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THE IMPACT OF MEAT CONSUMPTION ON THE URINARY CONCENTRATION OF DIALKYL PHOSPHATE METABOLITES

BY

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Degree to be awarded: MPH
Executive MPH

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An abstract of
A Thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
in partial fulfillment of the requirements of the degree of
Master of Public Health in the Executive MPH program
2015

Abstract

THE IMPACT OF MEAT CONSUMPTION
ON THE URINARY CONCENTRATION OF
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Background: Organophosphorus pesticides (OPs) are frequently used globally in agricultural and residential settings. Diet may contribute to chronic low-level exposure to OPs. The contribution of some dietary components, such as fruit, to OP exposure is well understood; limited previous research has identified the role of meat product consumption in OP exposure.
Methods: Data from five National Health and Nutrition Examination Survey (NHANES) cycles (from 1999 – 2008) were used to conduct a retrospective cross-sectional analysis to examine the association between meat consumption and the urinary concentration of four different dialkyl phosphate (DAP) metabolites of OPs. Using a linear regression analysis, the beta coefficients and 95% confidence intervals for meat consumption were estimated adjusting for covariates such as fruit and vegetable consumption. This analysis was also run on a subset of samples that had metabolite values above the limit of detection (LOD). A secondary analysis was conducted using logistic regression with an outcome of metabolite values either above or below the LOD. Each of these analyses was also run with and without the population weights provided in NHANES.
Results: The mean level of the sum of all DAP metabolites in the population was 160.7 nanomolar (sd = 639.8) and the mean level of meat consumption was 2.2 servings in 24 hours (sd = 2.3). Linear regression analysis showed a significant inverse association between consumption of meat and DAP metabolite levels ($p = 0.0020$). The association varied in significance for the individual metabolites, but the association was inverse in all cases. Logistic regression also showed an inverse association (odds ratio = 0.984), but no significance in the unweighted model ($p = 0.2539$).
Conclusions: Meat consumption was inversely associated with urinary DAP metabolite levels among individuals participating in the NHANES environmental chemicals subset in five NHANES cycles from 1999-2008. This may reflect residual confounding by imperfectly measured consumption of foods that are inversely related to meat consumption. Future studies should more specifically measure amounts of meat consumed while accounting for OP exposure from other specific dietary sources, and examine the effects of different types of meat (beef, pork, other preparations, etc.) on DAP levels.

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ACKNOWLEDGEMENTS

I would like to thank Dr. Lyndsey Darrow and Dr. Dana Barr for their support and encouragement throughout this process, as well as their willingness to share their expertise and pushing me to search farther and work harder.

Thank you to Steve for putting up with me these last few years and to the rest of my family and friends for sticking with me and for their support of my educational, employment and geographic adventures over the years.

Thank you to my FSIS colleagues, without whose support and occasional on-call coverage I would not have found the time to complete this program.

And thank you to Camden.

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CHAPTER I

INTRODUCTION

Introduction and Rationale

Due to their broad spectrum of applications and low cost, organophosphorus pesticides (OPs) are among the most widely used pesticides in the United States. They are frequently used in both agricultural and residential settings (1). OPs have also been used to combat public health issues, particularly for mosquito control (specifically malathion and naled). These uses have decreased or been eliminated in the United States, but still occur in other countries (2). The US Environmental Protection Agency (EPA) has developed risk mitigation measures for approximately 30 individual OPs from risk management analyses in the early 2000s. These measures resulted in the 2006 update of the Organophosphate Cumulative Risk Assessment (3). As of 2007, OPs still accounted for approximately 35% of all pesticides used in the United States, or about 33 million pounds used in 2007 (4).

Exposure to OPs is typically through multiple routes. These include ingesting contaminated food, hand-to-mouth contact after touching surfaces or dust containing OPs, as well as inhalation and dermal contact, although this is less common. OPs typically have better gastrointestinal than dermal absorption (2). Because of the multiple exposure routes, quantification of exposure is often difficult.

Although the literature has reported that certain foods (such as fruits) are more likely to be contaminated with OPs, fewer studies have looked at the role of meat and poultry consumption as it relates to the levels of OP metabolites measured in an individual. The aim of this research is to explore the relationship between diet, specifically meat and poultry consumption, and urinary OP metabolite levels. This information may aid in further clarifying whether meat and poultry consumption is a major source of exposure to OPs.

Problem Statement

The acute high-dose effects of OPs include neurological dysfunction from inhibition of acetylcholinesterase (AChE), a neuroenzyme that acts to inactivate the neurotransmitter acetylcholine, thus playing an important role in neurological signaling (5). The inhibition of AChE results in an accumulation of acetylcholine in the central and peripheral nervous systems. Symptoms include nausea, vomiting, cholinergic effects, weakness, paralysis, seizures, and mild to severe peripheral neuropathies, along with residual deficits in neurocognitive functioning (2, 6, 7). Consequences of chronic exposure are less well understood. In studies evaluating farmers and insecticide applicators without past acute poisoning, significant reductions in blood cholinesterase activity have not typically been observed. Some reports have suggested that neurotoxicity occurs due to OPs interfering with neurodevelopment. Other study results have been inconsistent, but indicate the possibility of subtle or subclinical neurological effects including low performance in visual tests, problem solving, motor steadiness, reaction, and dexterity (2, 6, 7). Animal studies at high doses have shown similar effects as humans and few animal studies have addressed the potential for low environmental doses to have non-cholinergic effects (2).

In a 1993 report, the National Research Council looked at dietary pesticide exposure in infants and children and scrutinized the use of OPs because of the potential consequences of childhood exposures. The Food Quality Protection Act (FQPA) of 1996 required the EPA to reassess all pesticide residue tolerances on food and specifically consider the potential cumulative and aggregate exposures to children. OPs were the first pesticides to be reassessed due to their common mode of toxicity, widespread use, and unknown long-term health effects (1). The assessment was updated in 2006. Following passage of the FQPA, use of OPs in the

United States actually increased between 1996 and 1999 from 75 to 91 million pounds per year, due mostly to the United States Department of Agriculture's (USDA) cotton boll weevil eradication program. Usage decreased to 46 million pounds by 2004, but residential use likely declined more quickly because of the voluntary cancellation of residential uses of chlorpyrifos and diazinon in 2000 (8).

The measurements of six common urinary dialkyl phosphate (DAP) metabolites are used to identify recent exposure to OPs. However, the levels of these metabolites do not indicate which pesticide an individual was exposed to (1). In the general population, the intake of OPs is considered to be below regulatory thresholds, but there have been concerns raised for infants and children as their dietary consumption patterns may differ from adults and may not be properly measured (9). Farm workers, those who work with plants (e.g. gardeners, florists), and pesticide applicators and manufacturers may have greater exposure than the general population. Some states monitor cholinesterase activity in the blood of pesticide applicators as part of mandatory exposure monitoring. The EPA, the Food and Drug Administration, the US Department of Agriculture, and the Occupational Safety and Health Administration all have criteria on allowable levels of OPs in the environment, foods, and the workplace (2).

OPs are composed of a phosphate group and an organic group. They are enzymatically converted to their oxon form or are hydrolyzed to their organic group metabolite and dialkyl thionate metabolite once they enter the body. The oxon form reacts with cholinesterase. The metabolites and/or the glucuronide or sulfate conjugates of OPs are excreted in urine. About 75% of registered OPs are metabolized to measureable DAP metabolites. These metabolites are not considered toxic, but do act as markers of exposure. Presence of the metabolites reflects recent exposure, within the previous few days. The metabolites may occur in the environment due to

degradation of OPs, so there is the potential that an individual who has measureable metabolites was exposed to the metabolite itself (2).

The OPs share a common mode of action as an insecticide. They also share a mode of acute toxicity in humans and other animals. OPs are potent acetyl cholinesterase (AChE) inhibitors. AChE is an enzyme that acts by breaking down the neurotransmitter acetylcholine. The mammalian elimination half-life varies from hours to weeks (2). OPs are non-persistent in outdoor settings and are degraded by natural and microbiological actions. However, they may remain stable for months to years when used indoors or as part of structural treatments, resulting in there being a continual risk for exposure (10).

Purpose Statement

The purpose of this research was to evaluate the relationship between an individual's diet, in particular recent meat and poultry consumption, and levels of OP metabolites detected by urinalysis. Both dietary interview and OP metabolite data are derived from the 1999-2008 National Health and Nutrition Examination Survey (NHANES). Specific research questions included:

1. Does an individual's reported amount of meat and poultry consumption impact the urinary concentration of DAP metabolites of OPs?
2. Does an individual's reported amount of meat and poultry consumption impact the urinary concentration of different DAP metabolites in the same way?
3. Do meat consumption levels contribute to changes in DAP metabolite levels as much as other foods, such as fruit, water, vegetables, or dairy products?

   Null Hypothesis: Our overarching null hypothesis is the urinary concentrations of specific DAP metabolites of OPs do not vary by consumption level of meat and poultry products.

Significance Statement

It is well established that acute, high-level exposures to OPs may result in severe health consequences. Persons facing the likelihood of such exposures due to their occupation may be monitored and existing regulations determine allowable levels of OPs in the workplace. Less is known about the general population's ongoing chronic exposures to OPs.

This research to evaluate the impact of the consumption of meat and poultry products on the levels of DAP metabolites will provide information about an exposure that has not previously been fully examined. It is known that food is certainly a means of exposure, and that certain food groups are more likely to contribute to higher levels of OPs, but this particular relationship has not been examined. Exposure to OPs is of particular concern in children. The conclusions derived from this project will provide information that may determine whether particular products should be consumed at a lower level by certain population groups.

Definition of Terms

AChE – Acetylcholinesterase

CDC – Centers for Disease Control and Prevention

DAP – Dialkyl phosphate

DEP – Diethylphosphate

DETP - Diethylthiophosphate

DMP – Dimethylphosphate

DMTP – Dimethylthiophosphate

EPA – Environmental Protection Agency

FDA – Food and Drug Association

LOD – Limit of detection

nM – Nanomolar

NHANES – National Health and Nutrition Examination Survey

OP – Organophosphorus pesticide

USDA – United States Department of Agriculture

CHAPTER II

REVIEW OF LITERATURE

The published literature was reviewed to determine the current understanding of the relationship of meat and poultry products to intake of OPs. The methodology in reviewing the literature included: a PubMed search for published studies in databases; a Google Scholar search of journal websites; and a review of bibliographies of published studies identified by the searches. Key words used in the search were organophosphorus, organophosphate, pesticide, NHANES, diet, meat, and dialkyl phosphate.

Organophosphorus Pesticides in Meat

In 1993, Coulibaly and Smith examined the thermostability of six OPs (chlorpyrifos, famphur, fenthion, parathion, ronnel, and stirofos) and their primary (oxon) and secondary (alcohol) metabolites in beef muscle and in water. The OPs and their metabolites were added to beef and water samples at a concentration of 50 ppm and then heated in a water bath to 70 or 80 degrees Celsius. They analyzed the compounds extracted from the beef and water using high-performance liquid chromatography and gas chromatography-mass spectrometry. They retrieved the parent OPs at levels from 64.5 to 98.4% from raw meat, from 30.0 to 87.4% from cooked meat, and from 10.6 to 107.2% from water. Recovery of the oxon and alcohol metabolites varied from 56.0 to 103.0% in raw meat, 23.9 to 81.0% in cooked meat and 72.7 and 105.0% in water. They did find that both the OPs and their metabolites were thermally degraded, but that significant amounts of the substances were still present after the water and beef muscle were heated. This indicates that while cooking may induce a significant thermal decomposition of these compounds, it cannot be relied ton to completely eliminate OP parent compound residues and their metabolites (11).

Coulibaly and Smith also examined the effect of pH and cooking temperature on the stability of OPs in beef muscle in a slightly different group of OPs (ronnel, fenthion, coumaphos,

chlorpyrifos, famphur, and stirofos). Fenthion, coumaphos, and ronnel were significantly more stable ($p < 0.05$) in raw meat at pH 4.5 (70.1 to 84.5% recovery), and concentration decreased at pH 5.5 and 6.5 (46.5 to 78.3% recovery), while there was no significant difference in the recovery of famphur, stirifos, or chlorpyrifos between pH 4.5 and 5.5. At pH 6.5, the recovery of all parent OPs was much lower indicating that increasing pH level in raw beef produced a progressive degradation of the OPs. The combined effect of pH and temperature resulted in more efficient degradation of OPs in cooked meat, although the amount of degradation at each pH varied by the compound. With cooking to 71°C, fenthion and coumaphos were the only OPs to show a significant change ($p < 0.05$) between pH 4.5 (50.4 and 62.4% recovery, respectively) and pH 5.5 and 6.5 (between 62.8 and 78.4%) with cooking. At 77°C, the recoveries of famphur, fenthion, and chlorpyrifos at pH 5.5 (from 60.5 to 69.7%) were significantly higher ($p < 0.05$) than at pH 4.5 and 6.5 (from 24.4 and 57.4%). Overall, the effect of the combination of pH and temperature on the recovery of the OPs in meat was more significant that the effect of pH alone and cooking at 77°C was more efficient in reducing recover than cooking at 71°C. Additionally, all OPs were unstable in raw meat at low acid levels and susceptible to thermal hydrolysis and oxidation in cooked meat at high and low acid levels. However, at any of the pH levels tested, the pH alone or combined with cooking did not completely eliminate OPs and their metabolites in meat. The meat was spiked with a level of OP that would fall in the range of the tolerance level in meat and meat products defined by USDA at the time (12).

In 2010, Riederer, et al. reported on composite diet samples from adults in Atlanta for the OPs chlorpyrifos, diazinon, and malathion. They measured four days of 24-hour duplicate diet samples over two cycles in 2005-2006. Chlorpyrifos was found above the limit of detection (LOD) in the meat, fish and egg composite food group at a frequency of 41% in the 2005 cycle

and 13% in 2006. Diazinon was found less frequently, at 3% and 10%, and malathion was not seen at all in this food composite group. Median chlorpyrifos concentrations were generally quite low for the meat, fish and egg group, at 2.4 ng/g in the 2006 cycle and only slightly higher in 2005, compared to the highest level of 435.8 ng/g found in the 2006 grain sample. The median diazinon concentration was approximately 30.0 ng/g in 2005 based on one sample, but rose to approximately 120.0 ng/g in 2006 vs. the high level of 248.5 ng/g median for grain samples in 2005. Total daily intakes were below the EPA's oral reference doses except in 6% of cases where the sum of OP pesticides found was to be over the recommendation. This study was important because it looked at foods outside of the typical fruits and vegetables as possible contributors to OP exposure in diets. However, it does not allow for distinguishing of the source of the OP as meat, fish and eggs are all considered in one group (13).

Organophosphorus Pesticides in Other Foods

In 1984, Ishikura, et al., examined the impact of cooking on OP levels when OPs are added to rice. They found that residual OPs decrease when the rice is cooked, but that the amount of decrease varies with the kind of pesticide used in part due to the thermal stability of the compound and whether it was susceptible to steam distillation. They tested eight OPs with cooking and the extent of the decrease varied from 20% for dimethoate to 93.5% for Ronnel (14).

Lu, et al. looked at the presence of DAPs in both organic and conventional fresh fruit juices, both when bought and after 72 hours of storage. DAPs were found in both conventional and organic juices, with the levels being higher in conventional juices. For both orange and apple juice, DEP and DETP levels were higher in conventional juice and below the LOD in organic juice. Conventional apple juice also had higher DMP and DMTP levels than organic, where they

were either below or slightly above the LODs. It was also found that when a dimethyl OP and a diethyl OP were added to the juices, 12% and 36.2% of each, degraded to dimethyl and diethyl DAPs, respectively. This study also indicated that care must be used in using urinary DAPs to attribute OP exposures to the environment, as diet is also an important exposure (15).

Lu, et al. also reported levels of OPs in children's diets as part of the Children's Pesticide Exposure Study (CPES) by collecting duplicate food samples of conventional fruits, vegetables, and fruit juices eaten by children over a 24-hour period. They measured a total of 11 OPs and found that 14% of the food samples contained at least one OP residue. The levels were generally within the ranges reported by the national Pesticide Data Program (PDP), although several composite samples of a variety of fruits and vegetables did have higher levels than those reported by the PDP. Detected levels were also below the U.S. EPA tolerance levels. However, tolerance levels are established on a per chemical, per crop basis and do not consider the impact of consumption of multiple pesticides with a common mechanism of action. They are intended for monitoring residues in raw produce prior to washing, shipping, storage, marketing and preparation, indicating that levels prior to consumption should be lower. Although levels detected in this study were found to be below tolerance levels, the fact that children frequently consumed multiple foods containing OPs indicates the challenges in assessing dietary exposure (16).

Symptoms of Chronic Organophosphorus Pesticide Exposure

In 1991, Rosenstock, et al. reported on a retrospective study of agricultural workers in Nicaragua who had been admitted to the hospital for occupationally related OP intoxication. The agricultural workers were each tested approximately two years following their exposure. When compared with a control group, they performed significantly worse on neuropsychological

subtests assessing verbal and visual attention, visual memory, visuomotor speed, sequencing and problem solving, and motor steadiness and dexterity. Although these patients all began with an acute exposure as opposed to an ongoing chronic exposure, this study did show that OP exposure may lead to longer term consequences (6).

Ray and Richards defined low level OP exposure as exposure that does not evoke cholinergic symptoms such as lacrimation, salivation, meiosis or muscle fasciculation, also described as clinically obvious poisoning (17). These chronic organophosphate-induced neuropsychiatric disorders (COPIND) are not dependent on AChE inhibition. They usually have a delayed onset and persist for a long time, suggesting permanent damage to the central nervous system. Symptoms include cognitive deficits, mood change, chronic fatigue, autonomic dysfunction, peripheral neuropathy, and extrapyramidal symptoms such as dystonia, resting tremor, bradykinesia, postural instability, and rigidity of face muscles (18).

Epidemiologic studies have been conducted looking at farm workers and pesticide applicators. They have found neuropsychological damage accompanying damage to the peripheral nervous system, anxiety, and depression. Agricultural workers tested two years after a poisoning episode showed lower performance in verbal and visual attention, visual memory, visuomotor speed, sequencing and problem solving. At more mild levels, farm workers who did not require hospitalization performed worse on cognitive and psychomotor function tests that non-poisoned workers two years post exposure. One study found a link between exposure to OPs and increased suicide rate (18).

Organophosphorus Pesticide Dietary Exposure

Lu, et al. measured dietary OP exposure in a group of children participating in the CPES by measuring urinary metabolites. Conventional fresh and processed fruits and vegetables, juices,

and wheat- or corn-based foods were substituted with organic versions of those foods into children's diets for a period of time. These particular foods are those regularly reported to contain OPs. They found that the median urinary concentrations of metabolites specific to malathion and chlorpyrifos decreased to nondetectable levels immediately following the introduction of the organic diet and remained such until the conventional diet was resumed. The median concentrations for other OP metabolites were also lower when the organic diet was consumed, but not frequently enough to show significance. The study showed the impact of these dietary items on OP exposures in these children, indicating that they were mostly likely exposed to these particular OPs exclusively through their diet (19). In a later study, Lu, et al. performed a similar dietary switch in both the summer and fall seasons and saw similar results. They also looked at OP metabolites in urine for 7, 12, or 15 consecutive days during each of the four seasons and saw a seasonal effect on urinary metabolite levels. This seasonal effect corresponded with the varying intake of fresh product throughout the year (20).

A study by Jensen, et al. assessed the probable cumulative acute dietary exposure of the population of Denmark to organophosphorus and carbamate pesticides, as being AChE inhibiting pesticides, they have a common mode of action. They used residue data obtained from Danish monitoring programs in 2004-2007, which included samples of fruits, vegetables, and cereals. Food consumption data was provided by a nationwide dietary survey from 2000-2002. The relative potency factor approach was used to normalize the toxicity of the pesticides to the two index compounds chlorpyrifos and methamidophos. The cumulative acute exposure, meaning the total exposure from multiple sources, of chlorpyrifos was calculated to be 1.8% of the acute reference dose, or maximum acceptable dose per day, for children and 0.8% for adults. The greatest contributor to the cumulative acute exposure was apples. The results indicated that there

was no cumulative acute risk to the Dutch population for dietary exposure to AChE inhibiting pesticides (21).

Surveys of Organophosphorus Pesticide Metabolite Levels

Several studies have indicated much higher urinary DAP levels are typically higher in children compared to other age groups (15). Thus, much of the research and reporting on urinary OP metabolite levels has focused on children, but others have looked at broader populations through the National Health and Nutrition Examination Survey (NHANES). In addition to diet, children and others living in agricultural areas may be exposed to OPs through drift while pesticides are applied or through exposures if they are in contact with someone who works in a treated field (1, 22, 23).

Bradman, et al. measured six OP metabolites in urine samples from about 400 children in an agricultural community at ages 6, 12, and 24 months. Most participants had at least one DAP detected, with detection frequencies for the three age groups of 93%, 94%, and 95%, respectively. DMAP metabolite levels were found to be higher than DEAP levels, which is consistent with previous studies. The geometric mean for total DAP was 40.0 nmol/L at 6 months, 54.3 nmol/L at 12 months, and 66.3 nmol/L at 24 months, with DMAP levels increasing the most with age from 18.5, to 26.4, to 45 nmol/L, respectively, while DEAP levels were lower, moving from 8.6, to 14.2, and to 8.4 nmol/L at 24 months of age. This may be in part due to the cancellation during this time of most residential uses of chlorpyrifos and diazinon, which are both diethyl OPs. There is not a known similar increase in dimethyl OP use to explain the rise in DMAP levels, but there may have been an increase due to agricultural use or illegal use of these pesticides at home. Agricultural related determinants such as field proximity or occupational status of parents did not have any consistent association across ages or between DMAP or DEAP

metabolites. However, fruit and vegetable intake was consistently and positively associated with both DMAP and DEAP levels at all ages, again confirming that diet is an important pesticide exposure route in children (24).

Barr, et al. looked at population-based concentrations of DAP metabolites of multiple OPs using data measured in 1999 and 2000 as part of NHANES. Each DAP metabolite was detected in more than 50% of the samples and DEP was most frequently detected (71%). DMAP metabolite levels were found to be 72.8 nmol/L (95% CI 54.3-97.5 nmol/L) in children aged 6-11, 56.9 (40.2-80.7) in adolescents from 12-19 years of age, and 42.1 (33.6-52.8) in adults aged 20-59 years. The value for children was significantly different ($p < 0.05$) from that for adults. DEAP metabolite levels also varied significantly ($p < 0.05$) between children and adults, with DEAP metabolite levels at 17.3 nmol/L (11.1-27.3) for children and 10.0 (7.5-13.2) for adults. Total DAP metabolite measurements varied significantly ($p < 0.05$) between children and adults and adolescents and adults. For children, the geometric mean was 109.6 nmol/L (88.3-144.3), 89.3 (65.2-122.2) for adolescents, and 66.9 (54.3-82.5 for adults). There was no significant difference found in DAP concentrations based on sex or race/ethnicity. A review of literature included in this paper also indicates that concentrations in the U.S. population are lower than those of other reference populations (1).

Federal Regulation of Organophosphorus Pesticides

Regulatory tolerance and residue monitoring for OPs are performed by three federal agencies in the U.S. The EPA approves pesticides for particular uses and establishes tolerances for OPs in food. The FDA enforces these tolerances in imported foods and in domestic foods shipped into interstate commerce, except for meat, poultry and certain egg products, for which the Food Safety and Inspection Service of the USDA is responsible. The FDA also carries out the Total

Diet Study, which determines the amount of a variety of pesticides in certain commodities. The Agricultural Marketing Service of the USDA also has a pesticide residue monitoring program, known as the Pesticide Data Program, which looks at raw agricultural products and various processed foods. This program imitates consumer practices in its analyses to provide data as close to actual consumption data as possible for use by the EPA in risk assessments (25).

Clune, et al. considered whether actions taken by the EPA to strengthen the regulation of pesticides were effective by looking at the concentration of urinary DAP metabolites in populations from 1988-1994 (prior to strengthening of regulations) and from 1999-2004 (after regulations had taken effect). Prior to 1996, pesticides were regulated through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA 1947) and the Federal Food, Drug, and Cosmetic Act (FFDCA 1938). The Food Quality Protection Act (FQPA) was signed into law in 1996 and amended FIFRA and FFDCA to include cumulative and aggregate exposure risk assessments in derivative food tolerance levels. OPs were selected as the first class of pesticides to reassess food tolerances and the chemical specific assessments of most of the OPs were completed by August 2006. These assessments resulted in the cancellation of nearly all residential uses of OPs. Clune, et al. found that all median DAP concentrations decreased significantly between the two populations except for diethyldithiophosphate, which was the least frequently detected DAP. The median concentration of DAP metabolites decreased on average by 84.0% (range 63.1-98.5%). Diethyl DAP metabolites had a greater average decrease than DMAP metabolites (92.1 vs. 73.9%, respectively). This decrease is likely related, in part, to EPA's efforts to phase out residential uses and limiting other uses of OPs, although other factors may have played a role as well (8).

CHAPTER III

METHODOLOGY

Study Type and Source of Data

This study is a retrospective cross-sectional analysis using data collected on participants in the 1999-2000, 2001-2002, 2003-2004, 2005-2006, and 2007-2008 cycles of NHANES. The NHANES is a series of surveys and examinations designed to evaluate the health and nutritional status of adults and children in the United States based on a representative sample. The survey combines interviews, physical examinations, and laboratory testing. It is administered by the National Center for Health Statistics, a part of the Centers for Disease Control and Prevention.

The NHANES surveys began in the early 1960s and became a continuous program in 1999. Each year, the survey examines a sample of approximately 5,000 people, selected to represent the U.S. population of all ages. Participants are located in counties around the country, 15 of which are visited each year. The interview includes demographic, socioeconomic, dietary, and health-related questions. The examination consists of medical, dental, and physiological measurements, and laboratory tests. NHANES findings are used to determine disease prevalence and risk factors. They provide a basis for national standards for measurements including height, weight, and blood pressure. Data collected are used for epidemiological studies and health sciences research, which help develop public health policy and programs and expand health knowledge.

Study Population

The study population includes participants in the NHANES who participated in the interview and laboratory portion of the survey, and who were included in the pesticide subsample. The pesticide subsample was a random 1/3 subset so representativeness of the survey was maintained. Participants in in 1999-2001 were from 6-59 years of age with an oversampling of 6-12 year olds, while the other survey cycles included participants age 6 years and over.

Analysis was limited to survey participants who had reliable food recall interviews and a measurement for at least one of the DAP metabolites. Additionally those analyzed had data available for all of the covariates examined.

Outcomes and Exposures

The primary outcomes of this study are the urinary levels of four commonly measured DAP metabolites – DMP, DEP, DMTP, and DETP. The sums of DMP and DMTP; DEP and DETP; and of all four metabolites were considered as outcomes as well. Values below the LOD were imputed as the LOD/$\sqrt{2}$ (26). Metabolite levels are represented as molar sums.

Variables: The primary exposure variable is number of servings of meat. This includes any foods produced from pork, beef, chicken, turkey, and other species. Survey participants reported their 24-hour food history as part of a food recall interview. Food types were recorded using the USDA Food Code and expressed as the number of grams consumed. The number of grams consumed was converted to the number of serving sizes consumed by each individual using the standard serving size of each type of meat. Serving size calculations were performed using the USDA reference amounts customarily consumed per eating occasion, which for most basic cuts of meat and poultry is 85 grams (27, 28).

The analysis also includes several other potential predictors of the DAP metabolites:

- Demographic Characteristics: **Age** was considered as a continuous variable. Age is an important consideration in the assessment of OP exposure, as children often have higher levels of DAP and are more seriously affected by that exposure when compared to adults (1). **Race/ethnicity** was categorized into five groups – non-Hispanic white, non-Hispanic black, Mexican American, non-Hispanic other race (including multi-racial), and other

Hispanic. **Gender** was considered as a dichotomous variable. **Education** was categorized into five levels – less than ninth grade, from ninth – eleventh grade (and twelfth with no diploma), high school graduate or equivalent, some college or associate degree, and college graduate or above**. Income level** was divided into four levels – from 0 - $19,999, from $20,000 - $54,999, from $55,000 - $74,999, and greater than $75,000. **Birthplace** had three categories – United States, Mexico, and elsewhere.

- Residential Pesticide Use: Two variables represented residential pesticide use, one regarding use in the yard and the other regarding use in the house. Both questions changed slightly between survey years with the first three surveys asking about the last month, while the second two surveys asked about the previous week. All questions regarding pesticide use in the yard were considered as one variable over time and all questions regarding pesticide use in the house were considered as one variable over time.

  From 1999-2004, NHANES included a question asking if the lawn or yard was treated in the last month with chemical products to kill insects, weeds, or plant diseases, while from 2005-2008, the question asked if in the past seven days chemical products were used in the lawn or garden to kill weeds. This variable, for pesticide use in the yard, was dichotomous.

  From 1999-2008, NHANES included a question regarding the use of chemicals in the house, specifically those to treat fleas, roaches, ants, termites or other insects. The questions were similar, but in 2006 switched to asking about a seven day period, instead of the month that was asked about previously. This variable was also dichotomous.

- Fruit and Vegetable Consumption: Fruits and fruit juices are known to be possible routes of exposure for both OPs and DAPs, so it was important to control for this in the analysis

(15, 16). Vegetable consumption was also included as a predictor. As with meat consumption, survey participants reported their 24-hour food history as part of a food recall interview. Food types were recorded using the USDA Food Code and expressed as the number of grams consumed. The number of grams consumed was converted to the number of serving sizes consumed by each individual using the standard serving size for fruits and vegetables. Serving size calculations were performed using the FDA reference amounts customarily consumed per eating occasion, which for most simple fruits is 140 grams and for most fresh vegetables is 85 g (29).

- Water Consumption: Water consumption is also a known source of OPs, and it was controlled for in the analysis (3). Again, survey participants reported their 24-hour food history as part of a food recall interview. Beverage types were recorded using the USDA Food Code and expressed as the number of grams consumed. The number of grams consumed was converted to the number of serving sizes consumed by each individual using the standard serving size for water. Serving size calculations were performed using the FDA reference amounts customarily consumed per eating occasion, which for water is 240 g (29).

  It should be noted that the collection of drinking water intake in NHANES only began as part of the 2005-2006 survey. Prior to this, information about water consumption was collected via questions asking the respondent using food-frequency type questions to estimate the amounts of tap and bottled water consumed the previous day. Starting in 2005-2006, based on a review of water consumption studies, a 24 hour recall method was determined to be a better method of data collection, meaning that the water consumption data for these final two cycles is not comparable to that from the

earlier cycles (30, 31). In this analysis, water consumption was treated the same regardless of the survey cycle for the overall analysis. Some analyses were also applied separately to the 2005-2006 and 2007-2008 survey years to examine the difference due to the method of measuring water consumption.

Weighting

The NHANES survey includes sample weights and other design variables ordinarily used to adjust statistical inferences to be representative of the entire U.S. population. In NHANES, a sample weight is assigned to each person in the sample. The weight is a measure of the number of people in the population that that sample person represents. This reflects the unequal probability of selection, nonresponse adjustment, and adjustment to independent population controls to produce an unbiased sample national estimate (32). These weights or other design adjustments were not applied in the primary analysis, and rather the analysis was considered to be representative of the group of people surveyed. There is some concern that due to the time of sampling and the geographic limitations of NHANES, that the survey may not always be completely representative of the U.S. population.

Weighting was used as a part of a secondary analysis, to analyze what differences were found when using weights in the study. The NHANES website provides descriptions as to which weights to use when looking at multiple variables and analyzing across several survey cycles. The weight of the smallest analysis subpopulation was used – in this case the weight associated with NHANES environmental chemical data.

Analysis Plan

The analyses described below were each performed for the following DAP metabolites and metabolite groups: DMP, DMTP, DEP, DETP, DMP+DMTP, DEP+DETP, and

DMP+DMTP+DEP+DETP, each reported in nM and the sums were summed on a molar basis. Each analysis was performed both with and without a weight variable in order to observe the effects of weighting on the analysis. Additionally, all analyses were performed both with the inclusion of observations at or below the LOD and not including those observations.

Descriptive Analyses: To describe the characteristics of the study population, demographic characteristics and other survey covariates are reported for the population. These categorical variables are reported by the frequency with which each category occurred. Servings of each of the food categories are continuous variables, and they are described by mean and standard deviation. Age is reported as both a categorical and continuous variable, to provide a clearer description of the population. Categorical variables were analyzed servings of meat and for each metabolite using ANOVA to determine whether there was a significant difference in concentration of the metabolite between each category of the variable.

Linear Regression Analysis: Linear regression analysis allows for the examination of the relationship between one or more independent variables and a continuous dependent variable. In this case the dependent variable was log transformed because the metabolite values did not have a normal distribution. Linear regression is well-suited for epidemiologic studies involving environmental chemicals, as the outcome variable – the level of the chemical – is a continuous outcome. It permits modeling of multiple variables (both categorical and continuous) and tests the significance of each covariate. All independent variables can be treated as exposures and potential confounders simultaneously. The β-estimate or effect of one unit of a variable on the levels of the metabolite of interest with the 95% confidence interval of this effect is reported. Wald p-values representing the significance of the effect of a variable are also provided. Several linear regression analyses were performed for each metabolite and the sums of metabolites. The

unweighted analyses were considered the primary analyses, but weighted analyses were also performed as secondary analyses to observe the effect of weighting.

Analyses were also conducted on just the observations above the LOD for the individual metabolites. Observations below the LOD comprised 4884 (55.9%) observations for DMP, 5044 (57.9%) for DEP, 2605 (29.8%) for DMTP, and 4827 (55.6%) for DETP. This analysis was motivated by concerns that the observations below the LOD lead to a violation of the assumption of normally distributed errors in linear regression. The LOD is an arbitrary set point determined by detection capabilities in the laboratory, so there is not necessarily clinical significance associated with this level, but since values below the LOD comprised such a large proportion of observations it was used as a cut point to differentiate between individuals with higher levels and lower levels of a metabolite and to examine the effects of the exposures on these two groups in logistic regression. To be consistent across years, the LOD selected for use in this study was the highest LOD fill value for all years of data being analyzed. This a conservative approach for selecting a consistent LOD (26).

Logistic Regression Analysis: Logistic regression analysis was also used as a secondary analysis to look at the differences in meat consumption between individuals when metabolite levels were categorized as a dichotomous dependent variable. The outcomes were either the metabolite level was at or below the LOD or above the LOD. Logistic regression is used for studies with dichotomous outcomes because it permits modeling of multiple variables and provides overall tests of significance. Again, all independent variables are treated as exposures and confounders simultaneously. For the logistic regression, crude and adjusted odds ratios (ORs) with 95% confidence intervals are reported. The adjusted ORs used the fully adjusted model. Wald p-values representing the combined significance across all levels of a variable are

also provided.

Model Selection: For these analyses, multiple models were specified, but in each case the final model used was the fully adjusted model:

Log (DAP metabolite) = β_0 + β_1(# meat servings) + β_2(# dairy servings) + β_3(# fruit servings) + β_4(# vegetable servings) + β_5(# water servings) + β_6(age in years) + β_7(surveyyr1) + β_8(surveyyr2) + β_9(surveyyr3) + β_{10}(surveyyr4) + β_{11}(surveyyr5) + β_{12}(gender) + β_{13}(educ1) + β_{14}(educ2) + β_{15}(educ3) + β_{16}(educ4) + β_{17}(educ5) + β_{18}(race1) + β_{19}(race2) + β_{20}(race3) + β_{21}(race4) + β_{22}(race5) + β_{23}(pesthome) + β_{24}(pestyard) + β_{25}(brthplc1) + β_{26}(brthplc2) + β_{27}(brthplc3)

Where surveyyr represents the 2-year cycle of NHANES, educ represents five levels of educational attainment (< 9^{th} grade, 9-11^{th} grade (and 12^{th} with no diploma), high school graduate, some college or AA degree, and college graduate or above), race represents race/ethnicity, pesthome and pestyard are indicators for pesticide use in these respective locations, and brthplc represents place of birth (United States, Mexico, or other). For the linear regression, initially, the crude model was run for each metabolite and metabolite sum examined as a baseline for the effects of the exposure variable, servings of meat. The next model was a fully adjusted model including all of the demographic, pesticide, and food consumption covariates. Backward elimination was performed on each metabolite model. Backward elimination started with the fully adjusted model and variables were eliminated one at a time based on their p-value. At each step, the parameter with the least significant p-value is removed, except for the exposure variable, which was left in the model regardless of its significance level. The stay level for the p-values in the backward elimination was 0.05. The most parsimonious model, which includes the minimal set of variables needed to describe the association between the exposure and the outcome was derived for each metabolite analyzed. This model included the exposure variable, any significant covariates, and the confounders of the exposure. For this model, if the difference between the adjusted effect when a covariate was removed and the

adjusted effect in the fully adjusted model was greater than 10%, then that covariate was considered a confounder and left in the model. Interaction was not assessed because it was decided a priori that there was no reason to expect that the influence of dietary factors on levels of OP metabolites would differ by demographics or other factors.

Although confounding was examined in the linear regression model, the fully adjusted model was used for the final analysis of each model. The backward elimination process revealed that each of the covariates was found to either have a significant p-value ($p < 0.05$) or to be a confounder in at least one of the models. Given the large number of analyses completed, examination of the results was simplified by using a consistent model throughout. Since the logistic regression was viewed as a secondary model, only the crude and fully adjusted forms of the model were analyzed.

Data Analysis: Statistical analyses were conducted using SAS version 9.4 software.

CHAPTER IV

RESULTS

Descriptive Analysis

The study population consisted of individuals participating in the pesticide subsample of NHANES surveys from 1999 through 2008, a total of 13,339 individuals. Of these, 12,111 (90.8%) had at least one nonmissing DAP metabolite value and had a reliable food recall interview. Individuals included in the analysis then needed valid answers for each of the demographic and pesticide use covariates, resulting in 8,735 (65.5%) with all results for DMP, 8,711 (65.3%) for DEP, 8,727 (65.45) for DMTP, and 8,678 (65.1%) for DETP. From this, for 8,727 (65.4%) individuals the sum of DMAP metabolites could be calculated, 8,654 (64.9%) had the sum of DEAP metabolites, and for 8,646 (64.8%) the sum of all DAP metabolites could be calculated. For the analyses where weighting was used, from 8,386 – 8,442 (62.9-63.3%) of these individuals had data for a weight variable. From the 2001-2002 survey, the weight variable used to calculate the overall weight variable was zero, resulting in an overall weight variable of zero and in these individuals not being included in the analyses where weighting was used. Data documentation for NHANES indicates that these individuals are non-respondents; however they have responses for all other necessary variables. This may have to do with a changing of the sampled subset in 2001 from oversampling of children, with a limited age range (6-59) to a sampled subset including all ages 6 and up, in 2002.

For each metabolite, the proportion of those tested that registered levels over the LOD varied. From the number analyzed for each metabolite, the number of subjects above LOD was 3,851 (44.1%) for DMP, 3,667 (42.1%) for DEP, 6,122 (70.2%) for DMTP, 3,851 (44.4%) for DETP, 3,097 (35.5%) for all DMAP metabolites, 2,162 (25.0%) for all DEAP metabolites, and 1,360 (15.7%) for all DAP metabolites. There may be some variation in these numbers from those reported in other studies, as the LOD was made to be consistent over all five NHANES

cycles, resulting in a greater proportion of values in some cycles being considered below the LOD. All metabolite observations are quantified in nM units. Looking at all the subjects (including those below LOD), the mean level for DMP was 47.4 nM (standard deviation = 142.1), for DEP 28.9 nM (sd = 363.3), for DMTP 77.7 nM (sd = 456.5), for DETP 7.0 nM (sd = 17.6), for the sum of DMP metabolites 125.0 nM (sd = 514.3), for the sum of DEP metabolites 35.9 nM (sd = 365.7), and for the sum of all four DAP metabolites 160.7 nM (sd = 639.8). The highest totals for the sum of all four DAP metabolites were seen in those involved in the 1999-2000 survey (240.7 nM, sd = 952.8) and in children aged 6-11 years (226.5 nM, sd = 1125.6). There was a wide range of metabolite levels, with some outliers for each metabolite, but one extreme outlier for DMTP was recoded as missing prior to analysis. The DMTP measurement for this outlier was 2,349,295.77 nM.

The characteristics of the final analyzed population of 8,735 individuals are described in Table 1. The number of individuals included for each survey year varies depending on how many individuals the NHANES includes in the pesticide subsample. The populations by cohort increased over the survey years from 1,034 (11.8%) for the 1999-2000 cohort, to 2005-2006, and 2007-2008 which had similar numbers at 2,268 (26.0%) and 2,165 (24.8%), respectively.

Demographic data were reported for all survey respondents and are described in Table 1. The mean age was 34.1 years. The largest age category was from 20-59 years with 3,543 subjects (40.6%). The population was nearly equal males (4,264, 48.8%) and females (4,471, 51.2%). NHANES populations oversample for minorities compared to the U.S. population. The breakdown of the population by race was 3,907 (44.7%) non-Hispanic white, 2,013 (23.1%) Mexican American, 2,001 (22.9%) non-Hispanic black, 463 (5.3%) other Hispanic, and 351 (4.0%) other race (including multi-racial). In terms of education, partially due to a large

	Mean (sd)	Number (%)	Mean (sd) servings of meat (n = 8735)	*p*-value	Mean (sd) DMP in nM (n = 8735)	*p*-value
Total population (n = 8,735)		**8735 (100.0%)**	**2.16 (2.34)**		**47.36 (142.12)**	
Survey Years (n = 8,735)				0.0175		< 0.0001
1999-2000		1034 (11.8%)	2.01 (2.15)		32.70 (74.92)	
2001-2002		1681 (19.2%)	2.08 (2.21)		31.69 (118.28)	
2003-2004		1586 (18.2%)	2.20 (2.44)		31.22 (60.34)	
2005-2006		2269 (26.0%)	2.15 (2.17)		61.63 (197.84)	
2007-2008		2165 (24.8%)	2.28 (2.59)		63.42 (152.77)	
Age (n = 8,735)	34.06 (22.67)			< 0.0001		0.0001
6-11 years		1389 (15.9%)	1.62 (1.71)		56.70 (138.29)	
12-19 years		2198 (25.2%)	2.14 (2.37)		49.68 (171.69)	
20-59 years		3543 (40.6%)	2.51 (2.59)		39.46 (108.20)	
60-85 years		1605 (18.4%)	1.88 (2.03)		53.57 (164.16)	
Gender (n = 8,735)				< 0.0001		0.0133
Male		4264 (48.8%)	2.55 (2.65)		43.51 (108.21)	
Female		4471 (51.2%)	1.79 (1.92)		51.04 (168.14)	
Race (n = 8,735)				0.0206		0.0003
Mexican American		2013 (23.1%)	2.19 (2.48)		45.93 (131.69)	
Other Hispanic		463 (5.3%)	2.17 (2.94)		59.39 (172.90)	
Non-Hispanic White		3907 (44.7%)	2.07 (2.25)		41.07 (116.50)	
Non-Hispanic Black		2001 (22.9%)	2.28 (2.11)		56.80 (147.68)	
Other Race - Including Multi-Racial		351 (4.0%)	2.25 (2.69)		56.05 (295.92)	
Education (n = 8,735)				< 0.0001		0.0206
< 9th grade		3027 (34.7%)	1.84 (2.10)		53.43 (157.95)	
9-11th grade (12th w/no diploma)		1717 (19.7%)	2.32 (2.45)		41.58 (122.01)	
High school grad/GED or equivalent		1399 (16.0%)	2.43 (2.38)		42.81 (120.25)	
Some College or AA degree		1541 (17.6%)	2.37 (2.50)		43.52 (133.03)	
College grad or above		1051 (12.0%)	2.19 (2.39)		51.01 (162.34)	
Income (n = 8,735)				0.0089		0.1637
0 - $19,999		1921 (22.0%)	2.00 (2.11)		50.32 (143.61)	
$20,000 - $54,999		3595 (41.2%)	2.21 (2.36)		45.01 (135.11)	
$55,000 - $74,999		1182 (13.5%)	2.24 (2.50)		42.38 (97.16)	
$75,000+		2037 (23.3%)	2.18 (2.38)		51.64 (171.61)	
Birthplace (n = 8,735)				0.0040		0.0033
US		7414 (84.9%)	2.13 (2.29)		45.48 (141.86)	
Mexico		752 (8.6%)	2.41 (2.55)		52.29 (119.50)	
Other		569 (6.5%)	2.26 (2.58)		65.44 (169.27)	
Pesticide use in home (n = 8,735)				0.0204		0.5519
Yes		1263 (14.5%)	2.30 (2.42)		45.16 (182.16)	
No		7472 (85.5%)	2.14 (2.32)		47.74 (134.19)	
Pesticide use in yard (n = 8,735)				0.9950		0.0029
Yes		1182 (13.5%)	2.16 (2.23)		35.91 (81.12)	
No		7553 (86.5%)	2.16 (2.35)		49.16 (149.36)	

Table 1. Characteristics of the study population.

	Mean (sd) DEP in nM (n = 8711)	*p*-value	Mean (sd) DMTP in nM (n = 8727)	*p*-value	Mean (sd) DETP in nM (n = 8678)	*p*-value
Total population (n = 8,735)	**28.90 (363.34)**		**77.68 (456.49)**		**6.97 (17.61)**	
Survey Years (n = 8,735)		0.3864		<0.0001		< 0.0001
1999-2000	25.61 (69.82)		176.69 (940.04)		5.69 (16.81)	
2001-2002	28.52 (262.00)		74.19 (611.03)		9.14 (21.72)	
2003-2004	45.44 (737.12)		61.74 (177.26)		6.65 (16.42)	
2005-2006	24.32 (274.76)		62.50 (258.72)		5.56 (12.89)	
2007-2008	23.61 (62.67)		60.67 (175.37)		7.61 (19.25)	
Age (n = 8,735)		0.0464		0.0075		0.2487
6-11 years	52.01 (799.00)		108.79 (729.27)		7.53 (23.27)	
12-19 years	31.90 (282.71)		88.66 (470.97)		6.81 (14.40)	
20-59 years	21.73 (155.99)		64.19 (390.90)		6.63 (17.53)	
60-85 years	20.58 (39.02)		65.47 (177.43)		7.46 (16.01)	
Gender (n = 8,735)		0.4368		0.5993		0.5987
Male	25.80 (204.05)		75.04 (463.55)		7.07 (16.79)	
Female	31.86 (467.17)		80.18 (449.70)		6.87 (18.35)	
Race (n = 8,735)		0.0113		0.6762		0.0159
Mexican American	22.63 (60.54)		89.05 (427.09)		6.87 (19.39)	
Other Hispanic	21.68 (52.49)		65.52 (194.75)		7.37 (16.60)	
Non-Hispanic White	20.76 (64.11)		71.70 (417.79)		6.49 (15.79)	
Non-Hispanic Black	54.56 (749.66)		80.56 (580.34)		8.09 (20.12)	
Other Race - Including Multi-Racial	18.44 (43.33)		78.53 (476.27)		6.01 (10.53)	
Education (n = 8,735)		0.2206		0.0016		0.2858
< 9th grade	41.07 (590.89)		100.95 (597.12)		6.95 (18.23)	
9-11th grade (12th w/no diploma)	27.34 (221.69)		70.18 (375.86)		6.91 (15.45)	
High school grad/GED or equivalent	21.81 (61.19)		52.53 (152.02)		6.68 (18.50)	
Some College or AA degree	21.13 (53.25)		54.44 (140.33)		6.61 (15.52)	
College grad or above	17.25 (40.24)		90.48 (642.06)		8.05 (20.50)	
Income (n = 8,735)		0.6783		0.1103		0.7747
0 - $19,999	31.29 (296.71)		76.93 (497.05)		6.88 (18.01)	
$20,000 - $54,999	25.87 (187.04)		74.10 (343.01)		6.80 (18.93)	
$55,000 - $74,999	22.08 (65.88)		57.36 (148.30)		7.34 (17.45)	
$75,000+	35.98 (647.87)		96.50 (663.29)		7.15 (14.64)	
Birthplace (n = 8,735)		0.6093		0.9483		0.0415
US	30.53 (393.78)		77.02 (479.93)		6.87 (17.41)	
Mexico	19.23 (47.86)		80.75 (334.98)		6.64 (17.32)	
Other	20.38 (45.67)		82.22 (223.14)		8.76 (20.25)	
Pesticide use in home (n = 8,735)		0.4861		0.7297		0.8189
Yes	22.30 (56.85)		81.78 (344.48)		6.86 (12.07)	
No	30.02 (392.11)		76.98 (472.84)		6.99 (18.38)	
Pesticide use in yard (n = 8,735)		0.4753		0.0019		0.9635
Yes	21.86 (48.62)		116.01 (832.49)		6.99 (13.03)	
No	30.00 (390.16)		71.67 (363.69)		6.97 (18.22)	

Table 1 (continued). Characteristics of the study population.

	Mean (sd) DMAP sum in nM (n = 8727)	*p*-value	Mean (sd) DEAP sum in nM (n = 8654)	*p*-value	Mean (sd) DAP sum in nM (n = 8646)	*p*-value
Total population (n = 8,735)	**124.97 (514.30)**		**35.88 (365.70)**		**160.69 (639.84)**	
Survey Years (n = 8,735)		< 0.0001		0.3644		0.0008
1999-2000	209.42 (945.28)		31.29 (73.35)		240.74 (952.80)	
2001-2002	105.89 (636.25)		37.68 (264.74)		143.41 (694.26)	
2003-2004	92.97 (210.48)		52.61 (750.09)		144.32 (789.35)	
2005-2006	123.79 (431.43)		29.90 (275.88)		153.78 (518.95)	
2007-2008	124.14 (307.96)		31.25 (70.40)		154.52 (330.03)	
Age (n = 8,735)		0.0009		0.0416		< 0.0001
6-11 years	165.53 (765.30)		59.72 (803.73)		226.52 (1125.55)	
12-19 years	138.37 (560.26)		38.80 (285.19)		176.86 (637.62)	
20-59 years	103.67 (431.02)		28.38 (158.69)		131.29 (462.57)	
60-85 years	118.52 (300.67)		27.87 (46.38)		146.73 (313.83)	
Gender (n = 8,735)		0.2432		0.4613		0.1996
Male	118.38 (496.61)		32.91 (206.68)		151.63 (546.02)	
Female	131.24 (530.59)		38.70 (469.31)		169.29 (717.62)	
Race (n = 8,735)		0.3625		0.0071		0.0154
Mexican American	135.03 (480.74)		29.40 (69.27)		163.22 (490.89)	
Other Hispanic	125.03 (349.04)		29.10 (61.63)		154.40 (372.00)	
Non-Hispanic White	112.54 (455.19)		27.24 (70.49)		139.57 (468.52)	
Non-Hispanic Black	137.39 (621.64)		62.84 (753.06)		201.25 (988.73)	
Other Race - Including Multi-Racial	134.58 (765.87)		24.45 (48.42)		159.03 (769.79)	
Education (n = 8,735)		0.0003		0.2317		0.0001
< 9th grade	154.42 (664.08)		48.12 (593.73)		202.29 (902.06)	
9-11th grade (12th w/no diploma)	111.76 (429.75)		34.30 (223.70)		145.84 (491.92)	
High school grad/GED or equivalent	94.72 (245.14)		28.49 (68.57)		123.68 (268.49)	
Some College or AA degree	97.98 (242.52)		27.70 (60.09)		126.34 (265.76)	
College grad or above	141.58 (675.44)		25.23 (49.79)		165.42 (680.58)	
Income (n = 8,735)		0.0567		0.6753		0.0265
0 - $19,999	127.28 (550.23)		38.15 (298.92)		165.57 (635.45)	
$20,000 - $54,999	118.88 (395.88)		32.66 (190.49)		150.96 (443.99)	
$55,000 - $74,999	99.74 (218.39)		29.36 (72.52)		127.19 (235.60)	
$75,000+	148.18 (737.55)		43.21 (650.61)		192.82 (997.79)	
Birthplace (n = 8,735)		0.4751		0.6420		0.8080
US	122.40 (534.93)		37.41 (396.19)		159.86 (674.84)	
Mexico	133.05 (385.43)		25.76 (55.99)		156.23 (389.60)	
Other	147.78 (368.37)		29.18 (54.57)		177.26 (387.09)	
Pesticide use in home (n = 8,735)		0.8824		0.4831		0.7745
Yes	126.95 (496.55)		29.16 (62.02)		155.89 (505.61)	
No	124.63 (517.28)		37.01 (394.46)		161.50 (659.81)	
Pesticide use in yard (n = 8,735)		0.0526		0.4699		0.2351
Yes	151.92 (844.47)		28.68 (54.85)		181.39 (855.51)	
No	120.74 (440.66)		37.00 (392.51)		157.46 (599.32)	

Table 1 (continued). Characteristics of the study population.

percentage of the population being children, the largest group of the population at 3,027 (34.7%) had less than a ninth grade education. Household income was reported in ranges with the largest category making from $20,000-$54,999 (3,595, 41.2%). Approximately equal proportions reported incomes of 0-$19,999, (1,921, 22.0%) and $75,000 or more (2,037, 22.3%). Most participants were born in the United States (7,414, 84.9%).

In this population, the majority of respondents did not use pesticides in the home (7,472, 85.5%) or in the yard (7,553, 86.5%). Those that did use pesticides in the home had a mean sum of DAP metabolites of 155.9 nM (sd = 505.6), which was similar, but lower than those who did not use pesticides in their home (mean sum DAP = 161.5 nM, sd = 659.8). Those who used pesticides in the yard also saw the opposite effect with a mean sum DAP of 181.4 nM (sd = 855.5), higher compared to those who did not use pesticides in their yard (mean sum DAP = 157.5 nM, sd = 599.3).

The variables most likely to have significant variation in the means of metabolites between variable categories were age, race and education, as seen in Table 1. For age, those aged 6-11 had the highest DAP observations for all metabolite measurements except for DETP, where those from 60-85 years had the same mean (7.5 nM, sd = 23.3 for aged 6-11, 16.0 nM for those aged 60-85). The widest range proportionally was for DMTP which was 2.5 times higher for those aged 6-11 than those aged 60-85 years, who had the lowest DMTP (52.0 nM, sd = 799.0 and 20.6 nM, sd = 39.0 nM, respectively) and 1.6 times higher than those aged 12-19, who had the closest DMTP to the youngest group (31.9 nM, sd = 282.7). For the other metabolite measurements, the 6-11 age group measured from 1.1 nM (for DETP) to 2.1 nM (for DEAP) times higher than the age group with the lowest measurement.

For race, the non-Hispanic black group had the highest mean observation for all

metabolites except DMP and DMTP (the other Hispanic group and the Mexican American group were highest, respectively). The widest range proportionally was seen in the mean DEP values, the non-Hispanic black group measured 3.0 times higher than the other race group, which was lowest (54.6 nM, sd = 749.7 and 18.4 nM, sd = 43.3 nM, respectively). Variation was not significant for DMTP or DMAP ($p = 0.6762$ and $p = 0.3625$, respectively).

Metabolite mean measurements varied significantly by education levels for DMP, DMTP, DMAP, and the DAP sum ($p = 0.0206$, $p = 0.0016$, $p = 0.0003$, $p = 0.0001$, respectively). Those with less than a ninth grade education have the highest level of each metabolite for all but DETP (6.9 nM, sd = 18.2) where those who are college graduates or above have the highest metabolite level (8.1 nM, sd = 20.5). The mean DAP for people with less than a ninth grade education was 202.3 nM (sd = 902.1), 1.6 times the mean observation for those with a high school diploma or equivalent (123.7 nM , sd = 265.8).

Survey year had significant variability between years for DMP, DMTP, DETP, DMAP, and DAP ($p < 0.0001$ for all but DAP, $p = 0.0008$). For the metabolites with significant variation between survey years, the mean was highest in 1999-2000 for DMTP (176.7 nM, sd = 940.0), DMAP (209.4 nM, sd = 945.3), and DAP (240.7 nM, sd = 952.8). The widest range proportionally was for DMTP where the mean for 1999-2000 was 2.9 times the mean for 2007-2008 (60.7 nM, sd = 175.4). Birthplace showed variation for DMP ($p = 0.0033$) and DETP ($p = 0.0415$). Pesticide use in the home showed no significant variation in metabolite levels between groups, while pesticide use in the yard had significant variation only for DMP ($p = 0.0029$) and DMTP ($p = 0.0019$).

The mean level of consumption of meat in the 24 hours prior to survey was 2.16 servings (sd = 2.34). Men reported eating the most meat with a mean of 2.55 servings (sd = 2.65) and

people from 20-59 years of age with 2.51 servings (sd = 2.59). Women and young children (age 6-11 year) consumed the least amount of meat (1.79 servings, sd = 1.92 and 1.62 servings, sd = 1.71, respectively). Mean fruit consumption was 1.21 servings (sd = 1.91), with those born in Mexico or in other countries besides the U.S. consuming more on average (1.54 servings, sd = 2.11 and 1.65 servings, sd = 2.17, respectively). Those who were at least college graduates also consumed more fruit (1.52 servings, sd = 1.97). The amount of meat consumed varied significantly ($p < 0.05$) between categories for all variables except for whether pesticides were used in the yard ($p = 0.9950$).

Mean water consumption was only considered using the 2005-2006 and 2007-2008 surveys, as in NHANES the collection of data on tap and bottled water consumed as a beverage did not begin until 2005 (30). The mean water consumption for these two survey populations was 3.49 servings (sd = 4.22), with again those have some college and those who were at least college graduates consuming the most water (4.48 servings, sd = 4.78, and 4.74 servings, sd = 4.41).

Linear Regression Analysis

A linear regression analysis with log-transformed metabolite levels as the outcome was used to examine the relationship between servings of meat (the exposure of interest), the other categorical and continuous exposures, and the four DAP metabolites and associated sums. For consistency the final model was also the fully adjusted model for all metabolites with both weighted and unweighted analysis, as backward elimination was completed for each model and demonstrated that each covariate was either a confounder or a significant factor in at least one of the models. The beta coefficient estimates for the effect of one serving of meat on DAP metabolite levels are shown in Table 2.

Metabolite		With all observations							
		Unweighted				Weighted			
		n	β estimate	95% C.I.†	*Wald p-value* ^	n	β estimate	95% C.I.†	*Wald p-value* ^
DMP	Crude	8735	-0.028	(-0.042, -0.014)	0.0001	8442	-0.027	(-0.041, -0.014)	< 0.0001
	*Fully-adjusted		-0.022	(-0.037, -0.008)	0.0021		-0.020	(-0.034, -0.007)	0.0036
DEP	Crude	8711	-0.020	(-0.034, -0.006)	0.0043	8418	-0.020	(-0.034, -0.007)	0.0033
	*Fully-adjusted		-0.014	(-0.028, 0.000)	0.0474		-0.013	(-0.027, 0.001)	0.0607
DMTP	Crude	8727	-0.017	(-0.031, -0.003)	0.0191	8434	-0.016	(-0.030, -0.002)	0.0273
	*Fully-adjusted		-0.013	(-0.028, 0.001)	0.0756		-0.013	(-0.027, 0.002)	0.0795
DETP	Crude	8678	-0.007	(-0.014, 0.000)	0.0623	8386	-0.004	(-0.010, 0.004)	0.3278
	*Fully-adjusted		-0.008	(-0.015, -0.001)	0.0279		-0.005	(-0.012, 0.002)	0.1473
DMP+DMTP	Crude	8727	-0.022	(-0.035, -0.009)	0.0011	8434	-0.019	(-0.032, -0.006)	0.0033
	*Fully-adjusted		-0.017	(-0.030, -0.004)	0.0124		-0.015	(-0.027, -0.002)	0.0262
DEP+DETP	Crude	8654	-0.017	(-0.028. -0.006)	0.0021	8362	-0.016	(-0.026, -0.005)	0.0032
	*Fully-adjusted		-0.015	(-0.026, -0.004)	0.0081		-0.014	(-0.024, -0.003)	0.0126
DMP+DMTP +DEP+DETP	Crude	8646	-0.023	(-0.035, -0.011)	0.0001	8354	-0.021	(-0.032, -0.009)	0.0004
	*Fully-adjusted		-0.019	(-0.030, -0.007)	0.0020		-0.017	(-0.029, -0.006)	0.0037

†95% C.I = 95% Confidence Interval

^Wald p-value = chunk test for overall significance of variable in the model

*Fully-adjusted models include these covariates: survey year, age, gender, education, race, pesticide use in the home, pestcide use in the yard, fruit consumption, dairy consumption, vegetable consumption, and water consumption.

Table 2. Effect of one serving of meat on log-transformed metabolite levels for each examined metabolite and metabolite sum. Crude and fully-adjusted results for both weighted and unweighted models, and both with and without observations at or below the LOD.

Metabolite		Without observations at or below LOD							
		Unweighted				Weighted			
		n	β estimate	95% C.I.†	*Wald p-value* ^	n	β estimate	95% C.I.†	*Wald p-value* ^
DMP	Crude	3851	-0.010	(-0.026, 0.006)	0.2190	3689	-0.018	(-0.033, -0.002)	0.0258
	*Fully-adjusted		-0.012	(-0.026, 0.002)	0.0852		-0.020	(-0.034, -0.006)	0.0059
DEP	Crude	3667	-0.004	(-0.020, 0.012)	0.6135	3512	-0.005	(-0.021, 0.011)	0.5617
	*Fully-adjusted		-0.014	(-0.029, 0.001)	0.0727		-0.016	(-0.032, -0.001)	0.0405
DMTP	Crude	6122	-0.017	(-0.031, -0.003)	0.0206	5950	-0.014	(-0.029, 0.000)	0.0482
	*Fully-adjusted		-0.009	(-0.024, 0.005)	0.1937		-0.008	(-0.022, 0.007)	0.2913
DETP	Crude	3851	-0.002	(-0.013, 0.009)	0.7302	3638	-0.006	(-0.017, 0.005)	0.2882
	*Fully-adjusted		-0.004	(-0.016, 0.007)	0.4422		-0.009	(-0.020, 0.003)	0.1291
DMP+DMTP	Crude	3097	-0.012	(-0.029, 0.005)	0.1549	2984	-0.017	(-0.034, 0.001)	0.0590
	*Fully-adjusted		-0.013	(-0.030, 0.004)	0.1230		-0.018	(-0.035, -0.001)	0.0390
DEP+DETP	Crude	2162	-0.006	(-0.023, 0.012)	0.5312	2032	-0.009	(-0.026, 0.009)	0.3485
	*Fully-adjusted		-0.011	(-0.027, 0.006)	0.2010		-0.010	(-0.027, 0.006)	0.2215
DMP+DMTP +DEP+DETP	Crude	1360	-0.014	(-0.038, 0.010)	0.2590	1283	-0.026	(-0.050, -0.001)	0.0386
	*Fully-adjusted		-0.014	(-0.037, 0.009)	0.2313		-0.023	(-0.047, 0.000)	0.0535

†95% C.I = 95% Confidence Interval

^Wald p-value = chunk test for overall significance of variable in the model

*Fully-adjusted models include these covariates: survey year, age, gender, education, race, pesticide use in the home, pestcide use in the yard, fruit consumption, dairy consumption, vegetable consumption, and water consumption.

Table 2 (continued). Effect of one serving of meat on log-transformed metabolite levels for each examined metabolite and metabolite sum. Crude and fully-adjusted results for both weighted and unweighted models, and both with and without observations at or below the LOD.

Meat consumption: In all analyses, one serving of meat was associated with a decrease in the level of urinary DAP metabolites. For the unweighted crude analyses with all observations, the beta coefficients for one serving of meat varied from -0.028 (95% confidence interval: -0.042 – -0.014, $p = 0.0001$) for DMP to -0.007 (-0.014 – 0.000, $p = 0.0623$) for DETP. The effect of one serving of meat was significant ($p < 0.05$) in the crude model for all metabolites except DETP. For the unweighted, fully adjusted model the beta coefficients varied from -0.022 (-0.037 – -0.008, $p = 0.0021$) for DMP to -0.008 (-0.015 – -0.001, $p = 0.0279$) for DETP. The effect of one serving of meat was significant in the unweighted, fully adjusted model for all metabolites except DMTP ($p = 0.0756$).

The secondary weighted analyses had similar findings. For the crude model, beta coefficients for 1 serving of meat varied from -0.027 (-0.041 – -0.014, $p < 0.0001$) for DMP to -0.004 (-0.010 – 0.004, $p = 0.3278$) for DETP. Again, the effect of one serving of meat was significant in the crude model for all metabolites except DETP. For the fully adjusted weighted model, the beta coefficients for one serving of meat varied from -0.020 (-0.034 – -0.007, $p = 0.0036$) for DMP to -0.005 (-0.012 – 0.002, $p = 0.1473$) for DETP. The effect of one serving of meat was significant in the fully adjusted model for DMP, and the sums of the DMAP, DEAP, and all DAP metabolites.

The analyses restricted to individuals with metabolite levels above the LOD also showed that the effect of one serving of meat was associated with a decrease in the level of the metabolite, although the decrease was generally smaller than that seen when looking at all observations. For the crude unweighted analyses, the effect of one serving of meat was only significant for DMTP ($\beta = -0.017$ (-0.041 – -0.014), $p = 0.0206$). For the fully-adjusted unweighted analyses, one serving of meat was not associated with a significant change in any of

the metabolites. For the crude weighted analyses, one serving of meat had a significant association with a decrease for DMP (β = -0.018 (-0.033 – -0.002), p = 0.0258), DMTP (β = -0.014 (-0.029 – 0.000), p = 0.0482), and the sum of the DAP metabolites (β = -0.026 (-0.050 – -0.001), p = 0.0386). This changed for the fully adjusted weighted analyses, where one serving of meat had a significant association with a decrease for DMP (β = -0.020 (-0.034 – -0.006), p = 0.0059), DEP (β = -0.016 (-0.032 – -0.001), p = 0.0405), and the sum of DMAP metabolites (β = -0.0390 (-0.035 – -0.001), p = 0.0390).

Consumption of other foods: Table 3 shows the beta coefficients, 95% confidence intervals, and *p*-values for the effects of one serving of dairy, fruit, vegetables, and water on DAP metabolite levels. Most notable was that in every model for all DAP metabolites, fruit consumption had a significant association ($p < 0.0001$ in all cases) with an increase in metabolite levels. Dairy products and vegetables did not show any consistent significant association with metabolite levels and their beta coefficient values where generally positive, indicating a contribution to a rise in metabolite levels.

In the models involving all observations, water was associated with a significant ($p < 0.05$) decrease in metabolite levels for all models except both DEP and DEAP sum models, and the weighted DETP model. There was less consistency in the association with water in the models involving only observations above the LOD. The association with water was also examined for the last two survey years separately and it was noted that water still had an inverse association with each of the metabolites. The association was significant for DMP (p = 0.0125), DMTP (p = 0.0005), DETP (p = 0.0428), the DMAP sum (p = 0.0002), and the sum of all DAPs (p = 0.0003).

Metabolite		With all observations					
		Unweighted			Weighted		
		β estimate	95% C.I.†	*Wald p-value* ^	β estimate	95% C.I.†	*Wald p-value* ^
DMP	Dairy	-0.008	(-0.022, 0.007)	0.3175	-0.005	(-0.019, 0.009)	0.4595
	Fruit	0.104	(0.087, 0.122)	< 0.0001	0.112	(0.095, 0.130)	< 0.0001
	Vegetables	0.017	(0.001, 0.033)	0.0367	0.005	(-0.009, 0.019)	0.5038
	Water	-0.013	(-0.024, -0.002)	0.0176	-0.014	(-0.024, -0.004)	0.0048
DEP	Dairy	-0.008	(-0.023, 0.006)	0.2666	-0.005	(-0.019, 0.009)	0.4666
	Fruit	0.077	(0.060, 0.094)	< 0.0001	0.075	(0.057, 0.092)	< 0.0001
	Vegetables	0.006	(-0.010, 0.022)	0.4581	0.003	(-0.011, 0.017)	0.6757
	Water	-0.008	(-0.019, 0.002)	0.1289	-0.005	(-0.015, 0.005)	0.3363
DMTP	Dairy	0.008	(-0.007, 0.023)	0.2986	-0.003	(-0.017, 0.012)	0.7127
	Fruit	0.128	(0.110, 0.145)	< 0.0001	0.152	(0.134, 0.170)	< 0.0001
	Vegetables	0.016	(0.000, 0.032)	0.0471	0.008	(-0.007, 0.022)	0.2870
	Water	-0.019	(-0.030, -0.008)	0.0010	-0.018	(-0.029, -0.008)	0.0004
DETP	Dairy	-0.004	(-0.012, 0.003)	0.2850	0.000	(-0.007, 0.007)	0.9836
	Fruit	0.071	(0.062, 0.079)	< 0.0001	0.075	(0.066, 0.084)	< 0.0001
	Vegetables	0.003	(-0.005, 0.011)	0.4417	0.009	(0.002, 0.016)	0.0104
	Water	-0.006	(-0.012, -0.001)	0.0243	-0.004	(-0.009, 0.001)	0.1282
DMP+DMTP	Dairy	0.001	(-0.013, 0.014)	0.9453	-0.004	(-0.017, 0.009)	0.5799
	Fruit	0.122	(0.106, 0.138)	< 0.0001	0.141	(0.125, 0.157)	< 0.0001
	Vegetables	0.017	(0.003, 0.032)	0.0217	0.005	(-0.008, 0.019)	0.4197
	Water	-0.018	(-0.029, -0.008)	0.0004	-0.020	(-0.030, -0.011)	< 0.0001
DEP+DETP	Dairy	-0.007	(-0.018, 0.005)	0.2532	-0.005	(-0.016, 0.006)	0.3866
	Fruit	0.083	(0.070, 0.096)	< 0.0001	0.082	(0.069, 0.096)	< 0.0001
	Vegetables	0.006	(-0.006, 0.018)	0.3402	0.008	(-0.003, 0.019)	0.1366
	Water	-0.008	(-0.016, 0.001)	0.0643	-0.005	(-0.013, 0.003)	0.1954
DMP+DMTP +DEP+DETP	Dairy	-0.002	(-0.015, 0.010)	0.7063	-0.007	(-0.019, 0.005)	0.2440
	Fruit	0.112	(0.098, 0.126)	< 0.0001	0.123	(0.109, 0.138)	< 0.0001
	Vegetables	0.015	(0.002, 0.028)	0.0381	0.008	(-0.004, 0.019)	0.2136
	Water	-0.016	(-0.025, -0.007)	0.0004	-0.017	(-0.026, -0.009)	< 0.0001

†95% C.I = 95% Confidence Interval

^Wald p-value = test for overall significance of variable in the model

*Fully-adjusted models include these covariates: survey year, age, gender, education, race, pesticide use in the home, pesticide use in the yard, fruit consumption, dairy consumption, vegetable consumption, and water consumption.

Table 3. Effect of one serving of each examined food on log-transformed metabolite levels for each examined metabolite and metabolite sum. Crude and fully-adjusted results for both weighted and unweighted models, and both with and without observations at or below the LOD.

Metabolite		Without observations at or below LOD					
		Unweighted			Weighted		
		β estimate	95% C.I.†	*Wald p-value* ^	β estimate	95% C.I.†	*Wald p-value* ^
DMP	Dairy	0.000	(-0.015, 0.015)	0.9591	-0.001	(-0.015, 0.014)	0.9346
	Fruit	0.053	(0.038, 0.069)	< 0.0001	0.059	(0.044, 0.075)	< 0.0001
	Vegetables	-0.009	(-0.023, 0.006)	0.2511	-0.007	(-0.021, 0.007)	0.3400
	Water	-0.012	(-0.024, 0.000)	0.0463	-0.018	(-0.029, -0.007)	0.0019
DEP	Dairy	-0.005	(-0.021, 0.010)	0.5144	-0.011	(-0.025, 0.004)	0.1480
	Fruit	0.045	(0.029, 0.061)	< 0.0001	0.051	(0.034, 0.068)	< 0.0001
	Vegetables	0.004	(-0.012, 0.021)	0.6106	0.009	(-0.006, 0.024)	0.2319
	Water	-0.012	(-0.025, 0.001)	0.0782	-0.012	(-0.024, 0.000)	0.0534
DMTP	Dairy	0.007	(-0.008, 0.021)	0.3729	0.004	(-0.011, 0.019)	0.6023
	Fruit	0.083	(0.067, 0.100)	< 0.0001	0.098	(0.082, 0.115)	< 0.0001
	Vegetables	0.013	(-0.003, 0.029)	0.1121	-0.002	(-0.016, 0.013)	0.8209
	Water	-0.013	(-0.023, -0.002)	0.0230	-0.009	(-0.019, 0.001)	0.0765
DETP	Dairy	-0.006	(-0.016, 0.005)	0.2701	0.002	(-0.008, 0.013)	0.6594
	Fruit	0.042	(0.031, 0.053)	< 0.0001	0.044	(0.032, 0.055)	< 0.0001
	Vegetables	-0.007	(-0.019, 0.005)	0.2387	-0.002	(-0.012, 0.009)	0.7500
	Water	0.000	(-0.009, 0.0010)	0.9561	0.003	(-0.006, 0.011)	0.5453
DMP+DMTP	Dairy	0.003	(-0.015, 0.021)	0.7253	0.002	(-0.016, 0.021)	0.8128
	Fruit	0.050	(0.033, 0.067)	< 0.0001	0.061	(0.044, 0.079)	< 0.0001
	Vegetables	-0.010	(-0.027, 0.008)	0.2766	-0.017	(-0.033, -0.001)	0.0410
	Water	-0.012	(-0.025, 0.002)	0.0972	-0.012	(-0.025, 0.002)	0.0895
DEP+DETP	Dairy	-0.014	(-0.029, 0.003)	0.0992	-0.015	(-0.030, -0.001)	0.0410
	Fruit	0.031	(0.016, 0.046)	< 0.0001	0.033	(0.018, 0.049)	< 0.0001
	Vegetables	-0.001	(-0.018, 0.016)	0.8792	0.007	(-0.008, 0.022)	0.3487
	Water	0.001	(-0.016, 0.019)	0.8852	0.005	(-0.011, 0.021)	0.5153
DMP+DMTP +DEP+DETP	Dairy	0.003	(-0.021, 0.026)	0.8312	-0.011	(-0.035, 0.014)	0.3932
	Fruit	0.043	(0.024, 0.062)	< 0.0001	0.042	(0.023, 0.061)	< 0.0001
	Vegetables	0.000	(-0.024, 0.024)	0.9949	0.007	(-0.014, 0.028)	0.5038
	Water	-0.011	(-0.034, 0.012)	0.3542	-0.016	(-0.038, 0.006)	0.1487

†95% C.I = 95% Confidence Interval

^Wald p-value = test for overall significance of variable in the model

*Fully-adjusted models include these covariates: survey year, age, gender, education, race, pesticide use in the home, pesticide use in the yard, fruit consumption, dairy consumption, vegetable consumption, and water consumption.

Table 3 (continued). Effect of one serving of each examined food on log-transformed metabolite levels for each examined metabolite and metabolite sum. Crude and fully-adjusted results for both weighted and unweighted models, and both with and without observations at or below the LOD.

Logistic Regression Analysis

The logistic regression analyses, both weighted and unweighted also found that meat consumption showed an inverse association with the metabolites with ORs less than 1.0. The ORs and 95% confidence intervals are shown in Table 4. The crude model showed that meat consumption had the largest inverse association with levels of DMP, with and without weighting (OR 0.965, 95% CI 0.948-0.983, $p = 0.0002$ and OR 0.969, 95% CI 0.969-0.969, $p < 0.0001$, respectively). The unweighted, fully-adjusted model showed the largest inverse association with the sum of DEAP metabolites (OR 0.969, 95% CI 0.951-0.987, $p = 0.0007$); when it was weighted, of the largest inverse association was with DMTP (OR 0.983, 95% CI 0.989-0.990, $p < 0.0001$). The weighted model most noticeably differed from the unweighted model in that in all cases the 95% confidence interval was very narrow and the *p*-value was < 0.0001, indicating significance in all cases, which was not true for the unweighted model. Since NHANES weighting treats each observation as multiple observations in order to achieve an unbiased national estimate of a parameter, it makes sense that more significance would be seen in the weighted model, as sample size has been artificially increased. As the sample size has been artificially increased, weighting provides an estimate of the national population, but the unweighted model provides more accurate findings for the specific individuals in the surveyed population studied here.

Metabolite		With all Observations					
		Unweighted			Weighted		
		OR*	95% C.I.†	*Wald p-value* ^	OR*	95% C.I.†	*Wald p-value* ^
DMP	Crude	0.965	(0.948, 0.983)	0.0002	0.969	(0.969, 0.969)	< 0.0001
	*Fully-adjusted	0.975	(0.956, 0.994)	0.0098	0.984	(0.984, 0.984)	< 0.0001
DEP	Crude	0.973	(0.955, 0.991)	0.0033	0.972	(0.972, 0.972)	< 0.0001
	*Fully-adjusted	0.986	(0.967, 1.005)	0.1540	0.990	(0.989, 0.990)	< 0.0001
DMTP	Crude	0.990	(0.971, 1.010)	0.3171	0.988	(0.988, 0.988)	< 0.0001
	*Fully-adjusted	0.988	(0.968, 1.008)	0.2339	0.983	(0.983, 0.983)	< 0.0001
DETP	Crude	0.978	(0.959, 0.997)	0.0219	0.996	(0.996, 0.996)	< 0.0001
	*Fully-adjusted	0.980	(0.962, 0.998)	0.0288	0.995	(0.995, 0.995)	< 0.0001
DMP+DMTP	Crude	0.985	(0.964, 1.006)	0.1698	0.993	(0.993, 0.993)	< 0.0001
	*Fully-adjusted	0.988	(0.966, 1.010)	0.2735	0.994	(0.994, 0.994)	< 0.0001
DEP+DETP	Crude	0.974	(0.955. 0.993)	0.0071	0.976	(0.976, 0.976)	< 0.0001
	*Fully-adjusted	0.969	(0.951, 0.987)	0.0007	0.984	(0.984, 0.985)	< 0.0001
DMP+DMTP +DEP+DETP	Crude	0.978	(0.957, 1.000)	0.0470	0.981	(0.981, 0.981)	<0.0001
	*Fully-adjusted	0.984	(0.958, 1.012)	0.2539	0.987	(0.986, 0.987)	<0.0001

*OR=Odds Ratio

†95% C.I = 95% Confidence Interval

^Wald p-value = chunk test for overall significance of variable in the model

*Fully-adjusted models include these covariates: survey year, age, gender, education, race, pesticide use in the home, pesticide use in the yard, fruit consumption, dairy consumption, vegetable consumption, and water consumption.

Table 4. Odds ratios expressing the effect of meat consumption on DAP metabolite and metabolite sum outcomes when the metabolite measurements are set to dichotomous levels (at or below LOD or above LOD). Crude and fully-adjusted results for both weighted and unweighted models.

CHAPTER V

CONCLUSION

Summary

OPs are a commonly used class of pesticides, particularly prior to the last decade or so during which the EPA has phased out most residential and many other uses. Toxicity due to OPs is a known health risk and mechanisms of acute toxicity for OPs as AChE inhibitors are well understood. The consequences of chronic, low-level exposure are less well understood, but studies have noted that OPs may disturb numerous other biological processes resulting in more varied neurologic signs (17, 33). Diet is known to be one of the major contributors to OP intake, but the complete role that all components of diet play in OP intake is not fully understood.

This study used data from several cycles of NHANES to examine the association between consumption of meat and levels of OP metabolites in participants' urine samples. Urinary OP metabolites are evidence of recent consumption of OPs. Meat consumption, as well as the consumption of other foods was reported in a dietary survey as part of NHANES. Demographic variables and other foods consumed were included as covariates in linear regression models. The fully adjusted model was used throughout the study as all factors were found to have a significant association with the outcome or to be a confounder in at least one of the models. Each model was run both using weighting from NHANES and without weighting, for comparison. Additionally, the model was repeated for each metabolite restricted to observations with metabolite values that were found to be above the LOD. This analysis was performed because for some metabolites, a large percentage of observations were at or below the LOD, potentially violating normality assumptions of linear regression. A logistic regression with a dichotomous outcome of metabolites at or below the LOD and those above the LOD was also performed for comparison. The overall results show that in the population observed, consumption of meat has a negative association with the levels of OP metabolites, noted more

strongly when observations below the LOD were included, in particular for DMP and the sum of all DAP metabolites analyzed.

Previous work on DAP metabolite levels have adjusted for urine creatinine concentrations in order to correct for variable urine dilutions caused by the different hydration states of sample donors (1, 34). In this study an a priori assumption was that meat consumption and creatinine consumption were correlated since creatinine is a product of muscle breakdown, so creatinine was not included in the analysis. However, this assumption was examined and it was found that urinary creatinine level and number of meat servings were only weakly positively correlated (Spearman correlation = 0.0629, $p < 0.0001$).

Limitations

When completing this study, several limitations were evident. The use of the NHANES survey as the source of data is overall a strength. The survey provides a large, diverse population to study and a relatively consistent application of study surveys, laboratory methods, and examinations. However, inherent in any dietary interview is the potential for information bias, where an individual either incorrectly remembers or incorrectly states what type or how much of a particular food they consumed. This is reduced in the NHANES survey through the use of measuring guides and specific wording of questions, but is not completely avoidable and may lead to the incorrect interpretation of results (35). Demographic data and pesticide exposure assessment data were used as covariates in the models analyzed, but not all survey participants had recorded responses to all questions. This resulted in those participants being left out of the study, both decreasing the power of the analysis by having fewer observations and potentially creating selection bias depending on the reason those questions were not answered.

The NHANES data includes a specific selected population that does not necessarily

represent the entire U.S. population and then includes sample weights used to make statistical inferences meant to represent the U.S. population. It was decided in this study to primarily focus on the relationships between OP metabolites and meat exposures in a convenient sample, although analyses were also performed with weighting and thus may be generalized to the U.S. population. However, in some weighted models the p-value was lower than in the equivalent non-weighted model due to weighting artificially increasing the number of subjects in the study population.

Urinary metabolite measurement for pesticide exposure assessment is inexact because there may be contributions from nonpesticide sources. Interperson variation in metabolism may also lead to inaccurate exposure assessment (36). Additionally, assessing the contribution of diet is not specific, as subjects may be exposed to pesticides from the environment and other sources. Including information from the pesticide use survey as covariates controls some of this, but not all potential pesticide sources are identified. In addition, questions about pesticide use in the yard changed slightly across cycles in terms of the length of time being asked about, likely resulting in an inconsistent estimate across survey years of the effect of pesticide use in the yard on DAP metabolite observations.

Pesticide metabolites are frequently found to have low concentrations. In this study, anywhere from 29.8% to 84.3% of the metabolite measurements were less than or equal to the LOD, depending on the metabolite analysis. This adds uncertainty to the confidence interval estimations and skews the distribution as for each metabolite or sum of metabolites, all these measurements are represented by the same imputed value.

Conclusions

Three related research questions were posed for this study each aimed at determining if,

in this population, there is an association between the exposure, the level of meat and poultry products consumed, and the outcome, the level of several DAP metabolites, and what the nature of the association is for each metabolite. It was determined that based on the primary, unweighted linear regression analysis looking at all observations that meat and poultry consumption is generally associated with a lower level of DAP metabolites, thus rejecting the null hypothesis that the level of consumption of meat and poultry products has no impact on the urinary concentration of DAP metabolites.

Many analyses were performed, weighted and unweighted, both looking at all observations and using only those observations that were above the LOD. In all linear regression analyses, the beta coefficient associated with meat consumption was negative and in all logistic regression analyses the OR was less than one, indicating a decrease in metabolite levels associated with meat consumption. For most of the metabolites and sums of metabolites, when considering all observations with linear regression, weighted and unweighted, the effect of meat consumption was significant ($p < 0.05$). When looking at just those outcomes over LOD, the effect of a serving of meat is generally not found to be significant, but these analyses had fewer observations and thus less statistical power and point estimates from these analyses still suggested an inverse relationship between meat consumption and OP metabolites. In the fully adjusted, unweighted logistic regression analysis, consumption of a serving of meat only has a significant effect on metabolite levels when associated with DMP, DETP or the sum of DEAP metabolites, while in the weighted logistic regression analysis, consumption of a serving of meat is all models, both crude and fully-adjusted. This difference is due the artificial increase in population due to weighting.

The association of servings of meat consumed and metabolite level differed for each

metabolite throughout the various analyses, leading to rejection of the second null hypothesis, as there is a difference in the effect of meat and poultry consumption for the different metabolites. Taking into account all analyses, meat and poultry consumption overall had the most consistent significant effect and largest on DMP levels. It follows that this effect was also seen, but to a lesser extent for the sum of DMAP metabolites and the sum of all DAP metabolites, as DMP is included in both of these. Meat consumption had less of an effect on individual DEAP metabolites, both in terms of the size of decrease associated with meat consumption and in terms of the level of significance, but it did have a comparatively strong effect on the sum of DEAP metabolites. These differences may be related to individuals being exposed to varying OPs, as the metabolites examined are not specific to any one particular OP (24). The difference may also have to do with how the meat was prepared, as differing preparation methods have been found to affect the levels of OPs found in meat products (12).

In order to fully understand the contribution that meat and poultry consumption makes to the concentration of urinary OP metabolites, several other foods were considered in the model. Of these, the number of servings of fruit consumed had by far the largest impact in the log transformed linear regression models on the concentration of DAP metabolites. Fruit consumption significantly contributed ($p < 0.0001$ in all analyses) to a higher level of DAP metabolites, having a beta coefficient for the effect of one serving of fruit that varied from 1.8 to 15 times that of the beta for one serving of meat in the same analysis, with most values being around 5-8 times as large. As detailed above, all meat effects were negative, while all fruit effects were positive, indicating that fruit consumption significantly contributes to a higher level of DAP metabolites. Fruit has been shown in multiple studies to be the most significant dietary contributor to OP dietary exposure, so this is consistent with previous findings. Vegetable

consumption was also examined as a possible factor in urinary DAP levels and was found to have a slight positive impact, but it was inconsistently significant. The possibility for measurement error in terms of the dietary survey must be considered as having some impact on these values, without having a dietary journal and instruments to measure food with, the survey can only estimate what people ate which may lead to inadequate control of other dietary components in the fully-adjusted model. Additionally, in this population, an individual eating above the average amount of meat (2.2 servings, sd = 2.3) typically ate less fruit (1.19 servings, sd = 1.88) than an individual eating less than the average amount of meat (1.22 fruit servings, sd = 1.93). The variation is slight, but it likely holds true that if an individual is eating more meat than average, they are likely eating less than average of other dietary components such as fruits and vegetables and vice versa. This may lead to the inverse association with meat consumption being confounded by an increased consumption of other food that is more likely to contain OPs, but it is imperfectly measured and thus not fully controlled in our models.

Interestingly, although in previous studies, drinking water has been found to be a source of OP exposure (10), in this study, water had a negative impact on DAP concentration, particularly significant for DMP and DMTP and their associated sums. One possibility for this is that drinking increased amounts of water led to more dilute urine and thus a lower concentration of DAP metabolites. The size of the effect for one serving of water was approximately equivalent to that of one serving of meat. Water was measured differently for the first three NHANES cycles and the measurements for the last two NHANES cycles are considered more accurate. However, even when those cycles where analyzed separately, consumption of one serving of water was still seen to have a negative effect on DAP metabolite levels, again particularly on DMP, DMTP, and their associated sums. As mentioned previously, creatinine has been used in

previous studies to control for the possible effect of differing levels of hydration between individuals. Creatinine was not included in the analysis for this study, but correlation between creatinine and water consumption was examined and it was found that urinary creatinine level and water consumption were only weakly positively correlated (Spearman correlation = -0.0754, $p < 0.0001$).

Future Directions

This study provided a basic answer as to the role meat and poultry consumption plays in the concentration of urinary DAP metabolites, but several limitations of this study could be addressed and further research may better clarify the association. A similar study could be done using a more controlled method of dietary recollection, such as food diaries over a specified period of time, with multiple measurements of DAP metabolites taken. Subjects may also be asked to more accurately measure the amount of particular foods that they ate, to eliminate any information bias from the food recall questionnaire. Similarly, a more specific questionnaire addressing pesticide use or water source may help better determine other exposures.

Although this survey did specify the types of meats eaten (poultry, beef, lamb, etc.), meat was grouped into one exposure to simplify this analysis. Future studies may look into whether particular species, preparations, or cuts (particular organ meats, such as liver, which may be more likely to harbor pesticide residues) may better elucidate whether one type of meat product contributes more to OP metabolite levels.

In addition to better specifying exposures, one limitation was that the levels of OP metabolites are often quite low and a large number of observations are at or below the LOD. This number was increased with the conservative approach used to select the LOD used for all years of NHANES. In some cases lower levels of the metabolite may be detected through laboratory

improvements. Additionally, a more refined analysis looking at metabolite levels using LODs for that survey year instead of the more conservative method of selection may more accurately define the relationship between urinary DAP metabolites and diet. Also, urine concentration was not taken into account as a factor in metabolite levels. Future studies may control for urine concentration and possible higher metabolite values due to concentration by including creatinine levels, osmolality, or urine flow rate in the analysis. However, the optimal to control for urine dilution in studies estimating the effect of diet on DAP metabolites is not clear since these measures can themselves be downstream effects of diet.

This work emphasizes one of the multiple factors that play a role in pesticide exposure and the difficulties in defining the effect of a specific exposure. This study alone is likely not enough to make a recommendation one way or another regarding the consumption of meat in relation to OP exposure, but it does suggest that meat intake is not likely to contribute to excessive OP exposure. Further, more refined studies may better develop the understanding of this relationship.

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