an interesting way of looking at the data on these two variables, but does not provide empirical evidence for the existence of a relationship between these variables. Such broad examinations are subject to the ecological fallacy whereby findings for a particular group are not found when specific data on each individual subject is evaluated.

Triazine herbicides are known to disturb energy metabolism (thiamin and riboflavin functions) (Extoxnet 1993). Adverse effects on metabolic systems have been noted in several studies and are often related to estrogen-like hormone disturbances (Belloni et al 2011; Langerveld et al 2009; Benachour et al 2007). The results of animal studies include decreased weight gain, increased white blood cell counts, decreased blood cholinesterase activity and deterioration and inflammation of the stomach (Extoxnet 1993). Prenatal exposure of male rats to atrazine led to preputia separation delay and significant increase in incidence and severity of inflammation of the prostate; there was a dose-dependent increase in epididymal fat masses and prostate foci in offspring

(Stanko et al 2010). Another study found that at doses higher than 50 mg/kg, body weight of adult male rats decreased and there was testis atrophy with a reduction in testosterone and an increase in estradiol levels (Victor-Costa et al 2010).

Simazine stimulates its own breakdown in the liver with some accumulation in fat (Extoxnet 1993). Short-term tests have investigated the mechanism for the estrogenic properties of exposure to triazines and their metabolites using human cell lines (Sanderson et al 2001). These substances were found to induce aromatase activity in H295R cells with a maximum induction of about 2.5-fold after 24 hours. There were similar increases in CYP19 mRNA levels determined by reverse-transcriptase polymerase chain reaction. Therefore, the estrogenic effects associated with triazines in vivo are not estrogen receptor-mediated, but are due to their ability to induce aromatase in vitro. These studies conflict in some ways with one another and with the general belief that triazines are endocrine disruptors. More laboratory research is needed to assess the true mechanisms by which triazines exert their effect on metabolism and thereby, weight. Studies have confirmed the presence of triazines and their metabolites in urine of agricultural workers during the application time period in Croatia (Mendas G, et al 2012).

Calabrese & Baldwin (1998) indicated that the most systematically assessed mechanism-based research has been in herbicide-induced stimulatory effects, with researchers assessing the molecular basis for the response. This is in keeping with the research so far on triazines which suggests potential stimulatory effects. They also

noted that mechanisms that have been proposed include the concept of adaptation and specific alterations in patterns of gene expression, which are of two classes: patterns associated with enhanced metabolic capacity for detoxification and patterns associated with a more general protection against cellular damage caused by a variety of agents (Calabrese & Baldwin 1998).

Grun & Blumberg (2006) discussed the existence of "obesogens", molecules that incorrectly regulate lipid metabolism and adipogenesis to promote obesity. They note that endocrine disruptors (EDs) promote adipocytes but indicate that it is unclear whether such effects of EDs are mediated solely through an estrogen receptor signaling pathway. This is because estrogens have also been shown to be antiadipogenic in some studies suggesting that increased obesity is associated with inhibition, rather than activation, of estrogen signaling (Grun & Blumberg 2006). Again, there is much laboratory research that can both support and deflect from the suggestion that triazines act as endocrine disruptors to induce weight gain in humans.

Other mechanisms have been hypothesized based on the results of specific animal studies, though none of these studies examined triazines. These hypotheses include adrenocortical hyperfunction (Picazo et al 1996), internally induced electric fields (Babbitt et al 2001), endocrine disturbances and/or changes in hepatic steroid metabolism (Hansson et al 1985), changes in cellular signal transduction pathways with inhibition of adipose tissue lipolysis and/or chronic inhibition of physiological beta-adrenergic and ACTH stimulation reducing energy expenditure (Irigaray et al 2006),

circulating metabolites increasing the deposition of total body lipids (Deichmann et al 1972), and a sex hormone related mechanism (Chen & Kelly 2005). This adds to the variety of hypotheses for how pesticides may induce effects on weight in humans. There is so much more that needs to be done to better define these mechanisms.

There is inconsistency in the human studies investigating rural populations versus non-rural populations in terms of BMI status in the two different groups. Similarly, studies investigating occupational groups with much physical activity compared to low physical activity jobs have also yielded inconsistent results. Many cross-sectional studies compare levels of environmental or occupational contaminants in human blood, urine, or tissues with BMI and/or prevalence of overweight or obesity. These findings are almost always positive (i.e., higher levels associated with higher weight). However, they are based on cross-sectional study designs and, therefore, cannot distinguish temporality and cannot provide evidence for causality. These studies rarely have information on socioeconomic status, which may confound these findings as poorer people are known to have higher BMIs. Only one human study discussed a possible mechanism. Pearson and Lewis (1998) found a high prevalence of obesity in rural residents who they observed to be "slow adopters" to positive health behaviors due to a high rate of poverty and low levels of education. They noted that rural families eat diets higher in calories and in fats with reduced energy expenditure due to the mechanization of modern farming. In other words, the occupation may be influencing activity patterns, which then influence weight gain. Activity patterns may be related to amount of pesticide exposure but we were unable to assess this potential interaction since our

energy output variable was not included in any of our regression models due to a lack of correlation with the outcome and the exposure.

5.5 Conclusions

We evaluated the relationship between BMI and pesticide functional classes (insecticides, fumigant, fungicides, herbicides, herbicides less triazines) and pesticide chemical classes (organochlorines, organophosphates, organothiophosphates, carbamates, and phenoxy and triazine herbicides). Factors that may have influenced our results include poorly defined energy input and output, potential selection bias due to our need to reduce our sample size to accommodate the measurement of energy input, the very small sample size for North Carolina which reduced the opportunity of identifying significant effects in some cases, and the use of self-reported information on weight, height, and medical conditions.

A number of significant associations were identified between pesticide class exposure and increase in BMI. None of these findings are as consistent as the results for triazine herbicides, which are intriguing, especially in light of their ability to eliminate all significant findings when included in the adjusted regression analyses and the ordinal logistic regression analyses. Referring back to the questions we listed in Chapter 1, Introduction, regarding our intended analyses, we have shown that (1) male pesticide applicators more highly exposed to triazine herbicides are more likely to have a higher BMI than those who are less exposed. (2) The effect of the triazine exposure on BMI is

consistent over time since we have found the same result for the enrollment cross-sectional, the prospective, and the follow-up cross-sectional analyses. (3) There is evidence of an exposure-response for increasing triazine herbicide exposure and increasing BMI. (4) We were not able to identify any mediating factors associated with the association between triazine herbicide exposure and BMI.

Chapter 2, Literature Review, evaluated some pesticide exposures relevant to our study of the AHS male cohort that may be associated with weight gain including DDT (organochlorine insecticide), organochlorine pesticides generally, TCDD (contaminant of phenoxy herbicides), parathion (organophosphate insecticide), chlorpyrifos (organophosphate insecticide), lindane (organochlorine insecticide), 2,4-D (phenoxy herbicide), and chlordane (organochlorine insecticide).. The most consistent evidence from this review suggests BMI may be related to TCDD and to organochlorine pesticides generally. Initially we identified significant findings for both phenoxy herbicide exposure and organochlorine exposure but these results were mitigated once we controlled for triazine herbicide exposure.

In January 2011, the National Toxicology Program (NTP) of the National Institute of Environmental Health Sciences (NIEHS) held a workshop to review the evidence on "The Role of Environmental Chemicals in the Development of Diabetes and Obesity". Pesticides were among the categories of chemicals reviewed. The overall review of the existing literature provided support for the developmental obesogen hypothesis that suggests chemicals may act to alter the differentiation of adipocytes or development of

neural circuits that regulate feeding behavior to result in a predisposition to obesity, especially under the influence of high-calorie, high fat diets (Thayer et al 2011, 2012).

The work group decided that with regard to pesticides, the epidemiological, animal, and mechanistic data support the biological plausibility that exposure to multiple classes of pesticides (primarily insecticides) may affect risk factors for diabetes and obesity. Insecticides impact neurotransmitter and/or ion channel systems that are also involved in regulating pancreatic function raising the possibility that these chemicals might affect glucose homeostasis at dose levels where they are effective as pesticides. Poisoning or intoxication of humans and animals with organophosphate insecticides causes hyperglycemia at high doses. Early life exposure to subtoxic levels of organophosphates results in subsequently-emerging pre-diabetes, abnormalities of lipid metabolism, and promotion of obesity due to increased dietary fat (Thayer et al. 2011, 2012).

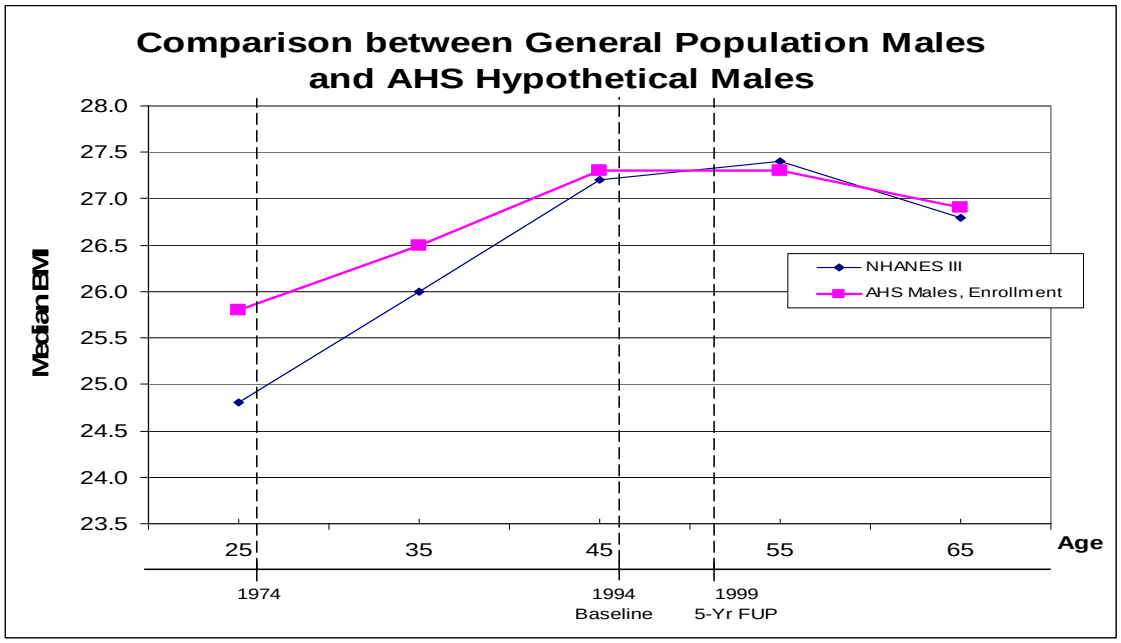
The NTP researchers had access to the Toxicity Reference Database (ToxRefDB) which contains data for pesticide registration for 474 chemicals that are not available in the peer-reviewed literature (Thayer et al. 2011, 2012). Querying this database revealed that 100 chemicals caused body weight gain, increased blood glucose, and/or pancreatic effects in various animal species. Preliminary analyses suggest some of these chemicals may impact biochemical or cellular targets that have not been identified in the peer-reviewed literature but support the biological plausibility for how they may exert effects on glucose homeostasis, insulin sensitivity, or adipocyte differentiation and function (Thayer et al. 2011, 2012).

Data from Tox21, a collaborative program between EPA, NIEHS/DNTP, NIH Chemical Genomics Center, and FDA designed to research, develop, validate and translate testing methods that characterize toxicity pathways, provided support for the biological plausibility of reported effects and often supported the mechanistic findings in the peer-reviewed literature (Thayer et al. 2011, 2012). While the overall workshop review of the existing literature supports the obesogen hypothesis, many research gaps were identified. These include: 1. deficiencies in human exposure data; 2. need for better biomarkers of exposure; 3. need for better understanding of the basic biology of critical cells; 4. information on how the biology that controls body weight and metabolic set points changes with life stage. The workshop group noted that non-monotonic dose response relationships are to be expected for environmental exposures and obesity and recognized the enormous complexity in this research overall (Thayer et al. 2011, 2012).

Despite the limitations of our study, it is the only human study utilizing correct temporality and detailed exposure information to provide evidence on the relationship between BMI and pesticide exposures for male pesticide applicators. On the heels of the NTP Workshop, our investigation may also spur additional epidemiologic research to shed further light on the relationship between pesticide exposures and BMI, particularly for individuals exposed to triazine herbicides. Future research should assess the relationship between pesticide exposure and BMI using anthropometrically measured BMI with detailed information on diet, exercise, and childhood exposures. Research on

groups of individuals exposed to triazine herbicides in non-agricultural settings and on women and children potentially exposed to triazines may also result from our findings. Our study will help to generate new hypotheses for both laboratory and epidemiologic research. Triazines are not insecticides, the pesticide class deems most likely to cause metabolic changes leading to weight gain. This may stimulate new and completely different hypotheses about how pesticides may effect human metabolism. In terms of epidemiologic studies, the first step would be to attempt to replicate these findings in other occupational settings. The low dose to which the general population is exposed creates a challenging opportunity for potential modeling using better defined exposure-response curves that look beyond the basic linear model.

Figure 5-1: Actual Median BMI by Age in AHS Males Compared to Median BMI by Age in NHANES III Males – Update to Hypothetical Results
(NHANES data and graph extracted from Roberts & Williamson 2002)



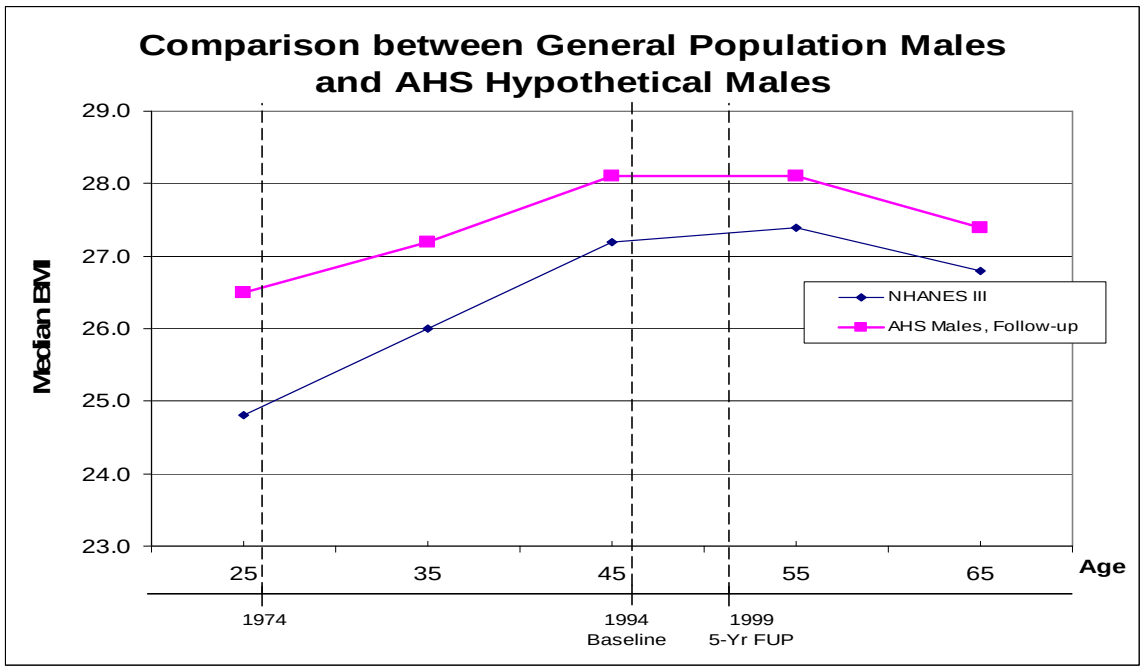


Table 5.1. Proportion of farmers who reported various activities by state at enrollment (Coble et al 2002)

Table 2. The percentage of farmers who reported various activities by state from the AHS enrollment questionnaire.

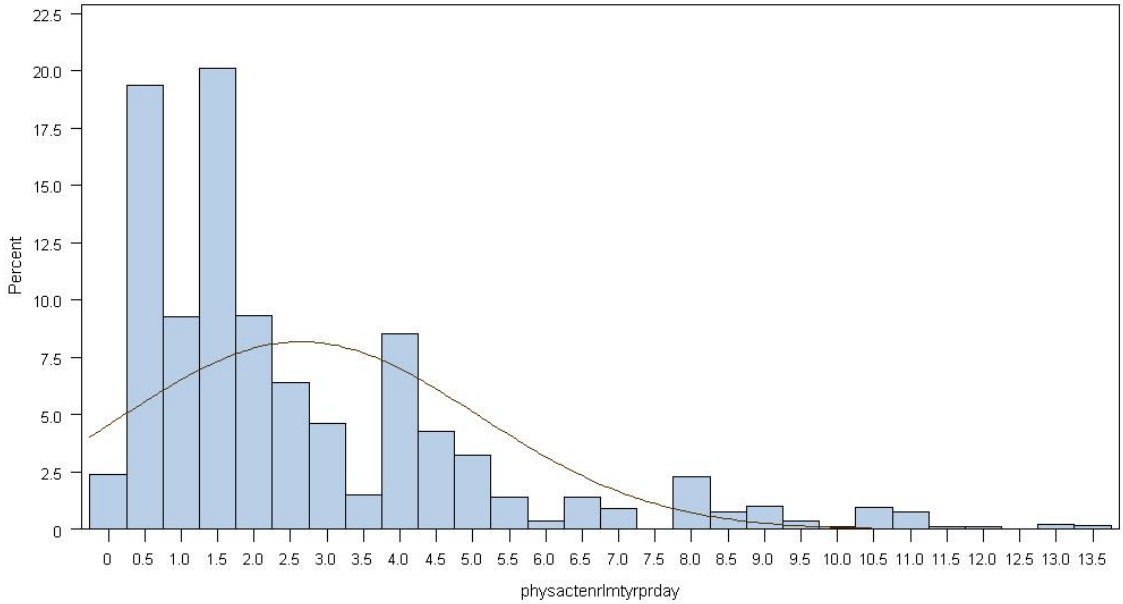
Which of the following activities do you perform at least once each year?	North Carolina (n=20,529) [%]	Iowa (n=31,598) [%]
Maintenance activities		
Paint	47	74
Weld	43	78
Repair pesticide application equipment	49	64
Repair engines	38	43
Replace asbestos brake linings	18	13
Crop handling		
Handle stored grain	32	86
Handle stored hay	36	64
Grind animal feed	17	59
Load or unload silage	6	29
Animal-related activities		
Perform veterinary services	25	65
Work in swine confinement areas	7	38
Butcher animals	14	13
Work in poultry confinement areas	6	2
Do not do any of above	7	1

Table 5.2. Proportion of farmers reporting exposures in nonfarm jobs by agent. (Coble et al 2002)

Table 8. Percentage of farmers reporting exposures in nonfarm jobs by agent.

Exposure agent	North Carolina (n=5,972) [%]	Iowa (n=8,739) [%]
Pesticides	7	7
Engine exhaust	20	21
Gasoline	16	15
Solvents	16	16
Welding fumes	16	16
Wood dust	14	8
Grain dust	4	10
Cotton dust	5	<1
Silica dust	5	4
Mineral dust	2	1
Asbestos	8	4
Lead solder	5	4
Lead	3	2
Cadmium	1	<1
Mercury	1	<1
Electroplating fumes	1	1
Other metals	4	3
Pneumatic drills	6	7
X-ray radiation	2	1
None of the above	21	15

Figure 5.2. Histograms for energy output at enrollment (physactenrlmtyrprday) and at follow-up (hrsliftprdy)



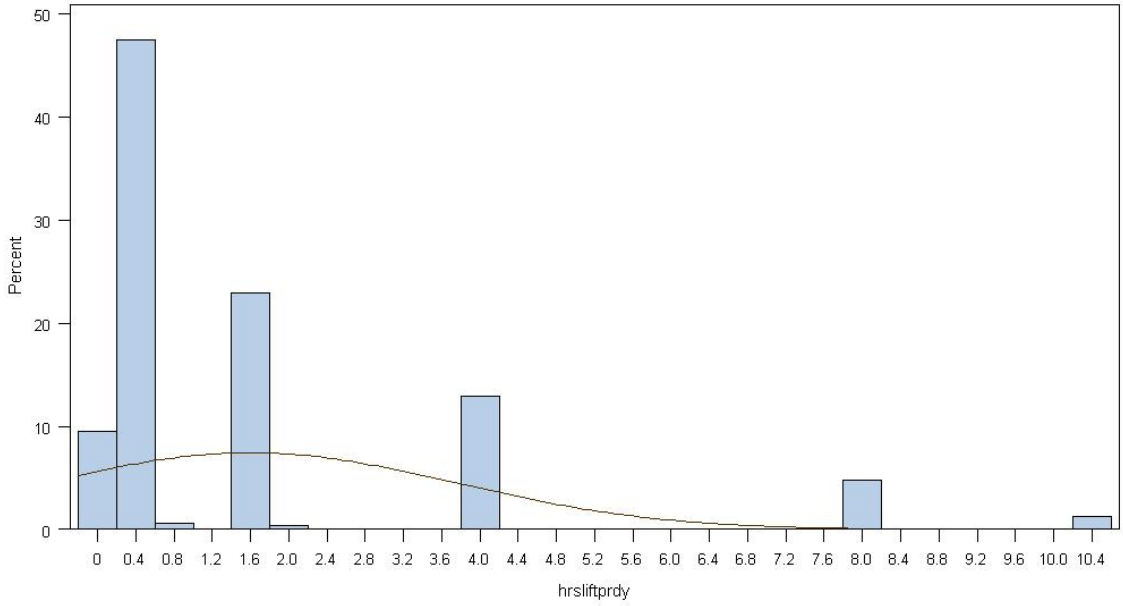
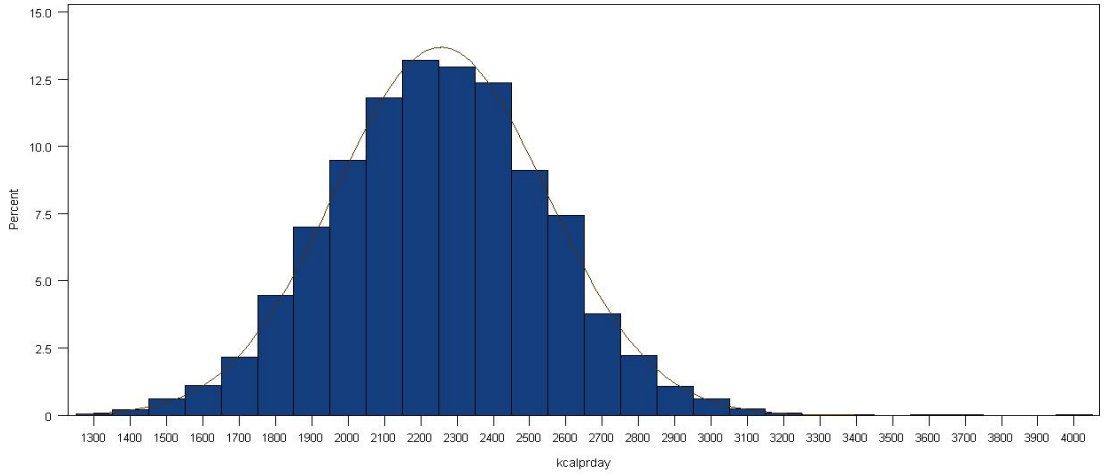


Figure 5.3. Histograms for energy input at enrollment (kcalprday) and at follow-up (food_energy___kcal)



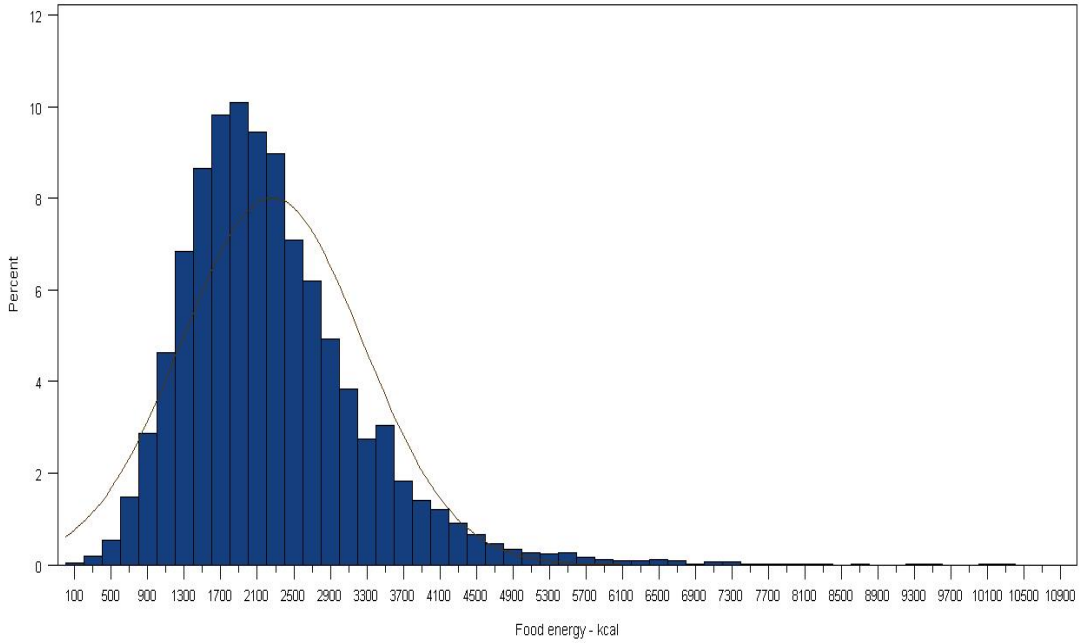
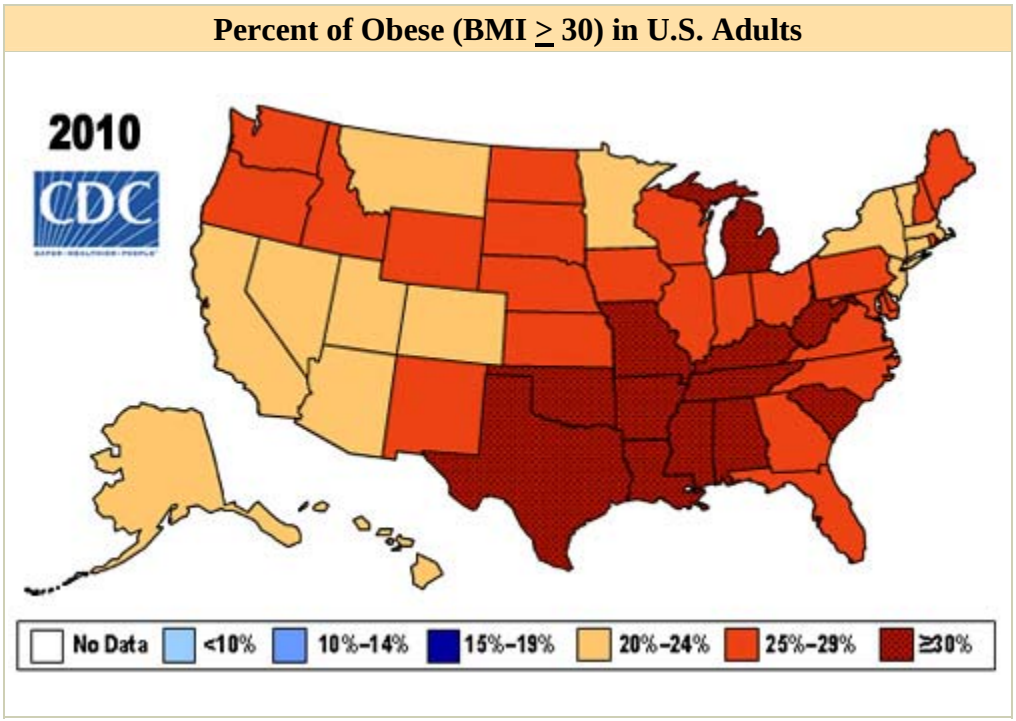
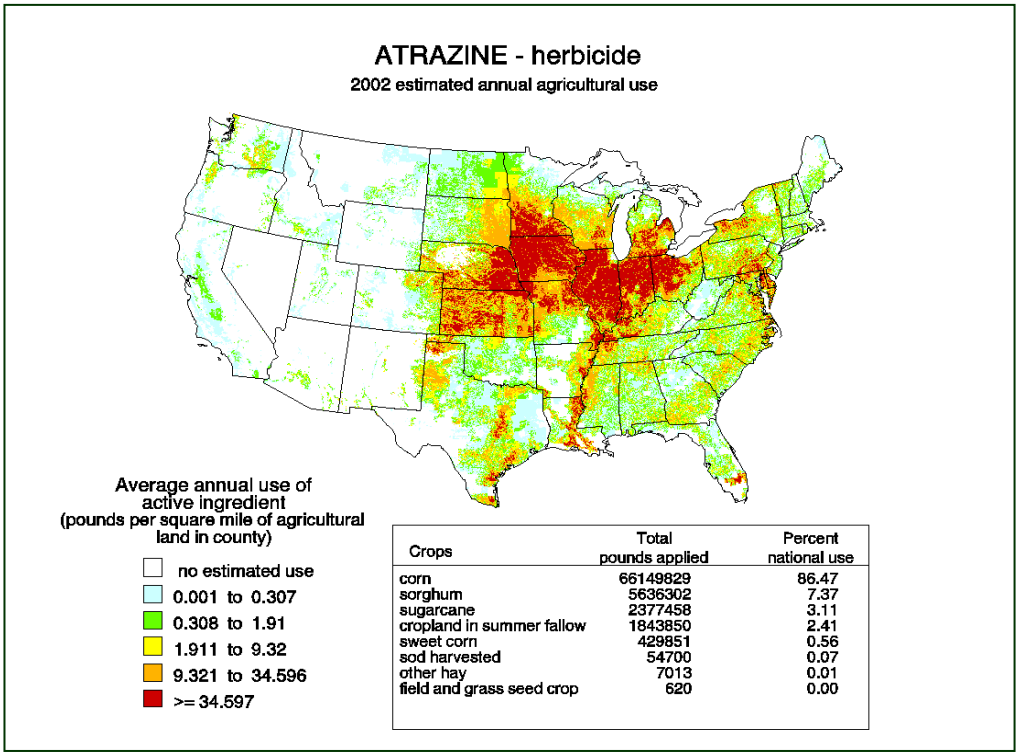


Figure 5.4. Percent of Obese in U.S. Adults by state, CDC, 2010; Usage estimates for atrazine by county in the U.S., USGS, 2002; from Lim et al. 2009





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APPENDIX

TABLES & FIGURES FOR CHAPTER 4

TABLE 4-1		
POPULATION NUMBERS FOR EACH SUBGROUP IN BMI STUDY		
all private applicators **= 9076**	private applicators excluding weight-related health conditions reported in Phase 1 **= 6952**	private applicators excluding weight-related health conditions reported in Phase 1 or Phase 2 **= 6367**
Iowa private applicators = **6754**	Iowa private applicators excluding weight-related health conditions reported in Phase 1 = **5245**	Iowa private applicators excluding weight-related health conditions reported in Phase 1 or Phase 2 = **4862**
North Carolina private applicators = **2322**	North Carolina applicators excluding weight-related health conditions reported in Phase 1 = **1707**	North Carolina private applicators excluding weight-related health conditions reported in Phase 1 or Phase 2 = **1505**

Table 4-2: Demographic Frequencies for Total Male Private Applicator Sample Aged 20 or More Years Versus Study Sample

TOTAL SUBJECTS: PARTICIPATED IN PHASE 1 ONLY OR BOTH PHASES (n=50641)			STUDY SAMPLE: COMPLETED PHASE 1, PHASE 2, AND DIET HISTORY WITH NUTRIENTS (n=9076)		
Variable	**Number**	**Percent**	**Variable**	**Number**	**Percent**
Age groups at phase 1	mean= 47.8	s.d.= 13.1	Age groups at phase 1	mean= 50.6	sd= 12.4
20-39 years	15843	31.3	20-39 years	2001	22.1
40-59 years	24573	48.5	40-59 years	4783	52.7
60-79 years	9998	19.7	60-79 years	2259	24.9
80+ years	227	0.5	80+ years	33	0.4
Age groups at phase 2	mean= 53.4	s.d.= 12.4	Age groups at phase 2	mean= 55.6	sd= 12.3
20-39 years	4657	14.4	20-39 years	992	10.9
40-59 years	17546	54.2	40-59 years	4606	50.8
60-79 years	9780	30.2	60-79 years	3346	36.9
80+ years	401	1.2	80+ years	132	1.5
Race at phase 1			Race at phase 1		
White	47906	97.1	White	8885	99.3
Black	1130	2.3	Black	49	0.6
Other	297	0.6	Other	18	0.2
Educational Level obtained at phase 1			Educational Level obtained at phase 1		
1-8 years through some high school	5017	10.4	1-8 years through some high school	578	6.6
high school grad/GED	23437	48.5	high school grad/GED	4188	47.5
1-3 years vocational education / some college	4452	9.2	1-3 years vocational education / some college	791	9.0
college graduate	8267	17.1	college graduate	1832	20.8
1+ years of graduate school	7204	14.9	1+ years of graduate school	1433	16.2
Marital Status, phase 1			Marital Status, phase 1		
Married / living as married	42630	84.6	married or living as married	7870	86.9
divorced, separated, widowed	2722	5.4	divorced, separated, widowed	369	4.1
never married	5041	10.0	never married	814	9.0
State at phase 1			State at phase 1		
Iowa	31261	61.7	Iowa	6754	74.4
North Carolina	19380	38.3	North Carolina	2322	25.6
Take-Home Enrollment Questionnaire, phase 1			Take-Home Enrollment Questionnaire, phase 1		
completed	21598	42.6	completed	8862	97.6
missing	29043	57.4	missing	214	2.4

Medical Exclusions at phase 1			Medical Exclusions at phase 1		
No weight-related health condition	42212	83.4	No weight-related health condition	6952	76.6
Weight-related health condition	8429	16.6	Weight-related health condition	2124	23.4
Medical Exclusions at phase 2			Medical Exclusions at phase 2		
No weight-related health condition	45527	89.9	No weight-related health condition	7576	83.5
Weight-related health condition	5114	10.1	Weight-related health condition	1500	16.5
Medical Exclusions at phase 1 or 2			Medical Exclusions at phase 1 or 2		
No weight-related health condition	39619	78.2	No weight-related health condition	6367	70.2
Weight-related health condition	11022	21.8	Weight-related health condition	2709	29.9
Smoked 100+ cigarettes over lifetime at phase 1			Smoked 100+ cigarettes over lifetime at phase 1		
No	25812	52.6	No	4975	55.6
Yes	23226	47.4	Yes	3967	44.4
Current smoker, phase 1			Current smoker, phase 1		
No	41120	84.1	No	8019	89.9
Yes	7764	15.9	Yes	902	10.1
Number of drinks/day over past year, phase 1			Number of drinks/day over past year, phase 1		
Didn't drink last year	15967	33.8	Didn't drink last year	2921	33.3
1 or 2 drinks	15177	32.1	1 or 2 drinks	4514	51.5
3 or 4 drinks	5972	12.7	3 or 4 drinks	1042	11.9
5-8 drinks	7206	15.3	5-8 drinks	245	2.8
9 or more drinks	2903	6.2	9 or more drinks	45	0.5
Frequency of drinking over past year, phase 1			Frequency of drinking over past year, phase 1		
Never	15967	33.8	Never	2920	33.2
< 1 time a month	7235	15.3	< one time a month	1540	17.5
1-3 times a month	7942	16.8	1-3 times a month	1543	17.5
1 time a week	5972	12.7	1 time a week	1051	12.0
2-4 times a week	7206	15.3	2-4 times a week	1227	14.0
almost every day	2311	4.9	almost every day	413	4.7
every day	592	1.3	every day	101	1.2

Table 4-3: Weight-related variables in total sample (50641) and study sample (9076)					
ALL PRIVATE APPLICATORS AGE 20 AND OLDER; PARTICIPATED IN PHASE 1 ONLY OR BOTH PHASES (n=50641)			**ALL PRIVATE APPLICATORS WHO COMPLETED PHASE 1, PHASE 2, AND DIET HISTORY WITH NUTRIENTS (n=9076)**		
Variable	**#**	**%**	**Variable**	**#**	**%**
Weight at enrollment based on phase 1 data	ẋ= 195.4	s.d.= 32.6	Weight at enrollment based on phase 1 data	ẋ= 195.0	s.d.= 31.9
90-149 pounds	898	4.2	90-149 pounds	346	3.9
150-169 pounds	3137	14.6	150-169 pounds	1349	15.2
170-189 pounds	5726	26.7	170-189 pounds	2401	27.0
190-209 pounds	4912	22.9	190-209 pounds	2013	22.6
210-229 pounds	3551	16.6	210-229 pounds	1489	16.7
230-269 pounds	2617	12.2	230-269 pounds	1084	12.2
270 or more pounds	599	2.8	270 or more pounds	219	2.5
Weight at 5-year follow-up, Phase 2 data	ẋ= 201.1	s.d.= 34.5	Weight at 5-year follow-up, Phase 2 data	ẋ= 198.5	s.d.= 33.4
90-149 pounds	967	3.0	90-149 pounds	296	3.3
150-169 pounds	3685	11.5	150-169 pounds	1179	13.0
170-189 pounds	7646	23.8	170-189 pounds	2236	24.7
190-209 pounds	7759	24.1	190-209 pounds	2241	24.7
210-229 pounds	5589	17.4	210-229 pounds	1504	16.6
230-269 pounds	5109	15.9	230-269 pounds	1289	14.2
270 or more pounds	1399	4.4	270 or more pounds	324	3.6
Height at enrollment based on phase 1 data	ẋ= 70.6	s.d.= 2.9	Height at enrollment based on phase 1 data	ẋ= 70.6	s.d.= 2.7
60-69 inches	6371	32.4	60-69 inches	2849	31.4
70-74 inches	11733	59.7	70-74 inches	5566	61.3
75-85 inches	1538	7.8	75-85 inches	661	7.3
Height at 5-year follow-up based on phase 2 data	ẋ= 70.8	s.d.= 2.7	Height at 5-year follow-up based on phase 2 data	ẋ= 70.6	s.d.= 2.6
60-69 inches	9223	28.7	60-69 inches	2879	31.7
70-74 inches	20730	64.4	70-74 inches	5542	61.1
75-85 inches	2233	6.9	75-85 inches	655	7.2
WHO Classifications for BMI at age 20 using phase 1 data	ẋ= 23.5	s.d.= 3.3	WHO Classifications for BMI at age 20 using phase 1 data	ẋ= 23.5	s.d.= 3.3
<16.00 = severe thinness	53	0.4	<16.00 = severe thinness	36	0.4
16.00-16.99 = moderate thinness	98	0.7	16.00-16.99 = moderate thinness	62	0.7

17.00-18.49 = mild thinness	439	3.2	17.00-18.49 = mild thinness	248	2.8
18.50-24.99 = normal range	9352	67.4	18.50-24.99 = normal range	6136	68.0
25.00-27.49 = overweight	2523	18.2	25.00-27.49 = overweight	1658	18.4
27.50-29.99 = pre-obese	835	6.0	27.50-29.99 = pre-obese	531	5.9
30.00-34.99=obese class I	495	3.6	30.00-34.99= obese class I	305	3.4
35.00-39.99=obese class II	73	0.5	35.00-39.99=obese class II	39	0.4
>=40.00 = obese class III	10	0.1	>=40.00 = obese class III	8	0.1
WHO Classifications for BMI at enrollment using phase 1 data	ẋ= 27.5	s.d.= 4.0	WHO Classifications for BMI at enrollment using phase 1 data	ẋ= 27.4	s.d.= 3.9
<16.00 = severe thinness	14	0.1	<16.00 = severe thinness	8	0.1
16.00-16.99 = moderate thinness	8	0.1	16.00-16.99 = moderate thinness	6	0.1
17.00-18.49 = mild thinness	27	0.2	17.00-18.49 = mild thinness	17	0.2
18.50-24.99 = normal range	3520	25.7	18.50-24.99 = normal range	2338	26.3
25.00-27.49 = overweight	4097	29.9	25.00-27.49 = overweight	2654	29.8
27.50-29.99 = pre-obese	2853	20.8	27.50-29.99 = pre-obese	1863	20.9
30.00-34.99 = obese class I	2603	19.0	30.00-34.99= obese class I	1647	18.5
35.00-39.99 = obese class II	483	3.5	35.00-39.99=obese class II	305	3.4
>=40.00 = obese class III	119	0.9	>=40.00 = obese class III	63	0.7
WHO Classifications for BMI at 5-yr FUP using phase 2 data	ẋ= 28.0	s.d.= 4.2	WHO Classifications for BMI at 5-yr FUP using phase 2 data	ẋ= 27.9	s.d.= 4.1
<16.00 = severe thinness	6	0.0	<16.00 = severe thinness	4	0.0
16.00-16.99 = moderate thinness	5	0.0	16.00-16.99 = moderate thinness	2	0.0
17.00-18.49 = mild thinness	22	0.2	17.00-18.49 = mild thinness	11	0.1
18.50-24.99 = normal range	3096	22.1	18.50-24.99 = normal range	2044	22.5
25.00-27.49 = overweight	3937	28.1	25.00-27.49 = overweight	2558	28.2
27.50-29.99 = pre-obese	3152	22.5	27.50-29.99 = pre-obese	2067	22.8
30.00-34.99 = obese class I	3014	21.5	30.00-34.99= obese class I	1916	21.1
35.00-39.99 = obese class II	620	4.4	35.00-39.99=obese class II	381	4.2
>=40.00 = obese class III	157	1.1	>=40.00 = obese class III	88	1.0

change in BMI class from age 20 to enrollment, phase 1 data			change in BMI class from age 20 to enrollment, phase 1 data		
-1 = reduced 1-- 5 categories	318	2.3	-1 = reduced 1 - 5 categories	217	2.4
0 = no change in category	4108	30.2	0 = no change in category	2727	30.8
1 = increased one category	4891	36.0	1 = increased one category	3158	35.7
2 = increased 2 categories	2862	21.1	2 = increased 2 categories	1873	21.2
3 = increased 3 categories	1198	8.8	3 = increased 3 categories	752	8.5
4 = increased 4 - 6 categories	216	1.5	4 = increased 4 - 6 categories	124	1.3
positive change in BMI from age 20 to enrollment, phase 1 data			positive change in BMI from age 20 to enrollment, phase 1 data		
No	4426	32.6	No	2944	33.3
Yes	9167	67.4	Yes	5907	66.7
change in BMI class from age 20 to 5-yr follow-up based on phase 1 and phase 2 data			change in BMI class from age 20 to 5-yr follow-up based on phase 1 and phase 2 data		
-1 = reduced 1 - 6 categories	327	2.4	-1 = reduced 1 - 6 categories	220	2.5
0 = no change in category	3559	25.7	0 = no change in category	2348	26.0
1 = increased one category	4754	34.3	1 = increased one category	3086	34.2
2 = increased 2 categories	3337	24.1	2 = increased 2 categories	2182	24.2
3 = increased 3 categories	1579	11.4	3 = increased 3 categories	998	11.1
4 = increased 4 - 7 categories	309	2.2	4 = increased 4 - 7 categories	184	2.1
positive change in BMI from age 20 to 5-yr FUP based on phase 1 and phase 2 data			positive change in BMI from age 20 to 5-yr FUP based on phase 1 and phase 2 data		
No	3886	28.0	No	2568	28.5
Yes	9979	72.0	Yes	6450	71.5
change in BMI class from enrollment to 5-yr follow-up based on phase 1 & phase 2 data			change in BMI class from enrollment to 5-yr follow-up based on phase 1 & 2 data		
-2= reduced 2 - 8 categories	134	0.9	-2=reduced 2-8 categories	93	1.0

-1 = reduced 1 category	1225	8.9	-1 = reduced 1 category	775	8.7
0 = no change in category	9138	66.7	0 = no change in category	5946	66.8
1 = increased one category	2953	21.5	1 = increased one category	1914	21.5
2 = increased 2 - 6 categories	261	1.9	2 = increased 2 - 6 categories	168	1.8
positive change in BMI from enrollment to 5-yr follow-up based on phase 1 & phase 2 data			positive change in BMI from enrollment to 5-yr follow-up based on phase 1 & 2 data		
No	10497	76.6	No	6814	76.6
Yes	3214	23.4	Yes	2082	23.4
consistently overweight or obese over 3 time periods based on Phase 1 & Phase 2 data			consistently overweight or obese over 3 time periods based on Phase 1 & 2 data		
No	9902	72.9	No	6475	73.2
Yes	3678	27.1	Yes	2371	26.8

TABLE 4-4					
BMI UNIVARIABLES AND CORRELATIONS FOR 3 TIME PERIODS					
BMI at Age 20		**BMI at Enrollment**		**BMI at 5-year follow-up**	
ALL MALE PRIVATE APPLICATORS		ALL MALE PRIVATE APPLICATORS		ALL MALE PRIVATE APPLICATORS	
Number	9023	Number	8900	Number	9069
Mean (SD)	23.5 (3.3)	Mean (SD)	27.4 (3.9)	Mean (SD)	27.9 (4.1)
Range	10.2 - 43.3	Range	13.1 - 56.3	Range	15.4 - 57.2
Percentiles		Percentiles		Percentiles	
25%	21.3	25%	24.7	25%	25.1
50%	23.1	50%	27.0	50%	27.4
75%	25.1	75%	29.7	75%	30.2
Missing Values	53 (0.6%)	Missing Values	176 (1.9%)	Missing Values	7 (0.1%)
Correlation, BMI2	0.57 (p<.0001)	Correlation, BMI1	0.58 (p<.0001)	Correlation, BMI1	0.53 (p<.0001)
Correlation, BMI3	0.53 (p<.0001)	Correlation, BMI3	0.90 (p<.0001)	Correlation, BMI2	0.90 (p<.0001)

TABLE 4-5 ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY				
UNIVARIABLE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, PESTICIDE FUNCTIONAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, MISSING VALUES EXCLUDED FROM ZERO SCORES				
Pesticide Class	**Number of Subjects**	**Percent**	**mean (sd)**	**quantiles: 25%, 50%, 75%**
Insecticides				
non-exposed	368	4.1		
exposed	6382	70.3	626.9 (1794.27)	56.7, 184.5, 532.8
missing	2326	25.6		
Fumigants				
non-exposed	6483	71.4		
exposed	1613	17.8	106.7 (577.7)	0, 0, 0
missing	980	10.8		
Fungicides				
non-exposed	5441	60.0		
exposed	2183	24.1	208.5 (1307.0)	0, 0, 1.4
missing	1452	16.0		
Herbicides				
non-exposed	65	0.7		
exposed	6793	74.9	1651.7 (3563.3)	280.1, 730.9, 1714.2
missing	2218	24.4		
Herbicides less triazines				
non-exposed	77	0.9	1578.6 (3602.3)	244.5, 664.9, 1598.7
exposed	6891	75.9		
missing	2108	23.2		

TABLE 4-5 ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY				
UNIVARIABLE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT FOLLOWUP, PESTICIDE FUNCTIONAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 2, MISSING VALUES EXCLUDED FROM ZERO SCORES				
Pesticide Class	**Number of subjects**	**Percent exposed & unexposed; percent missing of total subjects**	**mean (sd)**	**quantiles: 25%, 50%, 75%**
Insecticides				
Non-exposed	316	3.5		
Exposed	6453	71.1	654.0 (1812.3)	67.0, 201.5, 560.8
missing	2307	25.4		
Fumigants				
non-exposed	6476	71.4		
exposed	1620	17.9	107.3 (578.3)	0, 0, 0
missing	980	10.8		
Fungicides				
non-exposed	5329	58.7		
exposed	2322	25.6	222.5 (1342.7)	0, 0, 1.9
missing	1425	15.7		
Herbicides				
non-exposed	45	0.5		
exposed	6906	76.1	1767.3 (3619.8)	344.9, 837.3, 1864.2
missing	2125	23.4		
Herbicides less triazines				
non-exposed	55	0.6		
exposed	7003	77.2	1691.4 (3645.2)	315.5, 774.3, 1760.4
missing	2018	22.2		

TABLE 4-5 ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY				
UNIVARIABLE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, PESTICIDE CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, MISSING VALUES EXCLUDED FROM ZERO SCORES				
Pesticide Class	**Number of subjects**	**Percent**	**mean (sd)**	**quantiles: 25%, 50%, 75%**
Organochlorines				
non-exposed	3995	44.0		
exposed	4006	44.1	339.8 (1934.2)	0, 3.0, 160.7
missing	1075	11.8		
Organophosphates				
non-exposed	666	7.3		
exposed	6562	72.3	847.3 (2576.0)	69.0, 234.1, 686.7
missing	1848	20.4		
Organothiophosphates				
non-exposed	1479	16.3		
exposed	5996	66.1	727.5 (2069.5)	33.3, 196.9, 664.4
missing	1601	17.6		
Carbamates				
non-exposed	3174	35.0		
exposed	4548	50.1	676.9 (2477.5)	0, 49.6, 332.2
missing	1354	14.9		
Phenoxy herbicides				
non-exposed	1221	13.5		
exposed	6984	77.0	2371.1 (7440.5)	132.4, 676.7, 2141.3
missing	871	9.5		
Triazine herbicides				
nonexposed	1298	14.3		
exposed	6667	73.5	2130.5 (5201.4)	140.0, 678.3, 2135.0
missing	1111	12.2		

TABLE 4-5 ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY				
UNIVARIATE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT FOLLOW-UP, PESTICIDE CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 2, MISSING VALUES EXCLUDED FROM ZERO SCORES				
Pesticide Class	**Number of subjects**	**Percent**	**mean (sd)**	**quantiles: 25%, 50%, 75%**
Organochlorines				
non-exposed	3953	43.6		
exposed	4048	44.6	341.5 (1934.3)	0, 6.0, 168.8
missing	1075	11.8		
Organophosphates				
non-exposed	608	6.7		
exposed	6637	73.1	876.7 (2599.7)	78.8, 254.4, 721.9
missing	1831	20.2		
Organothiophosphates				
non-exposed	1411	15.6		
exposed	6070	66.9	748.5 (2082.0)	38.1, 215.3, 698.3
missing	1595	17.6		
Carbamates				
non-exposed	3034	33.4		
exposed	4705	51.8	712.8 (2519.3)	0, 60.0, 380.6
missing	1337	14.7		
Phenoxy herbicides				
non-exposed	1041	11.5		
exposed	7213	79.5	2497.4 (7528.8)	195.1, 798.9, 2333.3
missing	822	9.1		
Triazine herbicides				
nonexposed	1180	13.0		
exposed	6799	74.9	2239.2 (5250.2)	186.7, 780.0, 2295.7
missing	1097	12.1		

TABLE 4-5
ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY

UNIVARIABLE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, PESTICIDE FUNCTIONAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, ZERO SCORES INCLUDED FOR MISSING VALUES

Pesticide Class	Number of Subjects	Percent	mean (sd)	quantiles: 25%, 50%, 75%
Insecticides				
Non-exposed	959	10.6	684.0 (2074.6)	46.8, 182.5, 567.6
Exposed	8117	89.4		
Fumigants				
non-exposed	7381	81.3	101.1 (563.9)	0, 0, 0
exposed	1695	18.7		
Fungicides				
non-exposed	6538	72.0	217.0 (1317.7)	0, 0, 1.4
exposed	2538	28.0		
Herbicides				
non-exposed	541	6.0	1569.1 (3509.6)	207.8, 642.2, 1619.9
exposed	8535	94.0		
Herbicides less triazines				
non-exposed	568	6.3	1485.2 (3511.9)	181.6, 583.3, 1493.0
exposed	8508	93.7		

TABLE 4-5 ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY				
UNIVARIABLE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT FOLLOW-UP, PESTICIDE FUNCTIONAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 2, ZERO SCORES INCLUDED FOR MISSING VALUES				
Pesticide Class	**Number of subjects**	**Percent**	**mean (sd)**	**quantiles: 25%, 50%, 75%**
Insecticides				
Non-exposed	775	8.5	645.2 (1898.4)	52.3, 184.5, 541.7
Exposed	8301	91.5		
Fumigants				
non-exposed	7371	81.2	101.6 (564.4)	0, 0, 0
exposed	1705	18.8		
Fungicides				
non-exposed	6390	70.4	229.6 (1350.7)	0, 0, 1.9
exposed	2686	29.6		
Herbicides				
non-exposed	285	3.1	1672.9 (3551.2)	269.0, 745.8, 1764.8
exposed	8791	96.9		
Herbicides less triazines				
non-exposed	568	6.3	1589.3 (3555.3)	238.0, 687.3, 1647.3
exposed	8508	93.7		

TABLE 4-5
ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY

UNIVARIABLE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, PESTICIDE CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, ZERO SCORES INCLUDED FOR MISSING VALUES

Pesticide Class	Number of subjects	Percent	mean (sd)	quantiles: 25%, 50%, 75%
Organochlorines				
non-exposed	4717	52.0	338.5 (2014.3)	0, 0, 150.4
exposed	4359	48.0		
Organophosphates				
non-exposed	1331	14.7	793.6 (2537.9)	43.9, 197.1, 625.2
exposed	7745	85.3		
Organothiophosphates				
non-exposed	2265	25.0	675.8 (1989.2)	3.1, 162.9, 597.3
exposed	6811	75.0		
Carbamates				
non-exposed	4098	45.2	658.2 (2438.5)	0, 26.3, 297.0
exposed	4978	54.8		
Phenoxy herbicides				
non-exposed	1824	20.1	2286.8 (7720.3)	77.8, 585.6, 2000.4
exposed	7252	79.9		
Triazine herbicides				
non-exposed	1939	21.4	1988.3 (5088.0)	73.9, 562.7, 1955.5
exposed	7137	78.6		

TABLE 4-5
ALL PRIVATE APPLICATORS COMPLETING PHASE 1, PHASE 2, & DIET HISTORY

UNIVARIATE STATISTICS FOR INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT FOLLOW-UP, PESTICIDE CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 2, ZERO SCORES INCLUDED FOR MISSING VALUES

Pesticide Class	Number of subjects	Percent	mean (sd)	quantiles: 25%, 50%, 75%
Organochlorines				
non-exposed	4674	51.5	340.1 (2014.5)	0, 0, 154.3
exposed	4402	48.5		
Organophosphates				
non-exposed	1189	13.1	820.9 (2559.3)	55.1, 215.4, 663.9
exposed	7887	86.9		
Organothiophosphates				
non-exposed	2138	23.6	696.4 (2001.5)	13.8, 178.0, 630.3
exposed	6938	76.4		
Carbamates				
non-exposed	3870	42.6	695.7 (2482.2)	0, 45.9, 351.9
exposed	5206	57.4		
Phenoxy herbicides				
non-exposed	1506	16.6	2420.4 (7817.8)	127.7, 701.1, 2179.1
exposed	7570	83.4		
Triazine herbicides				
non-exposed	1729	19.1	2090.9 (5138.2)	110.0, 655.4, 2100.3
exposed	7347	81.0		

TABLE 4-6: AGE-ADJUSTED CORRELATION COEFFICIENTS ASSOCIATING PESTICIDE CLASSES AND BMI; MISSING VALUES EXCLUDED FROM PESTICIDE ZERO SCORES

CORRELATIONS BETWEEN BMI AT ENROLLMENT (CROSS-SECTIONAL ANALYSIS) OR AT 5-YEAR FOLLOWUP (PROSPECTIVE ANALYSIS) WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, FUNCTIONAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, MISSING VALUES EXCLUDED FROM ZERO SCORES

	BMI2, enrollment (p value)	BMI3, 5-year follow-up (p value)
insecticides	**0.04 (0.0098)**	0.03 (0.0244)
fumigants	-0.01 (0.6906)	-0.01 (0.5663)
fungicides	-0.01 (0.4539)	-0.02 (0.1183)
herbicides	**0.08 (<0.0001)**	**0.07 (<0.0001)**
herbicides less triazines	**0.07 (<0.0001)**	**0.06 (p<0.0001)**

CORRELATIONS BETWEEN BMI AT 5-YEAR FOLLOW-UP WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT FOLLOW-UP (CROSS-SECTIONAL ANALYSIS), ALGORITHM 1, VERSION 2, PHASE 2, MISSING VALUES EXCLUDED FROM ZERO SCORES

	BMI2, enrollment, not relevant to phase 2 data	BMI3, 5-year follow-up (p value)
insecticides		0.02 (0.0875)
fumigants		-0.01 (0.6158)
fungicides		-0.03 (0.0116)
herbicides		**0.06 (<0.0001)**
herbicides less triazines		**0.06 (<0.0001)**

CORRELATIONS BETWEEN BMI AT ENROLLMENT (CROSS-SECTIONAL ANALYSIS) OR 5-YEAR FOLLOWUP (PROSPECTIVE ANALYSIS) WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, MISSING VALUES EXCLUDED FROM PESTICIDE ZERO SCORES

	BMI2, enrollment (p value)	BMI3, 5-year follow-up (p value)
organochlorines	0.02 (0.1256)	0.01 (0.4137)
organophosphates	**0.05 (<0.0001)**	**0.05 (0.0002)**
organothiophosphates	**0.05 (<0.0001)**	**0.04 (0.0005)**
carbamates	-0.003 (0.7861)	-0.02 (0.1911)
phenoxy herbicides	**0.07 (<0.0001)**	**0.06 (<0.0001)**
triazine herbicides	**0.08 (<0.0001)**	**0.07 (<0.0001)**

CORRELATIONS BETWEEN BMI AT 5-YEAR FOLLOW-UP WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT 5-YEAR FOLLOW-UP (CROSS-SECTIONAL ANALYSIS). CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 2, MISSING VALUES EXCLUDED FROM PESTICIDE ZERO SCORES

	BMI2, enrollment, not relevant to phase 2 data	BMI3, 5-year follow-up (p value)
organochlorines		0.01 (0.4137)
organophosphates		**0.05 (0.0002)**
organothiophosphates		**0.04 (0.0005)**
carbamates		-0.02 (0.1911)
phenoxy herbicides		**0.06 (<0.0001)**
triazine herbicides		**0.07 (<0.0001)**

TABLE 4-6: AGE-ADJUSTED CORRELATION COEFFICIENTS ASSOCIATING PESTICIDE CLASSES AND BMI; MISSING VALUES AS ZERO PESTICIDE SCORES INCLUDED		
CORRELATIONS BETWEEN BMI AT ENROLLMENT (CROSS-SECTIONAL ANALYSIS) OR AT 5-YEAR FOLLOWUP (PROSPECTIVE ANALYSIS) WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, FUNCTIONAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, MISSING VALUES INCLUDED AS ZERO SCORES		
	BMI2, enrollment (p value)	**BMI3, 5-year follow-up (p value)**
insecticides	**0.03 (0.0035)**	**0.03 (0.0099)**
fumigants	-0.01 (0.3288)	-0.01 (0.2646)
fungicides	-0.01 (0.4353)	-0.02 (0.0833)
herbicides	**0.08 (<0.0001)**	**0.08 (<0.0001)**
herbicides less triazines	**0.08 (<0.0001)**	**0.07 (<0.0001)**
CORRELATIONS BETWEEN BMI AT 5-YEAR FOLLOW-UP WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT FOLLOW-UP (CROSS-SECTIONAL ANALYSIS), ALGORITHM 1, VERSION 2, PHASE 2, MISSING VALUES AS ZERO PESTICIDE SCORES INCLUDED		
	BMI2, enrollment, not relevant to phase 2 data	**BMI3, 5-year follow-up (p value)**
insecticides		0.02 (0.0462)
fumigants		-0.01 (0.3015)
fungicides		-0.02 (0.0190)
herbicides		**0.07 (<0.0001)**
herbicides less triazines		**0.07 (<0.0001)**
CORRELATIONS BETWEEN BMI AT ENROLLMENT (CROSS-SECTIONAL ANALYSIS) OR 5-YEAR FOLLOWUP (PROSPECTIVE ANALYSIS) WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT ENROLLMENT, CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 1, MISSING VALUES AS ZERO PESTICIDE SCORES INCLUDED		
	BMI2, enrollment (p value)	**BMI3, 5-year follow-up (p value)**
organochlorines	0.02 (0.0500)	0.02 (0.1186)
organophosphates	**0.04 (<0.0001)**	**0.04 (<0.0001)**
organothiophosphates	**0.04 (<0.0001)**	**0.05 (<0.0001)**
carbamates	-0.003 (0.7776)	-0.01 (0.2529)
phenoxy herbicides	**0.07 (<0.0001)**	**0.06 (<0.0001)**
triazine herbicides	**0.08 (<0.0001)**	**0.08 (<0.0001)**
CORRELATIONS BETWEEN BMI AT 5-YEAR FOLLOW-UP WITH PESTICIDE INTENSITY WEIGHTED CUMULATIVE EXPOSURE DAYS AT 5-YEAR FOLLOW-UP (CROSS-SECTIONAL ANALYSIS). CHEMICAL CLASSES, ALGORITHM 1, VERSION 2, PHASE 2, MISSING VALUES AS PESTICIDE ZERO SCORES INCLUDED		
	BMI2, enrollment, not relevant to phase 2 data	**BMI3, 5-year follow-up (p value)**
organochlorines		0.01 (0.1740)
organophosphates		**0.04 (<0.0001)**
organothiophosphates		**0.04 (<0.0001)**
carbamates		-0.02 (0.0639)
phenoxy herbicides		**0.06 (<0.0001)**
triazine herbicides		**0.07(<0.0001)**

SUMMARY TABLE 4-7a								
MULTIPLE REGRESSION, UNADJUSTED								
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT ENROLLMENT								
DEPENDENT VARIABLE: BMI AT ENROLLMENT; INDEPENDENT VARIABLE: CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1, MISSING VALUES EXCLUDED FROM ZERO SCORES								
Number of subjects	**F value**	**p-value**	**R^2**	**Variable: Functional class, intens-wtd cum. exp. days**	**Parameter Est.: β = chg in BMI (kg/m^2) per day**	**Std Error**	**t Value**	**p-value**
6642	2.48	0.1153	0.0004	Insecticide cumulative exposure	0.00004193	0.00002663	1.57	0.1153
7944	1.40	0.2360	0.0002	Fumigant cumulative exposure	0.00009003	0.00007596	1.19	0.2360
7497	0.37	0.5430	0.0000	Fungicide cumulative exposure	-0.00002079	0.00003417	-0.61	0.5430
6752	13.16	**0.0003**	0.0019	Herbicide cumulative exposure	0.00004832	0.00001332	3.63	**0.0003**
6860	10.32	**0.0013**	0.0015	Herbicides less triazines cumulative exposure	0.00004194	0.00001306	3.21	**0.0013**
				Variable: Chemical class, intens-wtd cum. exp. days				
7860	8.24	**0.0041**	0.0010	Organochlorine cumulative exposure	0.00006551	0.00002282	2.87	**0.0041**
7107	1.39	0.2391	0.0002	Organophosphate cumulative exposure	0.00002101	0.00001784	1.18	0.2391
7344	8.05	**0.0046**	0.0011	Organothiophosphate cumulative exposure	0.00006188	0.00002181	2.84	**0.0046**
7593	0.89	0.3444	0.0001	Carbamate cumulative exposure	0.00001741	0.00001841	0.95	0.3444
8056	9.52	**0.0020**	0.0012	Phenoxy herbicide cumulative exposure	0.00001801	0.00000584	3.08	**0.0020**
7829	38.98	**<0.0001**	0.0050	Triazine herbicide cumulative exposure	0.00005307	0.00000850	6.24	**<0.0001**

SUMMARY TABLE 4-7a									
MULTIPLE REGRESSION, UNADJUSTED									
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT ENROLLMENT									
DEPENDENT VARIABLE: BMI AT ENROLLMENT; INDEPENDENT VARIABLE: CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1, MISSING VALUES INCLUDED AS ZERO SCORES									
Number of subjects	**F value**	**p-value**	**R^2**	**Variable: Functional class, intens-wtd cum. exp. days**	**Parameter Est.: β = chg in BMI (kg/m^2) per day**	**Std Error**	**t Value**	**p-value**	**PERCENT DIFFERENCE WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**
8901	2.27	0.1323	0.0003	Insecticide cumulative exposure	0.00003024	0.00002009	1.51	0.1323	16.19786615
8901	0.49	0.4855	0.0001	Fumigant cumulative exposure	0.00005135	0.00007362	0.70	0.4855	27.35889093
8901	0.25	0.6196	0.0000	Fungicide cumulative exposure	-0.00001561	0.00003145	-0.50	0.6196	14.23076923
8901	25.85	**<0.0001**	0.0029	Herbicide cumulative exposure	0.00006007	0.00001181	5.08	**<0.0001**	-10.84048344
8901	17.72	**<0.0001**	0.0020	Herbicides less triazines cumulative exposure	0.00004967	0.00001180	4.21	**<0.0001**	-8.437943456
				Variable: Chemical class, intens-wtd cum. exp. days					
8901	6.65	**0.0099**	0.0007	Organochlorine cumulative exposure	0.00005329	0.00002066	2.58	**0.0099**	10.28619529
8901	0.83	0.3636	0.0001	Organophosphate cumulative exposure	0.00001485	0.00001635	0.91	0.3636	17.17791411
8901	8.22	**0.0041**	0.0009	Organothiophosphate cumulative exposure	0.00005978	0.00002085	2.87	**0.0041**	1.726121979
8901	0.25	0.6186	0.0000	Carbamate cumulative exposure	0.00000867	0.00001741	0.50	0.6186	33.51226994
8901	6.11	0.0135	0.0007	Phenoxy herbicide cumulative exposure	0.00001328	0.00000538	2.47	0.0135	15.11665069
8901	42.83	**<0.0001**	0.0048	Triazine herbicide cumulative exposure	0.00005366	0.00000820	6.54	**<0.0001**	-0.552796777

SUMMARY TABLE 4-7b
MULTIPLE REGRESSION, UNADJUSTED
PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 & BMI AT FOLLOW-UP
DEPENDENT VARIABLE: BMI AT FOLLOW-UP; INDEPENDENT VARIABLE: CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1, MISSING VALUES EXCLUDED AS ZERO SCORES

Number of subjects	F value	p-value	R^2	Variable: Functional class, intens-wtd cum. exp. days	Parameter Est.: β = chg in BMI (kg/m2) per day	Std Error	t Value	p-value
6749	1.67	0.1963	0.0002	Insecticide cumulative exposure	0.00003519	0.00002793	1.29	0.1963
8093	1.25	0.2637	0.0002	Fumigant cumulative exposure	0.00008790	0.00007864	1.12	0.2637
7622	1.07	0.3017	0.0001	Fungicide cumulative exposure	-0.00003641	0.00003525	-1.03	0.3017
6856	15.45	**<0.0001**	0.0022	Herbicide cumulative exposure	0.00005432	0.00001382	3.93	**<0.0001**
6966	12.11	**0.0005**	0.0017	Herbicides less triazines cumulative exposure	0.00004720	0.00001356	3.48	**0.0005**
				Variable: Chemical class, intens-wtd cum. exp. days				
7998	8.13	**0.0044**	0.0010	Organochlorine cumulative exposure	0.00006701	0.00002351	2.85	**0.0044**
7227	2.08	0.1497	0.0003	Organophosphate cumulative exposure	0.00002666	0.00001851	1.44	0.1497
7473	7.10	**0.0077**	0.0009	Organothiophosphate cumulative exposure	0.00006044	0.00002268	2.66	**0.0077**
7720	0.00	0.9685	0.0000	Carbamate cumulative exposure	0.00000073	0.00001847	0.04	0.9685
8201	6.75	**0.0094**	0.0008	Phenoxy herbicide cumulative exposure	0.00001572	0.00000605	2.60	**0.0094**
7962	36.00	**<0.0001**	0.0045	Triazine herbicide cumulative exposure	0.00005239	0.00000873	6.00	**<0.0001**

SUMMARY TABLE 4-7b									
MULTIPLE REGRESSION, UNADJUSTED									
PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 & BMI AT FOLLOW-UP									
DEPENDENT VARIABLE: BMI AT FOLLOW-UP; INDEPENDENT VARIABLE: CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1, MISSING VALUED INCLUDED AS ZERO SCORES									
Number of subjects	**F value**	**p-value**	**R^2**	**Variable: Functional class, intens-wtd cum. exp. days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Std Error**	**t Value**	**p-value**	**PERCENT DIFFERENCE WHEN EXCLUDING VERSUS INCLUDING MISSING VALUES AS ZERO SCORES**
9071	2.62	0.1054	0.0003	Insecticide cumulative exposure	0.00003362	0.00002076	1.62	0.1054	2.28164511
9071	0.50	0.4785	0.0001	Fumigant cumulative exposure	0.00005413	0.00007637	0.71	0.4785	23.7766669
9071	0.35	0.5541	0.0000	Fungicide cumulative exposure	-0.00001934	0.00003269	-0.59	0.5541	30.61883408
9071	24.53	**<0.0001**	0.0027	Herbicide cumulative exposure	0.00006070	0.00001226	4.95	**<0.0001**	-5.546861415
9071	16.79	**<0.0001**	0.0018	Herbicides less triazines cumulative exposure	0.0000502	0.00001225	4.10	**<0.0001**	-3.080082136
				Variable: Chemical class, intens-wtd cum. exp. days					
9071	6.58	0.0103	0.0007	Organochlorine cumulative exposure	0.00005485	0.00002138	2.57	0.0103	9.978664041
9071	2.22	0.1359	0.0002	Organophosphate cumulative exposure	0.00002531	0.00001697	1.49	0.1359	2.597652492
9071	7.94	**0.0048**	0.0009	Organothiophosphate cumulative exposure	0.00006100	0.00002164	2.82	**0.0048**	-0.46113307
9071	0.05	0.8183	0.0000	Carbamate cumulative exposure	-0.00000406	0.00001766	-0.23	0.8183	-143.8438438
9071	3.88	0.0489	0.0004	Phenoxy herbicide cumulative exposure	0.00001099	0.00000558	1.97	0.0489	17.70872332
9071	40.39	**<0.0001**	0.0044	Triazine herbicide cumulative exp	0.00005368	0.00000845	6.36	**<0.0001**	-1.216177996

SUMMARY TABLE 4-7c								
MULTIPLE REGRESSION, UNADJUSTED								
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 2 & BMI AT FOLLOW-UP								
DEPENDENT VARIABLE: BMI AT FOLLOW-UP; INDEPENDENT VARIABLE: CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 2, MISSING VALUES EXCLUDED FROM ZERO SCORES								
# subjects	**F value**	**p-value**	**R^2**	**Variable: Functional class, intens-wtd cum. exp. days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Std Error**	**t Value**	**p-value**
6768	1.55	0.2130	0.0002	Insecticide cumulative exposure	0.00003364	0.00002701	1.25	0.2130
8093	1.21	0.2721	0.0001	Fumigant cumulative exposure	0.00008628	0.00007856	1.10	0.2721
7649	1.67	0.1967	0.0002	Fungicide cumulative exposure	-0.00004422	0.00003425	-1.29	0.1967
6949	15.02	**0.0001**	0.0022	Herbicide cumulative exposure	0.00005231	0.00001350	3.88	**0.0001**
7056	12.07	**0.0005**	0.0017	Herbicides less triazines cumulative exposure	0.00004622	0.00001330	3.47	**0.0005**
				Variable: Chemical class, intens-wtd cum. exp. days				
7998	8.14	**0.0043**	0.0010	Organochlorine cumulative exposure	0.00006706	0.00002350	2.85	**0.0043**
7244	1.69	0.1941	0.0002	Organophosphate cumulative exposure	0.00002378	0.00001831	1.30	0.1941
7479	6.47	0.0110	0.0009	Organothiophosphate cumulative exposure	0.00005731	0.00002254	2.54	0.0110
7737	0.03	0.8652	0.0000	Carbamate cumulative exposure	0.00000310	0.00001824	0.17	0.8652
8250	6.64	0.0100	0.0008	Phenoxy herbicide cumulative exposure	0.00001534	0.00000595	2.58	0.0100
7976	34.47	**<0.0001**	0.0043	Triazine herbicide cumulative exposure	0.00005077	0.00000865	5.87	**<0.0001**

SUMMARY TABLE 4-7c									
MULTIPLE REGRESSION, UNADJUSTED									
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 2 AND BMI AT FOLLOW-UP									
DEPENDENT VARIABLE: BMI AT FOLLOW-UP; INDEPENDENT VARIABLE: CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 2, MISSING VALUES INCLUDED AS ZERO SCORES									
Number of subjects	**F value**	**p-value**	**R^2**	**Variable: Functional class, intens-wtd cum. exp. days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Std Error**	**t Value**	**p-value**	**PERCENT DIFFERENCE WHEN EXCLUDING VERSUS INCLUDING MISSING VALUES AS ZERO SCORES**
9071	2.36	0.1241	0.0003	Insecticide cumulative exposure	0.00003489	0.00002269	1.54	0.1241	-1.824018678
9071	0.48	0.4879	0.0001	Fumigant cumulative exposure	0.00005293	0.00007630	0.69	0.4879	23.95661231
9071	0.64	0.4226	0.0001	Fungicide cumulative exposure	-0.00002557	0.00003189	-0.80	0.4226	26.72302622
9071	23.86	**<0.0001**	0.0026	Herbicide cumulative exposure	0.00005917	0.00001211	4.88	**<0.0001**	-6.153570147
9071	16.34	**<0.0001**	0.0018	Herbicides less triazines cumulative exposure	0.00004892	0.00001210	4.04	**<0.0001**	-2.837923061
				Variable: Chemical class, intens-wtd cum. exp. days					
9071	6.59	0.0103	0.0007	Organochlorine cumulative exposure	0.00005488	0.00002138	2.57	0.0103	9.988518944
9071	1.96	0.1614	0.0002	Organophosphate cumulative exposure	0.00002357	0.00001683	1.40	0.1614	0.443505808
9071	7.42	**0.0064**	0.0008	Organothiophosphate cumulative exposure	0.00005861	0.00002151	2.72	**0.0064**	-1.121463078
9071	0.12	0.7250	0.0000	Carbamate cumulative exposure	-0.00000610	0.00001735	-0.35	0.7250	-306.6666667
9071	3.80	0.0513	0.0004	Phenoxy herbicide cumulative exposure	0.00001073	0.00000551	1.95	0.0513	17.68316072
9071	39.32	**<0.0001**	0.0043	Triazine herbicide cumulative exp	0.00005245	0.00000836	6.27	**<0.0001**	-1.627591552

SUMMARY TABLE 4-8a

MULTIPLE REGRESSION, BACKWARD ELIMINATION

CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT ENROLLMENT

DEPENDENT VARIABLE =BMI AT ENROLLMENT; INDEPENDENT VARIABLE =CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1

INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI-SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT ENROLLMENT, NUMBER OF CIGARETTES SMOKED PER DAY AT ENROLLMENT, EDUCATIONAL LEVEL AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, NUMBER OF ALCOHOLIC DRINKS/MONTH AT ENROLLMENT, TOTAL YEARS FARMING AT ENROLLMENT

RESULTS OF FINAL MODELS--MISSING VALUES AS ZERO EXPOSURE SCORES EXCLUDED

Number of sub-jects	Original Model ANOVA F-value	Original Model ANOVA p-value	Original Model R^2	Variable: Functional class, intensity-weighted cumulative exposure days	Parameter Est.: β = chg in BMI (kg/m2) per day	Standard Error	F Value	p-value	R^2 for model including pesticide class	step drop-ped pesti-cide / range of steps	Variables in final model	Variables dropped from model
6144	42.09	**<0.0001**	0.0267	Insecticide cumulative exposure	0.00000275	0.00002706	0.01	0.9189	0.0267	1 / 0-1	Education, smoking, energy input	insecticide exposure
7723	30.70	**<0.0001**	0.0118	Fumigant cumulative exposure	0.00002021	0.00007792	0.07	0.7953	0.0118	1 / 0 - 1	Education, smoking	fumigant exposure
7300	32.58	**<0.0001**	0.0132	Fungicide cumulative exposure	-0.00003392	0.00003423	0.98	0.3217	0.0132	1 / 0 - 1	Education, smoking	fungicide exposure
6484	30.71	**<0.0001**	0.0186	**Herbicide cumulative exposure**	0.00003991	0.00001350	2.95	**0.0031**	0.0180	final model	Herbicides, education, smoking, years of farming	none
6243	36.55	**<0.0001**	0.0285	Herbicides less triazines cumulative exposure	0.00002375	0.00001355	1.75	0.0797	0.0275	final model	herbicides less triazines, education, energy input, smoking	years of farming

				Variable: Chemical class, intensity-weighted cumulative exposure days								
7545	29.12	**<0.0001**	0.0152	Organo-chlorine cumulative exposure	0.00004523	0.00002313	1.96	0.0506	0.0147	final model	Organo-chlorine exposure, education, smoking, years of farming	none
6461	34.93	**<0.0001**	0.0263	Organo-phosphate cumulative exposure	-0.00001013	0.00001838	0.30	0.5817	0.0263	1 / 0 - 1	education, smoking, energy input	organophos phates, years of farming
6768	43.59	**<0.0001**	0.0251	Organothio-phosphate cumulative exposure	0.00003216	0.00002310	1.94	0.1639	0.0251	1 / 0 - 1	education, smoking, energy input	Organo-thiophos-phates exposure
7383	32.94	**<0.0001**	0.0132	Carbamate cumulative exposure	0.00000397	0.00001884	0.04	0.8333	0.0132	1 / 0 - 1	education, smoking	carbamate exposure
7286	31.13	**<0.0001**	0.0168	Phenoxy herbicide cumulative exposure	0.00001271	0.00000618	2.06	0.0398	0.0161	final model	phenoxy herbicide exposure, education, energy input	years of farming
7180	36.17	**<0.0001**	0.0198	**Triazine herbicide cumulative exposure**	0.00004640	0.00000912	5.09	**<0.0001**	0.0191	final model	triazine exposure, education, energy input	years of farming

SUMMARY TABLE 4-8a

MULTIPLE REGRESSION, BACKWARD ELIMINATION

CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT ENROLLMENT

DEPENDENT VARIABLE =BMI AT ENROLLMENT; INDEPENDENT VARIABLE=CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1

INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI-SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT ENROLLMENT, NUMBER OF CIGARETTES SMOKED PER DAY AT ENROLLMENT, EDUCATIONAL LEVEL AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, NUMBER OF ALCOHOLIC DRINKS/MONTH AT ENROLLMENT, TOTAL YEARS FARMING AT ENROLLMENT

RESULTS OF FINAL MODELS--MISSING VALUES AS ZERO EXPOSURE SCORES INCLUDED

Number of subjects	ANOVA F-value	ANOVA p-value	R^2	Variable: Functional class, intensity-weighted cumulative exposure days	Parameter Est.: β = chg in BMI (kg/m2) per day	Standard Error	F Value	p-value	R^2 for model with pesticide	step dropped pesticide / range of steps	Variables in final model	Variables dropped from model
7921	18.71	**<0.0001**	0.0254	Insecticide cumulative exposure	0.0000000863	0.00002271	0.00	0.9970	0.0254	1 / 0 - 2	education, smoking, energy input	insecticide exposure, years of farming
8536	11.12	**<0.0001**	0.0116	Fumigant cumulative exposure	-0.00002104	0.00007557	-0.28	0.7807	0.0116	1 / 0 - 1	education, smoking	fumigant exposure
8536	11.19	**<0.0001**	0.0117	Fungicide cumulative exposure	-0.000026256	0.00003156	-0.83	0.4054	0.0117	1 / 0 - 1	education, smoking	fungicide exposure
7921	19.51	**<0.0001**	0.0264	**Herbicide cumulative exposure**	0.00003738	0.00001228	3.04	**0.0023**	0.0259	final model	herbicide exposure, education, smoking, energy input	years of farming
8008	15.30	**<0.0001**	0.0188	Herbicides less triazines cumulative exposure	0.00002989	0.00001213	2.46	0.0104	0.0137	2 / 0 - 2	education, energy input	herbicide less triazine exposure, years of farming

				Variable: Chemical class, intensity-weighted cumulative exposure days								
9076	11.47	**<0.0001**	0.0135	Organochlorine cumulative exposure	0.00003758	0.00002088	1.80	0.0719	0.0135	1 / 0 - 1	education, smoking, years of farming	Organo-chlorine exposure
7821	37.20	**<0.0001**	0.0232	Organo-phosphate cumulative exposure	-0.00001167	0.00001689	0.48	0.4896	0.0232	1 / 0 - 1	education, smoking, years of farming, energy input	Organo-phos-phates exposure
7821	37.39	**<0.0001**	0.0234	Organothiophos-phate cumulative exposure	0.00002635	0.00002231	1.39	0.1877	0.2376	1 / 0 - 1	education, smoking, energy input, years of farming	Organo-thio-phos-phates exposure
7821	37.24	**<0.0001**	0.0233	Carbamate cumulative exposure	-0.00001570	0.00001931	0.66	0.4163	0.0233	1 / 0 - 1	education, smoking, energy input, years of farming	Carba-mates exposure
7911	30.98	**<0.0001**	0.0154	Phenoxy herbicide cumulative exposure	0.00000712	0.00000565	1.59	0.2073	0.0154	1 / 0 - 1	education, energy input, years of farming	phenoxy herbicide exposure
7911	36.79	**<0.0001**	0.0183	**Triazine herbicide cumulative exposure**	0.00004465	0.00000879	5.08	**<0.0001**	0.0176	final model	triazine exposure, education, energy input	years of farming

Table 8a: Cross-sectional Enrollment Analysis Summary of Differences when Excluding vs Including Missing Values as Zero Scores			
PESTICIDE CATEGORY	**PERCENT CHANGE IN PARAMETER ESTIMATE WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**	**DIFFERENCES IN COVARIATES WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**	
		EXCLUDING	**INCLUDING**
Insecticides	93.91460706	none	years of farming
Fumigants	-4969.879518	none	none
Fungicides	12.73597447	none	none
Herbicides	3.273385949	none	energy input
Herbicides less triazines	-11.44668158	smoking	none
Organochlorines	9.235376515	none	none
Organophosphates	-7.064220183	none	none
Organothiophosphates	9.929926508	none	years of farming
Carbamates	-167.6896846	none	energy input, years of farming
Phenoxy Herbicides	28.1896117	none	none
Triazine Herbicides	1.922020868	none	none

SUMMARY TABLE 4-8b												
MULTIPLE REGRESSION, BACKWARD ELIMINATION												
PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT FOLLOW-UP												
DEPENDENT VARIABLE=BMI AT FOLLOW-UP; MAIN INDEPENDENT VARIABLE=CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1												
INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT ENROLLMENT, NUMBER OF CIGARETTES SMOKED PER DAY AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, NUMBER OF ALCOHOLIC DRINKS/MONTH AT ENROLLMENT, TOTAL YEARS FARMING AT ENROLLMENT												
RESULTS OF FINAL MODELS---ZERO SCORES EXCLUDED												
Number of sub-jects	**ANOVA F value**	**ANOVA p-value**	**Original Model R^2**	**Variable: Functional class, intensity-weighted cumulative exposure days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Standard Error**	**t Value**	**p-value**	**R^2 for model with pesti-cide class**	**step dropped / range of steps**	**Variables in final model**	**Variables dropped from model**
6328	31.62	**<0.0001**	0.0198	Insecticide cumulative exposure	0.00001159	0.00002756	0.18	0.6742	0.0196	1 / 0 - 1	Enrollment age, smoking, energy input	insecticide exposure
7992	29.12	**<0.0001**	0.0108	Fumigant cumulative exposure	0.00004376	0.00007900	0.31	0.5796	0.0108	1 / 0 - 1	enrollment age, smoking	fumigant exposure
7529	25.52	**<0.0001**	0.0067	Fungicide cumulative exposure	-0.00005375	0.00003513	2.34	0.1261	0.0067	1 / 0 - 1	smoking	fungicide exposure
6679	25.67	**<0.0001**	0.0152	**Herbicide cumulative exposure**	0.00004907	0.00001392	3.52	**0.0004**	0.0146	final model	herbicides years of farming, smoking, enrollment age	none
6425	31.25	**<0.0001**	0.0238	Herbicides less triazines cumulative exposure	0.00003117	0.00001400	2.23	0.0261	0.0230	final model	herbicides less triazines, energy input, enrlmt age, smoking, yrs of farming	None

				Variable: Chemical class, intens-wtd cum. exp. days								
7789	30.81	**<0.0001**	0.0156	**Organochlorine cumulative exposure**	0.00007071	0.00002355	3.00	**0.0027**	0.0151	final model	Organo-chlorines, age at enrollment, smoking, years of farming	none
6662	30.15	**<0.0001**	0.0221	Organophosphate cumulative exposure	0.00000158	0.00001902	0.01	0.9337	0.0221	1 / 0 - 1	age at enrollment, smoking, years of farming, energy input	Organo-phosphate exposure
6986	38.23	**<0.0001**	0.0214	Organothio-phosphate cumulative exposure	0.00003548	0.00002383	2.22	0.1366	0.0214	1 / 0 -1	age at enrollment, smoking, energy input	Organo-thio-phosphate exposure
7623	29.58	**<0.0001**	0.0115	Carbamate cumulative exposure	-0.00000397	0.00001864	0.05	0.8311	0.0115	1 / 0 - 1	age at enrollment, smoking	carbamate exposure
7520	27.28	**<0.0001**	0.0143	Phenoxy herbicide cumulative exposure	0.000014	0.00000643	2.18	0.0294	0.0138	final model	phenoxy herbicide exposure, age at enrollment, years of farming, energy input	none
7404	30.55	**<0.0001**	0.0162	**Triazine herbicide cumulative exposure**	0.00004842	0.00000924	5.24	**<0.0001**	0.0157	final model	triazine exposure, age at enrollment, years of farming, energy input	none

SUMMARY TABLE 4-8b

MULTIPLE REGRESSION, BACKWARD ELIMINATION

PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT FOLLOW-UP

DEPENDENT VARIABLE = BMI AT FOLLOW-UP; MAIN INDEPENDENT VARIABLE = CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1

INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT ENROLLMENT, NUMBER OF CIGARETTES SMOKED PER DAY AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, NUMBER OF ALCOHOLIC DRINKS/MONTH AT ENROLLMENT, TOTAL YEARS FARMING AT ENROLLMENT

RESULTS OF FINAL MODELS---ZERO SCORES EXCLUDED

Num-ber of sub-jects	**ANOVA F value**	**ANOVA p-value**	**Original Model R^2**	**Variable: Functional class, intensity-weighted cumulative exposure days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Standard Error**	**t Value**	**p-value**	**R^2 for model with pesti-cide class**	**step drop-ped / range of steps**	**Variables in final model**	**Variables dropped from model**
6328	31.62	**<0.0001**	0.0198	Insecticide cumulative exposure	0.00001159	0.00002756	0.18	0.6742	0.0196	1 / 0 - 1	age at enrollment, smoking, energy input	insecticide exposure
7992	29.12	**<0.0001**	0.0108	Fumigant cumulative exposure	0.00004376	0.00007900	0.31	0.5796	0.0108	1 / 0 - 1	Enrollment age, smoking	fumigant exposure
7529	25.52	**<0.0001**	0.0067	Fungicide cumulative exposure	-0.00005375	0.00003513	2.34	0.1261	0.0067	1 / 0 - 1	smoking	fungicide exposure
6679	25.67	**<0.0001**	0.0152	**Herbicide cumulative exposure**	0.00004907	0.00001392	3.52	**0.0004**	0.0146	final model	herbicides, years of farming, smoking, enrollment age	none
6425	31.25	**<0.0001**	0.0238	Herbicides less triazines cumulative exposure	0.00003117	0.00001400	2.23	0.0261	0.0230	final model	herbicides less triazines, energy input, age at enrollment, smoking, years of farming	none

				Variable: Chemical class, intens-wtd cum. exp. days								
7789	30.81	**<0.0001**	0.0156	**Organo-chlorine cumulative exposure**	0.00007071	0.00002355	3.00	**0.0027**	0.0151	final model	Organochlorines, age at enrollment, smoking, years of farming	none
6662	30.15	**<0.0001**	0.0221	Organo-phosphate cumulative exposure	0.00000158	0.00001902	0.01	0.9337	0.0221	1 / 0 - 1	age at enrollment, smoking, years of farming, energy input	Organo-phosphate exposure
6986	38.23	**<0.0001**	0.0214	Organothio-phosphate cumulative exposure	0.00003548	0.00002383	2.22	0.1366	0.0214	1 / 0 -1	age at enrollment, smoking, energy input	Organo-thiophos-phate exposure
7623	29.58	**<0.0001**	0.0115	Carbamate cumulative exposure	-0.00000397	0.00001864	0.05	0.8311	0.0115	1 / 0 - 1	age at enrollment, smoking	carbamate exposure
7520	27.28	**<0.0001**	0.0143	Phenoxy herbicide cumulative exposure	0.000014	0.00000643	2.18	0.0294	0.0138	final model	phenoxy herbicide exposure, age at enrollment, years of farming, energy input	none
7404	30.55	**<0.0001**	0.0162	**Triazine herbicide cumulative exposure**	0.00004842	0.00000924	5.24	**<0.0001**	0.0157	final model	triazine exposure, age at enrollment, years of farming, energy input	none

SUMMARY TABLE 4-8b											
MULTIPLE REGRESSION, BACKWARD ELIMINATION											
PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 AND BMI AT FOLLOW-UP											
DEPENDENT VARIABLE = BMI AT FOLLOW-UP; MAIN INDEPENDENT VARIABLE = CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 1											
INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT ENROLLMENT, NUMBER OF CIGARETTES SMOKED PER DAY AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, NUMBER OF ALCOHOLIC DRINKS/MONTH AT ENROLLMENT, TOTAL YEARS FARMING AT ENROLLMENT											
RESULTS OF FINAL MODELS---ZERO SCORES INCLUDED											
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**R^2**	**Variable: Functional class, intensity-weighted cumulative exposure days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Standard Error**	**t Value**	**p-value**	**step eliminated/ range of steps**	**Variables in final model**	**Variables dropped from model**
8184	41.04	**<0.0001**	0.0245	Insecticide cumulative exposure	0.00001902	0.00002319	0.82	0.4122	1 / 0 - 1	age at enrollment, cigarettes/day, years of farming, kcal/day	insecticide exposure
8835	35.06	**<0.0001**	0.0118	Fumigant cumulative exposure	0.00000615	0.00007643	0.08	0.9359	1 / 0 - 1	age at enrollment, cigarettes/day	fumigant exposure
8835	35.32	**<0.0001**	0.0119	Fungicide cumulative exposure	-0.00002820	0.00003242	-0.87	0.3844	1 / 0 - 1	age at enrollment, cigarettes/day	fungicide exposure
8184	43.48	**<0.0001**	0.0259	**Herbicide cumulative exposure**	0.00004454	0.00001257	3.54	**0.0004**	final model	herbicide exposure, age at enrollment, cigarettes/day, kcal per day, years of farming	---
8274	36.03	**<0.0001**	0.0171	**Herbicides less triazines cumulative exposure**	0.00003611	0.00001246	2.90	**0.0038**	final model	herbicides less triazines exposure, age at enrollment, kcal/day, years of farming	----

				Variable: Chemical class, intens-wtd cum. exp. days							
8705	35.27	**<0.0001**	0.0160	**Organochlorine cumulative exposure**	0.00006218	0.00002132	2.92	**0.0035**	final model	organochlorine exposure, age at enrollment, cigarettes/day, years of farming	----
8184	40.98	**<0.0001**	0.0244	Organophosphate cumulative exposure	0.00001086	0.00001741	0.62	0.5328	1 / 0 - 1	age at enrollment, cigarettes/day, years of farming, kcal/day	organophosphate exposure
8184	39.03	**<0.0001**	0.0187	Organothiophosphate cumulative exposure	0.00003155	0.00002202	1.43	0.1519	1 / 0 - 2	cigarettes/day, kcal/day	organo-thiophosphate exposure, years of farming
8184	41.03	**<0.0001**	0.0245	Carbamate cumulative exposure	-0.00001468	0.00001866	-0.79	0.4316	1 / 0 - 1	age at enrollment, cigarettes/day, kcal/day, years of farming	carbamate exposure
8274	34.50	**<0.0001**	0.0164	Phenoxy herbicide cumulative exposure	0.000008763	0.00000565	1.55	0.1211	1 / 0 - 1	age at enrollment, kcal/day, years of farming	phenoxy herbicide exposure
8274	44.02	**<0.0001**	0.0157	**Triazine herbicide cumulative exposure**	0.00004739	0.00000883	5.37	**<0.0001**	final model	triazine exposure, kcal/day	years of farming

Table 4-8b. Prospective Analysis, Percent difference when excluding versus including missing values as zero scores			
PESTICIDE CATEGORY	**PERCENT CHANGE IN PARAMETER ESTIMATE WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**	**DIFFERENCES IN COVARIATES IN MODELS WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**	
		EXCLUDED	**INCLUDED**
Insecticides	-24.27311336	none	years of farming
Fumigants	75.35564015	none	none
Fungicides	31.17754728	none	age
Herbicides	4.839226578	none	energy input
Herbicides less triazines	-7.342449465	smoking	none
Organochlorines	6.422846822	none	none
Organophosphates	-74.59807074	none	none
Organothiophosphates	5.863046397	age	years of farming
Carbamates	-57.42627346	none	energy input, years of farming
Phenoxy herbicides	23.00663357	none	none
Triazine herbicides	1.075044359	age	none

SUMMARY TABLE 4-8c												
MULTIPLE REGRESSION, BACKWARD ELIMINATION												
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 2 AND BMI AT FOLLOW-UP												
DEPENDENT VARIABLE=BMI AT FOLLOWUP; MAIN INDEPENDENT VARIABLE=CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 2												
INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT FOLLOW-UP, SMOKING STATUS AT FOLLOWUP, ENERGY INPUT AT FOLLOWUP, ALCOHOL USE AT FOLLOWUP												
RESULTS OF FINAL MODELS---MISSING VALUES AS ZERO EXPOSURE SCORES EXCLUDED												
Num-ber of sub-jects	**ANOVA F value**	**ANOVA p-value**	**R^2**	**Variable: Functional class, intensity-weighted cumulative exposure days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Standard Error**	**t Value**	**p-value**	**R^2 model with pest-icide included**	**step drop-ped / range of steps**	**Variables in final model**	**Variables dropped from model**
6720	14.91	**<0.0001**	0.0088	Insecticide cumulative exposure	0.00003485	0.00002719	1.64	0.2000	0.0088	1 / 0 - 1	followup age, energy input, smoking	insecticide exposure
8092	23.89	**<0.0001**	0.0088	Fumigant cumulative exposure	0.00008661	0.00007846	1.22	0.2697	0.0088	1 / 0 - 1	followup age, smoking	fumigant exposure
7590	21.59	**<0.0001**	0.0113	Fungicide cumulative exposure	-0.00005288	0.00003425	2.38	0.1226	0.0113	1 / 0 - 1	followup age, smoking, energy input	fungicide exposure
6899	20.43	**<0.0001**	0.0117	**Herbicide cumulative exposure**	0.00005160	0.00001361	3.79	**0.0002**	0.0111	final model	Herbicidesfollow up age, smoking, energy input	none
7003	20.14	**<0.0001**	0.0114	**Herbicides less triazines cumulative exposure**	0.00004572	0.00001335	3.42	**0.0006**	0.0108	final model	herbicides less triazines, energy input, followup age, smoking	none

				Variable: Chemical class, intensity-weighted cumulative exposure days								
7996	27.34	**<0.0001**	0.0102	**Organochlorine cumulative exposure**	0.00008106	0.00002355	3.44	**0.0006**	0.0098	final model	Organo-chlorines, age at followup, smoking	none
7195	8.65	**<0.0001**	0.0036	Organophosphate cumulative exposure	0.00002335	0.00001843	1.61	0.2052	0.0036	1 / 0 - 1	energy input, age at followup	Organo-phosphate exposure
7426	20.94	**<0.0001**	0.0112	Organothiophos-phate cumulative exposure	0.00005319	0.00002257	2.36	0.0184	0.0106	1 / 0 - 1	Organo-thiophos-phate exposure, age at follow-up, energy input, smoking	none
7735	25.25	**<0.0001**	0.0097	Carbamate cumulative exposure	0.00000870	0.00001826	0.23	0.6339	0.0097	1 / 0 - 1	smoking status, age at followup	carbamate exposure
8187	24.08	**<0.0001**	0.0116	**Phenoxy herbicide cumulative exposure**	0.00001765	0.00000598	2.95	**0.0031**	0.0112	1 / 0 - 1	phenoxy herbicide exposure, age at follow-up, smoking, energy input	none
7917	28.45	**<0.0001**	0.0107	**Triazine herbicide cumulative exposure**	0.00005068	0.00000889	5.70	**<0.0001**	0.0103	final model	triazines, energy input, smoking	none

SUMMARY TABLE 4-8c

MULTIPLE REGRESSION, BACKWARD ELIMINATION

CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 2 AND BMI AT FOLLOW-UP

DEPENDENT VARIABLE=BMI AT FOLLOWUP; MAIN INDEPENDENT VARIABLE=CUMULATIVE PESTICIDE EXPOSURE INTENSITY-WEIGHTED DAYS AT PHASE 2

INDEPENDENT VARIABLES BASED ON CORRELATION COEFFICIENTS OR CHI SQUARE VALUES FOR OUTCOME AND PARTICULAR PESTICIDE CLASS: AGE AT FOLLOW-UP, SMOKING STATUS AT FOLLOWUP, ENERGY INPUT AT FOLLOWUP, ALCOHOL USE AT FOLLOWUP

RESULTS OF FINAL MODELS--MISSING VALUES ZERO EXPOSURE SCORES INCLUDED

Num-ber of sub-jects	**ANOVA F value**	**ANOVA p-value**	**R^2**	**Variable: Functional class, intensity-weighted cumulative exposure days**	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Standard Error**	**t Value**	**p-value**	**step elimin ated/ range of steps**	**Variables in final model**	**Variables dropped from model**
8994	25.83	**<0.0001**	0.0142	Insecticide cumulative exposure	0.00003992	0.00002262	1.76	0.0777	1 / 0 - 1	age at followup, smoking status, energy input	insecticide exposure
9066	13.64	**<0.0001**	0.0045	Fumigant cumulative exposure	0.00004199	0.00007600	0.55	0.5806	1 / 0 - 1	smoking status	fumigant exposure
9066	27.89	**<0.0001**	0.0122	Fungicide cumulative exposure	-0.000022457	0.00003160	-0.71	0.4773	1 / 0 - 1	smoking status, age at followup	fungicides exposure
8994	23.45	**<0.0001**	0.0103	**Herbicide cumulative exposure**	0.000055917	0.00001213	4.61	**<0.0001**	final model	herbicide exposure, energy input, smoking status	----
8997	18.72	**<0.0001**	0.0041	**Herbicides less triazines cumulative exposure**	0.00004462	0.00001211	3.69	**0.0002**	final model	herbicide exposure less triazines, energy input	----

				Variable: Chemical class, intensity-weighted cumulative exposure days							
8997	20.42	**<0.0001**	0.0068	**Organochlorine cumulative exposure**	0.000067204	0.00002137	3.14	**0.0017**	final model	organochlorine exposure, age at followup, energy input	----
8997	17.67	**<0.0001**	0.0059	Organophosphate cumulative exposure	0.00002193	0.00001682	1.30	0.1924	1 / 0 - 1	age at followup, energy input	organophosphate exposure
8997	19.26	**<0.0001**	0.0064	Organothiophosphate cumulative exposure	0.000054528	0.00002148	2.54	0.0112	1 / 0 - 1	age at enrollment, energy input	organothiophosphate exposure
9066	27.77	**<0.0001**	0.0121	Carbamate cumulative exposure	0.000002985	0.00001728	0.17	0.8628	1 / 0 - 1	age at followup, smoking status	carbamate exposure
8897	18.75	**<0.0001**	0.0062	Phenoxy herbicide cumulative exposure	0.000012222	0.00000551	2.22	0.0266	1 / 0 - 1	age at enrollment, energy input	phenoxy herbicide exposure
8997	30.22	**<0.0001**	0.0067	**Triazine herbicide cumulative exposure**	0.0000517	0.00000856	6.04	**<0.0001**	final model	triazine exposure, energy input	-----

Table 4-8c: Cross-sectional analysis of follow-up data, Percent difference when excluding versus including missing values as zero scores			
PESTICIDE CATEGORY	**PERCENT CHANGE IN PARAMETER ESTIMATE WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**	**DIFFERENCES IN COVARIATES IN MODELS WHEN EXCLUDING VS INCLUDING MISSING VALUES AS ZERO SCORES**	
		EXCLUDED	INCLUDED
Insecticides	-6.780794436	none	none
Fumigants	34.69463927	age	none
Fungicides	40.38254775	energy input	none
Herbicides	-4.015178995	age	none
Herbicides less triazines	1.217622316	age, smoking	none
Organochlorines	9.345491825	smoking	energy input
Organophosphates	3.136042403	none	none
Organothiophosphates	-1.242132234	smoking	none
Carbamates	48.90885751	none	none
Phenoxy Herbicides	18.17086235	smoking	none
Triazine herbicides	-0.996288338	smoking	none

Table 4-9			
ALL APPLICATORS, PHASE 2, BMI3, ADJUSTED CROSS-SECTIONAL ANALYSES			
ORIGINAL PESTICIDE PARAMETER ESTIMATE AND ESTIMATES ADJUSTED FOR TRIAZINES, MISSING VALUES INCLUDED AS ZERO SCORES			
Variable: Functional class, intensity-weighted cumulative exposure days	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Percent Change in Original Parameter Estimate**	**p-value**
ORIGINAL PARAMETER ESTIMATE: Insecticide cumulative exposure	0.00003992	N/A	0.0777
Insecticides controlling for triazines	-0.000005121	129.4318802	0.8289
ORIGINAL PARAMETER ESTIMATE: Fumigant cumulative exposure	0.00004199	N/A	0.5806
Fumigants controlling for triazines	-0.000006890	139.2570224	0.9280
ORIGINAL PARAMETER ESTIMATE: Fungicide cumulative exposure	-0.000022457	N/A	0.4773
Fungicides controlling for triazines	-0.000030107	-14.5536869	0.3398
ORIGINAL PARAMETER ESTIMATE: Herbicides less triazines cumulative exposure	0.00004462	N/A	**0.0002**
Herbicides less triazines controlling for triazines	-0.000000293461	101.3240871	0.9847
ORIGINAL PARAMETER ESTIMATE: Organochlorines cumulative exposure	0.000067204	N/A	**0.0017**
Organochlorines controlling for triazines	0.000044932	19.8615966	0.0382
ORIGINAL PARAMETER ESTIMATE: Organophosphates cumulative exposure	0.00002193	N/A	0.1924
Organophosphates controlling for triazines	-0.000014838	518.4433164	0.4050
ORIGINAL PARAMETER ESTIMATE: Organothiophosphates cumulative exposure	0.000054528	N/A	0.0112
Organothiophosphates controlling for triazines	-0.000005199	121.0788786	0.8276
ORIGINAL PARAMETER ESTIMATE: Carbamates cumulative exposure	0.000002985	N/A	0.8628
Carbamates controlling for triazines	-0.000009798	-187.6265962	0.5722
ORIGINAL PARAMETER ESTIMATE: Phenoxy herbicides cumulative exposure	0.000012222	N/A	0.0266
Phenoxy herbicides controlling for triazines	-0.000004674	223.8473768	0.4515

Table 4-9.			
ALL APPLICATORS, PHASE 2, BMI3, ADJUSTED CROSS-SECTIONAL ANALYSES			
ORIGINAL PESTICIDE PARAMETER ESTIMATE AND ESTIMATES ADJUSTED FOR TRIAZINES, MISSING VALUES EXCLUDED AS ZERO SCORES			
Variable: Functional class, intensity-weighted cumulative exposure days	**Parameter Est.: β = chg in BMI (kg/m2) per day**	**Percent Change in Original Parameter Estimate**	**p-value**
ORIGINAL PARAMETER ESTIMATE: Insecticide cumulative exposure	0.00003485	N/A	0.2000
Insecticides controlling for triazines	-0.00004471	-806.8965517	0.1456
ORIGINAL PARAMETER ESTIMATE: Fumigant cumulative exposure	0.00008661	N/A	0.2697
Fumigants controlling for triazines	0.00000060	98.6285662	0.9942
ORIGINAL PARAMETER ESTIMATE: Fungicide cumulative exposure	-0.00005288	N / A	0.1226
Fungicides controlling for triazines	-0.00005912	-5.5714286	0.0856
ORIGINAL PARAMETER ESTIMATE: Herbicides less triazines cumulative exposure	0.00004572	N / A	**0.0006**
Herbicides less triazines controlling for triazines	-0.00000339	116.0170092	0.8484
ORIGINAL PARAMETER ESTIMATE: Organochlorines cumulative exposure	0.00008106	N / A	**0.0006**
Organochlorines controlling for triazines	0.00001742	64.6222583	0.5913
ORIGINAL PARAMETER ESTIMATE: Organophosphates cumulative exposure	0.00002335	N / A	0.2052
Organophosphates controlling for triazines	-0.00001752	701.0291595	0.3844
ORIGINAL PARAMETER ESTIMATE: Organothiophosphates cumulative exposure	0.00005319	N / A	0.0184
Organothiophosphates controlling for triazines	-0.00000929	142.3234624	0.7225
ORIGINAL PARAMETER ESTIMATE: Carbamates cumulative exposure	0.00000870	N / A	0.6339
Carbamates controlling for triazines	-0.00001024	-1229.8701299	0.5939
ORIGINAL PARAMETER ESTIMATE: Phenoxy herbicides cumulative exposure	0.00001765	N / A	**0.0031**
Phenoxy herbicides controlling for triazines	-0.00000014	101.5990862	0.9844

SUMMARY TABLE 4-10a

EXPOSURE RESPONSE EVALUATION USING PROC GLM (GENERAL LINEARIZED MODEL)

CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 (MAIN INDEPENDENT VARIABLE) AND BMI AT ENROLLMENT (DEPENDENT VARIABLE)

ADDITIONAL INDEPENDENT VARIABLES INCLUDED IF RELATED TO BOTH EXPOSURE CLASS AND OUTCOME: AGE AT ENROLLMENT, NUMBER OF CIGARETTES PER DAY AT ENROLLMENT, EDUCATIONAL LEVEL AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, AND YEARS OF FARMING AT ENROLLMENT

INSECTICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Total Model, Exposure F-value (p-value), Type 1 SS	Total Model, Exposure F-value (p-value), Type 111 SS	Additional variables in model
7921	15.41	**<0.0001**	Insecticides, intensity-weighted cumulative exposure days in 5 categories	0.026569	4.78 **(0.0008)**	2.44 (0.0444)	smoking, education, energy input, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	BMI2	Exposure Category	insecticide cumulative exposure days	p-values	Model Parameter Estimate for each exposure category	Standard Error	t-value (p-value)
1	27.6	0	0	0.8864	0.16020	0.17274	0.93 (0.3537)
2	27.1	1	> 0 AND ≤ 42.5	0.0908	-0.36122	0.14452	-2.50 (0.0125)
3	27.4	2	> 42.5 AND ≤ 165.9	0.9240	-0.10128	0.12322	-0.82 (0.4111)
4	27.3	3	> 165.9 AND ≤ 516.0	0.8077	-0.13486	0.12283	-1.10 (0.2723)
5	27.5	4	> 516.0	referent	referent	referent	referent

FUMIGANTS

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-Value (p-value) Type 1 SS	Exposure F-Value (p-value) Type III SS	Additional variables in model
8536	10.23	**<0.0001**	Fumigants, intensity-weighted cumulative exposure days in categories	0.01186	1.32 (0.2676)	1.15 (0.3182)	smoking, education

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure Variable Effects in Model		
LSMEAN	BMI2	Exposure Category	fumigant cumulative exposure days	p-values	Variable Parameter Estimate	Standard Error	t-value (p-value)
1	27.4	0	0	0.8461	0.07971	0.14472	0.55(0.5818)
2	27.1	1	> 0 and ≤ 98.0	0.7546	-0.14101	0.19722	-0.71(0.4746)
3	27.3	2	>98.0	referent	referent	referent	referent

FUNGICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8536	10.09	**<0.0001**	Fungicides, intensity-weighted cumulative exposure days in 3 categories	0.011701	0.13 (0.8739)	0.46 (0.6314)	smoking, education

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	BMI2	Exposure Category	fungicide cumulative exposure days	p-values	Model Parameter Estimate for Exposure Variable	Standard Error	t-value (p-value)
1	27.3	0	0	0.6662	0.098639	0.11484	0.86 (0.3904)
2	27.4	1	> 0 AND ≤ 1.4	0.7568	0.184076	0.25876	0.71 (0.4769)
3	27.2	2	> 1.4	referent	referent	referent	referent

HERBICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
7921	17.65	**<0.0001**	Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.030310	16.49 **(<0.0001)**	10.08 **(<0.0001)**	education, energy input, smoking, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	BMI2	Exposure Category	herbicide cumulative exposure days	p-values	Model Parameter Estimate for Exposure Variable	Standard Error	t-value (p-value)
1	27.8	0	0	0.9992	0.055363	0.22660	0.24 (0.8070)
2	27.0	1	> 0 AND ≤ 207.8	**<0.0001**	-0.783050	0.13545	**-5.78 (<0.0001)**
3	27.2	2	> 207.8 AND ≤ 642.2	**0.0005**	-0.497203	0.12344	**-4.03 (<0.0001)**
4	27.4	3	> 642.2 AND ≤ 1619.9	0.0275	-0.360014	0.12258	**-2.94 (0.0033)**
5	27.7	4	> 1619.9	referent	referent	referent	referent

HERBICIDES LESS TRIAZINE HERBICIDES

Number of subjects	ANOVA F value	p-value	Variable: Functional class, intensity weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional Variables in Model
8008	14.44	**<0.0001**	Herbicides less triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.022937	14.80 **(<0.0001)**	9.93 **(<0.0001)**	education, years of farming energy input

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	BMI2	Exposure Category	Herbicide less triazine herbicides cumulative exposure days	p-values	Model Parameter Estimate for Exposure Variable	Standard Error	t-value (p-value)

1	27.7	0	0	1.0000	-0.026629	0.21977	-0.12 (0.9036)
2	27.0	1	> 0 AND ≤ 181.6	**<0.0001**	-0.737393	0.13543	-5.45 **(<0.0001)**
3	27.2	2	> 181.6 AND ≤ 583.3	**<0.0001**	-0.564212	0.12296	-4.59 **(<0.0001)**
4	27.5	3	> 583.3 AND ≤ 1492.8	0.1835	-0.267531	0.12216	-2.19 (0.0286)
5	27.7	4	> 1492.8	referent	referent	referent	referent

ORGANOCHLORINES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional Variables in Model
8412	10.48	**<0.0001**	Organochlorines, intensity-weighted cumulative exposure days in 5 categories	0.013541	3.33 (0.0358)	1.91 (0.1483)	smoking, education, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	**BMI2**	**Exposure Category**	**organochlorine cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.3	0	0	0.6493	-0.09382	0.10593	-0.89 (0.3758)
2	27.2	1	> 0 AND ≤ 150.4	0.1284	-0.23851	0.12314	-1.94 (0.0528)
3	27.4	2	> 150.4	referent	referent	referent	Referent

ORGANOPHOSPHATES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
7921	15.52	**<0.0001**	Organophosphates, intensity-weighted cumulative exposure days in 5 categories	0.026750	5.67 **(0.0001)**	2.81 (0.0240)	education, energy input, years of farming, smoking

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	**BMI2**	**Exposure Category**	**organophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.4	0	0	0.9955	-0.057809	0.15153	-0.38 (0.7028)
2	27.0	1	> 0 AND ≤ 43.9	0.0289	-0.470632	0.16116	**-2.92 (0.0035)**
3	27.2	2	> 43.9 AND ≤ 197.1	0.2513	-0.249589	0.12290	-2.03 (0.0423
4	27.4	3	> 197.1 AND ≤ 625.2	0.9811	-0.068349	0.12263	-0.56 (0.5773)
5	27.5	4	> 625.2	referent	referent	referent	referent

ORGANOTHIOPHOSPHATES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model

7921	15.33	**<0.0001**	Organothiophosphates, intensity-weighted cumulative exposure days in 5 categories	0.026424	5.08 **(0.0004)**	2.15 (0.0721)	education, energy input, smoking, years of farming
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**organothiophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.4	0	0	0.9369	-0.09888	0.12703	-0.78 (0.4364)
2	26.0	1	> 0 AND ≤ 3.1	0.9306	-1.55969	1.94933	-0.80 (0.4237)
3	27.2	2	> 3.1 AND ≤ 162.9	0.0502	-0.33573	0.12310	**-2.73 (0.0064)**
4	27.3	3	> 162.9 AND ≤ 597.3	0.5078	-0.19434	0.12269	-1.58 (0.1133)
5	27.5	4	> 597.3	referent	referent	referent	referent
CARBAMATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
7921	16.00	**<0.0001**	Carbamates, intensity-weighted cumulative exposure days in 4 categories	0.025625	1.01 (0.3860)	0.70 (0.5500)	education, smoking, energy input, years of farming
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**carbamate cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.4	0	0	0.9828	0.040824	0.11045	0.37 (0.7117)
2	27.1	1	> 0 AND ≤ 26.3	0.7442	-0.21899754	0.21707	-1.01 (0.3131)
3	27.3	2	> 26.3 AND ≤ 297.0	0.9527	-0.06491158	0.12233	-0.53 (0.5986)
4	27.4	3	> 297.0	referent	referent	referent	referent
PHENOXY HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8008	12.92	**<0.0001**	Phenoxy Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.020573	9.18 **(<0.0001)**	5.09 **(0.0004)**	education, energy input, years of farming
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.3	0	0	0.0132	-0.431548	0.13610	**-3.17 (0.0008)**

2	27.1	1	> 0 AND ≤ 77.8	0.0600	-0.570863	0.21452	**-2.66 (0.0055)**
3	27.2	2	> 77.8 AND ≤ 585.6	**0.0004**	-0.501808	0.12306	**-3.97 (<0.0001)**
4	27.4	3	> 585.6 AND ≤ 2000.4	0.0483	-0.337607	0.12316	**-2.79 (0.0052)**
5	27.7	4	> 2000.4	referent	referent	referent	referent
TRIAZINE HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8008	14.02	**<0.0001**	Triazine Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.022292	13.28 **(<0.0001)**	8.61 **(<0.0001)**	education, energy input, years of farming
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**triazine herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.3	0	0	**0.0056**	-0.46084	0.13459	**-3.42 (0.0006)**
2	26.9	1	> 0 AND ≤ 73.9	**0.0047**	-0.84922	0.24439	**-3.47 (0.0005)**
3	27.1	2	> 73.9 AND ≤ 562.7	**<0.0001**	-0.66511	0.12286	**-5.41 (<0.0001)**
4	27.4	3	> 562.7 AND ≤ 1955.5	**0.0059**	-0.41677	0.12231	**-3.41 (0.0007)**
5	27.8	4	> 1955.5	referent	referent	referent	referent

Table 4-10a							
EXPOSURE RESPONSE EVALUATION USING PROC GLM (GENERAL LINEARIZED MODEL)							
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 (MAIN INDEPENDENT VARIABLE) AND BMI AT ENROLLMENT (DEPENDENT VARIABLE) WITH CONTROL FOR TRIAZINE EXPOSURE							
ADDITIONAL INDEPENDENT VARIABLES INCLUDED IF RELATED TO BOTH EXPOSURE CLASS AND OUTCOME: AGE AT ENROLLMENT, NUMBER OF CIGARETTES PER DAY AT ENROLLMENT, EDUCATIONAL LEVEL AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, AND YEARS OF FARMING AT ENROLLMENT							
INSECTICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Total Model, Exposure F-value (p-value), Type 1 SS**	**Total Model, Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
7921	13.65	**<0.0001**	Insecticides, intensity-weighted cumulative exposure days in 5 categories	0.030158	4.79 **(0.0007)**	2.03 (0.0867)	smoking, education, energy input, years of farming, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**insecticide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.7	0	0	0.1827	0.40058	0.18275	2.19 (0.0284)
2	27.2	1	> 0 AND ≤ 42.5	0.9769	-0.09022	0.15341	-0.59 (0.5565)
3	27.4	2	> 42.5 AND ≤ 165.9	0.9554	0.09091	0.12889	0.71 (0.4806)
4	27.2	3	> 165.9 AND ≤ 516.0	0.9955	-0.04744	0.12419	-0.38 (0.7025)
5	27.3	4	> 516.0	referent	referent	referent	referent
FUMIGANTS							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-Value (p-value) Type 1 SS**	**Exposure F-Value (p-value) Type III SS**	**Additional variables in model**
8536	11.29	**<0.0001**	Fumigants, intensity-weighted cumulative exposure days in categories	0.018207	1.33 (0.2655)	1.69 (0.1855)	smoking, education, triazines
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure Variable Effects in Model**		
LSMEAN	**BMI2**	**Exposure Category**	**fumigant cumulative exposure days**	**p-values**	**Variable Parameter Estimate**	**Standard Error**	**t-value (p-value)**
1	27.3	0	0	0.6413	0.130234	0.14495	0.90 (0.3690)
2	27.0	1	> 0 and ≤ 98.0	0.8024	-0.124523	0.19697	-0.63 (0.5273)
3	27.1	2	>98.0	referent	referent	referent	referent

FUNGICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8536	11.13	**<0.0001**	Fungicides, intensity-weighted cumulative exposure days in 3 categories	0.017953	0.14 (0.8733)	0.58 (0.5575)	smoking, education, triazines
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY							
LSMEAN	**BMI2**	**Exposure Category**	**fungicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.2	0	0	0.6276	0.10616	0.11539	0.92 (0.3576)
2	27.4	1	> 0 AND ≤ 1.4	0.6587	0.22628	0.25981	0.87 (0.3838)
3	27.1	2	> 1.4	referent	referent	referent	referent
HERBICIDES (CANNOT ADJUST FOR TRIAZINES)							
HERBICIDES LESS TRIAZINE HERBICIDES							
Number of subjects	**ANOVA F value**	**p-value**	**Variable: Functional class, intensity weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional Variables in Model**
8008	11.68	**<0.0001**	Herbicides less triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.024247	14.81 **(<0.0001)**	4.00 **(0.0030)**	education, years of farming, energy input, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**Herbicide less triazine herbicides cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.7	0	0	0.9579	0.17521	0.25260	0.69 (0.4879)
2	27.1	1	> 0 AND ≤ 181.6	0.0222	-0.48194	0.16026	-3.01 **(0.0026)**
3	27.2	2	> 181.6 AND ≤ 583.3	0.0768	-0.35791	0.13947	-2.57 (0.0103)
4	27.4	3	> 583.3 AND ≤ 1492.8	0.7742	-0.14901	0.12846	-1.16 (0.2461)
5	27.5	4	> 1492.8	referent	referent	referent	referent
ORGANOCHLORINES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional Variables in Model**

8412	10.81	**<0.0001**	Organochlorines, intensity-weighted cumulative exposure days in 5 categories	0.018948	3.35 (0.0352)	1.02 (0.3611)	smoking, education, years of farming, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**organochlorine cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.3	0	0	0.8791	0.05226	0.10808	0.48 (0.6287)
2	27.1	1	> 0 AND ≤ 150.4	0.6932	-0.10157	0.12449	-0.82 (0.4146)
3	27.2	2	> 150.4	referent	referent	referent	referent
ORGANOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
7921	13.42	**<0.0001**	Organophosphates, intensity-weighted cumulative exposure days in 5 categories	0.029658	5.69 **(0.0001)**	1.02 (0.3972)	education, energy input, years of farming, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**organophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.5	0	0	0.8801	0.153023	0.16228	0.94 (0.3457)
2	27.1	1	> 0 AND ≤ 43.9	0.7406	-0.207481	0.17027	-1.22 (0.2231)
3	27.3	2	> 43.9 AND ≤ 197.1	0.9979	-0.041047	0.13028	-0.32 (0.7527)
4	27.3	3	> 197.1 AND ≤ 625.2	0.9983	0.037322	0.12499	0.30 (0.7653)
5	27.3	4	> 625.2	referent	referent	referent	Referent
ORGANOTHIOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
7921	13.38	**<0.0001**	Organothiophosphates, intensity-weighted cumulative exposure days in 5 categories	0.029568	5.10 **(0.0004)**	0.83 (0.5038)	education, energy input, smoking, years of farming, triazine exp
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**organothiophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**

1	27.4	0	0	0.8566	0.137069	0.137407	1.00 (0.3185)
2	25.9	1	> 0 AND ≤ 3.1	0.9614	-1.31834	1.94748	-0.68 (0.4985)
3	27.2	2	> 3.1 AND ≤ 162.9	0.9818	-0.073639	0.13349	-0.55 (0.5812)
4	27.3	3	> 162.9 AND ≤ 597.3	0.9994	-0.028781	0.12761	-0.23 (0.8216)
5	27.3	4	> 597.3	referent	referent	referent	referent
CARBAMATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
7921	14.14	**<0.0001**	Carbamates, intensity-weighted cumulative exposure days in 4 categories	0.029516	1.02 (0.3844)	0.97 (0.4062)	education, energy input, years of farming, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**carbamate cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.4	0	0	0.5454	0.14878	0.112076	1.33 (0.1844)
2	27.2	1	> 0 AND ≤ 26.3	0.9911	-0.06466	0.21947	-0.29 (0.7683)
3	27.2	2	> 26.3 AND ≤ 297.0	0.9997	-0.011667	0.12503	-0.09 (0.9257)
4	27.2	3	> 297.0	referent	referent	referent	Referent
PHENOXY HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8008	11.06	**<0.0001**	Phenoxy Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.022998	9.20 **(<0.0001)**	1.44 (0.2171)	education, energy input, years of farming, triazine exp
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI2**	**Exposure Category**	**Phenoxy herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for Exposure Variable**	**Standard Error**	**t-value (p-value)**
1	27.3	0	0	0.4117	-0.260969	0.15032	-1.74 (0.0826)
2	27.3	1	> 0 AND ≤ 77.8	0.6186	-0.315768	0.22331	-1.41 (0.1574)
3	27.2	2	> 77.8 AND ≤ 585.6	0.1767	-0.292039	0.13226	-2.21 (0.0273)
4	27.3	3	> 585.6 AND ≤ 2000.4	0.4450	-0.213451	0.12688	-1.68 (0.0926)
5	27.5	4	> 2000.4	referent	referent	referent	referent
TRIAZINE HERBICIDES (CANNOT ADJUST FOR TRIAZINES)							

SUMMARY TABLE 4-10b

EXPOSURE RESPONSE EVALUATION USING PROC GLM (GENERAL LINEARIZED MODEL)

PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 (MAIN INDEPENDENT VARIABLE) AND BMI AT FOLLOW-UP (DEPENDENT VARIABLE)

ADDITIONAL INDEPENDENT VARIABLES INCLUDED IF RELATED TO BOTH EXPOSURE CLASS AND OUTCOME: AGE AT ENROLLMENT, NUMBER OF CIGARETTES PER DAY AT ENROLLMENT, EDUCATIONAL LEVEL AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, YEARS OF FARMING AT ENROLLMENT

INSECTICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Insecticides F-value (p-value), Type 1 SS	Insecticides F-value (p-value), Type 111 SS	Additional variables in model
8184	27.49	**<0.0001**	Insecticides, intensity-weighted cumulative exposure days in 5 categories	0.0262	3.76 **(0.0047)**	3.77 **(0.0045)**	age, smoking, energy input, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**insecticide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.3	0	0	0.4395	0.29585	0.17495	1.69 (0.0909)
2	27.6	1	> 0 AND ≤ 42.5	0.0460	-0.40914	0.14833	**-2.76 (0.0058)**
3	27.9	2	> 42.5 AND ≤ 165.9	0.9310	-0.10061	0.12594	-0.80 (0.4244)
4	27.9	3	> 165.9 AND ≤ 516.0	0.9728	-0.07709	0.12541	-0.61 (0.5387)
5	28.0	4	> 516.0	referent	referent	referent	referent

FUMIGANTS

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8835	27.71	**<0.0001**	Fumigants, intensity-weighted cumulative exposure days in 2 categories	0.0124	2.59 (0.0751)	2.81 (0.0604)	age, smoking

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORIES					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**fumigant cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.0	0	0	0.3831	0.19349	0.1464	1.32 (0.1863)
2	27.7	1	> 0 AND ≤ 98	0.7959	-0.12879	0.2001	-0.64 (0.5198)
3	27.8	2	> 98	referent	referent	referent	referent

FUNGICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8835	27.74	**<0.0001**	Fungicides, intensity-weighted cumulative exposure days in 3 categories	0.012410	1.31 (0.2696)	2.86 (0.0575)	smoking, age

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**fungicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.0	0	0	0.0489	0	0.0000	0
2	28.0	1	> 0 AND ≤ 1.4	0.423	0.27219	0.1157	2.35 (0.0186)
3	27.7	2	> 1.4	referent	0.32828	0.2624	1.25 (0.2109)

HERBICIDES

Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8184	29.27	**<0.0001**	Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.02785	12.01 **(<0.0001)**	7.25 **(<0.0001)**	energy input, years of farming, age, smoking

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.2	0	0	0.9946	-0.09224	0.2309	-0.40 (0.6895)
2	27.6	1	> 0 AND ≤ 207.8	**<0.0001**	-0.65898	0.1375	**-4.79 (<0.0001)**
3	27.8	2	> 207.8 AND ≤ 642.2	**0.0008**	-0.49392	0.1258	**-3.93 (<0.0001)**
4	28.0	3	> 642.2 AND ≤ 1619.9	0.3046	-0.24029	0.1249	-1.92 (0.0544)
5	28.3	4	> 1619.9	referent	referent	referent	Referent

HERBICIDES LESS TRIAZINES

Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable: Functional class, intensity weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8274	24.05	**<0.0001**	Herbicides less triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.01996	11.87 **(<0.0001)**	8.08 **(<0.0001)**	energy input, years of farming, age

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**Herbicide less triazine herbicides cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.2	0	0	0.9961	-0.082162	0.2236	-0.37 (0.7133)
2	27.6	1	> 0 AND ≤ 110.0	**<0.0001**	-0.682082	0.1377	**-4.95 (<0.0001)**
3	27.8	2	> 110.0 AND ≤ 655.4	**0.0010**	-0.486327	0.1252	**-3.88 (0.0001)**

4	28.1	3	> 655.4 AND ≤ 2100.3	0.6616	-0.167990	0.1247	-1.35 (0.1780)
5	28.2	4	> 2100.3	referent	referent	referent	referent
ORGANOCHLORINES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8705	28.14	**<0.0001**	Organochlorines, intensity-weighted cumulative exposure days in 3 categories	0.01592	**4.66 (0.0095)**	4.08 (0.0170)	age, smoking, years of farming
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEANS	**BMI3**	**Exposure Category**	**organochlorine cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.3991	-0.14696	0.1137	-1.29 (0.1960)
2	27.7	1	> 0 AND ≤ 150.4	0.0129	-0.35526	0.1255	**-2.83 (0.0047)**
3	28.1	2	> 150.4	referent	referent	referent	referent
ORGANOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8184	27.19	**<0.0001**	Organophosphates, intensity-weighted cumulative exposure days in 5 categories	0.025915	4.92 **(0.0006)**	3.18 (0.0127)	age, energy input, years of farming, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEANS	**BMI3**	**Exposure Category**	**organophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.0	0	0	0.9731	-0.093884	0.1532	-0.61 (0.5401)
2	27.6	1	> 0 AND ≤ 43.9	0.02200	-0.494308	0.1642	**-3.01 (0.0026)**
3	27.8	2	> 43.9 AND ≤ 197.1	0.0936	-0.311649	0.1253	-2.49 (0.0129)
4	28.0	3	> 197.1 AND ≤ 625.2	0.9336	-0.098748	0.1250	-0.79 (0.4295)
5	28.1	4	> 625.2	referent	referent	referent	referent
ORGANOTHIOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8184	23.66	**<0.0001**	Organothiophosphates, intensity-weighted cumulative exposure days in 5 categories	0.01986	5.72 **(0.0001)**	2.87 (0.0219)	energy input, years of farming, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		

LSMEANS	BMI3	Exposure Category	organothiophosphate cumulative exposure days	p-values	Model Parameter Estimate for each exposure category	Standard Error	t-value (p-value)
1	28.0	0	0	0.6082	-0.184859	0.1293	-1.43 (0.1527)
2	26.3	1	> 0 AND ≤ 3.1	0.8907	-1.854607	2.0238	-0.92 (0.3595)
3	27.7	2	> 3.1 AND ≤ 162.9	0.0109	-0.405681	0.1256	**-3.23 (0.0012)**
4	27.9	3	> 162.9 AND ≤ 597.3	0.2426	-0.257241	0.1255	-2.05 (0.0405)
5	28.2	4	> 597.3	referent	referent	referent	referent

CARBAMATES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8184	30.11	**<0.0001**	Carbamates, intensity-weighted cumulative exposure days in 4 categories	0.02513	2.05 (0.1049)	2.04 (0.1057)	age, smoking, energy input, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY — **Exposure variable Effects in model**

LSMEANS	BMI3	Exposure Category	carbamate cumulative exposure days	p-values	Model Parameter Estimate for each exposure category	Standard Error	t-value (p-value)
1	28.1	0	0	0.3463	0.187669	0.1132	1.66 (0.0974)
2	27.7	1	> 0 AND ≤ 26.3	0.8776	-0.164418	0.2198	-0.75 (0.4545)
3	27.8	2	> 26.3 AND ≤ 297.0	0.9975	-0.024234	0.1258	-0.19 (0.8472)
4	27.9	3	> 297.0	referent	referent	referent	referent

PHENOXY HERBICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8274	21.88	**<0.0001**	Phenoxy Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.01819	5.46 **(0.0002)**	4.34 **(0.0016)**	age, energy input, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY — **Exposure variable Effects in model**

LSMEANS	BMI3	Exposure Category	phenoxy herbicide cumulative exposure days	p-values	Model Parameter Estimate for each exposure category	Standard Error	t-value (p-value)
1	27.8	0	0	0.0222	-0.41545	0.1382	**-3.01 (0.0026)**
2	27.7	1	> 0 AND ≤ 77.8	0.0666	-0.57775	0.2204	**-2.62 (0.0088)**
3	27.8	2	> 77.8 AND ≤ 585.6	**0.0028**	-0.45781	0.1268	**-3.61 (0.0003)**
4	27.9	3	> 585.6 AND ≤ 2000.4	0.0373	-0.35653	0.1259	**-2.83 (0.0046)**
5	28.3	4	> 2000.4	referent	referent	referent	referent

TRIAZINE HERBICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8274	22.33	**<0.0001**	Triazine Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.01595	11.22 **(<0.0001)**	7.32 **(<0.0001)**	energy input, years of farming

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**triazine herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	**0.0006**	-0.550433	0.1370	**-4.02 (<0.0001)**
2	27.7	1	> 0 AND ≤ 73.9	0.1031	-0.607384	0.2482	-2.45 (0.0144)
3	27.7	2	> 73.9 AND ≤ 562.7	**<0.0001**	-0.626884	0.1255	**-4.99 (<0.0001)**
4	27.9	3	> 562.7 AND ≤ 1955.5	0.0165	-0.387588	0.1249	**-3.10 (0.0019)**
5	28.3	4	> 1955.5	referent	referent	referent	

Table 4-10b

EXPOSURE RESPONSE EVALUATION USING PROC GLM (GENERAL LINEARIZED MODEL)

PROSPECTIVE ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 1 (MAIN INDEPENDENT VARIABLE) AND BMI AT F (DEPENDENT VARIABLE) WITH ADJUSTMENT FOR TRIAZINES

ADDITIONAL INDEPENDENT VARIABLES INCLUDED IF RELATED TO BOTH EXPOSURE CLASS AND OUTCOME: AGE AT ENROLLMENT, NUMBER OF CIGARETTES ENROLLMENT, EDUCATIONAL LEVEL AT ENROLLMENT, ENERGY INPUT AT ENROLLMENT, YEARS OF FARMING AT ENROLLMENT

INSECTICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8184	20.78	**<0.0001**	Insecticides, intensity-weighted cumulative exposure days in 5 categories	0.02962	3.77 **(0.0046)**	3.77 **(0.0046)**	age, smoking, energy input, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**fumigant cumulative exposure days**	**p-values**	**Model Parameter Estimate fo category**	**Standard Error**	**t-value (p-value)**
1	28.4	0	0	0.0162	0.57488	0.1849	**3.11 (0.00**
2	27.7	1	> 0 AND ≤ 42.5	0.8523	-0.15859	0.1574	-1.01 (0.31
3	27.9	2	> 42.5 AND ≤ 165.9	0.9763	0.07796	0.1316	0.59 (0.55
4	27.9	3	> 165.9 AND ≤ 516.0	1.0000	0.00361	0.1267	0.03 (0.97
5	27.9	4	> 516.0	referent	referent	referer	referent

FUMIGANTS

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8835	21.01	**<0.0001**	Fumigants, intensity-weighted cumu days in 2 categories	0.018687	2.61 (0.0739)	3.53 (0.0293)	age, smoking, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposu**	**fumigant cumulative exposure days**	**p-values**	**Model Parameter Estimate category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.2504	0.23301	0.1466	1.59 (0.1121)
2	27.6	1	> 0 AND ≤ 98	0.825	-0.11811	0.1999	-0.59 (0.5545)
3	27.7	2	> 98	referent	referent	referent	referent

FUNGICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8835	20.80	**<0.0001**	Fungicides, intensity-weighted cu days in 3 categories	0.018509	1.32 (0.2675)	2.73 (0.0652)	smoking, age, triazine expos

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Catego**	**fungicide cumu days**	**p-values**	**Model Parameter Estimate fo category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.0583	0.26549	0.1163	2.28 (0.0225)
2	28.0	1	> 0 AND ≤ 1.4	0.383	0.34833	0.2635	1.32 (0.1862)
3	27.6	2	> 1.4	referent	referent	referent	referent

HERBICIDES (CANNOT ADJUST FOR TRIAZINES)

HERBICIDES LESS TRIAZINES

Number of subjects	ANOVA F value	ANOVA p-value	Variable: Functional class, intensity weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8274	16.30	**<0.0001**	Herbicides less triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.021243	11.88 **(<0.0001)**	3.15 (0.0133)	energy input, years of farming, age, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**Herbicide less triazine herbicides cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard error**	**t-value (p value)**
1	28.2	0	0	0.9598	0.175602	0.2564	0.68 (0.4934)
2	27.6	1	> 0 AND ≤ 110.0	0.0747	-0.419569	0.1628	**-2.58 (0.0100)**
3	27.8	2	> 110.0 AND ≤ 655.4	0.3178	-0.269229	0.1418	-1.90 (0.0576)
4	28.0	3	> 655.4 AND ≤ 2100.3	0.9979	-0.0413973	0.1311	-0.32 (0.7522)
5	28.1	4	> 2100.3	referent	referent	referent	referent

ORGANOCHLORINES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8705	20.28	**<0.0001**	Organochlorines, intensity-weighted cumulative exposure days in 3 categories	0.020558	**4.68 (0.0093)**	2.43 (0.0880)	age, smoking, years of farming, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Cate**	**organochlorine cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.9932	0.0129399	0.1164	0.11 (0.9115)
2	27.7	1	> 0 AND ≤ 150.4	0.1933	-0.220013	0.1270	-1.73 (0.0833)
3	27.9	2	> 150.4	referent	referent	referent	referent

ORGANOPHOSPHATES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in Model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure F-value (p-value), Type 1 SS	Exposure F-value (p-value), Type 111 SS	Additional variables in model
8184	19.94	**<0.0001**	Organophosphates, intensity-weighted cumul in 5 categories	0.028456	4.93 **(0.0006)**	1.32 (0.2613)	age, energy input, years of farming, smoking, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEANS	**BMI3**	**Exposure Category**	**organophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.1	0	0	0.9218	-0.093884	0.1532	0.83 (0.4073)
2	27.7	1	> 0 AND ≤ 43.9	0.5780	-0.494308	0.1642	-1.48 (0.1399)
3	27.8	2	> 43.9 AND ≤ 197.1	0.8926	-0.311649	0.1253	-0.91 (0.3620)
4	27.9	3	> 197.1 AND ≤ 625.2	1.0000	-0.098748	0.1250	-0.02 (0.9842)
5	28.0	4	> 625.2	referent	referent	referent	referent

ORGANOTHIOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8184	16.99	**<0.0001**	Organothiophosphates, intensity-weighted cumulative exposure days in 5 categories	0.022363	5.73 **(0.0001)**	1.13 (0.3384)	energy input, years of farming, smoking, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEANS	**BMI3**	**Exposure Category**	**organothiophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.0	0	0	0.9951	0.05447332	0.1399	0.39 (0.6971
2	26.4	1	> 0 AND ≤ 3.1	0.9325	-1.60546	2.0225	-0.79 (0.4273)
3	27.8	2	> 3.1 AND ≤ 162.9	0.6715	-0.18138274	0.1362	-1.33 (0.1831)
4	27.9	3	> 162.9 AND ≤ 597.3	0.9049	-0.405681	0.1256	-0.88 (0.3798)
5	28.0	4	> 597.3	referent	referent	referent	referent

CARBAMATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8184	22.13	**<0.0001**	Carbamates, intensity-weighted cumulative exposure days in 4 categories	0.028927	2.05 (0.1040)	3.08 (0.0.0264)	age, smoking, energy input, years of farming, triazine exposure

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEANS	**BMI3**	**Exposure Category**	**carbamate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.0	0	0	0.0552	0.29063766	0.1148	2.53 (0.0114)
2	27.7	1	> 0 AND ≤ 26.3	0.9991	-0.03017056	0.2221	-0.14 (0.8920)
3	27.8	2	> 26.3 AND ≤ 297.0	0.9904	0.03855782	0.1274	0.30 (0.7622)
4	27.8	3	> 297.0	referent	referent	referent	referent

PHENOXY HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in Model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure F-value (p-value), Type 1 SS**	**Exposure F-value (p-value), Type 111 SS**	**Additional variables in model**
8274	15.57	**<0.0001**	Phenoxy Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.020307	5.47 **(0.0002)**	1.18 (0.3177)	age, energy input, yrs farming, triazines

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEANS	**BMI3**	**Exposure Category**	**phenoxy herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.6125	-0.217430	0.1528	-1.42 (0.1547)
2	27.8	1	> 0 AND ≤ 77.8	0.605	-0.32964	0.2297	-1.43 (0.1513)
3	27.8	2	> 77.8 AND ≤ 585.6	0.3647	-0.24734	0.1363	-1.82 (0.0695)
4	27.8	3	> 585.6 AND ≤ 2000.4	0.3770	-0.23259	0.1296	-1.79 (0.0728)
5	28.1	4	> 2000.4	referent	referent	referent	**referent**

TRIAZINE HERBICIDES (CANNOT CONTROL FOR TRIAZINES)

SUMMARY TABLE 4-10c

EXPOSURE RESPONSE EVALUATION USING PROC GLM (GENERAL LINEARIZED MODEL)

CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 2 (MAIN INDEPENDENT VARIABLE) AND BMI AT FOLLOW-UP (DEPENDENT VARIABLE)

ADDITIONAL INDEPENDENT VARIABLES CONSIDERED IF RELATED TO BOTH EXPOSURE CLASS AND OUTCOME: AGE AT FOLLOW-UP, SMOKING STATUS AT FOLLOWUP, ENERGY INPUT AT FOLLOWUP

INSECTICIDES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model
8994	18.15	**<0.0001**	Insecticides, intensity-weighted cumulative exposure days in 5 categories	0.0159	3.50 **(0.0073)**	4.74 **(0.0008)**	age, smoking, energy input
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY						**Exposure variable Effects in model**	
LSMEAN	**BMI3**	**Exposure Category**	**Insecticide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.2	0	0	0.7470	0.20604	0.170624	1.21 (0.2272)
2	27.5	1	> 0 AND ≤ 52.3	**0.0051**	-0.47588	0.137839	**-3.45 (0.0006)**
3	27.8	2	> 52.3 AND ≤ 184.5	0.3256	-0.23062	0.122363	-1.88 (0.0595)
4	27.8	3	> 184.5 AND ≤ 541.7	0.6546	-0.16489	0.121405	-1.36 (0.1744)
5	28.0	4	> 541.7	referent	referent	referent	referent

FUMIGANTS

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model
9066	11.63	**<0.0001**	Fumigants, intensity-wtd cum. exp. days in 2 categories	0.00511	2.48 (0.0837)	2.95 (0.0523)	smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**fumigant cumulative exposure days**		**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**

1	27.9	0	0	0.6383	0.13018	0.1441	0.90 (0.3665)
2	27.5	1	> 0 AND ≤ 98.4	0.4896	-0.22590	0.1982	-1.14 (0.2544)
3	27.8	2	> 98.4	referent	referent	referent	referent
FUNGICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
9066	23.54	**<0.0001**	Fungicides, intensity-weighted cumulative exposure days in 3 categories	0.012827	3.26 (0.0383)	3.30 (0.0371)	age3, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**fungicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.0450	0.237896	0.0997	2.39 (0.0171)
2	28.0	1	> 0 AND ≤ 1.9	0.2011	0.369485	0.2159	1.71 (0.0871)
3	27.7	2	> 1.9	referent	referent	referent	referent
HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	17.09	**<0.0001**	Herbicides, intensity-weighted cumulative exposure days in 5 categories	0.013139	**12.51 (<0.0001)**	**11.73 (<0.0001)**	smoking, energy input
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**		**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.6	0	0	0.0973	-0.63998	0.2590	-2.47 (0.0135)
2	27.5	1	> 0 AND ≤ 269.0	**<0.0001**	-0.78099	0.1259	**-6.21 (<0.0001)**
3	27.7	2	> 269.0 AND ≤ 745.8	**<0.0001**	-0.58573	0.1212	**-4.83 (<0.0001)**
4	28.0	3	> 745.8 AND ≤ 1764.8	0.1839	-0.265085	0.1211	-2.19 (0.0286)
5	28.2	4	> 1764.8	referent	referent	referent	referent
HERBICIDES LESS TRIAZINE HERBICIDES							

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model
8994	15.84	**<0.0001**	Herbicides less Triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.012192	**10.28 (<0.0001)**	**9.56 (<0.0001)**	energy input, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**Herbicides less triazine herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.7	0	0	0.1844	-0.54394	0.2486	-2.19 (0.0287)
2	27.5	1	> 0 AND ≤ 238.0	**<0.0001**	-0.73117	0.1264	**-5.78 (<0.0001)**
3	27.7	2	> 238.0 AND ≤ 687.3	**0.0005**	-0.48819	0.1212	**-4.03 (<0.0001)**
4	28.0	3	> 687.3 AND ≤ 1647.3	0.2600	-0.24379	0.1212	-2.01 (0.0442)
5	28.2	4	> 1647.3	referent	referent	referent	referent

ORGANOCHLORINES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model
8994	22.66	**<0.0001**	Organochlorines, intensity-weighted cumulative exposure days in 3 categories	0.014905	**5.49 (0.0041)**	**4.93 (0.0072)**	age, smoking, energy input
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**organochlorine cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.1688	-0.20133	0.1117	-1.80 (0.0715)
2	27.6	1	> 0 AND ≤ 154.3	**0.0048**	-0.38910	0.1239	**-3.14 (0.0017)**
3	28.0	2	> 154.3	referent	referent	referent	referent

ORGANOPHOSPHATES

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Functional class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model

8997	12.44	**<0.0001**	Organophosphates, intensity-weighted cumulative exposure days in 5 categories	0.008235	**5.80 (0.0001)**	**5.81 (0.0001)**	age, energy input
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**organophosphate cumulative exposure days**	**p-values**	Model Parameter Estimate for each exposure category	Standard Error	t-value (p-value)
1	28.0	0	0	0.4829	-0.23858	0.1471	-1.62 (0.1047)
2	27.6	1	> 0 AND ≤ 55.1	0.0004	-0.62109	0.1522	**-4.08 (<0.0001)**
3	27.7	2	> 55.1 AND ≤ 215.4	0.0018	-0.45474	0.1220	**-3.73 (0.0002)**
4	28.0	3	> 215.4 AND ≤ 663.9	0.5678	-0.18152	0.1217	-1.49 (0.1358)
5	28.2	4	> 663.9	referent	referent	referent	referent
ORGANOTHIOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	18.62	**<0.0001**	Organothiophosphates, intensity-weighted cumulative exposure days in 5 categories	0.016306	**6.12 (<0.0001)**	**5.67 (0.0001)**	age, kcal/day, never smoker, exsmoker, current smoker
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**organothiophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.1124	-0.29684	0.1234	-2.41 (0.0161)
2	27.3	1	> 0 AND ≤ 13.8	0.1396	-0.83877	0.3621	-2.32 (0.0205)
3	27.6	2	> 13.8 AND ≤ 178.0	**<0.0001**	-0.54087	0.1216	**-4.45 (<0.0001)**
4	27.8	3	> 178.0 AND ≤ 630.3	0.0337	-0.34808	0.1211	**-2.87 (0.0041)**
5	28.1	4	> 630.3	referent	referent	referent	referent
CARBAMATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**

9066	19.40	**<0.0001**	Carbamates, intensity-weighted cumulative exposure days in 4 categories	0.012689	2.60 (0.0503)	1.77 (0.1500)	age, smoking
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**carbamate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.6432	0.127954	0.1090	1.17 (0.2403)
2	27.6	1	> 0 AND ≤ 45.9	0.6883	-0.199045	0.1806	-1.10 (0.2705)
3	27.8	2	> 45.9 AND ≤ 351.9	0.9755	-0.050461	0.1209	-0.42 (0.6765)
4	27.8	3	> 351.9	referent	referent	referent	referent
PHENOXY HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	19.15	**<0.0001**	Phenoxy herbicides, intensity-weighted cumulative exposure days in 5 categories	0.016762	**4.91 (0.0006)**	**6.71 (<0.0001)**	age, smoking, kcal/day
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**phenoxy herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.6	0	0	**0.0002**	-0.57996	0.1361	**-4.26 (<.0001)**
2	27.6	1	> 0 AND ≤ 127.7	**0.0048**	-0.595255	0.1715	**-3.47 (0.0005)**
3	27.7	2	> 127.7 AND ≤ 701.1	**0.0005**	-0.49635	0.1221	**-4.06 (<0.0001)**
4	27.9	3	> 701.1 AND ≤ 2179.1	0.0649	-0.31902	0.1212	**-2.63(0.0085)**
5	28.2	4	> 2179.1	referent	referent	referent	referent
TRIAZINE HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	17.19	**<0.0001**	Triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.013216	**12.26 (<0.0001)**	**11.91 (<0.0001)**	smoking, energy input

RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					Exposure variable Effects in model		
LSMEAN	**BMI3**	**Exposure Category**	**triazine herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.6	0	0	**<0.0001**	-0.73575	0.1307	**-5.63 (<0.0001)**
2	27.4	1	> 0 AND ≤ 110.0	**<0.0001**	-0.933333	0.1976	**-4.72 (<0.0001)**
3	27.7	2	> 110.0 AND ≤ 655.4	**<0.0001**	-0.631594	0.1211	**-5.22 (<0.0001)**
4	27.9	3	> 655.4 AND ≤ 2100.3	**0.0013**	-0.46101	0.1210	**-3.81 (0.0001)**
5	28.3	4	> 2100.3	referent	referent	referent	referent

SUMMARY TABLE 10c							
EXPOSURE RESPONSE EVALUATION USING PROC GLM (GENERAL LINEARIZED MODEL)							
CROSS-SECTIONAL ANALYSIS: EXPOSURE TO PESTICIDE FUNCTIONAL OR CHEMICAL CLASSES THROUGH PHASE 2 (MAIN INDEPENDENT VARIABLE) AND BMI AT FOLLOW-UP (DEPENDENT VARIABLE) WITH ADJUSTMENT FOR TRIAZINES							
ADDITIONAL INDEPENDENT VARIABLES CONSIDERED IF RELATED TO BOTH EXPOSURE CLASS AND OUTCOME: AGE AT FOLLOW-UP, SMOKING STATUS AT FOLLOWUP, ENERGY INPUT AT FOLLOWUP							
INSECTICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	16.17	**<0.0001**	Insecticides, intensity-weighted cumulative exposure days in 5 categories	0.021154	3.52 **(0.0071)**	4.21 **(0.0021)**	age, smoking, energy input, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY						**Exposure variable Effects in model**	
LSMEAN	**BMI3**	**Exposure Category**	**Insecticide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.3	0	0	0.0144	0.56436261	0.179480	**3.14 (0.0017)**
2	27.6	1	> 0 AND ≤ 52.3	0.7984	-0.16257313	0.145727	-1.12 (0.2646)
3	27.7	2	> 52.3 AND ≤ 184.5	0.9999	-0.01952280	0.127264	-0.15 (0.8781)
4	27.7	3	> 184.5 AND ≤ 541.7	0.9781	-0.07097	0.122347	-0.58 (0.5623)
5	27.8	4	> 541.7	referent	referent	referent	referent
FUMIGANTS							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
9066	12.41	**<0.0001**	Fumigants, intensity-wtd cum. exp. days in 2 categories	0.01084	2.49 (0.0826)	3.54 (0.0289)	smoking, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**fumigant cumulative exposure days**		**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.4778	0.16756327	0.1446	1.16 (0.2465)

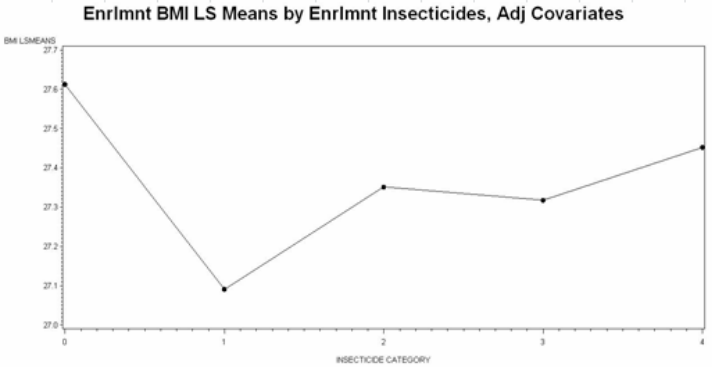
2	27.4	1	> 0 AND ≤ 98.4	0.5280	-0.21340706	0.1980	-1.08 (0.2812)
3	27.7	2	> 98.4	referent	referent	referent	referent
FUNGICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
9066	19.05	**<0.0001**	Fungicides, intensity-weighted cumulative exposure days in 3 categories	0.018577	3.28 (0.0376)	3.48 (0.0307)	age, smoking, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**fungicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.0319	0.25179072	0.1001	2.52 (0.0119)
2	27.9	1	> 0 AND ≤ 1.9	0.2321	0.35384272	0.2168	1.63 (0.1027)
3	27.6	2	> 1.9	referent	referent	referent	referent
HERBICIDES (CANNOT ADJUST FOR TRIAZINES)							
HERBICIDES LESS TRIAZINE HERBICIDES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	11.62	**<0.0001**	Herbicides less Triazine herbicides, intensity-weighted cumulative exposure days in 5 categories	0.014029	**10.29 (<0.0001)**	**1.85 (0.1162)**	smoking, energy input, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**Herbicides less triazine herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.9678	-0.17809	0.2766	-0.64 (0.5197)
2	27.6	1	> 0 AND ≤ 238.0	0.0821	-0.38932	0.1533	-2.54 (0.0111)
3	27.7	2	> 238.0 AND ≤ 687.3	0.4292	-0.23529	0.1378	-1.71 (0.0877)

4	27.9	3	> 687.3 AND ≤ 1647.3	0.9527	-0.09165	0.1279	-0.72 (0.4735)
5	27.9	4	> 1647.3	referent	referent	referent	referent
ORGANOCHLORINES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8994	18.26	**<0.0001**	Organochlorines, intensity-weighted cumulative exposure days in 3 categories	0.019926	**5.52 (0.0040)**	2.78 (0.0619)	age, smoking, energy input, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**organochlorine cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.8	0	0	0.9706	-0.02672	0.1148	-0.23 (0.8159)
2	27.6	1	> 0 AND ≤ 154.3	0.1023	-0.25578	0.1252	-2.04 (0.0411)
3	27.8	2	> 154.3	referent	referent	referent	referent
ORGANOPHOSPHATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Functional class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
8997	10.61	**<0.0001**	Organophosphates, intensity-weighted cum. exp. days in 5 categories	0.011667	**5.81 (0.0001)**	2.12 (0.0754)	age, energy input, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**organophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	28.0	0	0	0.9975	0.05191	0.1579	0.33 (0.1047)
2	27.6	1	> 0 AND ≤ 55.1	0.2292	-0.33452	0.1609	-2.08 (0.7423)
3	27.7	2	> 55.1 AND ≤ 215.4	0.3254	-0.24284	0.1288	-1.89 (0.0376)
4	27.9	3	> 215.4 AND ≤ 663.9	0.9781	-0.18152	0.1217	-1.49 (0.0595)
5	28.0	4	> 663.9	referent	referent	referent	**referent**
ORGANOTHIOPHOSPHATES							

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model
8994	15.41	**<0.0001**	Organothiophosphates, intensity-weighted cumulative exposure days in 5 categories	0.020172	**6.15 (<0.0001)**	**1.95 (0.0987)**	age, energy input, smoking, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**organothiophosphate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.0	0	0	referent	0.00597	0.1342	0.04 (0.9645)
2	26.5	1	> 0 AND ≤ 13.8	0.5659	-0.46870	0.3670	-1.28 (0.2016)
3	26.8	2	> 13.8 AND ≤ 178.0	0.2736	-0.26805	0.1307	-2.05 (0.0404)
4	26.9	3	> 178.0 AND ≤ 630.3	0.9937	-0.17602	0.1256	-1.40 (0.1612)
5	27.3	4	> 630.3	0.1137	referent	referent	referent
CARBAMATES							
Number of subjects	**ANOVA F value**	**ANOVA p-value**	**Variable in model: Chemical class, intensity-weighted cumulative exposure days**	**R^2**	**Exposure variable F-value (p-value) for model with Type 1 SS**	**Exposure variable F-value (p value) for model with Type III SS**	**Additional variables in model**
9066	17.30	**<0.0001**	Carbamates, intensity-weighted cumulative exposure days in 4 categories	0.01875	2.62 (0.0493)	2.86 (0.0357)	age, smoking, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**carbamate cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.9	0	0	0.1096	0.248574	0.1103	2.25 (0.0243)
2	27.6	1	> 0 AND ≤ 45.9	0.9919	-0.052072	0.1827	-0.28 (0.7757)
3	27.7	2	> 45.9 AND ≤ 351.9	1.0000	0.002869	0.1222	0.02 (0.9813)
4	27.7	3	> 351.9	referent	referent	referent	referent
PHENOXY HERBICIDES							

Number of subjects	ANOVA F value	ANOVA p-value	Variable in model: Chemical class, intensity-weighted cumulative exposure days	R^2	Exposure variable F-value (p-value) for model with Type 1 SS	Exposure variable F-value (p value) for model with Type III SS	Additional variables in model
8994	15.20	**<0.0001**	Phenoxy herbicides, intensity-weighted cumulative exposure days in 5 categories	0.019908	**4.93 (0.0006)**	**1.35 (0.2494)**	age, smoking, energy input, triazine exposure
RESULTS OF LEAST SQUARE MEANS BY EXPOSURE CATEGORY					**Exposure variable Effects in model**		
LSMEAN	**BMI3**	**Exposure Category**	**phenoxy herbicide cumulative exposure days**	**p-values**	**Model Parameter Estimate for each exposure category**	**Standard Error**	**t-value (p-value)**
1	27.6	0	0	0.2715	-0.30174	0.1517	-1.99 (0.0468)
2	27.7	1	> 0 AND ≤ 127.7	0.5209	-0.28347	0.1813	-1.56 (0.1179)
3	27.7	2	> 127.7 AND ≤ 701.1	0.3140	-0.24935	0.1308	-1.91 (0.0567)
4	27.8	3	> 701.1 AND ≤ 2179.1	0.5936	-0.18061	0.1243	-1.45 (0.1464)
5	27.9	4	> 2179.1	referent	referent	referent	referent
TRIAZINE HERBICIDES (CANNOT ADJUST FOR TRIAZINES)							

FIGURE 4-1: DOSE-RESPONSE GRAPHS FOR BMI AT ENROLLMENT (BMI2) USING PHASE 1 EXPOSURE DATA ON PESTICIDE FUNCTIONAL AND CHEMICAL CLASSES -- CUMULATIVE EXPOSURE AND LSMEANS FOR CROSS-SECTIONAL ANALYSIS



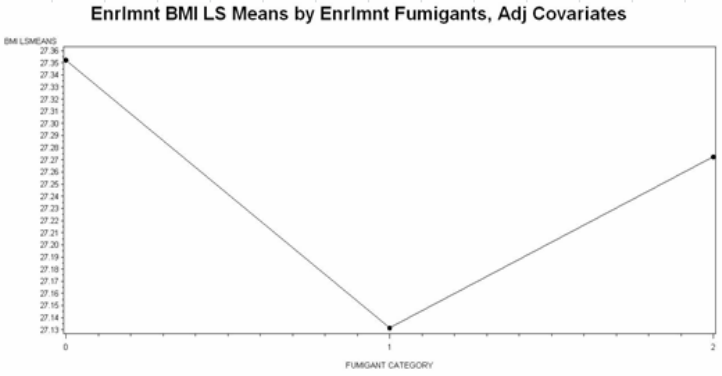
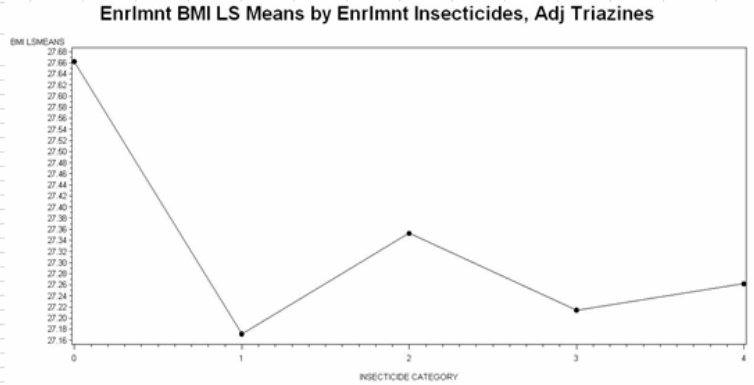
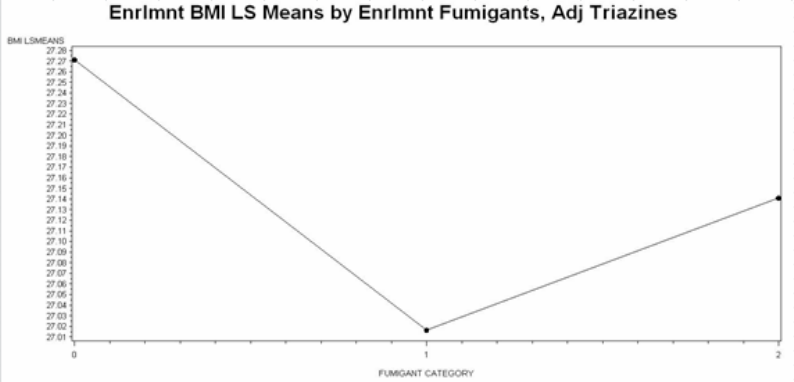
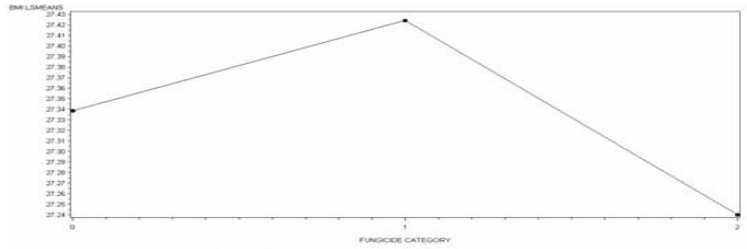


FIGURE 4-1: DOSE-RESPONSE GRAPHS FOR BMI AT ENROLLMENT (BMI2) USING PHASE 1 EXPOSURE DATA ON PESTICIDE FUNCTIONAL AND CHEMICAL CLASSES -- CUMULATIVE EXPOSURE AND LSMEANS FOR CROSS-SECTIONAL ANALYSIS CONTROLLING FOR TRIAZINES





Enrlmnt BMI LS Means by Enrlmnt Fungicides, Adj Covariates



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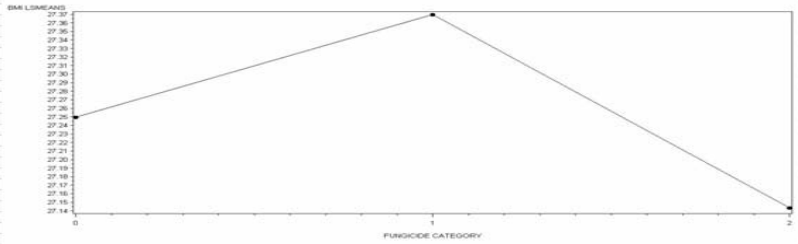
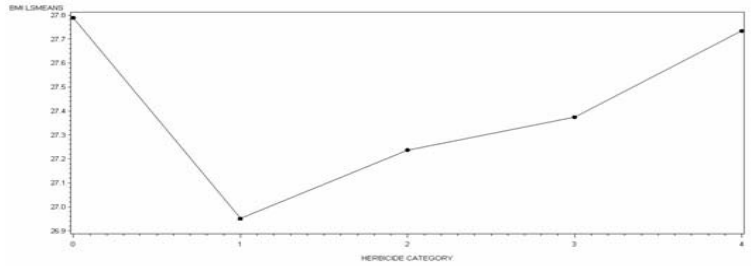
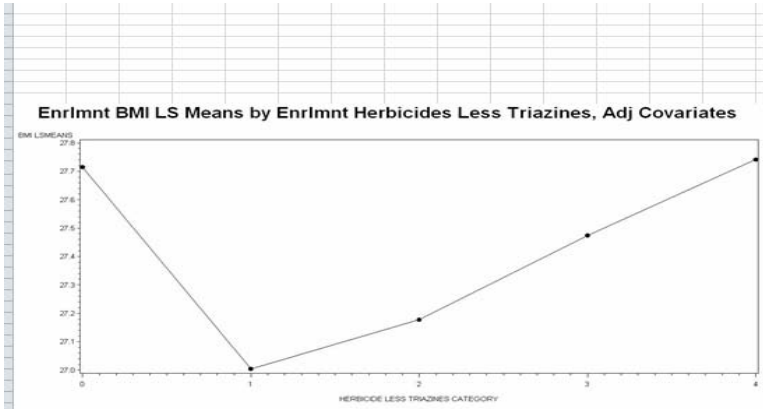
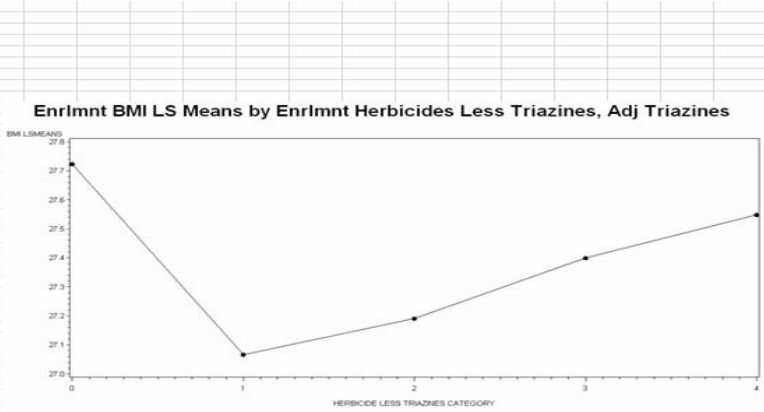


Enrlmnt BMI LS Means by Enrlmnt Herbicides, Adj Covariates



CANNOT ADJUST HERBICIDES FOR TRIAZINE EXPOSURE


Enrlmnt BMI LS Means by Enrlmnt Herbicides Less Triazines, Adj Covariates
BMI LSMEANS
HERBICIDE LESS TRIAZINES CATEGORY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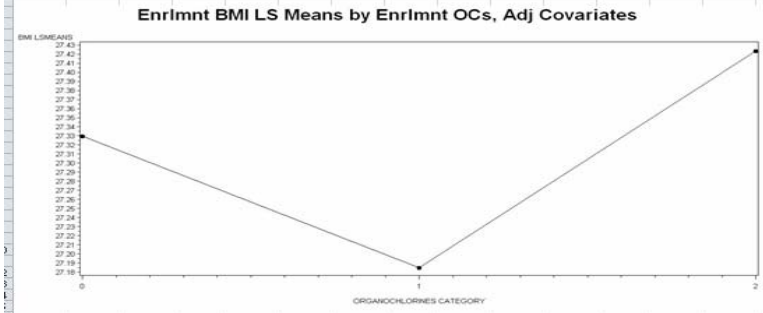
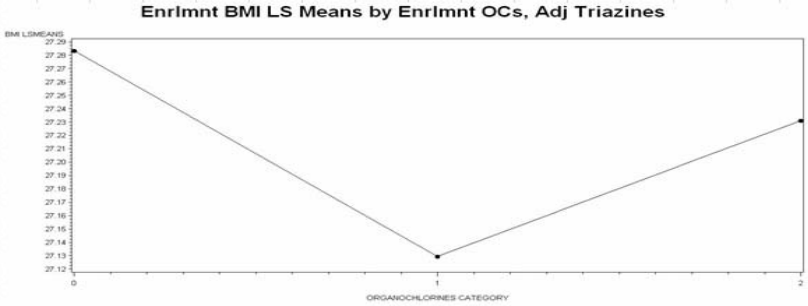
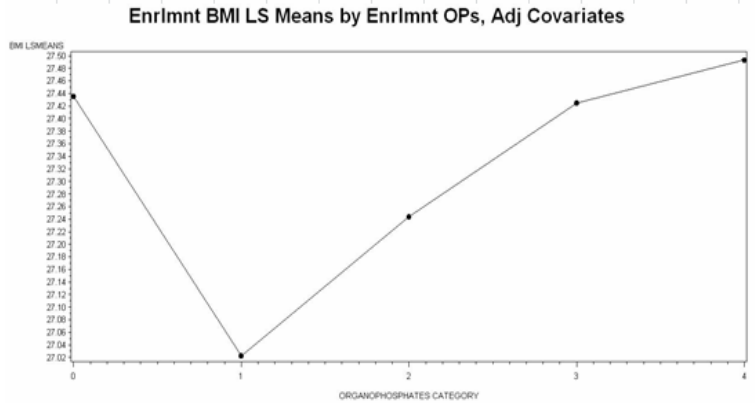
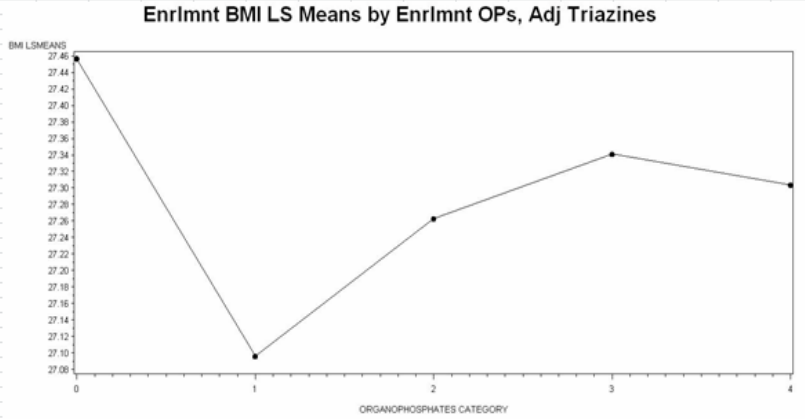
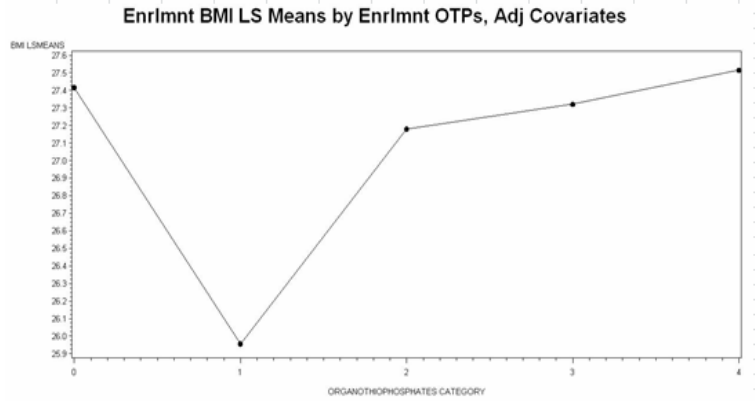
BMI LSMEANS
HERBICIDE LESS TRIAZINES CATEGORY

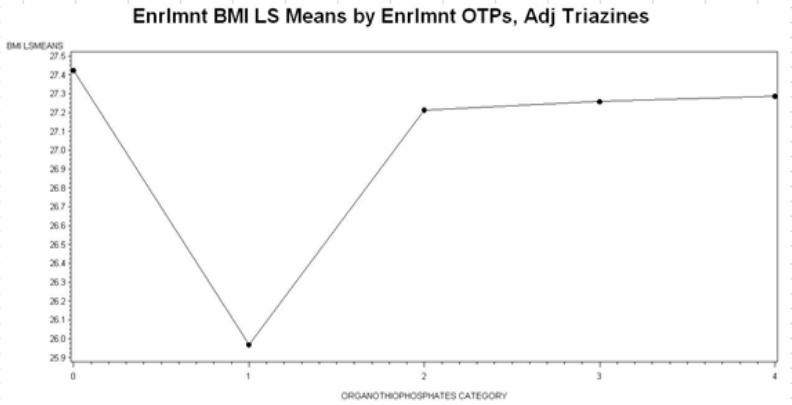

Enrlmnt BMI LS Means by Enrlmnt OCs, Adj Covariates
BMI LSMEANS
ORGANOCHLORINES CATEGORY

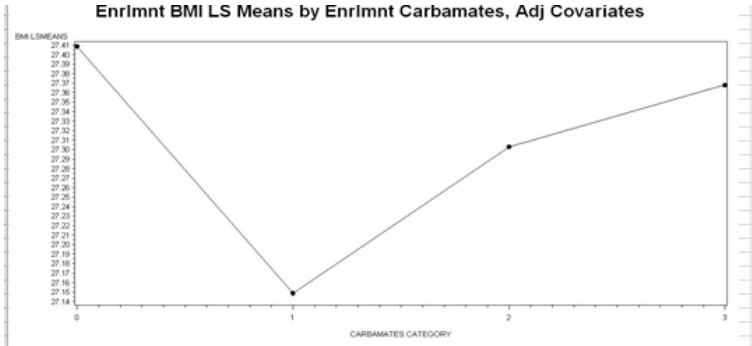

Enrlmnt BMI LS Means by Enrlmnt OCs, Adj Triazines
BMI LSMEANS
ORGANOCHLORINES CATEGORY

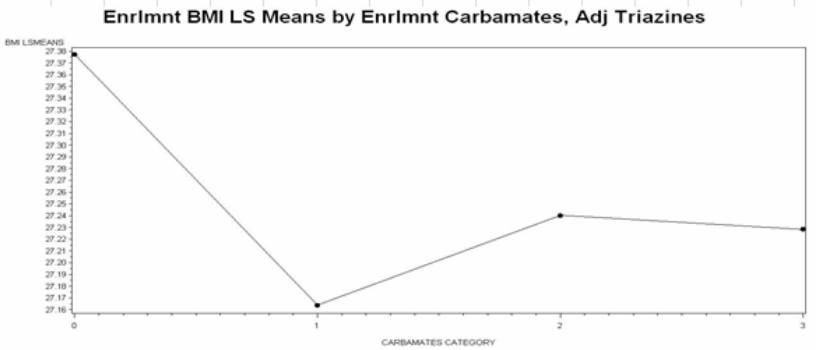

Enrlmnt BMI LS Means by Enrlmnt OPs, Adj Covariates
BMI LSMEANS
ORGANOPHOSPHATES CATEGORY

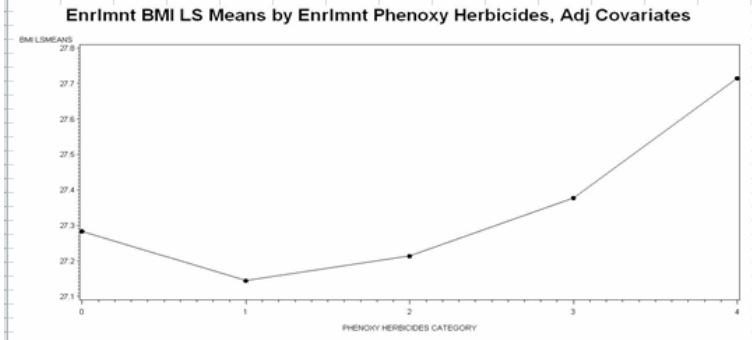

Enrlmnt BMI LS Means by Enrlmnt OPs, Adj Triazines
BMI LSMEANS
ORGANOPHOSPHATES CATEGORY

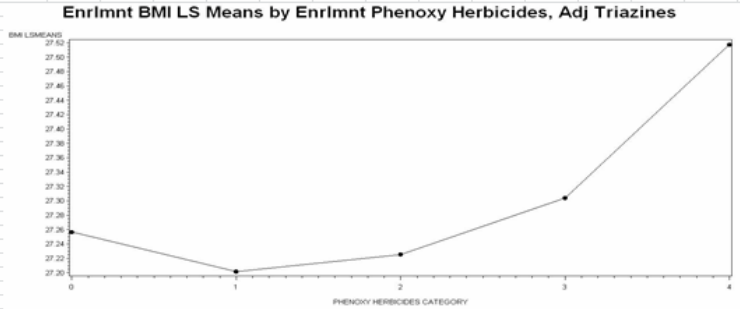

Enrlmnt BMI LS Means by Enrlmnt OTPs, Adj Covariates
BMI LSMEANS
ORGANOTHIOPHOSPHATES CATEGORY

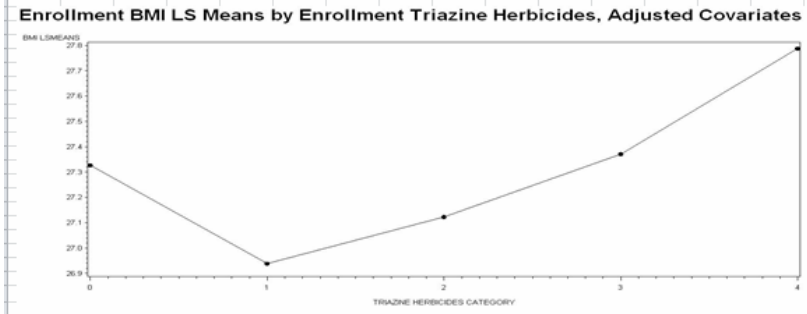

Enrlmnt BMI LS Means by Enrlmnt OTPs, Adj Triazines
BMI LSMEANS
ORGANOTHIOPHOSPHATES CATEGORY





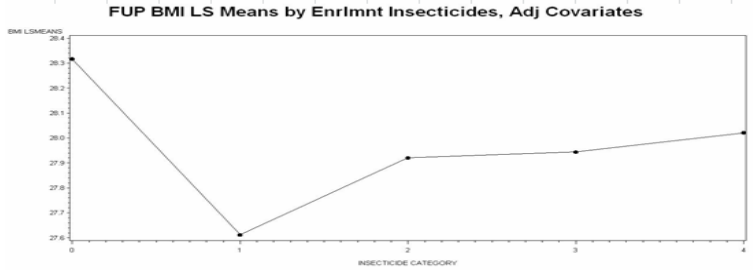






CANNOT ADJUST TRIAZINES FOR TRIAZINES

FIGURE 4-2: DOSE-RESPONSE GRAPHS FOR BMI AT FOLLOWUP (BMI3) BASED ON PHASE 1 EXPOSURE TO PESTICIDE FUNCTIONAL AND CHEMICAL CLASSES, PROSPECTIVE ANALYSIS



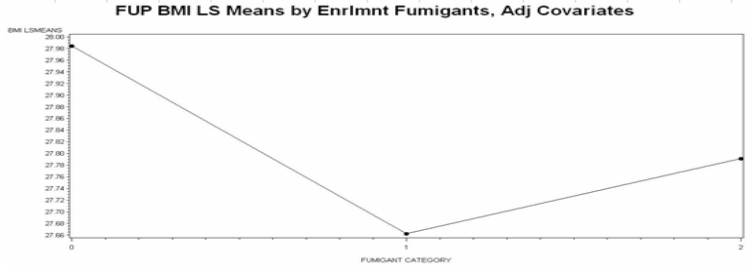
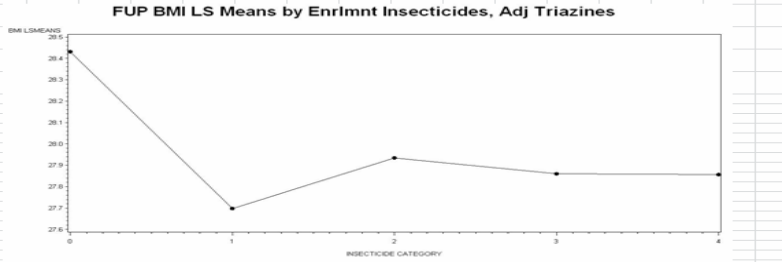
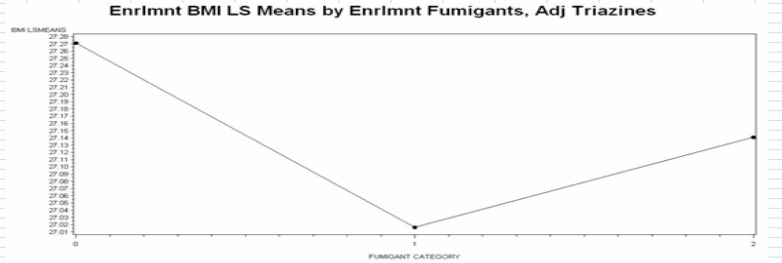
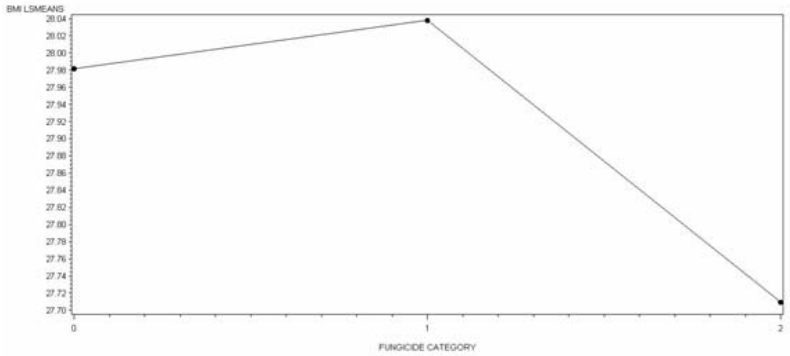


FIGURE 4-2: DOSE-RESPONSE GRAPHS FOR BMI AT FOLLOWUP (BMI3) BASED ON PHASE 1 EXPOSURE TO PESTICIDE FUNCTIONAL AND CHEMICAL CLASSES, PROSPECTIVE ANALYSIS CONTROLLING FOR COVARIATES AND TRIAZINES

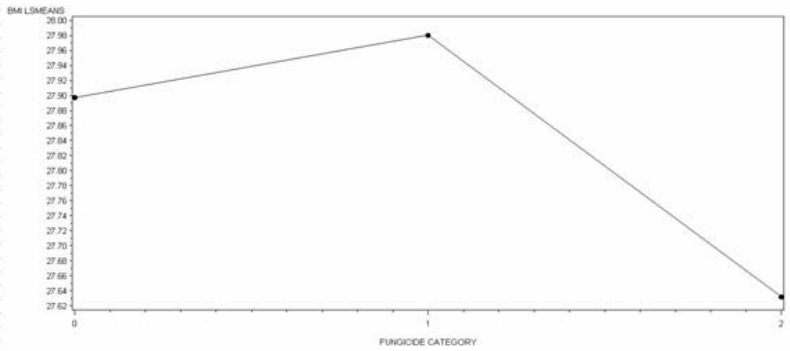




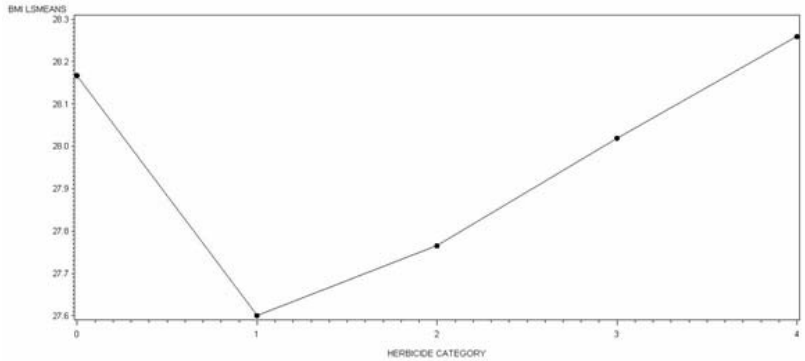
FUP BMI LS Means by Enrlmnt Fungicide Category, Adj Covariates



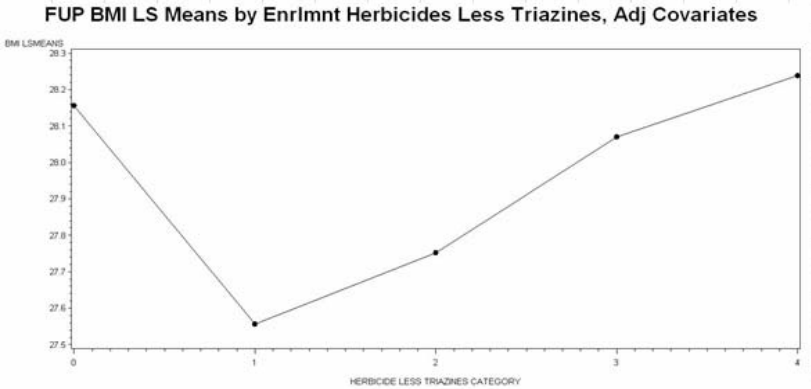
FUP BMI LS Means by Enrlmnt Fungicide Category, Adj Triazines

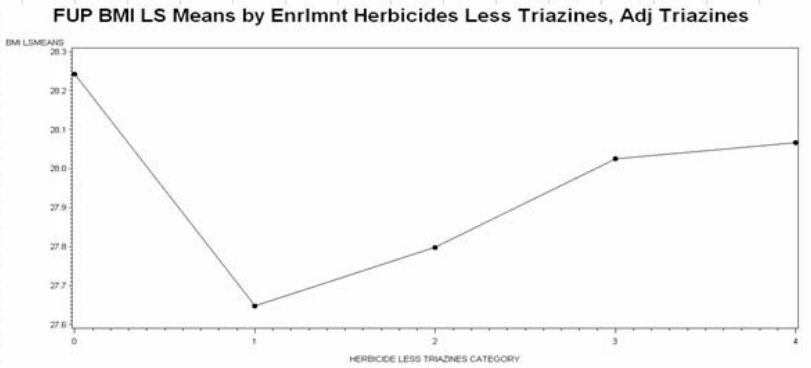


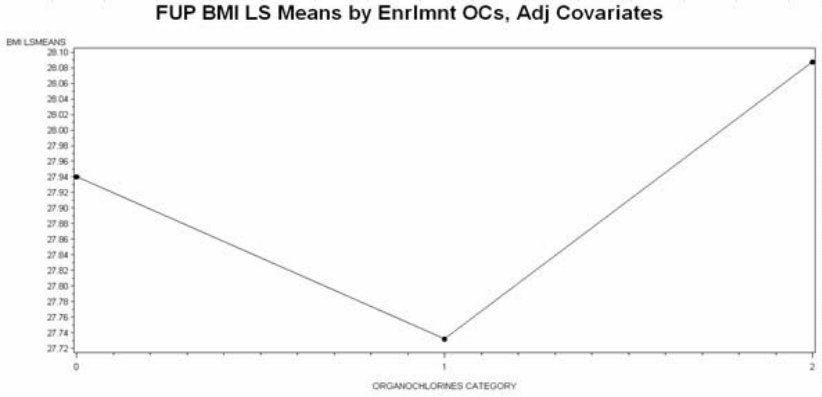
FUP BMI LS Means by Enrlmnt Herbicides, Adj Covariates



CANNOT ADJUST HERBICIDES FOR TRIAZINES


FUP BMI LS Means by Enrlmnt Herbicides Less Triazines, Adj Covariates
BMI LSMEANS
28.3
28.2
28.1
28.0
27.9
27.8
27.7
27.6
27.5
0
1
2
3
4
HERBICIDE LESS TRIAZINES CATEGORY


FUP BMI LS Means by Enrlmnt Herbicides Less Triazines, Adj Triazines
BMI LSMEANS
28.3
28.2
28.1
28.0
27.9
27.8
27.7
27.6
0
1
2
3
4
HERBICIDE LESS TRIAZINES CATEGORY


FUP BMI LS Means by Enrlmnt OCs, Adj Covariates
BMI LSMEANS
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28.06
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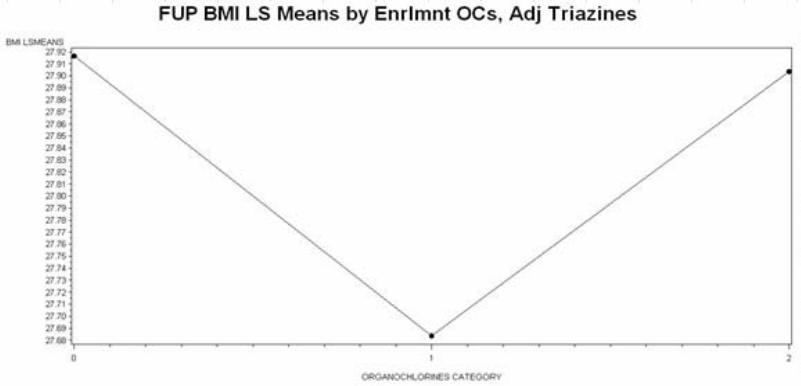
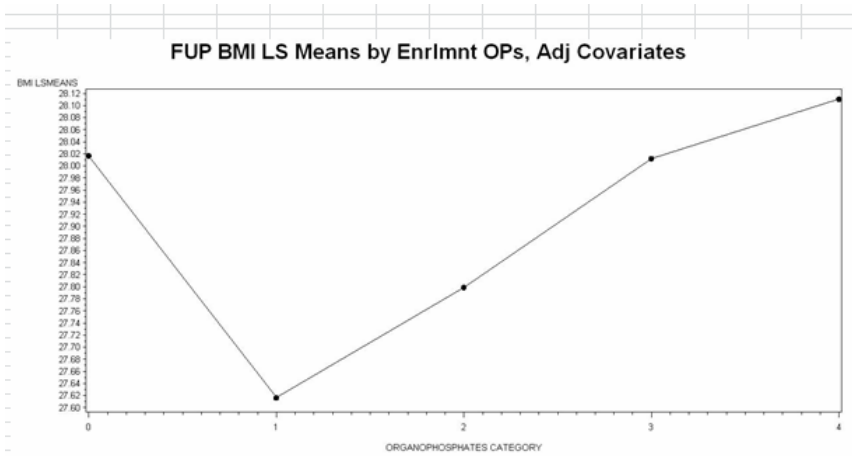
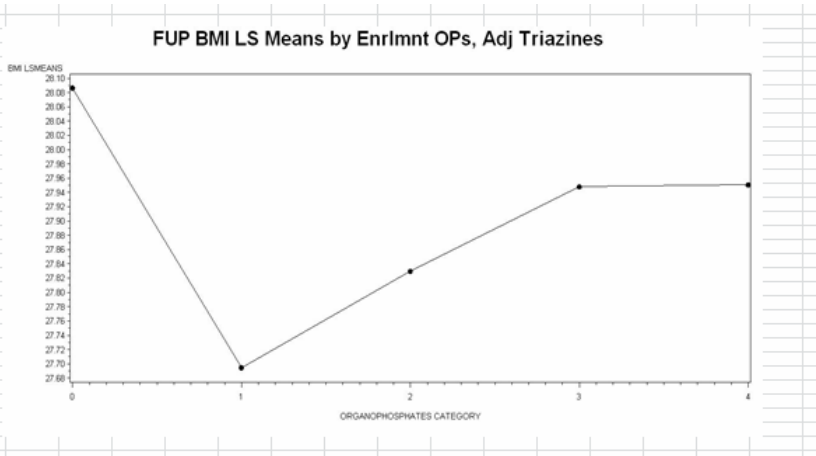
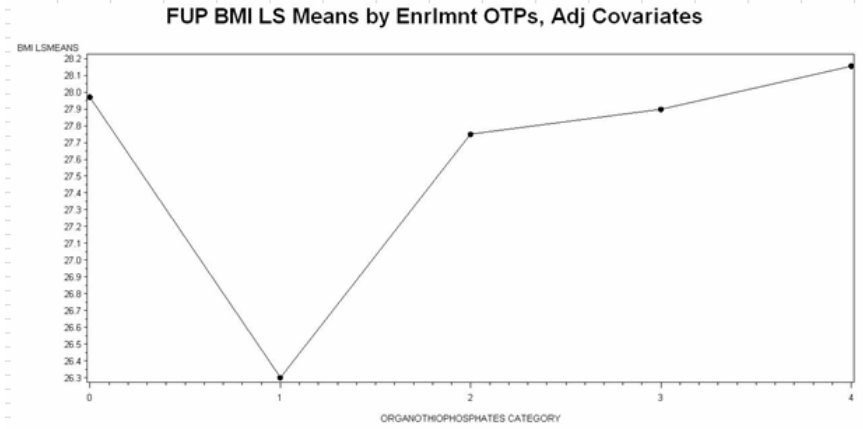
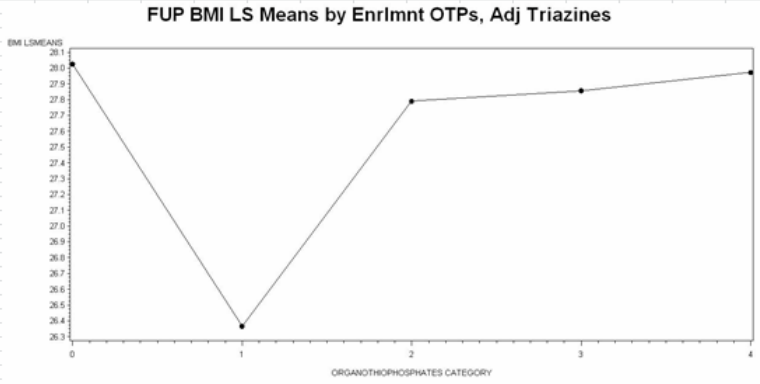
28.02
28.00
27.98
27.96
27.94
27.92
27.90
27.88
27.86
27.84
27.82
27.80
27.78
27.76
27.74
27.72
0
1
2
ORGANOCHLORINES CATEGORY

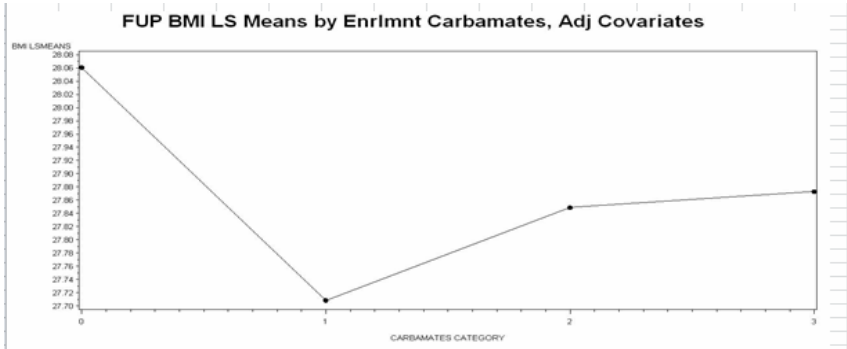

FUP BMI LS Means by Enrlmnt OCs, Adj Triazines
BMI LSMEANS
27.92
27.91
27.90
27.89
27.88
27.87
27.86
27.85
27.84
27.83
27.82
27.81
27.80
27.79
27.78
27.77
27.76
27.75
27.74
27.73
27.72
27.71
27.70
27.69
27.68
0
1
2
ORGANOCHLORINES CATEGORY

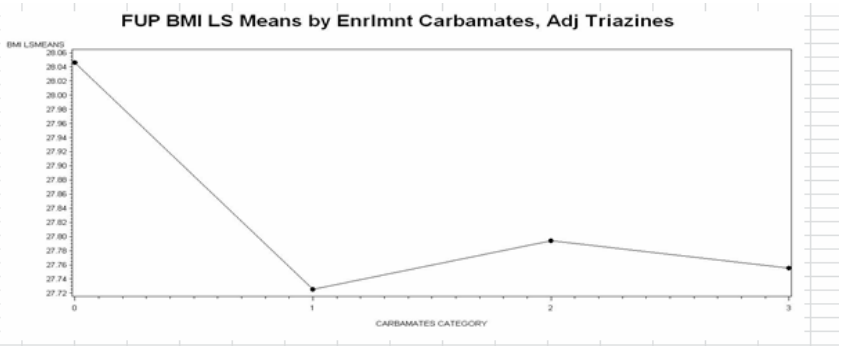

FUP BMI LS Means by Enrlmnt OPs, Adj Covariates
BMI LSMEANS
ORGANOPHOSPHATES CATEGORY

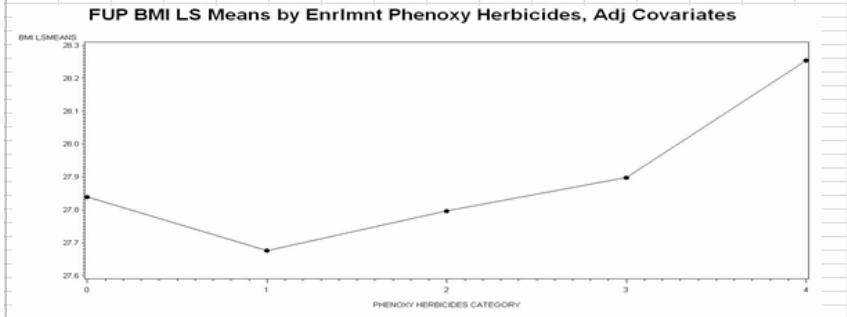

FUP BMI LS Means by Enrlmnt OPs, Adj Triazines
BMI LSMEANS
ORGANOPHOSPHATES CATEGORY

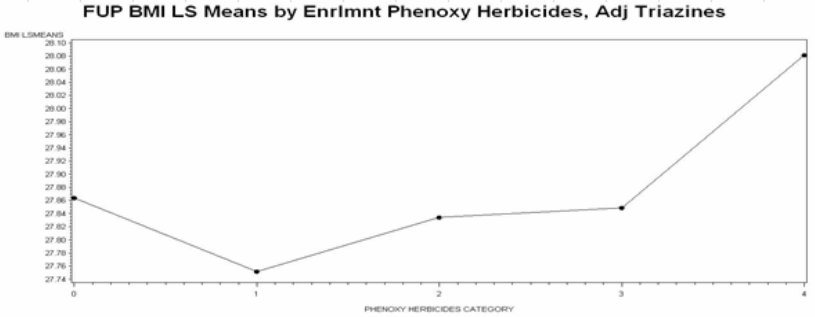

FUP BMI LS Means by Enrlmnt OTPs, Adj Covariates
BMI LSMEANS
ORGANOTHIOPHOSPHATES CATEGORY

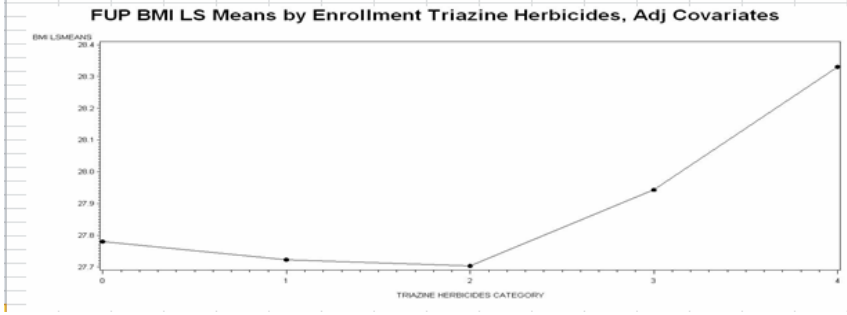

FUP BMI LS Means by Enrlmnt OTPs, Adj Triazines
BMI LSMEANS
ORGANOTHIOPHOSPHATES CATEGORY





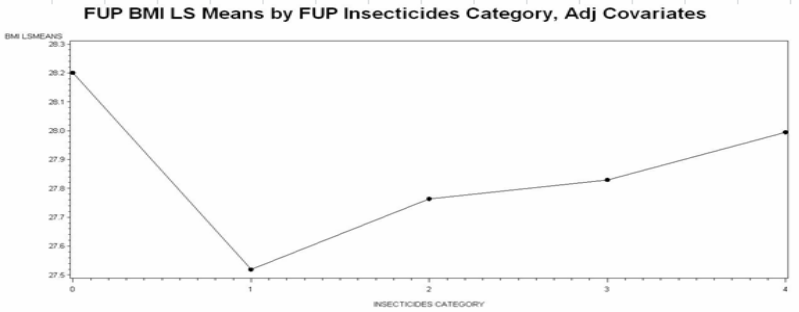


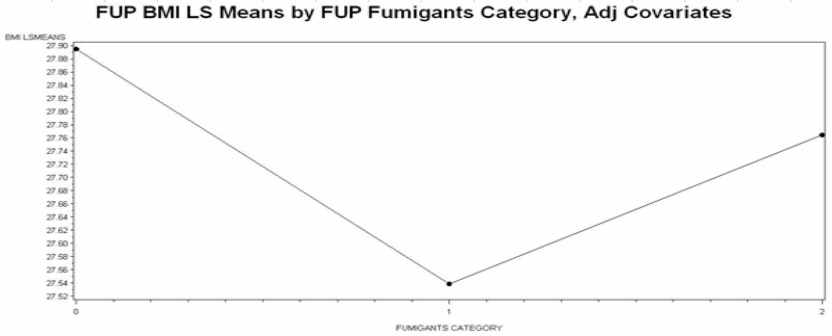




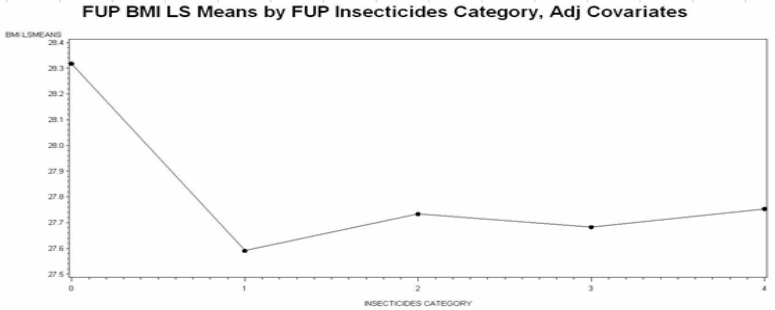
CANNOT ADJUST TRIAZINES FOR TRIAZINES

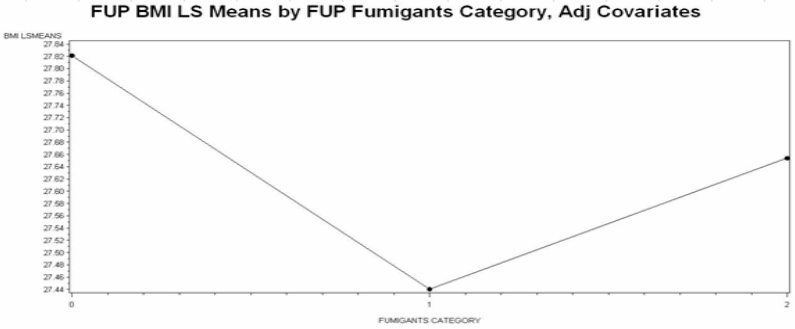
FIGURE 4-3: DOSE-RESPONSE GRAPHS FOR BMI3 (FOLLOW-UP) BASED ON PHASE 2 EXPOSURE TO FUNCTIONAL AND CHEMICAL CLASSES, CROSS-SECTIONAL ANALYSIS

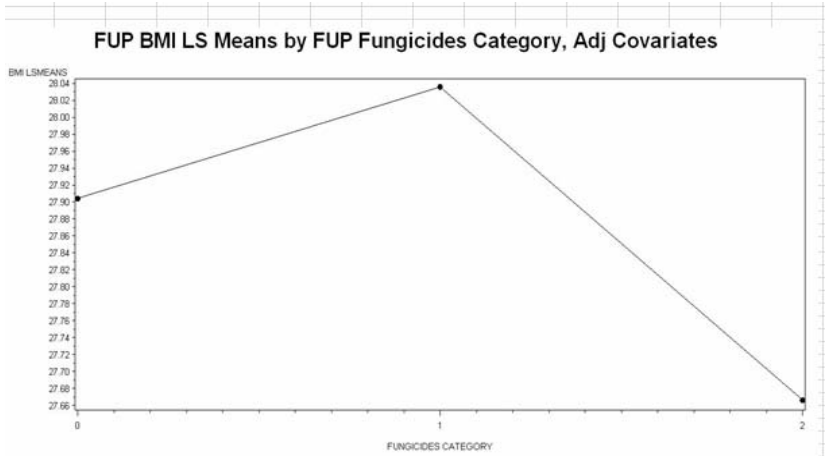


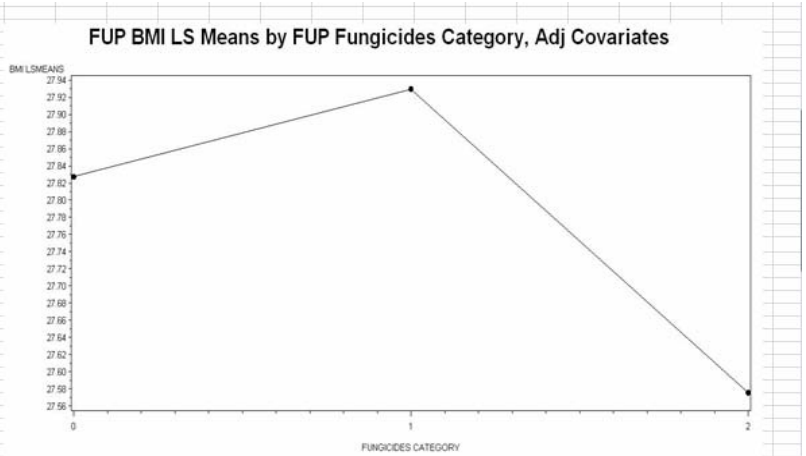


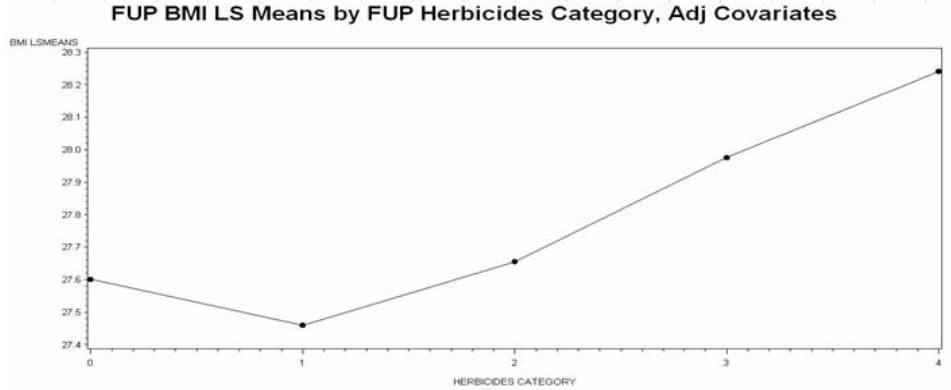
FUNCTIONAL AND CHEMICAL CLASSES, CROSS-SECTIONAL ANALYSIS CONTROLLING FOR TRIAZINE EXPOSURE



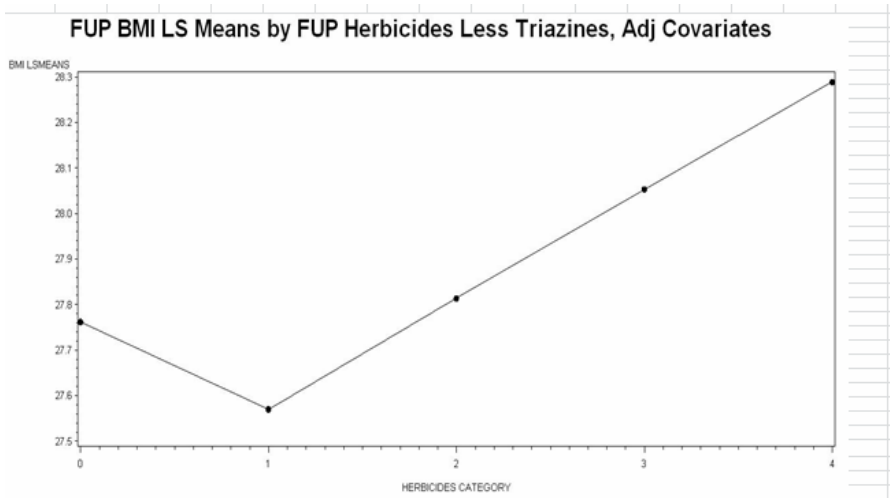


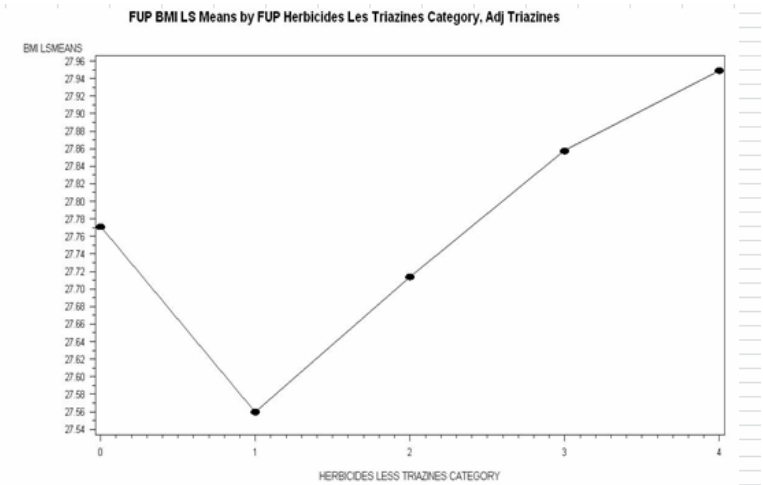


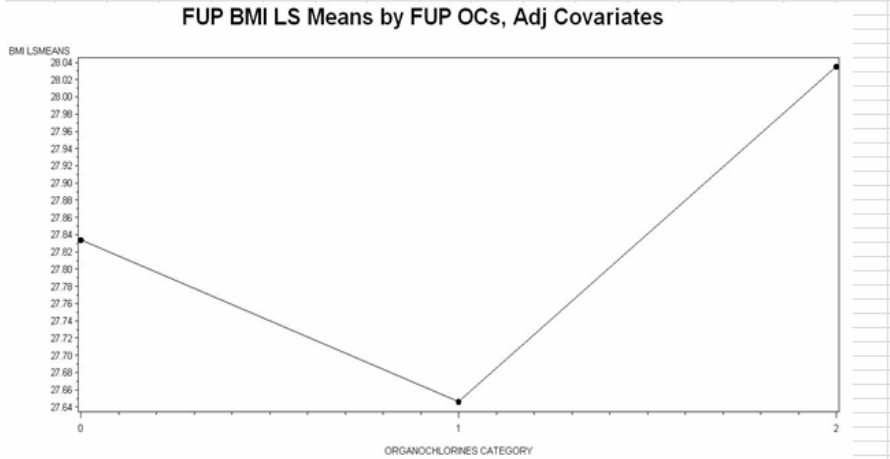


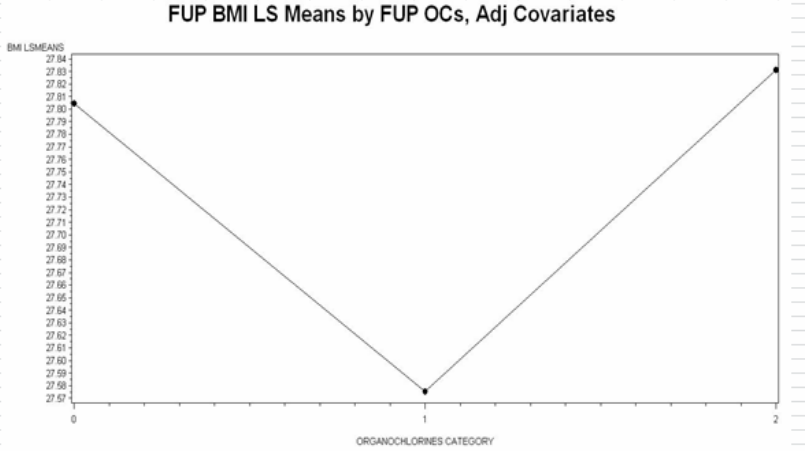


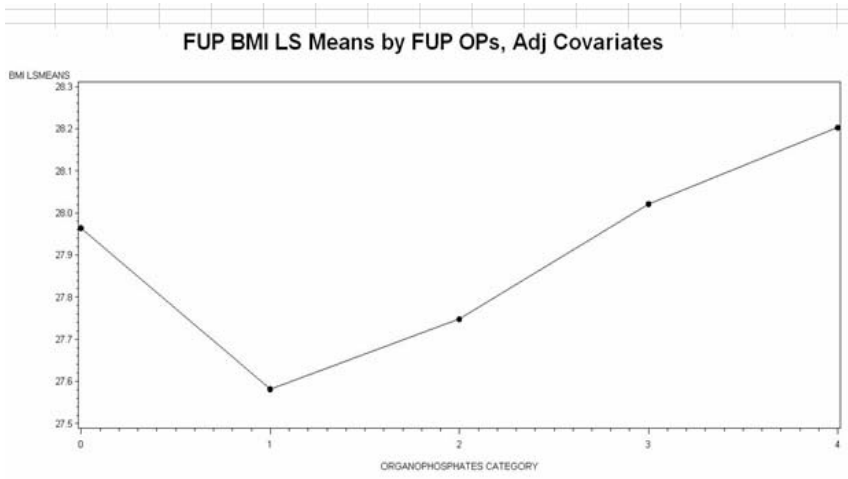
CANNOT ADJUST HERBICIDES FOR TRIAZINES

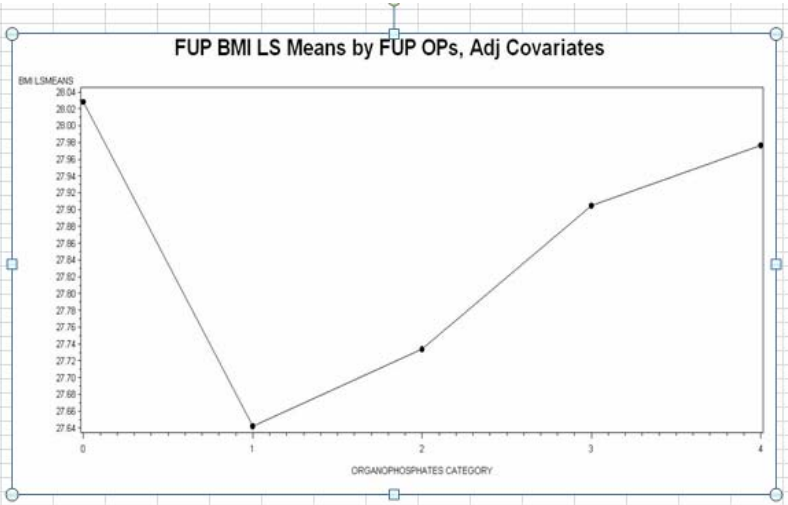

FUP BMI LS Means by FUP Herbicides Less Triazines, Adj Covariates
BMI LSMEANS
28.3
28.2
28.1
28.0
27.9
27.8
27.7
27.6
27.5
0
1
2
3
4
HERBICIDES CATEGORY

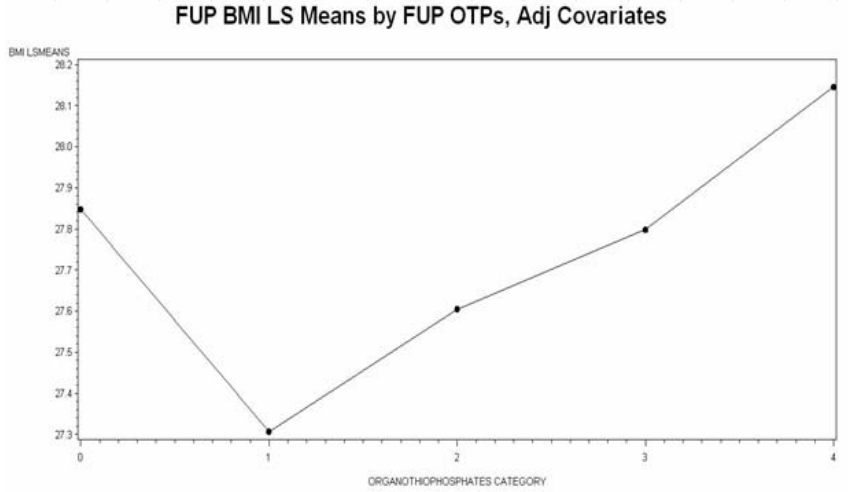

FUP BMI LS Means by FUP Herbicides Les Triazines Category, Adj Triazines
BMI LSMEANS
27.96
27.94
27.92
27.90
27.88
27.86
27.84
27.82
27.80
27.78
27.76
27.74
27.72
27.70
27.68
27.66
27.64
27.62
27.60
27.58
27.56
27.54
0
1
2
3
4
HERBICIDES LESS TRIAZINES CATEGORY

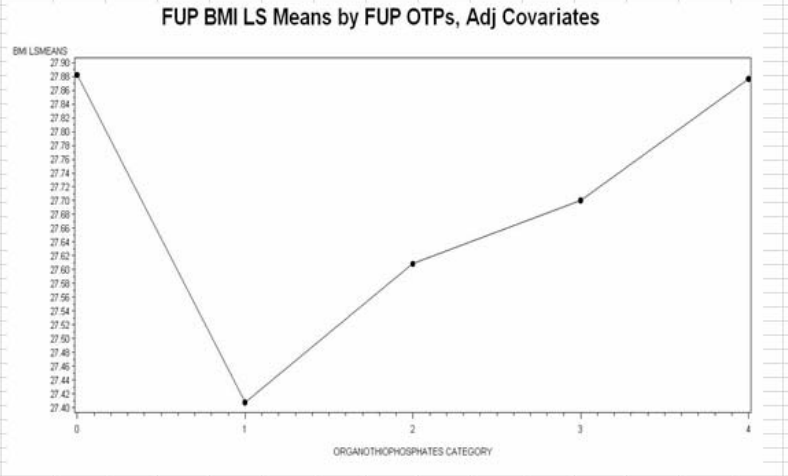

FUP BMI LS Means by FUP OCs, Adj Covariates
BMI LSMEANS
28.04
28.02
28.00
27.98
27.96
27.94
27.92
27.90
27.88
27.86
27.84
27.82
27.80
27.78
27.76
27.74
27.72
27.70
27.68
27.66
27.64
0
1
2
ORGANOCHLORINES CATEGORY


FUP BMI LS Means by FUP OCs, Adj Covariates
BMI LSMEANS
27.84
27.83
27.82
27.81
27.80
27.79
27.78
27.77
27.76
27.75
27.74
27.73
27.72
27.71
27.70
27.69
27.68
27.67
27.66
27.65
27.64
27.63
27.62
27.61
27.60
27.59
27.58
27.57
0
1
2
ORGANOCHLORINES CATEGORY


FUP BMI LS Means by FUP OPs, Adj Covariates
BMI LSMEANS
ORGANOPHOSPHATES CATEGORY


FUP BMI LS Means by FUP OPs, Adj Covariates
BMI LSMEANS
ORGANOPHOSPHATES CATEGORY


FUP BMI LS Means by FUP OTPs, Adj Covariates
BMI LSMEANS
ORGANOTHIOPHOSPHATES CATEGORY


FUP BMI LS Means by FUP OTPs, Adj Covariates
BMI LSMEANS
ORGANOTHIOPHOSPHATES CATEGORY

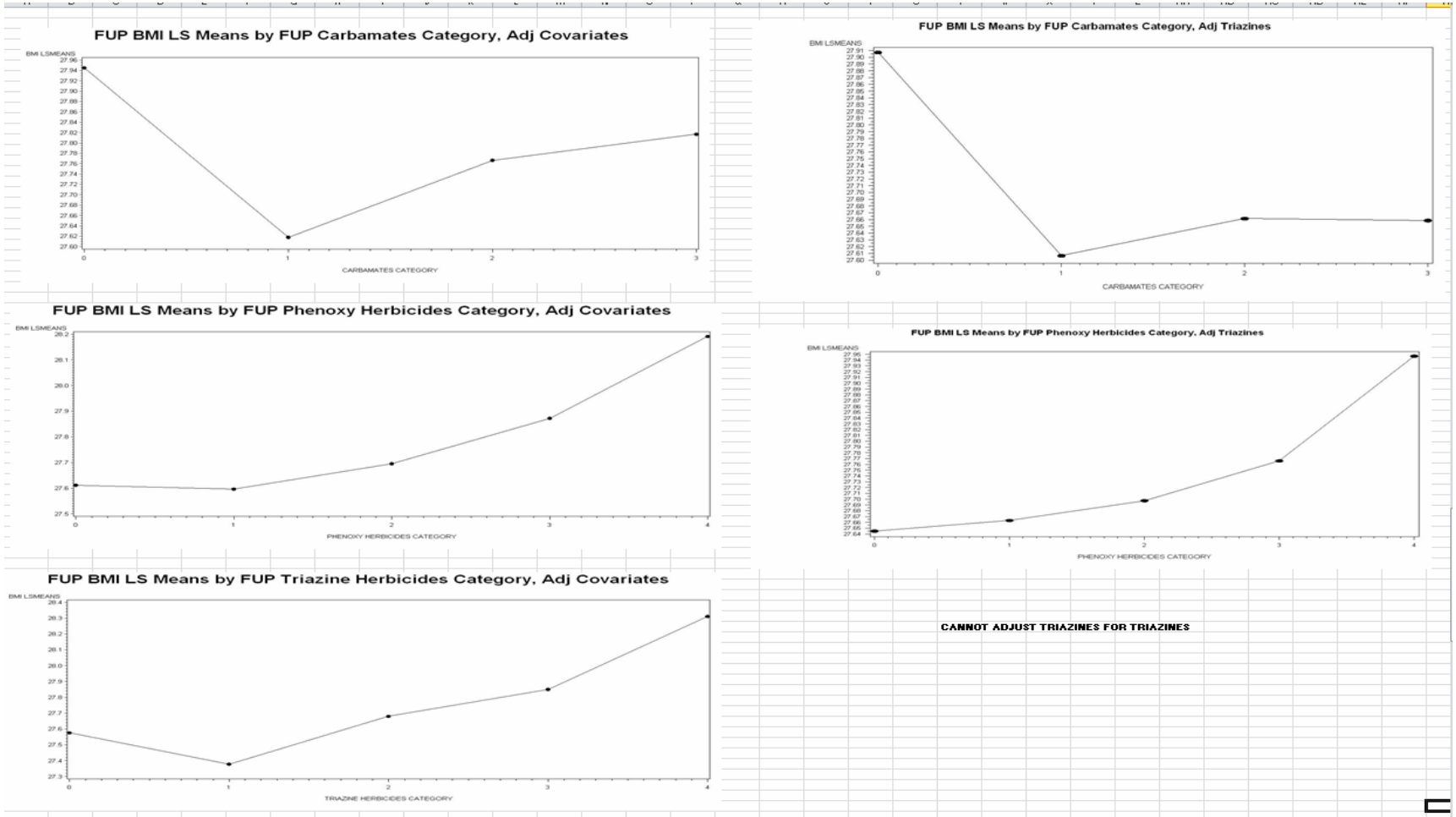

FUP BMI LS Means by FUP Carbamates Category, Adj Covariates
BMI LSMEANS
CARBAMATES CATEGORY
FUP BMI LS Means by FUP Carbamates Category, Adj Triazines
BMI LSMEANS
CARBAMATES CATEGORY
FUP BMI LS Means by FUP Phenoxy Herbicides Category, Adj Covariates
BMI LSMEANS
PHENOXY HERBICIDES CATEGORY
FUP BMI LS Means by FUP Phenoxy Herbicides Category, Adj Triazines
BMI LSMEANS
PHENOXY HERBICIDES CATEGORY
FUP BMI LS Means by FUP Triazine Herbicides Category, Adj Covariates
BMI LSMEANS
TRIAZINE HERBICIDES CATEGORY
CANNOT ADJUST TRIAZINES FOR TRIAZINES

TABLE 4-11a: LOGISTIC ORDINAL REGRESSION FOR CALCULATION OF ADJUSTED ODDS RATIOS USING PREDICTORS IDENTIFIED FOR ALL ANALYSES WITH ZERO SCORES INCLUDED

ODDS RATIOS REPRESENT: odds of exposure category having a higher BMI versus a lower BMI compared to the odds of zero exposure having a higher BMI versus a lower BMI

OUTCOME VARIABLE = BMI, 4 levels: 1 = underweight, 2 = normal weight, 3 = overweight, 4 = obese (see Table 3.3, WHO 2007)

	Cross-sectional Enrollment			**Prospective**				**Cross-sectional Follow-up**		
	Adjusted Model			**Adjusted Model**				**Adjusted Model**		
EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through enrollment	**OR (99% CI)**			**OR (99% CI)**			**EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through follow-up**	**OR (99% CI)**		
insecticides level zero	referent			referent*			insecticides level zero	referent^		
insecticides level one = >0 to ≤ 42.5	**0.785**	**(0.621,**	**0.993)**	**0.729**	**(0.579,**	**0.918)**	insecticides level one = >0 to ≤ 52.3	0.861	(0.654,	1.123)
insecticides level two = 42.5 to ≤ 165.9	0.875	(0.705,	1.086)	0.841	(0.681,	1.038)	insecticides level two = 52.3 to ≤ 184.5	1.013	(0.781,	1.315)
insecticides level three = 165.9 to ≤ 588.1	0.838	(0.675,	1.041)	0.834	(0.675,	1.031)	insecticides level three = 184.5 to ≤ 541.7	0.937	(0.724,	1.214)
insecticides level four = > 588.1	0.897	(0.722,	1.115)	0.875	(0.707,	1.082)	insecticides level four = >541.7	1.041	(0.802,	1.352)
fumigants level zero	referent				referent		fumigants level zero		referent	
fumigants level one = >0 to ≤ 98.0	0.865	(0.717,	1.044)	0.847	(0.704,	1.019)	fumigants level one = >0 to ≤ 98.4	0.837	(0.697,	1.004)
fumigants level two = > 98.0	0.911	(0.761,	1.091)	0.907	(0.760,	1.082)	fumigants level two = > 98.4	0.933	(0.785,	1.109)
fungicides level zero	referent				referent		fungicides level zero		referent*	
fungicides level one = >0 to ≤ 1.4	0.945	(0.700,	1.276)	0.970	(0.722,	1.303)	fungicides level one = >0 to ≤ 1.9	0.998	(0.780,	1.275)
fungicides level two = > 1.4	0.912	(0.791,	1.052)	**0.853**	**(0.742,**	**0.980)**	fungicides level two = > 1.9	**0.868**	**(0.770,**	**0.979)**
herbicides level zero	referent*				referent*		herbicides level zero		referent*	
herbicides level one = >0 to ≤ 207.8	**0.705**	**(0.527,**	**0.942)**	0.810	(0.609,	1.079)	herbicides level one = >0 to ≤ 269.0	0.974	(0.712,	1.332)
herbicides level two = 207.8 to ≤ 642.2	0.825	(0.621,	1.095)	0.859	(0.649,	1.136)	herbicides level two = 269.0 to ≤ 745.8	1.066	(0.781,	1.455)
herbicides level three = > 642.2 to ≤ 1619.9	0.922	(0.694,	1.225)	0.977	(0.738,	1.292)	herbicides level three = 745.8 to ≤ 1764.8	1.250	(0.916,	1.706)
herbicides level four = > 1619.9	1.009	(0.759,	1.342)	1.080	(0.816,	1.430)	herbicides level four = >1764.8	**1.415**	**(1.037,**	**1.932)**
herbicides less triazines level zero		referent*			referent*		herbicides less triazines level zero		referent*	
herbicides less triazines level one= >0 to ≤ 181.6	**0.751**	**(0.567,**	**0.995)**	0.779	(0.590,	1.027)	herbicides less triazines level one= >0 to ≤ 238.0	0.956	(0.708,	1.292)
herbicides less triazines level two= 181.6 to ≤ 583.3	0.846	(0.643,	1.112)	0.852	(0.650,	1.115)	herbicides less triazines level two= 238.0 > to ≤ 687.3	1.060	(0.787,	1.428)
herbicides less triazines level three= 583.3 to ≤ 1492.8	1.009	(0.767,	1.327)	0.986	(0.753,	1.291)	herbicides less triazines level three= 687.3 to ≤ 1647.3	1.200	(0.891,	1.617)
herbicides less triazines level four= > 1492.8	1.055	(0.801,	1.390)	1.060	(0.809,	1.390)	herbicides less triazines level four= > 1647.3	**1.351**	**(1.002,**	**1.821)**
organochlorines level zero	referent				referent		organochlorines level zero		referent	
organochlorines level one = >0 to ≤ 150.4	0.963	(0.843,	1.101)	0.937	(0.819,	1.073)	organochlorines level one = >0 to ≤ 154.3	0.953	(0.836,	1.088)
organochlorines level two = > 150.4	1.056	(0.926,	1.205)	1.064	(0.928,	1.220)	organochlorines level two = >154.3	1.099	(0.961,	1.256)
organophosphates level zero	referent				referent		organophosphates level zero		referent*	
organophosphates level one = >0 to ≤ 43.9	0.806	(0.640,	1.013)	0.854	(0.683,	1.070)	organophosphates level one = >0 to ≤ 55.1	0.877	(0.713,	1.078)
organophosphates level two = 43.9 to ≤ 197.1	0.907	(0.751,	1.097)	0.919	(0.764,	1.105)	organophosphates level two = 55.1 to ≤ 215.4	0.946	(0.793,	1.127)
organophosphates level three = 197.1 to ≤ 625.2	0.980	(0.810,	1.186)	1.030	(0.855,	1.240)	organophosphates level three = 215.4 to ≤ 663.9	1.078	(0.904,	1.285)
organophosphates level four = > 625.2	1.010	(0.835,	1.222)	1.041	(0.864,	1.253)	organophosphates level four = > 663.9	1.144	(0.959,	1.364)
organothiophosphates level zero	referent				referent*		organothiophosphates level zero		referent*^	
organothiophosphates level one = >0 to ≤ 3.1	0.822	(0.071,	9.505)	0.315	(0.026,	3.875)	organothiophosphates level one = >0 to ≤ 13.8	1.115	(0.642,	1.936)
organothiophosphates level two = 3.1 to ≤ 162.9	0.903	(0.771,	1.058)	0.870	(0.745,	1.016)	organothiophosphates level two = 13.8 to ≤ 178.0	0.968	(0.807,	1.161)
organothiophosphates level three = 162.9 to 597.3	0.961	(0.820,	1.127)	0.962	(0.823,	1.124)	organothiophosphates level three = >178.0 to ≤ 630.3	1.090	(0.906,	1.311)
organothiophosphates level four = > 597.3	1.105	(0.942,	1.296)	1.114	(0.953,	1.303)	organothiophosphates level four = > 630.3	**1.371**	**(1.132,**	**1.661)**
carbamates level zero	referent				referent		carbamates level zero		referent	
carbamates level one = >0 to ≤ 26.3	0.821	(0.632,	1.067)	0.836	(0.647,	1.080)	carbamates level one = >0 to ≤ 45.9	0.872	(0.710,	1.072)
carbamates level two = 26.3 to ≤ 297.0	0.949	(0.827,	1.089)	0.940	(0.821,	1.075)	carbamates level two = 45.9 to ≤ 351.9	0.938	(0.825,	1.068)
carbamates level three = > 297.0	0.937	(0.815,	1.076)	0.892	(0.777,	1.023)	carbamates level three = > 351.9	0.906	(0.794,	1.032)
phenoxy herbicides level zero	referent*				referent*		phenoxy herbicides level zero		referent*	
phenoxy herbicides level one = >0 to ≤ 77.8	0.956	(0.726,	1.258)	0.931	(0.710,	1.220)	phenoxy herbicides level one = >0 to ≤ 127.7	1.058	(0.850,	1.317)
phenoxy herbicides level two = 77.8 to ≤ 585.6	1.027	(0.869,	1.214)	1.003	(0.852,	1.181)	phenoxy herbicides level two = 127.7 to ≤ 701.1	1.079	(0.916,	1.270)
phenoxy herbicides level three = 585.6 to ≤ 2000.4	1.167	(0.985,	1.383)	1.106	(0.937,	1.305)	phenoxy herbicides level three = 701.1 to ≤ 2179.1	**1.201**	**(1.020,**	**1.415)**
phenoxy herbicides level four = > 2000.4	**1.270**	**(1.071,**	**1.506)**	**1.225**	**(1.037,**	**1.448)**	phenoxy herbicides level four = > 2179.1	**1.354**	**(1.150,**	**1.596)**
triazine herbicides level zero	referent*				referent*		triazine herbicides level zero		referent*	
triazine herbicides level one = >0 to ≤ 73.9	0.832	(0.610,	1.134)	1.017	(0.751,	1.378)	triazine herbicides level one = >0 to ≤ 110.0	0.919	(0.720,	1.173)
triazine herbicides level two = 73.9 to ≤ 562.7	0.950	(0.805,	1.122)	0.986	(0.838,	1.160)	triazine herbicides level two = 110.0 to ≤ 655.4	1.076	(0.919,	1.258)
triazine herbicides level three = 562.7 to ≤ 1955.5	1.106	(0.936,	1.307)	1.142	(0.969,	1.345)	triazine herbicides level three = 655.4 to ≤ 2100.3	**1.201**	**(1.027,**	**1.406)**
triazine herbicides level four = > 1955.5	**1.263**	**(1.067,**	**1.495)**	**1.333**	**(1.130,**	**1.574)**	triazine herbicides level four = > 2100.3	**1.481**	**(1.265,**	**1.733)**

*Significant relationship between exposure to pesticide class and BMI classification based on Wald Chi-sq value, p<0.01

^BMI modelled as binary variable due to results of score test of proportional odds assumption rejecting null hypothesis

Table 4-11a: LOGISTIC ORDINAL REGRESSION FOR CALCULATION OF ADJUSTED ODDS RATIOS USING PREDICTORS IDENTIFIED FOR ALL ANALYSES WITH ZERO SCORES INCLUDED & ADJUSTING FOR TRIAZINE EXPOSURE (red indicates differences from original analyses)

ODDS RATIOS REPRESENT: odds of exposure category having a higher BMI versus a lower BMI compared to the odds of zero exposure having a higher BMI versus a lower BMI

OUTCOME VARIABLE = BMI, 4 levels: 1 = underweight, 2 = normal weight, 3 = overweight, 4 = obese (see Table 3.3, WHO 2007)

	Cross-sectional Enrollment			Prospective				Cross-sectional Follow-up		
	Adjusted Model			Adjusted Model				Adjusted Model		
EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through enrollment	**OR (99% CI)**			**OR (99% CI)**			**EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through follow-up**	**OR (99% CI)**		
insecticides level zero	referent			referent*			insecticides level zero	referent		
insecticides level one = >0 to ≤ 42.5	0.784	(0.614,	1.000)	**0.709**	**(0.558,**	**0.900)**	insecticides level one = >0 to ≤ 52.3	**0.833**	**(0.629,**	**1.104)**
insecticides level two = 42.5 to ≤ 165.9	0.831	(0.663,	1.043)	**0.785**	**(0.629,**	**0.980)**	insecticides level two = 52.3 to ≤ 184.5	**0.918**	**(0.701,**	**1.203)**
insecticides level three = 165.9 to ≤ 588.1	**0.758**	**(0.604,**	**0.953)**	**0.744**	**(0.595,**	**0.929)**	insecticides level three = 184.5 to ≤ 541.7	**0.795**	**(0.607,**	**1.042)**
insecticides level four = > 588.1	**0.783**	**(0.622,**	**0.985)**	**0.751**	**(0.599,**	**0.940)**	insecticides level four = >541.7	**0.840**	**(0.638,**	**1.106)**
fumigants level zero	referent			referent			fumigants level zero	referent		
fumigants level one = >0 to ≤ 98.0	0.852	(0.706,	1.028)	0.835	(0.694,	1.004)	fumigants level one = >0 to ≤ 98.4	**0.826**	**(0.688,**	**0.992)**
fumigants level two = > 98.0	0.894	(0.746,	1.071)	0.892	(0.747,	1.065)	fumigants level two = > 98.4	0.919	(0.772,	1.093)
fungicides level zero	referent			referent			fungicides level zero	referent*		
fungicides level one = >0 to ≤ 1.4	0.955	(0.707,	1.291)	0.980	(0.729,	1.318)	fungicides level one = >0 to ≤ 1.9	0.978	(0.765,	1.252)
fungicides level two = > 1.4	0.916	(0.793,	1.058)	**0.860**	**(0.747,**	**0.990)**	fungicides level two = > 1.9	**0.866**	**(0.767,**	**0.977)**
NO ADJUSTMENT FOR TRIAZINES	NO ADJ FOR TRIAZINES			NO ADJ FOR TRIAZINES				NO ADJ FOR TRIAZINES		
herbicides level zero	referent*			referent*			herbicides level zero	referent*		
herbicides level one = >0 to ≤ 207.8	**0.705**	**(0.527,**	**0.942)**	0.810	(0.609,	1.079)	herbicides level one = >0 to ≤ 269.0	0.974	(0.712,	1.332)
herbicides level two = 207.8 to ≤ 642.2	0.825	(0.621,	1.095)	0.859	(0.649,	1.136)	herbicides level two = 269.0 to ≤ 745.8	1.066	(0.781,	1.455)
herbicides level three = > 642.2 to ≤ 1619.9	0.922	(0.694,	1.225)	0.977	(0.738,	1.292)	herbicides level three = 745.8 to ≤ 1764.8	1.250	(0.916,	1.706)
herbicides level four = > 1619.9	1.009	(0.759,	1.342)	1.080	(0.816,	1.430)	herbicides level four = >1764.8	**1.415**	**(1.037,**	**1.932)**
herbicides less triazines level zero		referent*			referent		herbicides less triazines level zero		referent	
herbicides less triazines level one= >0 to ≤ 181.6	0.761	(0.565,	1.024)	0.763	(0.570,	1.022)	herbicides less triazines level one= >0 to ≤ 238.0	0.932	(0.686,	1.280)
herbicides less triazines level two= 181.6 to ≤ 583.3	0.828	(0.613,	1.118)	0.806	(0.600,	1.082)	herbicides less triazines level two= 238.0 > to ≤ 687.3	0.984	(0.716,	1.354)
herbicides less triazines level three= 583.3 to ≤ 1492.8	0.948	(0.698,	1.289)	0.888	(0.653,	1.200)	herbicides less triazines level three= 687.3 to ≤ 1647.3	1.055	(0.764,	1.458)
herbicides less triazines level four= > 1492.8	0.949	(0.691,	1.302)	0.900	(0.660,	1.228)	herbicides less triazines level four= > 1647.3	1.103	(0.791,	1.537)
organochlorines level zero	referent			referent			organochlorines level zero	referent		
organochlorines level one = >0 to ≤ 150.4	0.956	(0.836,	1.094)	0.923	(0.806,	1.057)	organochlorines level one = >0 to ≤ 154.3	0.930	(0.815,	1.062)
organochlorines level two = > 150.4	0.986	(0.862,	1.129)	0.979	(0.850,	1.127)	organochlorines level two = >154.3	0.999	(0.870,	1.147)
organophosphates level zero	referent			referent			organophosphates level zero	referent		
organophosphates level one = >0 to ≤ 43.9	0.814	(0.642,	1.030)	0.848	(0.673,	1.068)	organophosphates level one = >0 to ≤ 55.1	0.872	(0.706,	1.077)
organophosphates level two = 43.9 to ≤ 197.1	0.885	(0.726,	1.080)	0.889	(0.733,	1.079)	organophosphates level two = 55.1 to ≤ 215.4	0.900	(0.750,	1.080)
organophosphates level three = 197.1 to ≤ 625.2	0.907	(0.742,	1.109)	0.946	(0.778,	1.151)	organophosphates level three = 215.4 to ≤ 663.9	0.968	(0.804,	1.165)
organophosphates level four = > 625.2	0.898	(0.732,	1.101)	0.916	(0.750,	1.117)	organophosphates level four = > 663.9	0.975	(0.806,	1.178)
organothiophosphates level zero	referent			referent			organothiophosphates level zero	referent		
organothiophosphates level one = >0 to ≤ 3.1	0.818	(0.071,	9.450)	0.314	(0.026,	3.865)	organothiophosphates level one = >0 to ≤ 13.8	1.153	(0.662,	2.006)
organothiophosphates level two = 3.1 to ≤ 162.9	0.903	(0.768,	1.063)	0.856	(0.731,	1.004)	organothiophosphates level two = 13.8 to ≤ 178.0	0.941	(0.780,	1.134)
organothiophosphates level three = 162.9 to 597.3	0.915	(0.776,	1.079)	0.909	(0.773,	1.068)	organothiophosphates level three = >178.0 to ≤ 630.3	1.002	(0.826,	1.215)
organothiophosphates level four = > 597.3	0.990	(0.833,	1.177)	0.990	(0.836,	1.173)	organothiophosphates level four = > 630.3	1.165	(0.946,	1.435)
carbamates level zero	referent			referent			carbamates level zero	referent		
carbamates level one = >0 to ≤ 26.3	0.834	(0.641,	1.084)	0.844	(0.653,	1.091)	carbamates level one = >0 to ≤ 45.9	0.881	(0.716,	1.083)
carbamates level two = 26.3 to ≤ 297.0	0.930	(0.810,	1.068)	0.917	(0.801,	1.051)	carbamates level two = 45.9 to ≤ 351.9	0.903	(0.793,	1.029)
carbamates level three = > 297.0	0.894	(0.776,	1.029)	**0.850**	**(0.739,**	**0.977)**	carbamates level three = > 351.9	**0.853**	**(0.747,**	**0.975)**
phenoxy herbicides level zero	referent			referent			phenoxy herbicides level zero	referent		
phenoxy herbicides level one = >0 to ≤ 77.8	0.990	(0.749,	1.309)	0.944	(0.717,	1.243)	phenoxy herbicides level one = >0 to ≤ 127.7	1.088	(0.855,	1.385)
phenoxy herbicides level two = 77.8 to ≤ 585.6	1.036	(0.869,	1.235)	0.998	(0.841,	1.184)	phenoxy herbicides level two = 127.7 to ≤ 701.1	1.049	(0.884,	1.246)
phenoxy herbicides level three = 585.6 to ≤ 2000.4	1.128	(0.942,	1.352)	1.052	(0.882,	1.254)	phenoxy herbicides level three = 701.1 to ≤ 2179.1	1.102	(0.924,	1.313)
phenoxy herbicides level four = > 2000.4	1.171	(0.970,	1.414)	1.101	(0.915,	1.324)	phenoxy herbicides level four = > 2179.1	1.155	(0.965,	1.382)
NO ADJUSTMENT FOR TRIAZINES	NO ADJ FOR TRIAZINES			NO ADJ FOR TRIAZINES				NO ADJ FOR TRIAZINES		
triazine herbicides level zero	referent*			referent*			triazine herbicides level zero	referent*		
triazine herbicides level one = >0 to ≤ 73.9	0.832	(0.610,	1.134)	1.017	(0.751,	1.378)	triazine herbicides level one = >0 to ≤ 110.0	0.919	(0.720,	1.173)
triazine herbicides level two = 73.9 to ≤ 562.7	0.950	(0.805,	1.122)	0.986	(0.838,	1.160)	triazine herbicides level two = 110.0 to ≤ 655.4	1.076	(0.919,	1.258)
triazine herbicides level three = 562.7 to ≤ 1955.5	1.106	(0.936,	1.307)	1.142	(0.969,	1.345)	triazine herbicides level three = 655.4 to ≤ 2100.3	**1.201**	**(1.027,**	**1.406)**
triazine herbicides level four = > 1955.5	**1.263**	**(1.067,**	**1.495)**	**1.333**	**(1.130,**	**1.574)**	triazine herbicides level four = > 2100.3	**1.481**	**(1.265,**	**1.733)**

*Significant relationship between exposure to pesticide class and BMI classification based on Wald Chi-sq value, p<0.01

^BMI modelled as binary variable due to results of score test of proportional odds assumption rejecting null hypothesis

TABLE 4-11b: LOGISTIC ORDINAL REGRESSION FOR CALCULATION OF ADJUSTED ODDS RATIOS USING PREDICTORS IDENTIFIED FOR EACH ANALYSIS WITH ZERO SCORES FOR MISSING VALUES EXCLUDED

ODDS RATIOS REPRESENT: odds of exposure category having a higher BMI versus a lower BMI compared to the odds of zero exposure having a higher BMI versus a lower BMI

OUTCOME VARIABLE = BMI, 4 levels: 1 = underweight, 2 = normal weight, 3 = overweight, 4 = obese (see Table 3.3, WHO 2007)

	Cross-sectional Enrollment			**Prospective**				**Cross-sectional Follow-up**		
	Adjusted Model			**Adjusted Model**				**Adjusted Model**		
EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through enrollment	**OR (99% CI)**			**OR (99% CI)**			**EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through follow-up**	**OR (99% CI)**		
insecticides level zero	referent			**referent***			insecticides level zero	referent		
insecticides level one	0.865	(0.731,	1.022)	**0.830**	**(0.703,**	**0.980)**	insecticides level one	**0.850**	**(0.726,**	**0.995)**
insecticides level two	0.947	(0.811,	1.107)	0.951	(0.816,	1.108)	insecticides level two	0.900	(0.777,	1.041)
insecticides level three	0.881	(0.753,	1.029)	0.877	(0.753,	1.022)	insecticides level three	0.910	(0.787,	1.053)
insecticides level four	1.011	(0.865,	1.182)	0.952	(0.817,	1.109)	insecticides level four	0.976	(0.844,	1.129)
fumigants level zero	referent				referent		fumigants level zero		referent	
fumigants level one	0.954	(0.836,	1.088)	0.917	(0.806,	1.043)	fumigants level one	0.939	(0.827,	1.067)
fungicides level zero	referent				referent		fungicides level zero		referent	
fungicides level one	0.979	(0.730,	1.313)	1.020	(0.766,	1.359)	fungicides level one	0.999	(0.787,	1.268)
fungicides level two	0.986	(0.871,	1.116)	0.909	(0.806,	1.026)	fungicides level two	**0.886**	**(0.786,**	**0.999)**
herbicides level zero	**referent***				**referent***		herbicides level zero		**referent***	
herbicides level one	**0.798**	**(0.681,**	**0.934)**	**0.793**	**(0.679,**	**0.926)**	herbicides level one	**0.852**	**(0.733,**	**0.991)**
herbicides level two	0.944	(0.808,	1.103)	0.895	(0.769,	1.043)	herbicides level two	0.967	(0.832,	1.122)
herbicides level three	1.055	(0.903,	1.233)	1.014	(0.870,	1.181)	herbicides level three	1.071	(0.922,	1.243)
herbicides level four	1.115	(0.953,	1.304)	1.051	(0.902,	1.225)	herbicides level four	1.110	(0.956,	1.290)
herbicides less triazines level zero		**referent***			**referent***		herbicides less triazines level zero		**referent***	
herbicides less triazines level one	**0.802**	**(0.679,**	**0.948)**	**0.796**	**(0.676,**	**0.938)**	herbicides less triazines level one	**0.832**	**(0.715,**	**0.968)**
herbicides less triazines level two	1.014	(0.861,	1.196)	0.930	(0.792,	1.093)	herbicides less triazines level two	0.940	(0.809,	1.092)
herbicides less triazines level three	1.002	(0.850,	1.180)	0.950	(0.808,	1.116)	herbicides less triazines level three	1.051	(0.905,	1.222)
herbicides less triazines level four	1.091	(0.925,	1.288)	1.024	(0.871,	1.205)	herbicides less triazines level four	1.117	(0.961,	1.299)
organochlorines level zero	referent				referent		organochlorines level zero		referent	
organochlorines level one	1.328	(0.201,	8.774)	0.785	(0.119,	5.192)	organochlorines level one	1.036	(0.527,	2.037)
organochlorines level two	0.967	(0.851,	1.098)	0.945	(0.833,	1.072)	organochlorines level two	0.967	(0.854,	1.095)
organochlorines level three	1.045	(0.919,	1.188)	1.027	(0.901,	1.170)	organochlorines level three	1.049	(0.923,	1.193)
organophosphates level zero	referent				referent		organophosphates level zero		referent	
organophosphates level one	**0.816**	**(0.682,**	**0.976)**	**0.825**	**(0.692,**	**0.984)**	organophosphates level one	0.879	(0.746,	1.036)
organophosphates level two	0.928	(0.793,	1.085)	0.91	(0.781,	1.061)	organophosphates level two	0.908	(0.786,	1.050)
organophosphates level three	0.973	(0.831,	1.139)	1.000	(0.857,	1.166)	organophosphates level three	1.018	(0.881,	1.176)
organophosphates level four	1.025	(0.875,	1.200)	0.995	(0.852,	1.161)	organophosphates level four	1.062	(0.919,	1.226)
organothiophosphates level zero	referent				**referent***		organothiophosphates level zero		**referent***	
organothiophosphates level one	0.914	(0.704,	1.187)	0.842	(0.651,	1.090)	organothiophosphates level one	0.853	(0.676,	1.077)
organothiophosphates level two	0.919	(0.794,	1.064)	0.902	(0.782,	1.041)	organothiophosphates level two	0.921	(0.803,	1.057)
organothiophosphates level three	0.963	(0.832,	1.115)	0.988	(0.856,	1.141)	organothiophosphates level three	0.993	(0.866,	1.139)
organothiophosphates level four	1.132	(0.977,	1.312)	1.128	(0.977,	1.304)	organothiophosphates level four	**1.170**	**(1.020,**	**1.342)**
carbamates level zero	referent				referent		carbamates level zero		referent	
carbamates level one	0.898	(0.741,	1.088)	0.877	(0.727,	1.057)	carbamates level one	0.856	(0.720,	1.019)
carbamates level two	0.946	(0.831,	1.078)	0.919	(0.809,	1.044)	carbamates level two	0.909	(0.801,	1.031)
carbamates level three	1.028	(0.902,	1.171)	0.956	(0.841,	1.087)	carbamates level three	0.953	(0.840,	1.082)
phenoxy herbicides level zero	**referent***				**referent***		phenoxy herbicides level zero		**referent***	
phenoxy herbicides level one	0.927	(0.757,	1.135)	0.896	(0.733,	1.095)	phenoxy herbicides level one	0.951	(0.793,	1.140)
phenoxy herbicides level two	1.031	(0.883,	1.205)	1.004	(0.861,	1.170)	phenoxy herbicides level two	1.016	(0.876,	1.179)
phenoxy herbicides level three	1.132	(0.969,	1.323)	1.071	(0.917,	1.249)	phenoxy herbicides level three	1.110	(0.957,	1.288)
phenoxy herbicides level four	**1.272**	**(1.087,**	**1.487)**	**1.183**	**(1.012,**	**1.383)**	phenoxy herbicides level four	**1.256**	**(1.083,**	**1.458)**
triazine herbicides level zero	**referent***				**referent***		triazine herbicides level zero		**referent***	
triazine herbicides level one	**0.784**	**(0.633,**	**0.969)**	0.824	(0.668,	1.016)	triazine herbicides level one	0.888	(0.735,	1.073)
triazine herbicides level two	0.983	(0.842,	1.148)	0.969	(0.833,	1.128)	triazine herbicides level two	1.075	(0.932,	1.240)
triazine herbicides level three	1.081	(0.926,	1.263)	1.040	(0.893,	1.211)	triazine herbicides level three	1.101,	(0.955,	1.269)
triazine herbicides level four	**1.196**	**(1.023,**	**1.495)**	**1.231**	**(1.056,**	**1.435)**	triazine herbicides level four	**1.381**	**(1.197,**	**1.593)**

*Significant relationship between exposure to pesticide class and BMI classification based on Wald Chi-sq value, p<0.01

^BMI modelled as binary variable due to results of score test of proportional odds assumption rejecting null hypothesis

Table 4-11b: LOGISTIC ORDINAL REGRESSION FOR CALCULATION OF ADJUSTED ODDS RATIOS USING PREDICTORS IDENTIFIED FOR EACH ANALYSIS WITH ZERO SCORES FOR MISSING VALUES EXCLUDED & ADJUSTING FOR TRIAZINE EXPOSURE (red indicates differences from original analyses)

ODDS RATIOS REPRESENT: odds of exposure category having a higher BMI versus a lower BMI compared to the odds of zero exposure having a higher BMI versus a lower BMI

OUTCOME VARIABLE = BMI, 4 levels: 1 = underweight, 2 = normal weight, 3 = overweight, 4 = obese (see Table 3.3, WHO 2007)

	Cross-sectional Enrollment			**Prospective**				**Cross-sectional Follow-up**		
	Adjusted Model			**Adjusted Model**				**Adjusted Model**		
EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through enrollment	**OR (99% CI)**			**OR (99% CI)**			**EXPOSURE VARIABLE = intensity-weighted cumulative exposure days through follow-up**	**OR (99% CI)**		
insecticides level zero	referent			**referent***			insecticides level zero	referent		
insecticides level one	0.893	(0.751,	1.061)	0.855	(0.720,	1.014)	insecticides level one	0.866	(0.735,	1.019)
insecticides level two	0.930	(0.791,	1.092)	0.932	(0.796,	1.091)	insecticides level two	0.868	(0.746,	1.011)
insecticides level three	**0.832**	**(0.708,**	**0.977)**	**0.820**	**(0.700,**	**0.960)**	insecticides level three	**0.836**	**(0.718,**	**0.973)**
insecticides level four	0.933	(0.793,	1.098)	0.861	(0.735,	1.010)	insecticides level four	0.864	(0.741,	1.006)
fumigants level zero	referent			referent			fumigants level zero		referent	
fumigants level one	0.945	(0.828,	1.079)	0.906	(0.796,	1.032)	fumigants level one	0.926	(0.815,	1.052)
fungicides level zero	referent			referent			fungicides level zero		referent	
fungicides level one	0.984	(0.733,	1.321)	1.021	(0.765,	1.361)	fungicides level one	0.980	(0.771,	1.245)
fungicides level two	0.972	(0.859,	1.101)	0.895	(0.793,	1.010)	fungicides level two	**0.875**	**(0.776,**	**0.986)**
NO ADJUSTMENT FOR TRIAZINES	NO ADJ FOR TRIAZINES			NO ADJ FOR TRIAZINES			herbicides level zero	NO ADJ FOR TRIAZINES		
herbicides group includes triazines	herbicides grp includes triazines			herbicides grp includes triazines			herbicides level one	herbicides grp includes triazines		
							herbicides level two			
							herbicides level three			
							herbicides level four			
herbicides less triazines level zero		referent			referent		herbicides less triazines level zero		referent	
herbicides less triazines level one	**0.839**	**(0.706,**	**0.997)**	**0.834**	**(0.704,**	**0.988)**	herbicides less triazines level one	**0.853**	**(0.727,**	**0.999)**
herbicides less triazines level two	1.017	(0.855,	1.210)	0.937	(0.790,	1.110)	herbicides less triazines level two	0.921	(0.784,	1.083)
herbicides less triazines level three	0.970	(0.815,	1.154)	0.912	(0.769,	1.082)	herbicides less triazines level three	0.980	(0.832,	1.153)
herbicides less triazines level four	1.017	(0.846,	1.222)	0.925	(0.773,	1.107)	herbicides less triazines level four	0.973	(0.820,	1.154)
organochlorines level zero	referent			referent			organochlorines level zero		referent	
organochlorines level one	1.439	(0.218,	9.522)	0.847	(0.128,	5.603)	organochlorines level one	1.129	(0.573,	2.224)
organochlorines level two	0.964	(0.849,	1.095)	0.938	(0.826,	1.066)	organochlorines level two	0.950	(0.838,	1.077)
organochlorines level three	0.990	(0.868,	1.128)	0.960	(0.839,	1.098)		0.968	(0.848,	1.105)
organophosphates level zero	referent			referent			organophosphates level zero		referent	
organophosphates level one	0.839	(0.698,	1.010)	0.853	(0.711,	1.022)	organophosphates level one	0.892	(0.752,	1.058)
organophosphates level two	0.920	(0.782,	1.083)	0.909	(0.775,	1.066)	organophosphates level two	0.885	(0.760,	1.031)
organophosphates level three	0.927	(0.787,	1.093)	0.954	(0.812,	1.120)	organophosphates level three	0.945	(0.811,	1.101)
organophosphates level four	0.951	(0.805,	1.124)	0.914	(0.776,	1.077)	organophosphates level four	0.942	(0.806,	1.100)
organothiophosphates level zero	referent			referent			organothiophosphates level zero		referent	
organothiophosphates level one	0.942	(0.723,	1.227)	0.863	(0.665,	1.120)	organothiophosphates level one	0.874	(0.690,	1.107)
organothiophosphates level two	0.915	(0.787,	1.064)	0.898	(0.775,	1.041)	organothiophosphates level two	0.909	(0.788,	1.048)
organothiophosphates level three	0.914	(0.784,	1.065)	0.940	(0.808,	1.092)	organothiophosphates level three	0.938	(0.812,	1.084)
organothiophosphates level four	1.032	(0.880,	1.209)	1.014	(0.868,	1.185)	organothiophosphates level four	1.031	(0.887,	1.198)
carbamates level zero	referent			referent			carbamates level zero		referent^	
carbamates level one	0.911	(0.750,	1.106)	0.883	(0.731,	1.067)	carbamates level one	0.980	(0.777,	1.237)
carbamates level two	0.914	(0.801,	1.043)	0.882	(0.775,	1.003)	carbamates level two	0.869	(0.736,	1.027)
carbamates level three	0.978	(0.856,	1.116)	0.898	(0.789,	1.023)	carbamates level three	**0.835**	**(0.707,**	**0.987)**
phenoxy herbicides level zero	referent			referent			phenoxy herbicides level zero		referent	
phenoxy herbicides level one	0.960	(0.781,	1.142)	0.930	(0.757,	1.142)	phenoxy herbicides level one	0.973	(0.806,	1.174)
phenoxy herbicides level two	1.030	(0.876,	1.189)	1.013	(0.863,	1.189)	phenoxy herbicides level two	1.006	(0.859,	1.177)
phenoxy herbicides level three	1.095	(0.928,	1.230)	1.045	(0.888,	1.230)	phenoxy herbicides level three	1.056	(0.900,	1.238)
phenoxy herbicides level four	**1.187**	**(1.000,**	**1.301)**	1.098	(0.926,	1.301)	phenoxy herbicides level four	1.127	(0.956,	1.330)
triazine herbicides level zero	NO ADJ FOR TRIAZINES			NO ADJ FOR TRIAZINES			triazine herbicides level zero	NO ADJ FOR TRIAZINES		
triazine herbicides level one	triazine grp includes triazines			triazine grp includes triazines			triazine herbicides level one	triazines grp includes triazines		
triazine herbicides level two							triazine herbicides level two			
triazine herbicides level three							triazine herbicides level three			
triazine herbicides level four							triazine herbicides level four			

*Significant relationship between exposure to pesticide class and BMI classification based on Wald Chi-sq value, p<0.01

^BMI modelled as binary variable due to results of score test of proportional odds assumption rejecting null hypothesis