2012

ASSOCIATIONS BETWEEN SERUM CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS, SERUM CAROTENOIDS, AND THE PROBABILITY OF METABOLIC SYNDROME IN THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2003-2004

Carolyn R. Hofe
University of Kentucky, carolyn.hofe@gmail.com

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Carolyn R. Hofe, Student

Dr. Lisa Gaetke, Major Professor

Dr. Howard P. Glauert, Director of Graduate Studies

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DISSERTATION

A dissertation submitted in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in the College of Medicine
at the University of Kentucky

By

Carolyn R. Hofe

Lexington, Kentucky

Director: Dr. Lisa Gaetke, Professor of Nutrition and Food Science

Lexington, Kentucky

ABSTRACT OF DISSERTATION

ASSOCIATIONS BETWEEN SERUM CONCENTRATIONS OF POLYCHLORINATED BIPHENYLS, SERUM CAROTENOIDS, AND THE PROBABILITY OF METABOLIC SYNDROME IN THE NATIONAL HEALTH AND NUTRITION EXAMINATION SURVEY 2003-2004

Diabetes and cardiovascular disease are leading causes of death and disability in the United States. These chronic diseases are clinical sequelae of metabolic syndrome (MetS), a condition that affects approximately one-third (1/3) of American adults. Metabolic syndrome occurs in response to environmental and genetic influences, among them food intake, a sedentary lifestyle, BMI, advancing age, and exposure to persistent organic pollutants (POPs). POPs are known to cause endocrine disruption and PCBs cause oxidative stress, disrupt endothelial cell integrity, and promote atherosclerosis. Nutrition plays a significant role in the prevention and management of these chronic diseases and has been shown to modulate the toxicity of PCBs. Serum carotenoid (SC) concentrations are the best biomarker indicative of fruit and vegetable intake and an improved nutritional status.

The purpose of this study was to investigate the relationship between serum carotenoid concentrations, serum concentrations of PCBs, and the probability of developing metabolic syndrome. The National Health and Nutrition Examination Survey (NHANES) is a program of the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), that utilizes a cross-sectional sample survey design to collect, maintain, and disseminate the health and nutrition data of persons residing in the United States. Carotenoids and PCBs share similar biological pathways due in part to lipophilicity. Both concentrate to lipids in blood, are stored primarily in adipose tissue, and may competitively bind nuclear receptors.

A statistical interaction was sought between the two variables for their combined effect on the probability of metabolic syndrome. An increase in probability

was observed in the first exposure quartile for many PCBs, individually and pooled, suggestive of a low dose endocrine effect. Statistical modeling consistently showed strong decreasing trends in the probability of metabolic syndrome with higher concentrations of serum carotenoids in the 3rd and 4th PCB exposure quartiles. These data suggest a protective effect of serum carotenoids, and therefore of fruit and vegetable intake, despite higher serum levels of PCBs, in the probability of developing metabolic syndrome.

Key words: Metabolic syndrome, Polychlorinated Biphenyls, Carotenoids, Nutrition, Environmental Health

Carolyn R. Hofe

January 11, 2012

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By

Carolyn R. Hofe

Lisa M. Gaetke, PhD RD
Director of Dissertation

Howard P. Glauert, PhD
Director of Graduate Studies

January 11, 2012

ACKNOWLEDGEMENTS

It is with sincere appreciation that I thank the Superfund Research Program of the University of Kentucky for funding this research initiative. Acknowledgement is further due the National Institutes of Health and National Institute of Environmental Health Sciences, Grant No. NIH/NIEHS P42ES007380, for making this work possible. I also offer sincere thanks to the College of Agriculture of the University of Kentucky for partially funding my work.

To a very great extent, I owe a debt of gratitude to my Dissertation Chair, Dr. Lisa Gaetke, for her dedicated, scholarly assistance, encouragement, persistence, and friendship. Her support and belief in the work never wavered and was crucial for many reasons. Further, I extend my appreciation to the following: My dissertation committee, Dr. Geza Bruckner, Dr. Bernhard Hennig, and Dr. Sandra Bastin, for their analytical guidance, encouragement, and ongoing support; Dr. Melissa Newman, for her valuable insights and willingness to serve as external committee member; Dr. Howard Glauert, Director of Graduate Studies, for his steady influence and friendship; and Dr. Lisa Cassis, Chair of the Graduate Center for Nutritional Sciences, for her efforts to establish and support a program of scientific rigor within the UK College of Medicine.

Sincere thanks are offered to Limin Feng, PhD candidate in the UK Department of Statistics, for her tireless efforts in the statistical modeling and analysis of the dissertation, as well as for her enduring kindness and patience. Thanks are also offered to Dr. Arnold Stromberg, Chair of the UK Department of Statistics, without whose statistical expertise portions of this work would not have been accomplished.

I thank my husband, Darryl, who has loved me sufficiently to allow me to pursue my life's endeavors. I thank my sons, Nathan, Elliott, and David, who have indulged my proclivity for work. A silent prayer of thanks I send with the beat of my heart to my mother and my father, who may know that it is accomplished, but who definitely share a portion. And I thank my grandmother, Mam, who showed great wisdom and was correct when she said, "Eat your vegetables".

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CHAPTER ONE

INTRODUCTION

1.1 Overview

The past thirty years have seen a dramatic and rapid increase in the prevalence of overweight and obesity. Data indicate that two-thirds of Americans are currently either overweight or obese (Ogden, et al. 2010). Both conditions are associated with the metabolic syndrome, which is characterized by a clustering of cardiometabolic risk factors that predict Type 2 diabetes mellitus (T2DM), cardiovascular disease (CVD), and mortality (Li, et al. 2006). In addition to body mass index (BMI), associated risk factors include advancing age, a sedentary lifestyle, and the excess calorie consumption so easily attained in today's environment of highly palatable, relatively inexpensive, energy-dense foods. Some environmental contaminants have been implicated in the pathogenesis of metabolic syndrome as well and are thought to interfere with normal functioning of the endocrine system (Lee DH, et al. 2011b, Ruzzin, et al. 2010, Lee DH, et al. 2007b).

One group of these contaminants is polychlorinated biphenyls (PCBs), a family of 209 manufactured compounds known for their persistence, lipophilicity, and damage to health (Safe, 1993). Although production was banned in the late 1970s, they have persisted in the environment and are ubiquitous. The primary vehicle of exposure today is through food, primarily marine, mammal, and dairy foods possessing a lipid compartment (Longnecker, 2001). This suggests analogous exposures for many, if not most, people. Importantly, food is also the primary and preferred vehicle for nutrition. Nutrition plays an important role in the prevention and treatment of metabolic syndrome, T2DM, and CVD, and has been shown to modulate the toxicity of PCBs (Ford, 2001, Joshipura, et al. 2001, Majkova, et al. 2008, Panagiotakos, et al. 2007). There is scant evidence to date on what foods and nutrients are associated with maintenance of health in the presence of PCB exposure. The studies presented in this dissertation reveal associations between specific nutrients known to be abundant in fruits and

vegetables, serum concentrations of PCBs, and the probability of developing metabolic syndrome. It is hoped this will contribute to a greater understanding which nutritional and overall food patterns may preserve the maintenance of health in a toxic world.

1.2 Metabolic Syndrome

In 1988, Dr. Gerald Reaven delivered the Banting Lecture, at which time he posited the theory of a pathogenic syndrome comprised of individual, interrelated risk factors leading to a heightened incidence of CVD and T2DM (Reaven, 1988). This "syndrome x" included specific cardiovascular disease risk factors with underlying, persistent insulin resistance. While hyperglycemia and central obesity were prevalent, patients did not always present with these symptoms. The National Cholesterol Education Program (NCEP) Guidelines followed in 2001 with proposals that based determination of syndrome x, now referred to as the metabolic syndrome, on common clinical measures, allowing for broad and simple diagnosis of the syndrome (3rd Report NCEP ATPIII). Other organizations have put forth their own criteria for a determination of metabolic syndrome. These include, but are not limited to, the World Health Organization (WHO) and the American Academy of Clinical Endocrinologists (AACE) (Li, et al. 2006). The NCEP Guidelines have been used in this dissertation.

Table 1.1

National Cholesterol Education Program, Adult Treatment Panel III (ATP III) Clinical Identification of the Metabolic Syndrome	
RISK FACTOR	DEFINING LEVEL
Abdominal obesity, given as waist circumference	
Men	>102 cm (>40 inches)
Women	>88 cm (>35 inches)
Triglycerides	≥ 150 mg/dL
HDL cholesterol	
Men	<40 mg/dL
Women	<50 mg/dL
Blood pressure	≥ 130/85 mm Hg
Fasting plasma glucose	100-125 mg/dL*
*Early determination of impaired fasting glucose was set at ≥110 mg/dL. The American Diabetes Association lowered the cutpoint to 100 mg/dL, at which point a person is considered to have prediabetes.	

A finding of three or more of the above five criteria constitutes a diagnosis of metabolic syndrome. Triglycerides, HDL-cholesterol, and blood pressure may be considered a positive marker for metabolic syndrome if medication is being taken to correct for levels beyond normal thresholds. In recent years, the definition of metabolic syndrome has been expanded to include a persistent proinflammatory state and prothrombotic state (Grundy SM, et al, 2005), although clinical diagnosis of metabolic syndrome does not include their identification and current therapies for them are limited, often to daily, low-dose aspirin and lifestyle modification.

Identification of metabolic syndrome offers an opportunity to clinicians beyond the first line treatment and prevention of atherosclerotic disease and diabetes. The Scientific Statement of the American Heart Association (AHA) and National Heart, Lung, and Blood Institute (NHLBI) stated in 2005 that the greatest benefit for individuals with MetS was to be found in lifestyle intervention (Grundy SM, et al. 2005). Major lifestyle interventions include (1) weight loss in the overweight and obese, (2) increased physical activity, (3) smoking cessation, if applicable, and (4) modification of an atherogenic diet, i.e. one high in total fat, saturated fat, trans fat, and cholesterol.

A uniform dietary approach has not been accepted for metabolic syndrome as of yet. The primary goal of preventing atherosclerotic disease, however, has led to a broad adoption of the NCEP ATPIII Therapeutic Lifestyle Change (TLC) guidelines for the lowering of LDL-cholesterol, which focuses primarily on macronutrient manipulation.

Table 1.2 ATP III Therapeutic Lifestyle Change (TLC) Dietary Recommendations

NCEP ATP III TLC Dietary Recommendations	
COMPONENT	RECOMMENDATION
Carbohydrate	50-60% of total calories
Protein	Approximately 15% of total calories
Total fat	25-35% of total calories
Polyunsaturated fat	≤ 10% of total calories
Monounsaturated fat	≤ 20% of total calories
Saturated fats	<7% total calories

Table 1.2 (continued)

Dietary cholesterol	Less than 200 mg/day
Dietary fiber	20-30 grams per day
Plant stanols/sterols	2 grams per day

In a March 2000 interview with Nutrition Action Healthletter, Dr. Reaven proposed a Syndrome X diet with a macronutrient distribution of 45% carbohydrate, 15% protein, and 40% fat, almost exclusively from unsaturated sources (Reaven, 2000). He reasoned that both carbohydrate and protein would stimulate insulin secretion and that protein foods in the western diet are often accompanied by saturated fat.

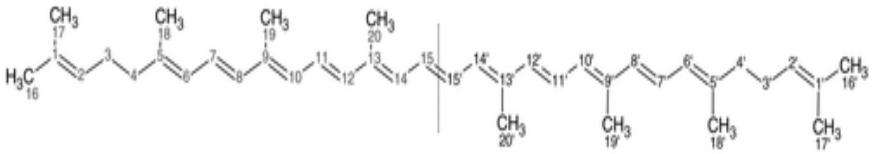
Other diets have looked beyond macronutrient distribution and quality. The Dietary Approaches to Stop Hypertension (DASH) and Mediterranean dietary patterns, both plentiful in plant foods rich in micronutrients – vitamins, minerals, phytochemicals - have shown benefits in reduced incidence of MetS, T2DM, and CVD (Bhupathiraju, et al. 2011, Carter, et al. 2010, Knoops, et al. 2004). One group of phytochemicals, the carotenoids, is a vast group of colorful plant pigments with associated health benefits. The National Academies for the Institute of Medicine has stated that serum carotenoids are the best biological markers for the assessment of fruit and vegetable consumption (DRIs for Vitamin C, Vitamin E, Selenium, & Carotenoids, IOM 2000). Higher blood concentrations of serum carotenoids have been associated with a healthy weight and reduced risk of several chronic diseases. Further, from a behavioral perspective, daily fruit and vegetable consumption at recommended intake levels requires an intentionality that can manifest in healthy behaviors in other areas of the lifestyle (Dietary Guidelines for Americans 2010, 7th Ed.).

1.3 Serum Carotenoids

Food carotenoids are a family of over 600 lipophilic plant pigments considered beneficial in the prevention of disease. They are most readily identified by their conjugated polyene chain, most commonly having eight isoprenoid units, and this

structure informs their physical and chemical characteristics of color, redox potential, and responsiveness to heat, light, and acids (Liaaen-Jensen S, 2004). The carotenoids currently known to be important to health and nutrition in the western diet are alpha-carotene, beta-carotene, alpha-cryptoxanthin, beta-cryptoxanthin, lycopene, lutein, and zeaxanthin.

Figure 1.1 Lycopene

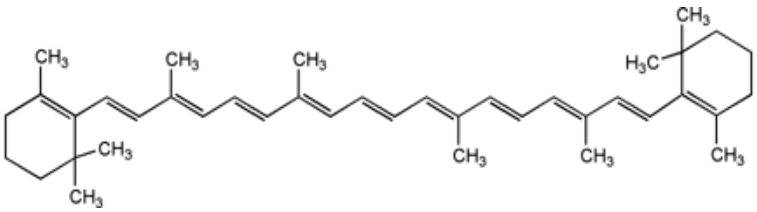


Namitha KK, Negi PS (2010). *Chemistry & Biotechnology of Carotenoids.* Critical Reviews in Food Science & Nutrition 50:8, 728-760.

Carotenoids are lipid-soluble and are absorbed with dietary fat and fat-soluble vitamins. Bioavailability is dependent on several factors, but solubility of carotenoids into mixed micelles and intestinal absorption is better facilitated with a small amount of fat in the meal. Carotenoids become incorporated into plasma lipoprotein particles during transport, and are primarily stored in adipose tissue (Rao, et al. 2007). No dietary reference intakes have been established for carotenoids (DRIs, 2000).

In the early 1900s, it was noted that the yellow color in plant foods was associated with vitamin A activity and subsequently, it was demonstrated that specific carotenoids were converted to colorless vitamin A in rats (Barua, 2004). Less than 10% of the carotenoids found in nature have properties allowing conversion to vitamin A. The predominant provitamin A carotenoids in the western diet include α- and β-carotene, and α- and β-cryptoxanthin. The structural requirement for provitamin A activity is one unsubstituted β-ionone ring attached to a polyene chain with at least five conjugated double bonds. Provitamin A activity is the only known physiologic function of carotenoids at this time. Although carotenoids may not convey essentiality, they do have biological actions that are becoming increasingly clear in the prevention of disease and in optimal biological functioning over the course of a lifetime.

Figure 1.2 Beta-Carotene



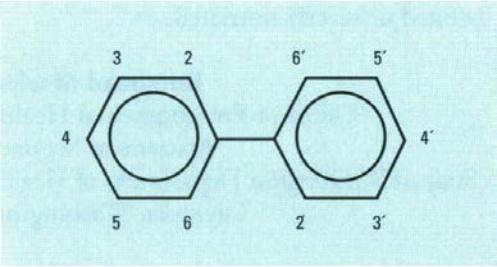
The common non-provitamin A carotenoids, lycopene, lutein, and zeaxanthin, are considered to be potent antioxidants (Di Mascio 1989). More than 80% of dietary lycopene is consumed in the form of processed tomatoes. Epidemiological evidence suggests that lycopene is effective against prostate cancer (Giovannucci, 2005), atherosclerosis (Palozza, et al. 2010), age-related macular degeneration (ARMD) (Sommerburg, et al. 1999), multiple sclerosis, and other diseases. Zeaxanthin and lutein have been associated with prevention of ARMD and cataracts.

Fruits and vegetables are the primary source of carotenoids in the diet. It has not yet been unequivocally established whether it is the consumption of fruits and vegetables in general, the effects of specific nutritional constituents within them, or a synergism between the two, that accounts for their health benefits. Data from epidemiological studies have generally supported a protective effect of fruit and vegetable consumption on lung cancer risk (Ziegler, 1996; Michaud, 2000). Studies attempting to prove this benefit was due to beta-carotene were abruptly halted because of clear evidence showing significant increases in both incidence and death due to lung cancer (ATBC Cancer Prevention Study Group 1994; Omen 1996). In terms of carotenoid supplementation, a pro-oxidant effect has been documented. No such effect has been noted with food-sourced carotenoids. Inverse associations have been observed between baseline dietary intake of fruits and vegetables, serum beta-carotene and subsequent occurrence of lung cancer.

1.4 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are a class of synthetic, organic chemicals that were produced in the United States and other industrialized countries for nearly fifty years, and used widely in a variety of applications. Chemically, PCBs are lipid soluble, aromatic compounds composed of two biphenyl rings with up to ten chlorine substituents. Based on the number and positioning of these chlorine atoms, 209 distinct congener PCBs are possible having the basic formula $C_{12}H_{10-n}Cl_n$, where n = 1-10.

Figure 1.3 Polychlorinated Biphenyl



Wolff M, Camann D, Gammon M, Stellman S (1997). Environmental Health Perspectives; 105(1): 13.

At ambient conditions, individual PCB congeners tend to be colorless, odorless crystals. However, PCBs were distributed commercially as mixtures as early as 1929, in the form of clear, viscous liquids. Aroclor 1254, a Monsanto product, referred to a refined PCB that was 54% chlorine by weight, and included several individual PCB congener classes (Erickson 2001). This structure yielded physicochemical properties that extended well beyond industrial utility to an ongoing, dynamic impact on environmental health (Hopf, et al. 2009; White, et al. 2009; Fischer, et al. 1998).

PCBs are persistent. PCBs have proven highly useful because of their chemical and physical stability. They demonstrate low flammability and high insulating properties, giving them wide commercial utility throughout much of the last century in electrical transformers and capacitors, hydraulic fluids, in paints, printing inks, adhesives and tapes, insulation materials, as a de-dusting agent, and more. It is precisely because of this stability, however, that the higher chlorinated PCBs, in particular, persist in the

environment today. Consumer products produced before 1977 may still contain PCBs, including caulk, microscope oil, fluorescent lighting fixtures, and other electrical devices (ATSDR 2000).

PCBs are ubiquitous. These chemicals are among a select group considered global environmental pollutants. Their hydrophobic nature causes them to adhere primarily to soil, but many congeners readily volatilize and are transported far from their site of origin. PCBs have been measured in remote areas, such as open oceans, deserts, the Arctic and Antarctic, without regard for climate or geography. Although it has been estimated that 99% of PCB mass is found in soil, atmospheric transport is believed to be the primary route of global dispersion. Heavier PCBs may also settle in river and coastal sediment, where they enter the food chain. While human exposures today may occur for several reasons, including improper disposal or storage, seepage from landfill or a closed system accident, the primary route of exposure by far is through food consumption.

PCBs are lipophilic. They can be absorbed through skin, the gastrointestinal tract, or lung. However, most are ingested with food, where they preferentially partition to body fat and reside in adipose tissue indefinitely with steady state equilibrium between adipose and blood. They resist excretion, although the mobilization of adipose tissue by weight loss has resulted in significantly increased serum levels, partitioning of PCBs into other tissues, including remaining adipose at higher concentrations. Serum levels of PCBs have been demonstrated to increase by 388.2% in obese individuals one year following roux-en-Y surgery (Hue, et al. 2006). Further, it has been hypothesized that current obesity rates could be a response to early metabolic programming that favors an adipogenic pathway over one favoring osteogenesis. Studies further suggest the body's response to environmental chemicals may well extend beyond increasing the number of fat cells to possibly affecting appetite regulation, resting metabolic rate, and regulation of fatty acid synthesis (Janesick, et al. 2011b). A favorable biological benefit in the short-term may be that adipose tissue sequesters lipophilic contaminants from

essential organs. Regardless, humans represent the highest trophic level in the food chain and have a vast capacity for fat storage with excess calorie consumption.

One method of classifying PCBs is by whether they exhibit dioxin-like properties. PCBs that possess a minimum of four chlorine atoms in the lateral positions, but do not have chlorine atoms in the ortho positions (2, 2', 6, 6'), are coplanar. This coplanarity restricts the biphenyl rings from rotating. The configuration gives them properties similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), the most toxic dioxin and one by which the toxicity of similar compounds is scored. The coplanar PCB class is the smallest, comprised of only four PCB congeners, but is considered to be the most toxic PCB group because of its high affinity for the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor in the cytosol of vertebrates. The AhR is believed to play important roles in development, aging, hypoxia, and circadian rhythms (White, et al. 2009). Activation of the AhR by exposure to dioxin-like compounds may be expected to disrupt cell signaling processes, expression of important metabolic enzymes, and generally alter patterns of metabolism. Binding of AhR to regulatory regions of specific genes, such as CYP1A1, may lead to increased and/or inappropriate transcription of that gene. Chronic exposures may inhibit the AhR in its role in homeostasis through a persistent activation of this receptor. Importantly, dioxins and dioxin-like compounds induce toxicity in animals and humans.

A second class, the mono-ortho-substituted PCBs, exhibit partial dioxin-like activity. As the name implies, this group has one chlorine atom in the ortho position. There are eight mono-ortho-substituted PCBs. Mono-ortho-substituted PCBs have a weaker affinity for the AhR, and are known to act via non-AhR pathways as well.

A third class, the non-dioxin-like PCBs (di-ortho-substituted PCBs), is the largest group at 197 of 209 congeners (Henry T, et al. 2003). This class varies in toxicity, but its chemical conformation does not allow binding of the AhR, so this biochemical pathway and the damage potentiated by it are not at issue with these chemicals. Research does suggest they cause oxidative stress, an inflammatory response, and may affect gene

expression via other nuclear receptor pathways. Evidence is increasing that they aggravate the dioxin-like compounds and may compound their activity within the body.

Distinguishing between the different PCB classes is important as this affects their mechanism of toxicity. Degree of chlorination is a consideration as the higher chlorinated congeners appear to have greater effects in the body. It is important to note that PCBs enter the environment as mixtures rather than as single congeners. Exposure to both dioxin-like and non-dioxin-like compounds is the more likely occurrence, a fact further complicated by various biodegradation rates or transport of substituents of mixtures in the environment over time, the conversion to hydroxyl or methyl sulfone metabolites in vivo, the route and duration of exposures, as well as individual responses of the host (Giesy, et al. 1998). These all affect exposure-risk relationships and impact efforts to establish specific guidelines for the exposed in the maintenance of their health.

PCBs are toxic to humans and wildlife. Human health effects have been examined following industrial and accidental exposures, but these represent largely acute exposures to high levels of PCBs. Documented effects include dermal changes, i.e. chloracne; elevated hepatic enzymes; dyslipidemia; and carcinogenicity. Although PCBs are not considered directly genotoxic, they are classified as a Group 2A carcinogen (probably carcinogenic) to humans by the IARC (IARC Monographs) and as reasonably anticipated to be carcinogens by the NTP (NTP ROC12). Two year rat gavage studies of PCBs 118, 126, and 153 conducted by the NTP showed clear evidence of cholangeocarcinomas at two years, with hepatocyte hypertrophy noted as early as 14 weeks, and non-neoplastic lesions at 31 weeks. Animal studies have also indicated immune suppression, neurotoxicity, liver cancer, and aberrations in thyroid and reproductive function.

Less is known about the health effects of chronic exposure to background levels of PCBs in the environment, but this is the reality for most people. PCBs have not left the environment. They have been modified in many cases via partial decomposition, incineration, or metabolism, but they still exist in the environment. If humans are the

highest trophic level in the food chain, it must be considered that humans have become one final repository for PCBs, as well as other lipophilic contaminants. And if so, it is plausible that their biological effects are being witnessed in the current rates of obesity and T2DM.

1.5 Endocrine Disruption

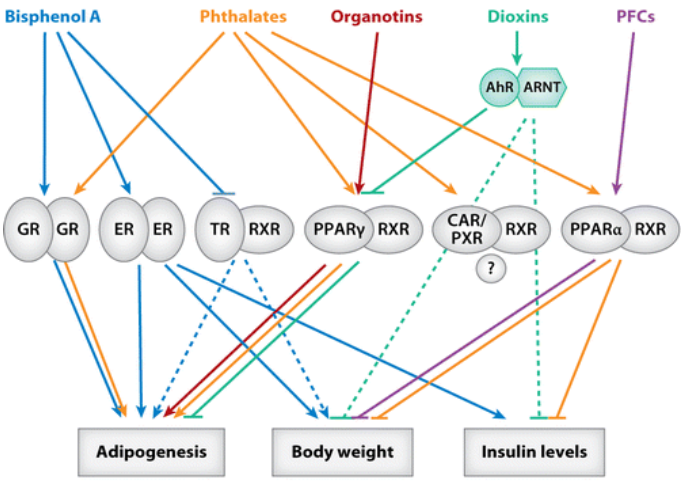
For the past two decades, scientists have been investigating the toxic effects of chemicals that act on the endocrine system. These "endocrine disrupting chemicals" (EDCs) interfere with the natural functioning of hormones and alter their ability to communicate with and respond to their environment. A common feature they share is small molecular mass, usually <1000 Daltons, but they otherwise may not be easily identifiable (Diamanti-Kandarakis, et al. 2009). Many are lipophilic and these will be persistent chemicals, stored in body fat. The persistent EDCs, such as PCBs, have a phenolic group which mimics natural hormones, allowing them to act as either agonists or antagonists. They may persist in body fat as the original compound or as a potentially more toxic metabolite for years.

The sharp increase in obesity rates over the past few decades has coincided with the widespread use of industrial chemicals, some of them EDCs. With increasing rates of obesity come the associated metabolic abnormalities of insulin resistance, hyperinsulinemia, hypertension, and dyslipidemia, commonly referred to as metabolic syndrome, a precondition of T2DM and CVD. Diabetes rates have also risen dramatically over recent decades, with approximately 311.4 million incident cases worldwide of T2DM (WHO Diabetes, 2011). The obesogen hypothesis states that EDCs actually promote obesity directly by increasing the number or size of fat cells, or indirectly by acting on the basal metabolic rate and/or hormonal control of appetite and satiety (Janesick & Blumberg, 2011).

Early understanding of EDC-mediated toxic pathways focused on nuclear receptors, a superfamily of about 48 members that act as master switches for specific genetic programs (Casals-Casas, et al. 2011). Nuclear receptors activated by

endogenous ligands can facilitate gene regulation for such functions as homeostasis, cell differentiation, proliferation, and apoptosis. Once activated, nuclear receptors translocate to the nucleus and bind as dimers to specific response elements near target gene promoters. Inappropriate expression or suppression by EDCs, however, disrupts these processes in ways that are still being understood.

Figure 1.4 Xenobiotic ligands, nuclear receptors, and identified biological endpoints



Casals-Casas C, Desvergne B. 2011.
Annu. Rev. Physiol. 73:135–62

EDCs (top row of figure) interact with nuclear receptors: AhR – aryl hydrocarbon receptor; ARNT – aryl hydrocarbon receptor nuclear translocator; GR – glucocorticoid receptor; ER – estrogen receptors; TR – thyroid hormone receptors; RXR – retinoid X receptor; peroxisome proliferator-activated receptor gamma; constitutive androstane receptor; pregnane X receptor; (dashed lines indicate mechanism not well understood).

Research has broadened the knowledge of EDCs' actions to include membrane and neurotransmitter receptor pathways, enzymatic pathways, as well as other mechanisms. There are important physiological consequences to the effects of EDCs that call for consideration, as outlined in the First Endocrine Society Statement (Diamanti-Kandarakis 2009).

1) The effects of exposure in the womb or shortly after birth may be very different than the effects of exposure as an adult;

2) The consequences of exposure during development may not manifest until later in life;
3) Different classes of EDCs within an individual may have additive or synergistic effects;
4) EDCs may exhibit non-traditional dose response curves, low dose exposures may exert more potent effects than high dose exposures, and this can yield non-traditional curves, such as U- or inverted U-shaped curves as seen with hormones and neurotransmitters; and
5) Later generations may be affected through epigenetic regulation causing either transcription or silencing of certain genes via DNA methylation and histone acetylation.

Diet may be crucial in this milieu. Plant foods provide a natural and plentiful source of ligands for specific nuclear receptors and should not be overlooked as an important factor in modulating endocrine signaling pathways. Phytochemicals, such as carotenoids and flavonoids, are important to health but lack the essentiality of vitamins and minerals. Their natural affinity for nuclear receptors could favorably influence critical pathways (Jeuken, et al. 2003, Fukuda, et al. 2004). Daily exposure to beneficial plant constituents may, through competitive binding or other mechanisms yet revealed, provide a pathway to health with a molecular modality.

1.6 National Health and Nutrition Examination Survey

The National Health and Nutrition Examination Survey (NHANES) is a program of the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC), that utilizes a cross-sectional sample survey design to collect, maintain, and disseminate the health and nutrition information of non-institutionalized, non-military persons residing in the United States. The collection of a broad range of data, including dietary intake of foods and fluids, biomonitoring of environmental pollutants, biochemical laboratory testing, and physical examinations, made the present

work possible. Approximately 5,000 persons are assessed each year. Although the program began in 1960, NHANES began collecting extensive environmental pollutant data in blood and urine in 1999. The 2003-2004 data release was utilized for this dissertation. These data are publicly available on-line (CDC 2003-2004).

1.7 Hypothesis

A diet rich in fruits and vegetables, as determined by serum carotenoid concentrations, mitigates the effects of PCB exposure on cardiometabolic disease, as defined by the reduced probability of developing metabolic syndrome.

1.8 Specific Aims

The aims of this research were to examine the associations between serum concentrations of PCBs; serum concentrations of specific nutrients, carotenoids, vitamins C and E; with related socioeconomic and lifestyle characteristics; on the probability of developing metabolic syndrome. Polychlorinated biphenyls have been studied in animal models and in epidemiological studies, and have been linked to the etiology of cardiovascular disease, insulin resistance, and T2DM. The carotenoids, considered a biomarker of fruit and vegetable consumption and important dietary antioxidants, are associated with the promotion and maintenance of human health. Ecological studies have shown inverse associations between food-sourced carotenoids, and cardiovascular disease and glucose tolerance. Research objectives address the variables more particularly, as each contained several factors requiring it.

1.8.1 Research Objectives: PCBs

1.8.1.1 To assess PCBs individually and as pooled subclasses, defined by the presence of one or more chlorine atoms in the ortho- position, which is adjacent to the biphenyl bridge;

1.8.1.2 To assess PCBs in concentrations and as ranks;

1.8.1.3 To assess PCBs in quartiles, continuous linear, and continuous quadratic;

1.8.1.4 To apply different modeling of covariates to use as surrogates for NHANES sample weights;

1.8.1.5 To analyze data results with and without the first exposure quartile, relating to the persistent first quartile increasing trend in the probability of metabolic syndrome. While this finding was compelling and suggested a biological mechanism, it obfuscated clear statistical analysis of trends in the short-term.

1.8.1.6 To assess PCBs in solitaire and in a combined interaction model with nutrients to consider their overall effect on the probability of MetS;

1.8.1.7 To consider analyzing models of PCBs as ng/g (ppb) whole weight and as lipid weight ng/g (ppb). PCBs concentrate to lipids in blood. Lipid-adjusted values are adjusted for total cholesterol and triglycerides by NHANES. Using of whole weight values would necessitate independently adjusting for total cholesterol and triglycerides. Fasting triglyceride is one criterion of MetS. Evaluating several models may be useful.

1.8.2 Research Objectives: Serum Carotenoids

1.8.2.1 To assess twelve serum carotenoids individually and as a pooled sub-class;

1.8.2.2 To assess serum vitamin C;

1.8.2.3 To assess three serum tocopherols: α, δ, and γ;

1.8.2.4 To assess serum carotenoids, vitamin C, and the tocopherols, as an antioxidant pool;

1.8.2.5 To assess all serum nutrients combined;

1.8.2.6 To assess dietary recall-sourced nutrients, i.e. carotenoids, potassium, magnesium, calcium, fiber, monounsaturated fat, polyunsaturated fat, vitamin K, and selenium;

1.8.2.7 To assess dietary recall-sourced fruit and vegetable intakes using NHANES-U.S.D.A. interfaced databases, i.e. MyPyramid Equivalents Database 2.0 and Food and Nutrient Database for Dietary Studies 2.0;

1.8.2.8 To assess nutrients singly and in a combined interaction model with PCBs to consider their overall effect on the probability of metabolic syndrome.

1.9 Research Questions

1.9.1 What was the prevalence of metabolic syndrome in this population?

1.9.2 What PCB congeners, subclasses, or mixtures were associated with metabolic syndrome in this population?

1.9.3 Were serum carotenoids, singly or pooled, associated with risk reduction of metabolic syndrome in a PCB exposed population?

1.9.4 Were serum nutrients, other than carotenoids, associated with risk reduction of metabolic syndrome in a PCB exposed population?

1.9.5 Were fruit and vegetable servings, as compiled from the sum of two 24-hour dietary recalls, associated with risk reduction of metabolic syndrome in a PCB exposed population?

1.9.6 Were specific nutrients, as compiled from the sum of two 24-hour dietary recalls, associated with risk reduction of metabolic syndrome in a PCB exposed population?

1.10 Justification

Cardiovascular disease is the leading cause of death in the United States and Kentucky. Diabetes mellitus ranks as the seventh leading cause of death according to the CDC's preliminary data release, 2009 National Vital Statistics Reports, (Kochanek, et al. 2011). Obesity rates have dramatically risen the past two to three decades, with 33.8% U.S. adults now considered obese. In 2010, Kentucky's obesity rates were documented at 31.3% (CDC, *Overweight & obesity*). Obesity, T2DM, and CVD are closely related. Metabolic syndrome is a pre-diabetic state that is easily diagnosable in a clinical setting.

A diagnosis of metabolic syndrome places an individual at five times the relative risk of T2DM and two times the risk of CVD (Wilson, et al. 2005). Lifestyle intervention has been shown to reduce the incidence of diabetes by 58% (DPPRG 2002).

Cross-sectional survey analyses of U.S. adult populations have revealed positive associations between persistent organic pollutants (POPs), including dioxin-like and non-dioxin-like PCBs, with T2DM (Patel, et al. 2010; Lee, et al. 2006; Lee, et al. 2007c), metabolic syndrome (Lee, et al. 2007b; Lim, et al. 2008), insulin resistance (Lee, et al. 2011b; Lee, et al. 2007a), hypertension (Ha, et al. 2009), cardiovascular disease (Ha, et al. 2007), and non-alcoholic fatty liver disease (Cave, et al. 2010). Animal model studies, including University of Kentucky Superfund Research Program (UK-SRP) bench studies of the aryl-hydrocarbon receptor ligand, PCB 77, have demonstrated disruption of arterial endothelial cell integrity (Hennig, et al. 2005; Hennig, et al. 2001) as well as adipose mass increases of gonadal, visceral, and liver tissues (Arsenescu, et al. 2008).

Analyses of broad population cross-sectional surveys have also indicated that food-sourced serum antioxidants, including carotenoids, are associated with normal glucose tolerance (Ford, et al. 1999), and inversely associated with metabolic syndrome (Suzuki, et al. 2011; Beydoun, et al. 2011) and C-reactive protein (Ford, et al. 2003a).

Importantly, communities are living with the effects of environmental pollutants in the present. While many of these damaging compounds are no longer being manufactured, they persist in the environment and in adipose tissue for decades. Evidence suggests that in utero exposures cause changes to the phenotype that may well be transgenerational and lead to the unwelcome progression of obesity, metabolic syndrome, T2DM, and CVD. If research can be undertaken to more clearly understand the environmental pollutant, nutrition, and disease/health paradigm, then it most certainly is justified.

1.11 Assumptions

For quantitative measurements, the sample was representative of the United States non-institutionalized, non-military, adult population according to the Census for

the years 2003-2004. That all procedures, quality control measures, questionnaires, and indices were executed correctly and by trained and certified professionals. The equipment was calibrated and tested accurately and routinely. That all participants responded honestly, and that all data were collected, reported, maintained, and disseminated in an ethical and responsible manner.

1.12 *Limitations*

The findings in this study must be interpreted with caution because of the cross-sectional nature of the study. It was not possible to determine cause and effect or to ascribe temporal characteristics to any part of the dataset. It would be possible, for instance, for the metabolic disturbances of disease to create differences in nutrient or pollutant concentrations.

Secondly, the relationships involving PCBs are complex. Most could not be interpreted by seeking a monotonic dose response curve and small p-value. Many of the PCBs showed a low dose effect that was further complicated by lack of a true reference group. Our statistical reference group would be provided by observations below the 60% limit of detection (LOD). However, there were no observations for many PCBs below the LOD. A chemical has a 95% probability of being greater than zero at the LOD and values above the LOD (from 60-100%) may be evaluated across quartiles. The 2003-2004 dataset had many PCBs with zero observations below the LOD, which confounded efforts to execute a standard multivariate regression adjusted odds-ratio analysis. The decision to seek a statistical interaction between PCBs and serum carotenoids influenced further attempts to analyze using multivariate regression. Rather, seeking consistency across several models and across similar studies was useful. We sought interactions between PCBs and serum carotenoids early and significant findings were meaningful and inferred significance for PCBs had carotenoids not been present.

A third limitation may have been due to the presence of pollutant mixtures in study participants rather than just PCBs. Also, analyzing the effect of participants'

overall dietary intakes on health outcomes was beyond the scope of this dissertation. Health outcomes could be related to exposure to chemical mixtures beyond PCBs, dietary patterns beyond fruit and vegetable intakes, or genotypes susceptible to T2DM and/or CVD. Any of these factors may have contributed to these results.

A fourth limitation also holds promise in future research. By seeking significant interactions between serum carotenoids and PCBs, multivariate regression analysis modeling of factors that may independently be associated with the three variables – PCBs, carotenoids, and metabolic syndrome – could not accommodated in the usual manner. Advancing age is known to have a strong independent association with both metabolic syndrome and PCBs. There is no age-related association with carotenoids. Analysis using age as a cut-point may be executed moving forward. Statistical and nutritional analyses are continuing in a prudent manner.

CHAPTER TWO

REVIEW OF LITERATURE

Metabolic Syndrome has been examined in relation to diet and nutrition. Diet and nutrition have been examined in relation to environmental chemical contamination. Environmental chemical contamination has been examination in relation to metabolic syndrome. The purpose of this chapter is to provide a foundation upon which to examine all three variables combined: the effects of nutrition, as assessed by serum carotenoid concentrations, in the presence of environmental chemical contamination, on the probability of developing metabolic syndrome.

2.1 *Metabolic Syndrome*

The NCEP Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Third Report, NCEP), has set metabolic syndrome as a secondary target of coronary heart disease risk reduction beyond the primary target of lowering of LDL-cholesterol. Stated goals of the clinical management of metabolic syndrome are to reduce the risk of atherosclerotic disease and risk for T2DM, where the latter has not yet manifested (Grundy, et al. 2005). Metabolic syndrome has been shown in prospective studies to increase the risk of CVD and T2DM. At 8-year follow-up of the Framingham Heart Study Offspring cohort, grouping of participants by metabolic syndrome trait clustering found that when impaired fasting glucose (IFG) was not one of three criteria, they were seen to have two times the relative risk (RR) of CVD events and five times the RR of T2DM as people without that trait grouping. Participants meeting the criteria for metabolic syndrome with IFG as one of the three traits plus any other two were seen to have a RR of 2.5 for CVD and a RR of 11.0 for T2DM (Wilson, et al. 2005). Giving clinicians the tools to identify the patients at significant risk does not answer the more prescient question of why there has been such a dramatic increase of this syndrome over the past three decades and what can be done to attenuate it.

The environmental factors involved in the etiology of metabolic syndrome include diet, sedentary lifestyle, and obesity (Miegs, 2002; Millen, et al. 2006). These collectively may be referenced as a state of energy imbalance and are characteristic of the modern lifestyle. The metabolic syndrome also has a genetic basis, as may be seen with most disease processes. The Third National Health and Nutrition Examination Survey (NHANES) 1988-1994 found that among 8814 participants, there was a 21.8% prevalence of metabolic syndrome unadjusted, with age-adjusted prevalence at 23.7% (Ford, et al., 2002). Among race/ethnicity, Mexican Americans had the highest prevalence at 31.9%. When compared to rates of metabolic syndrome in the subsequent NHANES release, 1999-2000 (n = 1,677), age-adjusted increases were observed of 23.5% in women and 2.2% in men, yielding overall metabolic prevalence of 27.0%. Importantly, obesity was an important determinant in both studies. Analysis of NHANES 2003-2006 data reveal 34% of adults met the criteria for metabolic syndrome (Ervin, 2009). As rates of obesity continue to climb, the incidence of metabolic syndrome, T2DM, and CVD, may be expected to follow.

Age remains a significant independent factor in the probability of metabolic syndrome. The prevalence of persons meeting the criteria of metabolic syndrome among NHANES 2003-2006 participants was found to be three times more likely in the 40-59 age range than in the 20-39 age range. Among those sixty years and older, men were over four times and women over six times more likely to have metabolic syndrome than the 20-39 age group (Ervin, 2009). The Framingham Offspring Study (FOS) cohort found an age-adjusted increase in the prevalence of metabolic syndrome at eight year follow-up of 56% among men (baseline mean age of 50) and 47% among women (baseline mean age of 51) (Wilson, et al. 2005). Analysis of NHANES III (1988-1994) participants found the prevalence increased from 6.7% through the 20's to 43.5% and 42.0% for participants in their sixties and ≥ 70, respectively (Ford, et al. 2002).

Therapeutic lifestyle changes remain at the forefront of treatment and prevention. An ancillary twelve year analysis of the FOS cohort diets and metabolic syndrome indicated that a lower diet quality was positively associated with increased

incident metabolic syndrome. The lower quality diet was higher in total fats, alcohol, and lower in total carbohydrate, fiber, and all micronutrients. The higher diet quality scores were more compliant with the NCEP ATP III dietary guidelines for total fat, cholesterol, carbohydrate, and fiber (Millen, 2006). A five year review of carbohydrate-associated constituents found lower insulin resistance with diets high in whole grains, and fruit and vegetable fibers, although only cereal fiber was found to be inversely associated with prevalence of metabolic syndrome. Fruit, vegetable, and legume fibers were not inversely related (McKeown, et al. 2004).

Obesity is considered a proinflammatory state (Krug, 2005; Lumeng, 2111). Inflammatory mechanisms in the body appear to interfere with insulin signal transduction causing insulin resistance, increases in free fatty acid concentrations, and further promoting oxidative stress and metabolic syndrome (Dandona, et al. 2005). Analysis of the dietary constituents that have a pro-oxidant effect on the body has implicated excessive macronutrient intake. Healthy, fasted individuals were randomized to one of three groups, then given a challenge of glucose, cream, or casein, and subsequently assayed at baseline, one, two, and three hours. Reactive oxygen species (ROS) were generated at significant levels in all three groups (Mohanty 2002; Mohanty 2000). A later study demonstrated similar results with a mixed macronutrient fast food meal. Nine non-diabetic, fasted individuals were given a 910 kcal egg/sausage/potato/muffin breakfast to consume within fifteen minutes and blood drawn at baseline, one, two, and three hours. As in earlier studies, the controls consumed only water. Significant increases were seen in intranuclear NF-kB binding activity at one and two hours, and in cellular $p47^{phox}$, the key protein component of NADPH oxidase involved in ROS generation. $p47^{phox}$ increases were seen within an hour of the meal and lasting for three or more hours, or essentially until the next meal for many people. Importantly, NF-kB is thought to bind the p65:p50 heterodimer that is responsible for transcription of proinflammatory genes (Aljada, 2004).

Examination of the associations between habitual dietary patterns and metabolic syndrome found stronger associations in women between metabolic

syndrome and a pattern replete with empty calorie foods and beverages, i.e. higher intakes of total fat, calories, and sugar, and low in fiber and vegetables (Sonnenberg, et al. 2005). This was true for obese and non-obese participants. Similarly, in a typical western dietary pattern (WDP) dominated by refined grains, french fries, cheese dishes, red and processed meats, and sweetened desserts, beverages, and snacks, stronger associations with metabolic syndrome were seen in 19-39 year olds than in participants consuming a prudent diet comprised more of fruits, vegetables, broth-based soups, salads, and poultry (Deshmukh-Taskar, et al. 2009). A Greek population consuming a Mediterranean dietary pattern rich in cereals, fish, legumes, fruits, and vegetables was found to have a 13% lower likelihood of having metabolic syndrome (Panagiotakos, et al., 2007), and in older participants at high cardiovascular risk (PREDIMED Study), quartile analysis of adherence to Mediterranean diet components was associated with a 56% lower risk of having metabolic syndrome than were those at lower quartile adherence (Babio, et al. 2009). Importantly, where weight loss may not be achievable or advisable, modifications to the nutrient profile of the diet may be sufficient.

Micronutrient modulation of the diet could present significant opportunities for the prevention of MetS and its complications. The Dietary Approaches to Stop Hypertension (DASH) diet, which emphasizes fruits, vegetables, whole grains, low fat dairy, and lean protein, has been effective in reducing hypertension, one marker of metabolic syndrome (U.S.D.H.H.S. 2006). A randomized controlled trial placing 81 individuals with metabolic syndrome on a calorie controlled DASH diet found that those further supplemented with vegetable juice lost significantly more weight, had significant decreases in serum leptin, and increased intakes of vitamin C, potassium, and vegetables, although neither systolic nor diastolic blood pressure were statistically significantly changed over the twelve week intervention. The only metabolic syndrome marker statistically affected was elevated triglyceride, with the juice-supplemented group found 10% less likely to have elevated triglycerides than those not consuming vegetable juice (40%) (Shenoy, et al., 2010). Notably, all participants were on the DASH diet. No analysis was included for diets other than DASH.

Studies utilizing cross-sectional surveys of antioxidant associations and metabolic syndrome found benefits of higher antioxidant status. Waist circumference, hypertriglyceridemia, and low HDL were most associated with antioxidant status in NHANES III, 1988-1994, (Ford, 2003b). Participants meeting all five criteria of metabolic syndrome had 40% lower vitamin C concentrations, 27% lower total carotenoid concentrations, and consumed fewer fruits and vegetables than participants not meeting the criteria for metabolic syndrome. Analysis of NHANES 2001-2006 data found a significant inverse relationship between beta-carotene and vitamin C for men and women, beta-cryptoxanthin for men, and lutein/zeaxanthin for women with metabolic syndrome. Prevalence of metabolic syndrome for these six years was found to be 32% in men and 29.5% in women (Beydoun, et al. 2011). A cross-sectional Japanese study found significantly lower levels of beta-carotene, but higher levels of beta-cryptoxanthin (significant only in women) in the metabolic syndrome group (Suzuki, et al. 2011). The criteria for metabolic syndrome differ in Japan, with waist circumference set at ≥ 85 cm for men and ≥ 90 cm for women, SBP ≥ 130 mm Hg, FPG ≥ 110, and HDL ≤ 40 for both genders. Prevalence of metabolic syndrome was 22.3% in men and 7.5% in women in this study.

Analysis of NHANES III data revealed differences in serum carotenoid status between participants with normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and newly diagnosed diabetes (Ford, et al. 1999). Lycopene and beta-carotene were both inversely related to glucose tolerance. In the newly diagnosed diabetes participants, the mean of serum cryptoxanthin was 23% lower than in normal glucose tolerance participants. All carotenoids were significantly inversely related to fasting serum insulin concentrations.

2.2 *Carotenoids and Related Antioxidants*

Oxidative damage is a key factor in disease processes, whether secondarily or causally. The balance between oxidants and antioxidants, altered in favor of oxidants in the disease state, may be favorably modulated by an antioxidant-rich diet. Carotenoids

are potent antioxidants and singlet oxygen quenchers and are known to be involved in many different cellular pathways. Individual carotenoids and fruit and vegetable consumption in general, are associated with an enhanced antioxidant capacity and the ability to inhibit disease processes, including features of metabolic syndrome, cardiovascular disease, or carcinogenesis (Hermsdorff, et al. 2010; Krinsky & Johnson 2005).

2.2.1 *Cancer*

Epidemiological studies have shown that lower levels of antioxidants, in particular vitamins C, E, carotenoids, and polyphenols, to be associated with a higher prevalence of cardiovascular diseases, cancer, and metabolic syndrome. Importantly, these studies considered antioxidants from fruits and vegetables.

An ecological study among 634 Japanese men found inverse associations for plasma levels of specific carotenoids, alpha-tocopherol, and vitamin C with risk of gastric cancer (Tsubono, et al. 1999). A two-year Swiss study measured plasma levels of Vitamins C, E, and carotene at baseline in healthy men and women. Review of mortality records at seventeen years revealed that 290 of 814 men had died of cancer. Cancer mortality was associated with low carotene and vitamin C levels at baseline (Eichholzer 1996). During a 25-year follow-up of a Finnish study that evaluated associations between fruit and vegetable antioxidant intake and lung cancer, significant inverse relationships were observed with alpha-carotene, fruits, and root vegetables (Knekt 1999). Similar associations were seen with lung cancer risk for dietary alpha- and beta-carotenes and lutein, with the strongest protection conferred by consumption of a variety of vegetables rather than one particular carotenoid-concentrated food (Le Marchand 1993). Pooled analyses of food carotenoids and risk of lung cancer in two U.S. cohorts found significant inverse associations between lung cancer risk and alpha-carotene and lycopene (Michaud 2000).

The two large randomized control studies supplementing the diet of smokers with beta-carotene and either alpha-tocopherol (ATBC study) or retinol (CARET)

observed an increased incidence of and mortality to lung cancer (Albanes, et al. 1995; Omenn, et al. 1996). ATBC was terminated on schedule at a median duration of 6.1 years, with an 18% statistically significant increase in lung cancer incidence noted in the β-carotene group at term. CARET was terminated 21 months early in light of a 28% increase in the number of lung cancers and a 17% increase in deaths among the intervention group only. In the ATBC study, subsequent analysis of participant diets found total fruit and vegetable intakes significantly associated with reduced risk of lung cancer. In particular, dietary lycopene, lutein/zeaxanthin, beta-cryptoxanthin, and total carotenoids, were associated with a 15-28% lower lung cancer risk (Holick, et al., 2002). A twelve year follow-up of CARET participants found a 44% lower lung cancer risk only among placebo participants consuming eleven servings of fruit per week, as opposed to those consuming only two servings per week. No statistically significant decrease in risk was seen in the intervention group, even with high fruit intake (Neuhouser, et al., 2003).

2.2.2 *Cardiovascular Disease*

A diet rich in fruits and vegetables has long been associated with cardiovascular health. Epidemiological studies have suggested that persons who consume more fruits and vegetables are more likely to maintain a healthy weight and have lower rates of cardiac and vascular diseases. Pooled analyses of two U.S. cohort studies with similar designs found for the highest quintiles of fruit and vegetable intake a 0.80 relative risk for coronary heart disease (CHD) and 0.69 relative risk of ischemic stroke, as compared to the lower quintiles of intake (Joshipura, 2001; Joshipura, 1999). Median intakes of total fruits and vegetables were 5.8 servings per day for women and 5.1 servings for men; however, 1 additional serving per day was associated with a 6% lower risk of ischemic stroke. The fruits and vegetables associated with lowest risks for CHD and ischemic stroke were green leafy vegetables, cruciferous vegetables, and vitamin C-rich fruits and vegetables. However, no inverse association was seen for legumes and potatoes. Neither was an inverse association seen for citrus juice, but only with CHD.

A cohort follow-up study from the NHANES I (1971-1975), examined 9608 participants for fruit and vegetable consumption and risk of cardiovascular disease, and found that fruit and vegetable intake ≥ 3 times/day as compared to < 1 time/day was associated with:

Table 2.1 Fruit and vegetable intake and CVD risk reduction in NHANES I follow-up cohort

27% lower incidence of stroke
42% lower mortality from stroke
24% lower mortality from ischemic heart disease
27% lower mortality from CVD
15% lower mortality from all causes

These data were collected by food frequency questionnaire in 1982-1984, 1986, 1987, and 1992, and were adjusted for diabetes, as well as significant demographic and lifestyle covariates (Bazzano, et al., 2002). Cross-sectional analysis of NHANES III data to determine associations between serum vitamins, carotenoids and angina pectoris found that after adjusting for multiple CVD-related risk factors, none of the serum vitamins (A, C, E, B12, serum folate, and red blood cell folate) suggested any predictive trend for angina pectoris. In contrast, all of the serum carotenoids showed significant inverse associations with angina pectoris (Ford, 2000). The highest quartiles of α-carotene, β-carotene, and β-cryptoxanthin were associated with a reduced risk of angina pectoris of 55%, 43%, and 43%, respectively, as compared to the lowest quartiles.

Reviews of carotenoids have sought to identify one or more single nutrient involved in protection against cardiovascular disease. In an elderly Dutch cohort, a significant reduced risk of myocardial infarction (MI) was seen in the highest tertile of beta-carotene intake as compared to the lowest (RR = 0.55), although no association was seen for food sourced vitamins C and E (Klipstein-Grobusch, et al., 1999). Similarly, reduction in the risk of acute myocardial infarction (AMI) was noted in the fourth intake quartile for α-carotene (OR=0.71), β-carotene (OR=0.71), and β-cryptoxanthin (OR=0.64) in a case-control study of an Italian population (Tavani, et al., 2006), suggesting a weak

inverse association with risk of nonfatal AMI. In a nested case-control study of healthy physicians evaluated for risk of ischemic stroke, participants with baseline plasma levels of α-carotene, β-carotene, and lycopene in the second through fifth quintiles tended to have lower risks of ischemic stroke throughout the study duration (mean 7.3 years). For β-carotene, a 43% lower risk of ischemic stroke was seen (Hak, et al. 2004). Conversely, no similar associations were seen in risk of ischemic stroke when levels of α-carotene, β-carotene, and lycopene were measured by food frequency questionnaires (Ascherio, et al. 1999), perhaps related to the subjective nature of data collection.

Lycopene has been studied for its ability to protect against atherosclerosis. Found predominantly in tomatoes, its properties confer a high bioavailability, heat resistant antioxidant activity, and the most powerful singlet oxygen quenching ability of the common plant carotenoids (Di Mascio, et al. 1989). Serum lycopene levels have been reported to be inversely related to intimal wall thickness and lesions in the carotid artery and aorta (Rissanen, et al. 2003), and may even limit oxidative stress caused by cigarette smoke by modulating molecular pathways (Palozza, et al. 2005). Other studies have indicated that lycopene may reduce macrophage foam cell formation (Napolitano, et al. 2007) by decreasing lipid synthesis in response to modified LDL, and to also inhibit 7-ketocholesterol-induced (7-KC) ROS production, as well as reduced levels of hsp70, hsp90, and oxidative DNA damage (Palozza, et al. 2010a). Importantly, 7-KC is an oxysterol found in high concentrations in atherosclerotic plaque. An earlier study suggested that lycopene activity may be dependent on the type of lipoprotein carrier (Moore, et al. 2004), citing enhanced lipid accumulation in macrophages when carried in chylomicron remnants, inhibition of oxidation when carried in LDL, and down-regulated expression of SR-A, a scavenger receptor known to endocytose modified LDL. Further, decreased expression of IL-10, an anti-inflammatory cytokine, was observed by macrophages treated with lycopene by up to 75%, whether LDL was present or not (Napolitano, et al. 2007). With its long polyene chain, lycopene is known to be an important reducing agent and has been shown to inhibit ROS production and pro-inflammatory cytokine secretion (Palozza, et al. 2010b).

Tomato extract components were shown to inhibit platelet aggregation in a time-course study of healthy humans (O'Kennedy, et al. 2006). A significant decrease in platelet activity was seen three hours after extract consumption, suggesting reduced risk of thrombotic and related proinflammatory events. Adipose tissue biopsies were analyzed in a case-control study (EURAMIC Study) for concentrations of α-carotene, β-carotene, and lycopene shortly after myocardial infarction (Kohlmeier, et al. 1997). While each carotenoid appeared to be protective when assessed individually, once pooled and adjusted for significant demographic and lifestyle covariates, only lycopene was found to be protective at significant levels with an adjusted odd-ratio of 0.57 (10^{th} to 90^{th} percentile). A Japanese population cohort study examined associations between serum levels of α-carotene, β-carotene, and lycopene, and cardiovascular disease mortality over 11.9 years (Ito, et al., 2006). Mortality records revealed significant inverse relationships between CVD and stroke for α-carotene, β-carotene, and total carotene; and for CHD and α-carotene and total carotene. Lycopene showed protective effects for CVD at significance ($p = 0.032$) in modeling adjusted for gender, age, and smoking.

A diet study of 412 U.S. adults with an average SBP of 120-159 mm Hg and DBP of 80-95 mm Hg randomized participants to either a typical western diet or the DASH diet for thirty days. The Dietary Approaches to Stop Hypertension (DASH) diet emphasizes plentiful fruits and vegetables, but also low fat dairy and protein. Further randomization occurred within the two diets providing low, medium, and high sodium intakes. The DASH diet lowered blood pressure at all sodium intake levels, but it had a more pronounced effect on both systolic and diastolic blood pressure at the high sodium levels ($p < 0.001$ for interaction) than it did at low levels (Sacks, et al., 2001). Adherence to a Mediterranean dietary plan, also abundant in fruits, vegetables, and legumes, was assessed in relation to overall mortality during a 44 month follow-up period (EPIC Study). Greater adherence to the diet was associated with reduced mortality to CHD with an adjusted hazard ratio of 0.67, although individual constituents within the diet were not found to be significant (Trichopoulou, et al., 2003). The

Mediterranean-Diet Scale scored beneficial foods consumed below the median at "0"; those consumed above median were scored "1". Detrimental foods were scored in an inverse order. A mere two point improvement in the overall score was associated with a 25% reduction in total mortality ($p < 0.001$).

2.2.3 *Metabolic Disease*

A prospective study of first and second degree relatives of type 2 diabetics were assessed for dietary and plasma antioxidant intake status, and glucose tolerance (Botnia Dietary Study). Plasma β-carotene concentrations were inversely correlated with insulin resistance ($p=0.003$) and BMI; in men only, total dietary carotenoids were inversely associated with fasting plasma glucose concentrations ($p <0.05$) (Ylönen, et al., 2003). Importantly, this population could be considered at higher risk of T2DM than the general population. A cohort 23-year follow-up study (Finnish Mobile Clinic Health Examination Survey) to assess dietary antioxidant intake and T2DM risk found strong inverse associations with β-cryptoxanthin, total vitamin E, α- and γ-tocopherols, and β-tocotrienol and risk of T2DM. The p-trend for the RR of T2DM and dietary total carotenoid intake was 0.07 (Montonen, et al., 2004). A longitudinal study of serum carotenoids, fat-soluble vitamins, and women with T1DM and preeclampsia, revealed lower α- and β-carotenes and vitamin D in women prior to developing preeclampsia (Azar 2011). T1DM occurs in response to the autoimmune destruction of pancreatic beta cells, but optimal antioxidant status may be expected to prevent or modulate comorbid conditions.

Analysis of the NHANES III cohort for associations between serum carotenoids and glucose tolerance found inverse relationships for all carotenoids and fasting serum insulin after adjustment for several cofounders (Ford, et al. 1999). Participants with IGT and newly diagnosed diabetes had mean β-carotene levels 13% and 20% lower, respectively, than participants with NGT. Mean serum lycopene levels were 6% and 17% lower in participants with IGT and newly diagnosed diabetes. Cryptoxanthin was about 23% lower in those with newly diagnosed diabetes as compared to those with NGT.

Additional analysis of the same survey population for metabolic syndrome found an age-adjusted prevalence of 23.7% MetS with lower concentrations of vitamin C, α- and β-carotenes, β-cryptoxanthin, and lutein/zeaxanthin (Ford, et al., 2003b). A NHANES 2001-2006 population was found to have a higher prevalence of 32% (men) and 29.5% (women) metabolic syndrome, but lower total carotenoid and vitamin C concentrations than those without metabolic syndrome (Beydoun, et al., 2011).

Several other population-based, cross-sectional studies have assessed the impact of antioxidants and carotenoids on the prevalence of metabolic syndrome. The mean dietary carotenoid intake of 440 middle-aged and elderly men was assessed at ~10 mg/day and their prevalence of metabolic syndrome at 22%. A significant inverse association was noted between higher carotenoid intake and metabolic syndrome, primarily lycopene and β-carotene. Among individual metabolic syndrome markers, higher lycopene was associated with lower triglyceride concentrations (p-trend = 0.04) and higher β-carotene was associated with smaller waist circumference (p-trend = 0.01) (Sluijs, et al., 2009). A cross-sectional Australian study found an overall prevalence of 24% metabolic syndrome, although men consuming four or more servings of vegetables per day were less like to have metabolic syndrome than those consuming one or less serving. Significantly lower concentrations of α-carotene, β-carotene, and total carotenoids were seen in participants with metabolic syndrome. As the number of individual metabolic syndrome components increased, serum carotenoid concentrations were observed to decrease (Coyne, et al. 2009). Significant gender differences were seen in a Japanese cross-sectional study of metabolic syndrome, with men presenting at 22.3% and women at 7.5% (Suzuki, et al., 2011). Serum β-carotene was significantly and inversely related to MetS in both genders, as was β-cryptoxanthin in women. Significant associations were observed between individual metabolic syndrome features and individual serum carotenoids.

In a NHANES III follow-up study, serum α-carotene was inversely associated with all-cause mortality, both adjusted and non-adjusted (Li, et al., 2010). This association was independent of demographic and lifestyle characteristics, and relevant health risk

factors. In vivo studies of α-carotene have shown it to be more effective than β-carotene in inhibiting tumor cell proliferation. The current study showed significant inverse association for α-carotene and death from all causes. Notably, α-carotene is not routinely found in supplements, but would be plentiful in yellow-orange and dark-green leafy and cruciferous vegetables. 13,293 NHANES III participants were examined for associations between serum carotenoid concentrations and all-cause mortality, using mortality records through 2006. Total carotenoids, α-carotene, and lycopene were inversely, significantly associated with all-cause mortality (Shardell, et al., 2011). Alpha-carotene was inversely, significantly associated with CVD mortality. High β-carotene concentration was not associated with reduced mortality from any cause. Neither were any carotenoids associated with reduced mortality from cancer.

2.2.4 *General*

A separate analysis of NHANES III participants examined relationships between carotenoids, vitamins, selenium, and inflammation (Ford, et al., 2003a). Age-adjusted concentrations of retinol, retinyl esters, vitamin C, serum folate, the carotenoids, and selenium were inversely related to C-reactive protein concentrations ($p < 0.001$). Analysis of 4,557 nonsmoking NHANES III participants yielded a 30% subsample with detectable C-reactive protein levels above 0.21 mg/dL (Kritchevsky, et al., 2000). All five serum carotenoid concentrations were statistically significantly lower in the 30% than in those without detectable C-RP levels. Beta-carotene and α-carotene were about 20% lower in the highest C-RP category. Neither fibrinogen nor white cell count was associated with overall carotenoid concentrations with similar results. Similar results were seen in the Nun's Study, where 11.5% (10 of 85 nuns) had detectable C-RP, defined as above 1.5 mg/dL (Boosalis, et al. 1996). While no significant relationship was seen between lutein/zeaxanthin or cryptoxanthin and C-RP, the remaining carotenoids individually and all carotenoids pooled were significantly, inversely associated.

Lycopene was found to be protective to the male reproductive system of healthy Sprague-Dawley rats when administered in combination with the PCB mixture, Aroclor

1254 (Ateşşahin, et al. 2010). PCBs have been shown to be damaging to the reproductive system in various ways, including decreased hormone levels, diminished gland weight and sperm count, decreased sperm motility, and altered gene expression. Significant improvements were observed in sperm and tissue quality and the antioxidant-oxidant balance in the lycopene treated rats.

Lifestyle covariates have been affected by carotenoid concentrations. Smoking was significantly associated with decreased levels of beta-carotene and vitamin C, and physical activity significantly associated with increased levels of the same. Oral contraceptive use was associated with lower beta-carotene only (Pincemail, et al., 2011). NHANES III participants were analyzed for associations between serum lycopene concentrations and various population characteristics (Ganji & Kafai, 2005). Lower concentrations were reported for women, the elderly, participants below the poverty income ratio and with less education, participants residing in the south, those with lowest total cholesterol, highest serum triglyceride, dietary fat intake, and, not surprisingly, low reported tomato and tomato-based product consumption. Analysis of 1990 Behavioral Risk Factor Surveillance System (BRFSS) responses of 21,892 participants correlating fruit and vegetable intake to cigarette smoking, leisure-time physical activity, alcohol consumption, and cholesterol screening, found inverse relationships between smoking status, alcohol consumption, and cholesterol screening and levels of fruit and vegetable consumption (Serdula, et al., 1996). Physical activity level and frequency of fruit and vegetable intake were directly related. Less than 1/3 of participants in any of the risk-behavior categories consumed five or more servings per day suggesting those at greatest risk of chronic disease are consuming the lowest levels of fruits and vegetables.

2.3 *Polychlorinated Biphenyls*

Although production of PCBs was banned in the United States in the late 1970s, about 70% of those are still present in the environment (Birnbaum, 2008), and their

complete degradation could take decades or centuries to complete (Li, et al., 2009). PCB production was at its height around 1970 and ended in 1993 when Russia closed its last plant (Brevik, et al., 2011). While levels in the U.S. are decreasing, air concentrations of PCBs in Asia and Africa, where PCBs were not used to any great extent, remain high.

More than 95% of human exposure to dioxin-like PCBs occurs through food. PCBs are persistent and concentrate to lipids within an organism, which results in biomagnification through the food chain. This further causes greater concentrations of PCBs at higher trophic levels (Ritter, et al.) Dioxin-like PCBs may account for up to 50% of the toxic equivalency factor (TEQ) in human milk, although this will vary somewhat by country. Breast milk has been monitored since the 1950s when concentrations of DDT were first detected in samples, but breastfeeding continues to be recommended for infants by the American Academy of Pediatrics, World Health Organization, and the Department of Health and Human Services (Am. Acad. Pediatric Comm. Environ. Health).

Research has shown that PCBs are toxic to wildlife and humans. Animal model studies of individual congeners have elicited wide toxic responses. PCB 126 was administered to female Harlan Sprague-Dawley rats to evaluate the effects of chronic exposure on the cardiovascular system (Lind, et al. 2004) and they found elevated serum cholesterol, increased blood pressure, and increased myocardial mass. The NTP rat gavage studies administered PCB 126 singly and in a binary mixture with PCB 118, then evaluated at 14, 31, 53, and 104 weeks. They demonstrated altered thyroid hormone concentrations at just 14 weeks; hepatic cell proliferation at 14, 31, and 53 weeks; increased CYP1A1, CYP1A2, and CYP2B1 activities at 14, 31, and 53 weeks; and detectable concentrations of PCB 126 in liver, fat, lung, and blood tissues. At two years, there was clear evidence of cholangiocarcinoma of the liver (NTP Technical Rpt PCB 126). PCB 126 is the most potent coplanar PCB with a TEF of 0.1, accounts for 40-90% of the total toxic potency of PCBs having dioxin-like activity, and has high bioaccumulation in the food chain. PCB 118 has a single chlorine atom in the ortho- position, exhibits partial dioxin-like activity with a TEF of 0.0001, and accounts for 14% of the PCB toxic

equivalency present in human tissues (NTP Technical Rpt PCB 118). PCB residues are present in fish, milk and other dairy products, meat and animal products, but have also been detected in vegetables.

PCB 153 is a di-ortho-substituted PCB, does not exhibit dioxin-like activity, and is a Phenobarbital-like inducer of hepatic cytochrome P-450. Nonplanar PCBs have been shown to induce neurobehavioral toxicity as well as endocrine alterations (Fischer, et al. 1998). PCB 153 has been shown to reduce long-term potentiation in Sprague-Dawley rats and may be a factor in reduced learning ability and IQ deficits (Hussain, et al. 2000). PCB 153 has been measured at the highest concentrations in human tissue on a molar basis of all PCBs due to its persistence (NTP Technical Rpt PCB 153).

A human study of a Swedish fishing community to determine PCB exposure and prevalence of T2DM found that 6% of men and 5% of women had T2DM, with PCB 153-associated ORs of 1.16 for those with diabetes (Rylander 2005). The diabetic participants had a significantly higher PCB 153 body burden than did the non-diabetic participants. A follow-up study composed of 38% of the wives was undertaken to confirm earlier findings (Rignell-Hydbom 2007). Age-adjusted modeling of PCB 153-associated T2DM corresponded to an OR of 1.4, confirming an increased risk of having T2DM with higher levels of PCB 153. A cohort study of Great Lakes sport fishermen consumers found significant negative associations between triiodothyronine (T_3), thyroxine (T_4), thyroid stimulating hormone (TSH), and sex-hormone binding globulin (SHBG)-bound testosterone and PCB concentrations (Turyk, et al. 2006). This cohort, evaluated in 1994-1995, was administered follow-up questionnaires in 1995-1996, 2001-2003, 2003, and 2004-2005, to assess incident diabetes (Turyk, et al. 2009). DDE exposure was positively associated with T2DM incidence, but no association was found between years of sport fish consumption and PCB 118, total PCBs, or DDE.

Another study examined fasting plasma glucose and insulin levels, seventeen serum PCDDs and PCDFs, and 12 PCBs of non-diabetic, pregnant women (Chen, et al. 2008). PCBs 123, 126, and 169 levels were significantly associated with a decrease in insulin sensitivity. Cross-sectional analysis of a Native-American Mohawk population

found positive associations between total PCBs, PCB 74, PCB 153, DDE, and HCB, and an elevated incidence of T2DM after adjustments for age, BMI, serum lipids, gender, and smoking (Codru, et al. 2007).

The Michigan PBB cohort began in 1976 with physical examination and biochemical indices, and concluded in 2001 by surveying participants on their health status, including presence of diabetes. They found that PCBs (not PBBs) were associated with an increased incidence of diabetes in women, but not men (Vasiliu, et al. 2006). An elderly, Swedish cohort (PIVUS Study) was evaluated at baseline for plasma concentrations of 21 POPs (14 PCBs) and at five years for incident diabetes (Lee, et al. 2011a). Only 5% (n=36) developed diabetes in that time, but plasma concentrations of PCBs strongly predicted future risk of diabetes. Cross-sectional survey analysis of a Japanese population found that of 29 dioxin-like chemicals (10 PCDDs, 7 PCDFs, 12 PCBs) examined in relation to prevalence of metabolic syndrome, the PCBs were significantly associated with each clinical marker of metabolic syndrome (Uemura, et al. 2009). The ORs within the highest quartiles of PCB 126 and PCB 105 were 9.1 and 7.3, respectively.

NHANES 1999-2002 cross-sectional survey data have been analyzed for associations between POPs and obesity (Elobeid, et al. 2010), cardiovascular disease (Ha, et al. 2007), hypertension (Ha, et al. 2009), insulin resistance (Lee, et al. 2007a), metabolic syndrome (Lee, et al. 2007b), and diabetes (Lee, et al. 2007c). In most cases, approximately 19 POPs were investigated above the 60% LOD, including PCDDs, PCDFs, PCBs, and organochlorine pesticides. The Elobeid study using obesity and waist circumference as a clinical endpoint was an exception as it evaluated only two dioxins and three pesticides, choosing not to evaluate PCBs at all. In the remaining studies, PCBs were positively associated with CVD, and both dioxin-like and non-dioxin-like PCBs were positively associated with hypertension (ORs of 2.3 & 2.8, respectively), but only among the men. Organochlorine pesticides showed a strong p-trend for a positive association with insulin resistance ($p < 0.01$). Individual analysis of PCBs 170 and 187 showed significant associations with HOMA-IR. Dioxin-like PCBs were most strongly associated with diabetes and organochlorine pesticides were most strongly associated

with metabolic syndrome. However, dioxin-like PCBs were positively associated and non-dioxin-like PCBs yielded an inverted-U curve suggestive of a low-dose response. Of individual metabolic syndrome markers, PCBs were associated with waist circumference, hypertriglyceridemia, and impaired fasting glucose.

Analysis of NHANES 2003-2004 data for associations between non-alcoholic fatty liver disease (NALFD) and POPs found significant positive associations between NALFD and dioxin-like and non-dioxin-like PCBs (p-trend < 0.001 for both) (Cave, et al. 2010), citing dose-dependent associations with PCB 126 and PCB 153, in particular.

CHAPTER THREE

METHODOLOGY

3.1 *Introduction*

The specific aim of this research was to examine associations between serum concentrations of PCBs; serum concentrations of specific nutrients, carotenoids, Vitamins C and E; related socioeconomic and lifestyle characteristics; and the probability of developing metabolic syndrome. This research used existing data from the 2003-2004 NHANES release. Data were analyzed using SAS 9.2©.

3.2 *Selection of Research Design*

A growing body of research exists on the effects of persistent organic pollutants, including PCBs, on health. Similarly, extensive research has been conducted on the effects of specific nutrients and/or foods on health. There exists scant evidence on the combined effects of pollutants and nutrition on human health and disease. Even individuals with similar exposures can have vastly different health profiles. Nutrition is associated with a healthy weight, absence of chronic disease, and/or modulation of disease progression.

NHANES uses a cross-sectional sample design for examining associations, patterns, and confounding influences between dependent and independent variables. Among its eight stated goals, NHANES includes the following:

- Monitor trends in risk behaviors and environmental exposures;
- Analyze risk factors for selected diseases;
- Study the relationship between diet, nutrition, and health.

While this design cannot establish cause and effect, it can be useful in assessing the interactions between these variables, the consistency of findings within individual exposures, as well as across statistical models, and any significant socioeconomic and lifestyle covariates. Findings such as these may contribute to development of new hypotheses, research on the effects of nutrition on POPs exposure and health, and may

contribute to the development and validation of nutritional recommendations for the exposed.

3.3 *Description of the Data Source*

NHANES is a continuous, population-based survey of the non-institutionalized, civilian U.S. population. It is one program of the National Center for Health Statistics (NCHS), Centers for Disease Control and Prevention (CDC). Data are available online to the public and periodic, ongoing updates and additions to the data are announced. NHANES applies a complex, probability random sampling method to yield a representative population of all ages for evaluation. The program combines interviews with physical examination and laboratory testing of blood, serum, and urine. Field staff receives extensive training and strict quality control standards are applied to all data collection and analyses.

The complex sample survey design requires proper use of sample weights and variance estimation to yield proper estimates and standard error of estimates. Sample weights correct for the probability of selection, participant non-response, oversampling of certain groups, and factors uniquely related to the sample characteristics. Variance estimation tests for sampling errors.

NHANES tested for 219 environmental chemicals in blood, serum, or urine between 1999 and the 2003-2004 data period. The Fourth National Report on Human Exposure to Environmental Chemicals presents biomonitoring data on these chemicals for the years 1999-2004 (CDC 4th Nat'l Rpt). Nutritional biochemistries were collected for retinyl palmitate, retinyl stearate, retinol, Vitamin B6, Vitamin B12, folate, Vitamin C, Vitamin D, α-tocopherol, β-tocopherol, γ-tocopherol, α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lycopene, lutein, zeaxanthin, phytoene, and phytofluene. Standard blood, serum, and urinary laboratory tests, such as complete blood counts (CBC), were executed. Additionally, two days of 24-hour dietary recall data and food frequency questionnaire were collected during the MEC interview process.

Initial health interviews were conducted in participant homes. The physical examination usually occurred within one to two weeks later at the MEC. MEC appointment duration was about 3.5 hours. Although the MEC was located within the PSU for easy access, transportation was available to participants requiring assistance and to minimize barriers to participation. The MEC laboratory staff was comprised of three certified medical technologists and/or phlebotomists who tested some samples on-site. All other specimen testing was conducted by federal, private, or university-based laboratories under contract to NCHS.

NHANES data were collected in an ethical manner with regard to informed consent and confidentiality. Institutional Review Board (IRB) approval for NHANES 1999-2004 was granted by the NCHS Research Ethics Review Board (ERB) under Protocol #98-12. Participants received remuneration for the MEC visit and additional remunerations for fasting, completing the 24-hour dietary telephone interview, and the food frequency questionnaire.

3.4 *Description of the Dataset*

While NHANES attempts to examine a nationally representative sample, they do so by evaluating approximately 5,000 individuals across 15 counties each year. Any disparities from actual population parameters are corrected for by using the complex survey design characteristics of stratification, clustering, and weighting. The subjects in the present study were male and female, 20 years and older, of diverse racial and ethnic backgrounds, and residing in the United States during 2003-2004. Importantly, while all MEC participants underwent extensive testing, not all participants were tested for all parameters. NHANES MEC Subsample C, which was comprised of individuals who were analyzed for serum concentrations of PCBs, provided the initial sample pool for this study.

10,122 individuals participated in the in-home interview process of NHANES 2003-2004. 95.3% of these participated in the MEC physical examination portion of the study, including biochemical lab analyses and the first of two 24-hour dietary recalls.

For analyses of environmental chemicals, 5-10 ml of serum was drawn and participants randomly assigned to subsample A, B, or C. This placement determined which classes of environmental chemicals for which they would be analyzed. The sample size tested for subsample C pollutants was 1,850. The final subpopulation for this study after removal of participants for necessary exclusions and missing data consisted of 1,058 persons.

3.5 *Exclusions from the Dataset*

3.5.1 Age.

Persons younger than twenty years of age were excluded from the study. The SRP Community Engagement Core (CEC) NIEHS grant (P42ES007380) does not permit children or adolescents to be participants in CEC research initiatives. Further, exposure to PCBs and the effects of nutrition and usual dietary intake patterns on the development of cardiometabolic illness have temporal implications that are best analyzed in an adult population.

3.5.2 Diabetes Mellitus

Persons diagnosed with diabetes mellitus were excluded from the dataset. Metabolic syndrome is a condition that is consistently associated with increased risks of T2DM and cardiovascular disease, but one which precedes an actual diagnosis of T2DM. A diagnosis of diabetes could be established from two different sections of NHANES data, the questionnaire or physical examination. Either a fasting plasma glucose value of ≥ 126 mg/dL (≥ 7.0 mmol/L) or a history of physician diagnosed diabetes would establish a basis for eliminating these participants from the study.

Table 3.1 NHANES Determination for Type 2 Diabetes Mellitus

ITEM LABEL	SAS LABEL	NHANES 2003-2004		COMMENT
		ITEM NUMBER	DATA FILE	
DIQ 010	Doctor told you have diabetes	174	DIQ_C	
LBXGLU	Plasma Glucose ≥ 126 mg/dL		L10AM_C	Count: 3169

3.5.3 Metabolic Syndrome Determinants

Persons with insufficient data to make a determination of metabolic syndrome were excluded from the study. The NCEP ATPIII criteria for metabolic syndrome is based on five measures routinely collected in clinical settings and by NHANES. Any three or more of the five criteria yield a finding of metabolic syndrome. Blood pressure and waist circumference were obtained during the MEC physical examination. HDL-cholesterol was a non-fasting laboratory analysis and could be collected at either the morning or afternoon lab draw. Accurate triglyceride and plasma glucose analyses for metabolic syndrome required an overnight fast. These participants were asked to fast for nine hours for the morning session or six hours for the afternoon and evening sessions. If the participant failed to fast for the required time, efforts were made to obtain the fasting measures at the end of the MEC visit if the participant was within thirty minutes of the required fasting time. As metabolic syndrome is based on the presence of three or more criteria, a stepwise method was used to eliminate participants, if necessary.

1) If fasting blood work was not done (plasma glucose and triglyceride values), but a participant met the definition of metabolic syndrome based on the remaining three criteria (low HDL-cholesterol, elevated blood pressure, and central adiposity), the participant met the requirement for metabolic syndrome and was included in the study. If, however, fasting lab work was not collected and one or more of the remaining three indices were missing, the subjects were excluded from the study, as a determination of metabolic syndrome could not conclusively be done.
2) If fasting plasma glucose and triglyceride values were collected and:
    a. All five markers were available a determination of metabolic syndrome could be made. No subjects were excluded in this scenario.
    b. One or two measures were missing from the remaining three indices, HDL-cholesterol, blood pressure, and waist circumference, the

participants were eliminated if a determination was dependent on the missing value(s).

i. One measurement missing:
    1. If zero or one criterion were met for metabolic syndrome, the participant still would not meet the definition of metabolic syndrome, even if the missing index had been collected and found positive for metabolic syndrome. These participants were not excluded from the study.
    2. If two criteria were met for metabolic syndrome, a single missing index would either meet or reject the definition of metabolic syndrome. These participants were excluded from the study.
    3. If three or four criteria were met for metabolic syndrome, these participants met the definition of metabolic syndrome, even in the absence of one marker. These participants were not excluded.

ii. Two measurements missing:
    1. If a determination of metabolic syndrome could be made with the remaining three markers, the subjects were included in the study.
    2. If a determination of metabolic syndrome was dependent on the missing values, the subjects were excluded from the sample.

3) If three or more measurements were missing, a determination of metabolic syndrome could not be made regardless of fasting laboratory measures. These participants were excluded from the sample.

3.5.4 24-Hour Dietary Recall Data

NHANES 2003-2004 collected two days of dietary intake data collection. Two days of food and fluid records have been shown to more closely correlate with usual intake patterns than one day's intake. Accurate analysis of food and fluid intakes with the specific aim of estimating the effect of diet on health must be targeted to usual intake patterns, as opposed to acute or unusual exposures. Subjects with two days of complete and reliable dietary intake data were included in the sample. If participants completed only one 24-hour dietary recall or if the data were unreliable or incomplete, the participants were excluded from the sample.

3.6 *Measurement of Independent Variables*

3.6.1 Polychlorinated Biphenyls

Serum specimens of PCBs were measured by high-resolution gas chromatography/isotope-dilution high-resolution mass spectrometry. Quality assurance measures included the measurement of samples in each analytical run for accuracy and precision, verification of calibration materials, and review of all operational parameters. All PCBs above the 60% LOD were analyzed. Lower limits of detection for PCBs varied as each analyte had its own limit. If no observations were seen above the 60% LOD, those observations were dropped from the sample. PCBs lacking observations below the LOD presented a challenge in that below-LOD observations offered the closest approximation to a reference group for environmental pollutant data, where no true reference group exists. The greater the sample volume the lower the detection limit. Nearly five times more blood volume was drawn in 2003-2004 than in NHANES 1999-2002. This resulted in many more PCB congeners in the 2003-2004 population with zero observations below the LOD.

Importantly, PCBs entered the environment as mixtures. Studies assessing background exposures to PCBs have estimated, as one example, Aroclor 1254 exposure by summing PCB congeners 99, 118, 138/163, 145, 132/153/105, 167, 170/190, 172/197, 177, 178, 180, 182/187, 194, 195/208, 201, 203/196, and 206 (Lang, 1992; Burse, et al.

1990 & 1994). It is known that Monsanto manufactured a variant of Aroclor 1254 in the 1970s (Frame, 2001), and that the effects of the environment on decomposition vary by congener. Chemical analyses of different Aroclor 1254 lots have revealed substantial differences in congener composition with challenges for determining biological endpoints (Kodavanti, et al., 2001).

3.6.1.1 Lipid-adjusted versus whole weight

NHANES reported PCBs as both grams per total lipid (pg/g lipid or ppt) and as grams per whole weight serum (ng/g or ppb). PCBs are lipophilic and gravitate to lipids in serum. Concentrations of lipid-adjusted PCBs are considered a reflection of PCB concentrations stored in body fat. Whole weight PCB measures are useful for comparison studies, although it has been suggested that wet weight PCBs (not lipid-adjusted) should be considered because PCBs may disturb normal lipid metabolism (Schisterman, et al., 2005). One recent study examining the low dose effects of some POPs on obesity, insulin resistance, and dyslipidemia, used whole weight POP measures after adjusting for total cholesterol and triglycerides (Lee, et al. 2010 & 2011b). Dyslipidemia has been demonstrated to precede clinical manifestation of T2DM, as is observed with metabolic syndrome. Adjusting PCBs for circulating lipid concentrations in this case could underestimate true risk associations. Conversely, not using lipid-adjusted PCBs would under-adjust at baseline further underestimating body burden and related associations to disease. Due to the NHANES recommendation to use lipid-adjusted values and to better facilitate comparison with related research almost exclusively using lipid-adjusted concentrations this dissertation relied on lipid-adjusted PCB values.

3.6.1.2 Rank versus Concentration

As participants were exposed to several PCB congeners, an order ranking method was used to provide a systematic way for assessing cumulative exposures. Participants were ranked according to their measured concentration of each individual

PCB, the ranks within a subclass summed, and a combined exposure ranking assigned. Participants could then be placed with one of four quartiles as one observation representing a cumulative exposure to several PCBs. PCB concentrations were applied in several models as well, using a similar method. Serum PCB concentrations are considered a reflection of body burden. The use of pollutant concentrations instead of rank gave a participant's placement within the quartile greater context, and yielded additional information.

3.6.1.3 Quartile versus Continuous

Models were executed primarily by ranking PCBs across four quartiles. For those PCBs having observations below the LOD, this range was assigned as reference group in addition to the quartiles. PCBs lacking a reference group relied on the first exposure quartile as the reference group. Even though use of quartiles does not facilitate analysis of the mean, quartile analysis is primarily used in environmental chemical studies. Skewed distributions and outliers are common in exposure assessment studies. Covariate analysis across quartiles may be expected to reveal a more enhanced analysis of the variables than would measurements of central tendency or dispersion. However, some modeling relied on placement of PCBs along a continuous scale to better understand the complex relationships between variables.

3.6.1.4 Four Covariate versus Full Covariate Modeling

Observations were adjusted for age, gender, race/ethnicity, and poverty income ratio (PIR) instead of applying one NHANES subsample weight. This method has been considered an acceptable compromise between bias and efficiency (Graubard & Korn, 1999; Korn & Graubard, 1991). As discussed previously, NHANES uses a random sampling method within specific predetermined population domains. Thus, certain groups are oversampled. Applying the sample weight corrects for this oversampling, in addition to correcting for probability of selection, non-response, and any unique characteristics related to a specific subsample. Several of these subsamples were used

in the present study and it was untenable to apply one weight to correct for disparate characteristics across samples. The four covariate model provided a good surrogate for sample weights.

The relevant demographic and behavioral characteristics that can affect the probability of developing metabolic syndrome, an individual's serum concentrations of PCBs, or their serum concentrations of carotenoids include age, gender, race/ethnicity, PIR, cigarette smoking, serum cotinine, alcohol consumption, leisure-time physical activity, BMI, use of dietary supplement, total cholesterol, and non-HDL cholesterol. For actual food intake records, total caloric intake would adjust for the ratio of nutrient dense intake per total calorie intake. A reasonable assumption was that exposure to PCBs, rather than being singly causative of metabolic syndrome, should be considered as one additional covariate across the full spectrum of demographic and lifestyle factors. For this reason, a full covariate model was executed in which all of the above characteristics were included *a priori.* Ultimately, the four covariate model was applied as a closer approximation to NHANES weighting.

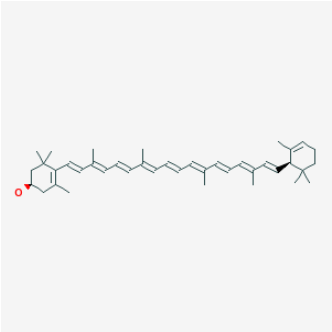
3.6.1.5 First Quartile: With and Without

The application of general linear models revealed that most PCBs did not respond in a standard toxicological dose-response manner, and that the relationships were complex. While variability occurred among congeners, one consistency was the increased probability of metabolic syndrome within the first quartile for all PCBs. Analysis was further compounded by the lack of a reference group for many congeners. Models were executed with and without the first quartile to assess relationships between variables across increasing exposure quartiles. This low dose effect has been documented in other literature and may be indicative of disruption of normal endocrine function. At physiologically active hormone levels, a mimic would be expected to either saturate or down-regulate receptor activity at relatively low levels.

3.6.2 Serum Carotenoids

Twelve carotenoids were measured in serum using high performance liquid chromatography with multiwave-length photodiode-array absorbance detection. Carotenoids were measured at an UV absorbance capacity of 450 nm. As fat soluble molecules, carotenoids have in common a conjugated double bond system of primarily eight isoprenoid units that interact with UV and visible light, yielding their characteristic yellow, orange, or red color.

Figure 3.1 Alpha-Cryptoxanthin



http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?sid=17425144&viewopt=Deposited; accessed 10-17-11.

The carotenoids analyzed in sera by NHANES 2003-2004 included alpha-carotene, alpha-cryptoxanthin, *cis*-beta-carotene, *trans*-beta-carotene, beta-cryptoxanthin, *cis*-lycopene, *trans*-lycopene, lutein, zeaxanthin, total lutein/zeaxanthin, phytoene, and phytofluene. Values were given in umol/L and ug/dL. The present study included alpha- and beta-carotenes, alpha- and beta-cryptoxanthins, total lycopene, and total lutein/zeaxanthin.

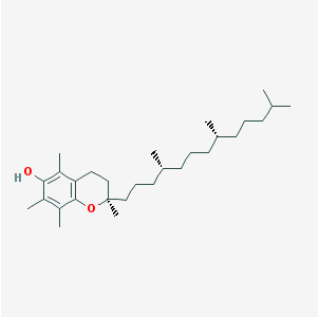
Carotenoids and PCBs share some similarities in that both are lipid-soluble, both concentrate to lipid fractions in serum, and both are considered to have an effect on the probability of metabolic syndrome, although opposing effects. For these reasons, a statistical interaction was first sought for carotenoids and PCBs in serum. If statistical significance for this interaction was revealed within the models for individual and pooled PCB subpopulations, this would imply significance for carotenoids and PCBs individually. The combined interactions were evaluated across four quartiles, and then

across sixteen moving quartiles, to examine their effect on the probability of developing metabolic syndrome.

3.6.3 Vitamin E

Serum concentrations of three forms of Vitamin E, alpha-tocopherol, delta-tocopherol, and gamma-tocopherol, were measured using high performance liquid chromatography with multiwave-length photodiode-array absorbance detection. The tocopherols were measured by UV absorbance at maxima of between 292 and 300 nm.

Figure 3.2 Alpha-Tocopherol



http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=14985&loc=ec_rcs; accessed 10-17-11

NHANES 2003-2004 measured three of the eight naturally occurring forms of Vitamin E. The RDA for Vitamin E is based on α-tocopherol because other forms are not converted to α-tocopherol in humans (DRIs 2000). Alpha-, gamma-, and delta-tocopherols were evaluated in the present study. NHANES values for Vitamin E were given in umol/L and ug/dL.

Vitamin E is lipid-soluble and concentrates to lipid fractions. Analysis of Vitamin E was undertaken as with carotenoids by seeking a significant interaction with PCBs first, then evaluating their combined effect on the probability of developing metabolic syndrome. Analyses were executed across four quartiles, and then across sixteen moving quartiles, for a more precise evaluation.

3.6.4 Vitamin C

Vitamin C is an essential, water-soluble vitamin. The term refers to ascorbic acid and dehydroascorbic acid (DHA), and it functions as a powerful reducing agent of reactive oxygen species (DRIs 2000).

Vitamin C in serum was measured by NHANES 2003-2004 by isocratic HPSC electrochemical detection at 650 mV. Peak height was quantified and based on a standard curve using three different concentrations, 0.005, 0.030, and 0.100 mg/dL. NHANES values for Vitamin C were given in umol/L and ug/dL.

A significant interaction was sought between PCBs and Vitamin C. For those with a significant result, analysis was executed of their combined effect across four quartiles, and then across sixteen moving quartiles, for a more refined evaluation of their overall effect in the probability of developing metabolic syndrome.

3.7 *Measurement of Dependent Variables*

3.7.1 Metabolic Syndrome

The five clinical criteria necessary for a determination of metabolic syndrome were accessed from the laboratory, examination, and questionnaire data files of NHANES 2003-2004.

3.7.1.1 Waist Circumference

Abdominal obesity is defined as a waist circumference greater than 40 inches (102 cm) for men and 35 inches (88 cm) for women.

Table 3.2 NHANES Determination for Waist Circumference

ITEM LABEL	SAS LABEL	DATA FILE	COUNT	MISSING
BMXWAIST	Waist Circumference (cm)	BMX_C	8397	1247

3.7.1.2 Elevated Blood Pressure

Elevated blood pressure is defined as average pressure greater than or equal to 130/85 or if participant answered "yes" to the question, "Are you currently taking antihypertensive medication?" Up to four blood pressure measurements were taken from participants. If three or four measurements were taken an average of the last two measurements were used to assess blood pressure status. If two measurements were taken the second measurement was used. If only one measurement was taken, it was used as a measurement of blood pressure status. If, however, the participant answered affirmatively that he or she was currently taking medication for elevated blood pressure, he/she was classified as having elevated blood pressure.

Table 3.3 NHANES Determination for Elevated Blood Pressure

ITEM LABEL	SAS LABEL	DATA FILE	COUNT	MISSING
BPXSY1	Systolic: blood pressure 1st reading mm Hg	BPX_C	6274	3369
BPXDI1	Diastolic: blood pressure 1st reading mm Hg	BPX_C	6274	3369
BPQ040A	Because of your high blood pressure, have you ever been told to take prescribed medicine?	BPQ_C	1452	4412

3.7.1.3 Elevated Fasting Triglycerides

Elevated fasting triglycerides are defined as ≥ 150 mg/dL (≥ 1.7 mmol/L). NHANES 2003-2004 measured triglycerides in participants who had fasted 8.5 hours or more. Triglycerides were measured enzymatically in serum using a series of coupled reactions that hydrolyzed triglyceride to glycerol. Absorbance was measured at 500 nm.

Table 3.4 NHANES Determination for Elevated Fasting Triglycerides

ITEM LABEL	SAS LABEL	DATA FILE	COUNT	MISSING
LBXTR	Triglyceride (mg/dL)	L13A M_C	3680	354

3.7.1.4 Low HDL-Cholesterol

Low HDL-cholesterol is defined as < 40 mg/dL (<0.9 mmol/L) for men and < 50 mg/dL (< 1.1 mmol/L) for women. HDL-cholesterol was measured directly in sera at absorbance maxima of 600 nm.

Table 3.5 NHANES Determination for Low HDL-Cholesterol

ITEM LABEL	SAS LABEL	DATA FILE	COUNT	MISSING
LBXHDD	Direct HDL Cholesterol (mg/dL)	L130C	7773	783

3.7.1.5 Fasting Plasma Glucose

Fasting plasma glucose is defined as greater than 100 mg/dL (> 5.6 mmol/L) but less than 126 mg/dL, which reaches the threshold of diabetes mellitus. Fasting plasma glucose was measured in participants fasting 8.5 hours or longer. Participants with fasting plasma glucose greater than 100 mg/dL but less than 126 mg/dL, or who responded "yes" to the question, "Have you ever been told by a doctor or health professional that you have diabetes or sugar diabetes?

Table 3.6 NHANES Determination for Fasting Plasma Glucose

ITEM LABEL	SAS LABEL	DATA FILE	COUNT	MISSING
LBXGLU	Systolic: blood pressure 1st reading mm Hg	L10AM_C	3169	187
DIQ010	Doctor told you have diabetes	DIQ_C	559	9645

The nicotinamide adenine dinucleotide (NADH) generated in the second step of glycolysis is proportional to glucose concentration, measurable at absorbance of 340 nm.

3.8 *Measurement of Covariates*

3.8.1 Smoking Status

Smoking status was determined from three variables from the questionnaire section of the NHANES data file. Participants were coded as "never smoker", "current smoker", or "former smoker".

Table 3.7 NHANES Determination for Cigarette Smokers

ITEM LABEL	SAS LABEL	DATA FILE	COMMENT
SMQ020	Smoked at least 100 cigarettes in life	SMQ_C	"No"; code as NEVER smoker
SMQ040	Do you now smoke cigarettes	SMQ_C	"Everyday" & "some days"'' code as CURRENT smoker
SMQ050Q	How long since quit smoking	SMQ_C	"Range of values"; code as FORMER smoker

3.8.2 Serum Cotinine

Cotinine is the primary metabolite of nicotine and has been considered the best biomarker of active and passive smoking. The plasma half-life of cotinine is approximately 16 hours, whereas the half-life of nicotine is less than 3 hours, making serum cotinine a better reflection of true smoking status. The detection limit for serum cotinine in the NHANES 2003-2004 data release was 0.015; the below limit of detection was 0.011. Nonsmoking has been defined as a serum cotinine level of ≤ 10 ng/mL. However, exposure to high levels of environmental tobacco smoke (ETS), commonly referred to as "second hand smoke", may exceed 10 ng/mL. Participants were coded as positive or negative for serum cotinine.

Table 3.8 NHANES Determination for Serum Cotinine

ITEM LABEL	SAS LABEL	DATA FILE	COUNT	MISSING
LBXCOT	Cotinine (ng/mL)	L06COT_C	6478	764

3.8.3 Alcohol Consumption

Alcohol consumption status was determined from the questionnaire section of the NHANES 2003-2004 data release. Participants were coded as "non-drinker", "non-excessive drinker", or "excessive drinker", using a scale adapted from a cross-sectional study correlating patterns of alcohol use, obesity, and elevated serum hepatic enzymes (Tsai, et al. 2011). Participants consuming no alcoholic beverages over the past month were coded as "non-drinkers". Males consuming ≤ 2 drinks per day (≤ 14 per week), and women consuming ≤ 1 drink per day (≤ 7 drinks per week) were coded as non-excessive

drinkers. Males exceeding two per day (> 14 per week) and women exceeding one per day (> 7 per week) were coded as excessive drinkers. If, however, a non-excessive drinker responded that they consumed ≥ 5 drinks on any one day, they were coded as an excessive drinker, as this meets the definition of binge drinking.

Table 3.9 NHANES Determination for Alcohol Consumption

ITEM LABEL	SAS LABEL	DATA FILE	COMMENT
ALQ120Q	In past 12 months, how often did you drink any type of alcoholic beverage	ALQ_C	"Zero"; code as NON-DRINKER
ALQ120U	Unit of measure	ALQ_C	Yields days per week, month, year
ALQ130	In past 12 months, on those days that you drank alcohol, on average, how many drinks did you have	ALQ_C	Drinks per day, on average
ALQ140Q	In past 12 months, on how many days did you have 5 or more drinks of any alcoholic beverages	ALQ_C	≥ 5 drinks per day; code as EXCESSIVE DRINKER

3.8.4 Leisure-Time Physical Activity

Leisure-time physical activity status was determined from participant self-reporting of specific activities. Participants' activity status was coded as "vigorous", "moderate", or "none". Initially, it was deemed reasonable that if no activities were identified for a respondent that the participant would be coded as "none", i.e. sedentary. This proved to be problematic however, as all 1058 participants coded at least one activity. The formatting of this data set prompted positive responses. Further, participants falling in the highest category for vigorous exercise were excessive, with many participants reporting many hours of vigorous exercise. It was noted that if a participant played a 4-hour football game, he/she recorded 4 hours of actual physical activity. Similarly, a 4-hour dance was reported as 4 hours of continuous dancing. Conversely, if a metabolic equivalent score (MET) for an activity was recorded as less than ten minutes, it was excluded by NHANES.

A simple method was adopted to report leisure-time physical activity. The U.S. Department of Health and Human Services recommendations for healthy adults are to achieve 150 minutes of moderate activity or 75 minutes of vigorous activity per week

U.S.D.H.H.S. 2008 Physical Activity Guidelines). Activities reported as moderate were weighted by a factor of one. Activities reported as vigorous were weighted by a factor of two. Participants reporting less than 90 minutes of leisure-time physical activity per week were scored as "none", or sedentary.

Table 3.10 NHANES Determination for Leisure-Time Physical Activity

ITEM LABEL	SAS LABEL	DATA FILE	COMMENT
PADACTIV	Leisure time activity	PAQIAF_C	Code all activities that apply
PADLEVEL	Activity level	PAQIAF_C	Moderate; vigorous
PADTIMES	Number of times did activity past thirty days	PAQIAF_C	Range of values
PADDURAT	Over past 30 days, on average about how long did you do activity each time	PAQIAF_C	Average duration of activity (minutes)

3.8.5 Body Mass Index

Body mass index (BMI), defined as weight (kg) divided by [height (m)]^2, has been correlated with overall mortality and nutritional risk. The Expert Panel on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults has endorsed the following classifications. Anthropometric measures were assessed in participants as part of the MEC physical examination.

Table 3.11 World Health Organization International Classification for Body Mass Index

Underweight	< 18.5 kg/m^2
Normal weight	18.5 – 24.9 kg/m^2
Overweight	25 – 29.9 kg/m^2
Obesity (Class I)	30 – 34.9 kg/m^2
Obesity (Class II)	35 – 39.9 kg/m^2
Extreme obesity (Class III)	≥ 40 kg/m^2

3.8.6 Dietary Supplement Use

Dietary supplement use was determined by participant response to the question, "Have you used or taken any vitamins, minerals, or other dietary supplements in the past month?" A separate analysis of NHANES 2003-2006 data found that 54% of adults reported dietary supplement use and that fewer obese individuals reported dietary supplement use than either overweight or normal weight individuals (Bailey, et al., 2011). Positive associations were also seen with higher educational status and non-Hispanic white race-ethnicity.

Table 3.12 NHANES Determination for Dietary Supplement Use

ITEM LABEL	SAS LABEL	DATA FILE	YES	NO	MISSING
DSD010	Any dietary supplements taken	DSQ1_C	3820	6273	14

All datasets were accessed before October 31, 2011.

CHAPTER FOUR

RESULTS

Project I. Serum concentrations of PCB 118, PCB 126, and PCB 153; serum carotenoids; and the probability of metabolic syndrome

Three PCBs were identified for initial analysis and a preliminary study undertaken to determine if significant differences existed between the mean serum carotenoid concentrations of those with and without metabolic syndrome across increasing PCB exposure quartiles. In other words, participants with similar PCB concentrations (by quartile), and defined by health status (i.e. with or without metabolic syndrome), could be assessed by differences in their mean serum carotenoid concentrations. Serum carotenoids have been defined by the IOM as the best biomarker of fruit and vegetable intake.

PCBs 118, 126, and 153 represent different congener subclasses based on the ortho positioning of their chlorine atoms. Further, all have previously been selected by the NTP for two-year rat gavage studies relating to their toxicity, persistence, and bioaccumulation in tissue. The analytic samples were maintained as three disparate samples at this time relating to differences in their chemistry and effects in vivo. Seven serum carotenoids were examined as a pool of "total carotenoids", which represented the sum of each participant's serum concentrations of α-carotene, β-carotene, α-cryptoxanthin, β-cryptoxanthin, lycopene, and lutein/zeaxanthin. A determination of metabolic syndrome was found if three or more NCEP ATPIII criteria were met. In this project, NHANES methodology was strictly followed with regard to sample weight and variance estimation. MEC Subsample C provided the smallest sample size for proper weight selection in accordance with the Continuous NHANES Tutorial (CDC, Cont Tutorial) and NHANES Environmental Chemical Data Tutorial (CDC, NHANES Env Chem Tutorial), as well as providing three sub-populations that had been tested for PCB 118, PCB 126, and PCB 153 concentrations.

A. PCB 118 - 2,3',4,4',5-Pentachlorobiphenyl

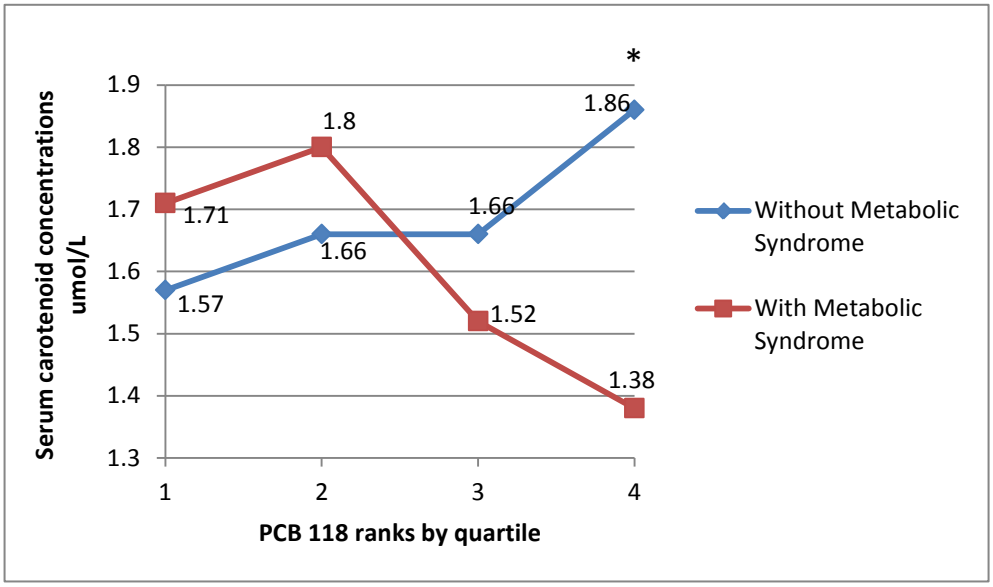
PCB 118 status was categorized by rank, n = 917. No observations were found below the LOD for PCB 118 in the 2003-2004 data release.

Table 4.1 PCB 118 rank analysis by quartile

Analysis Variable : LBX118LA PCB118 Lipid Adj (ng/g)						
Values of PCB118_p were Replaced by Ranks	N Obs	N	Mean	Std Dev	Minimum	Maximum
1	223	223	2.1838117	0.5279258	0.7600000	2.9500000
2	240	240	3.7319167	0.4994001	3.0000000	4.5100000
3	224	224	5.6838393	0.9866747	4.5300000	7.9000000
4	230	230	22.5208261	23.4045465	7.9100000	177.0000000

Observations were nearly equally distributed across the quartiles. The mean, minimum, and maximum do not reflect actual pollutant concentrations. A ranking system, as used in similar studies (Ha, et al. 2007; Cave, 2010), was applied. The mean and maximum in the highest quartile are indicative of outliers, a not uncommon occurrence when dealing with environmental pollutant measures.

Figure 4.1 Mean serum carotenoid concentrations across PCB 118 quartiles



* $p < 0.01$

The above graph tracks the serum carotenoid concentrations of participants with metabolic syndrome (red) and participants without metabolic syndrome (blue) across increasing exposure quartiles of PCB 118 by rank. A significant association was seen at the highest PCB 118 rank quartile ($p < 0.01$) for participants with high serum carotenoid concentrations. Individuals in the fourth quartile would have had similar PCB 118 levels in their serum at the highest observed concentrations. They were shown to have a significantly lower probability of developing metabolic syndrome based on their serum carotenoid concentrations. Serum carotenoids appeared to have no effect in the first exposure quartile. While both groups' carotenoid levels parallelled linearly in the first quartile, the participants with metabolic syndrome actually had the higher serum carotenoid concentrations. This trend ended abruptly at the second quartile, however.

Table 4.2 Multivariate Regression: PCB 118 and the probability of metabolic syndrome

Analyte	<25th percentile	25th-<50th percentile	50th-<75th percentile	>75th percentile	P_{trend}	$P_{covariate}$
Concentration (ng/g of lipid)	2.18/0.53	3.73/0.50	5.68/0.99	22.52/23.40		
Cases / n	21/223	34/240	42/224	55/230		
Prevalence (%)	8.61	12.41	15.79	19.30		
Model 0	referent	1.3 (0.7-2.5)	1.4 (0.7-2.5)	2.0 (1.1-3.6)	0.41	
Model 1 – age	referent	1.3 (0.7-2.5)	1.2 (0.6-2.2)	1.4 (0.8-2.7)	0.91	0.02
Model 2 – gender	referent	1.4 (0.7-2.7)	1.4 (0.7-2.6)	2.1 (1.1-4.0)	0.44	0.15
Model 3 – race/ethnicity	referent	1.3 (0.7-2.5)	1.4 (0.7-2.7)	1.9 (1.0-3.7)	0.44	0.28
Model 4 – PIR	referent	2.0 (1.0-4.0)	1.8 (0.9-3.5)	2.7 (1.4-5.1)	0.97	0.97
Model 5 – cigarette smoking	referent	1.3 (0.7-2.5)	1.4 (0.8-2.6)	2.0 (1.1-3.7)	0.44	<0.01
Model 6 – serum cotinine	referent	1.3 (0.7-2.5)	1.4 (0.7-2.5)	2.0 (1.1-3.6)	0.43	0.50
Model 7 – alcohol consumption	referent	0.5 (0.1-2.1)	1.6 (0.5-5.0)	2.3 (0.8-6.4)	0.04	0.50
Model 8 – leisure physical activity	referent	1.4 (0.7-2.6)	1.4 (0.7-2.5)	1.8 (1.0-3.3)	0.71	0.04
Model 9 - BMI	referent	1.2 (0.7-2.3)	1.3 (0.7-2.4)	1.8 (1.0-3.4)	0.39	<0.01

The demographic and behavioral characteristics thought to be involved in the etiology of metabolic syndrome were examined above. Carotenoids were not considered in this analysis. The first quartile of PCB 118 was used as reference group as no observations were detected below the LOD. Model 0 represented PCB 118 without other covariate influence. P-trend indicated there was no association between PCB 118 and the probability of metabolic syndrome. P-trend tests for any trend across quartiles, as compared to the reference group. The lack of a true reference group in this analysis may be expected to impact findings. Significance was seen, however, in modeling of PCB 118, alcohol consumption, and the probability of metabolic syndrome. P-covariate, which considers only the covariate's influence (in absence of PCB 118), revealed age,

cigarette smoking, leisure time physical activity, and BMI, as having a significant effect on the probability of metabolic syndrome.

Table 4.3 PCB 118 concentration as a continuous variable in the probability of metabolic syndrome

Model	PCB 118	$P_{covariate}$
Model 0	0.01	
Model 1 – age	0.09	0.05
Model 2 – gender	0.01	0.17
Model 3 – race/ethnicity	0.01	0.19
Model 4 – PIR	0.01	0.87
Model 5 – cigarette smoking	0.01	<0.01
Model 6 – serum cotinine	0.01	0.52
Model 7 – alcohol consumption	0.06	0.47
Model 8 – leisure-time physical activity	0.03	0.07
Model 9 - BMI	0.01	<0.01

PCB 118 was examined as a continuous variable in this analysis rather than as a categorical variable across quartiles. Significance was observed for several covariates in modeling with PCB 118 in the probability of developing metabolic syndrome. Age, which is known to have a strong independent association with metabolic syndrome, was not indicated as being significant in modeling with or without PCB 118 above. When evaluated as a continuous variable, PCB 118 was shown to have a significant association with the probability of metabolic syndrome.

B. PCB 126 - 3,3’,4,4’,5-Pentachlorobiphenyl

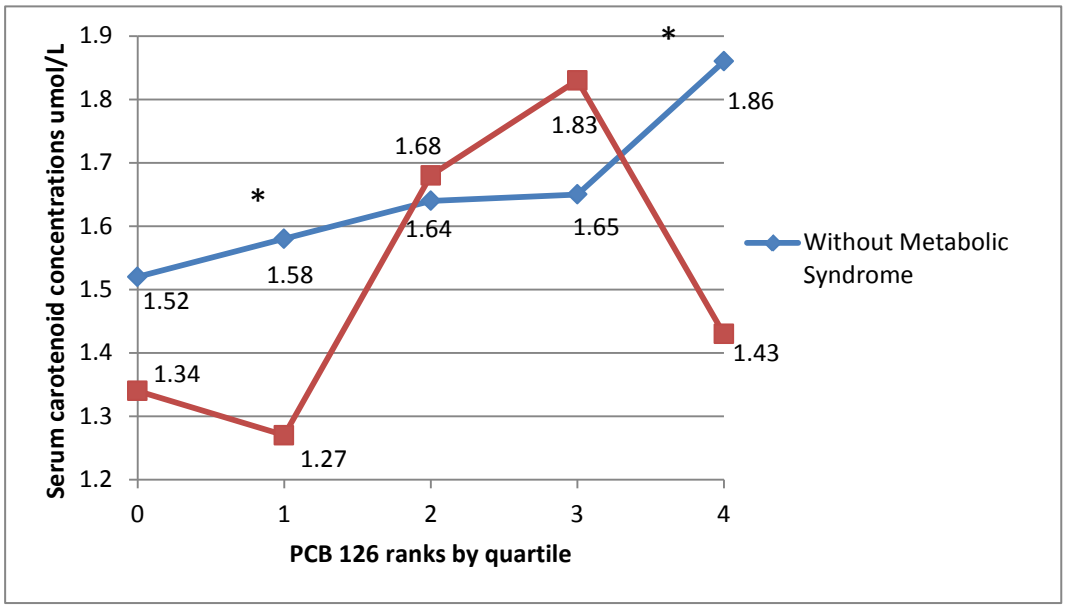
PCB 126 status was categorized by rank, n = 996. 69 observations were found below the LOD for PCB 126 in the 2003-2004 data release, allowing for quartile analysis with a reference group.

Table 4.4 PCB 126 rank analysis by quartile

Analysis Variable : LBXPCBLA 3,3',4,4',5-pcnb Lipid Adj (pg/g)						
Values of PCB126_p Were Replaced by Ranks	N Obs	N	Mean	Std Dev	Minimum	Maximum
0	69	69	4.8884058	1.1786753	2.2000000	7.6000000
1	233	233	6.8781116	1.7103619	2.2000000	9.2000000
2	231	231	11.3268398	1.2002058	9.3000000	13.2000000
3	227	227	16.7229075	2.0231852	13.3000000	20.6000000
4	236	236	53.2084746	51.8971759	20.7000000	341.0000000

Observations were fairly evenly distributed across PCB 126 quartiles, with 69 observations below the LOD. Fourth quartile values were skewed due to outliers with higher PCB 126 levels, resulting in the much higher values.

Figure 4.2 Mean serum carotenoid concentrations across PCB 126 quartiles



*p < 0.01

The above graph tracks the serum carotenoid concentrations of participants with metabolic syndrome (red) and participants without metabolic syndrome (blue) across increasing exposure quartiles of PCB 126 by rank. Significant associations were seen at the first and fourth quartiles ($p < 0.01$) for participants with higher serum carotenoid concentrations and a reduced probability of metabolic syndrome. Observations within the same PCB quartile would have had similar PCB concentrations in their serum. Serum carotenoid concentrations trended upward for participants without metabolic syndrome despite increasing concentrations of PCB 126, suggesting a protective influence. The carotenoid concentrations of participants with metabolic syndrome revealed no meaningful pattern.

Table 4.5 Multivariate Regression: PCB 126 and the probability of metabolic syndrome

Analyte	Not detectable	<25th percentile	25th-<50th percentile	50th-<75th percentile	>75th percentile	P_{trend}	$P_{covariate}$
Concentration (ng/g of lipid)	4.89/1.18	6.88/1.71	11.32/1.2	16.72/2.02	53.21/51.89		
Cases / n	8/69	22/233	28/231	45/227	56/236		
Prevalence (%)	10.39	8.63	10.81	16.54	19.18		
Model 0	referent	0.9 (0.4-2.3)	1.0 (0.4-2.5)	1.9 (0.8-4.6)	2.1 (0.9-5.1)	0.02*	
Model 1 – age	referent	0.9 (0.3-2.2)	0.9 (0.4-2.4)	1.8 (0.7-4.2)	1.7 (0.7-4.1)	0.04*	0.05
Model 2 – gender	referent	0.9 (0.4-2.4)	1.1 (0.4-2.6)	2.0 (0.8-4.8)	2.3 (0.9-5.5)	0.01*	0.11
Model 3 – race/ethnicity	referent	1.0 (0.4-2.5)	1.1 (0.5-2.7)	2.0 (0.8-5.0)	2.3 (1.0-5.4)	0.01*	0.28
Model 4 – PIR	referent	0.7 (0.3-1.8)	1.0 (0.4-2.5)	2.0 (0.8-4.9)	2.0 (0.9-4.8)	<0.01*	0.26
Model 5 – cigarette smoking	referent	0.9 (0.4-2.3)	1.2 (0.5-2.9)	2.3 (1.0-5.6)	2.6 (1.1-6.1)	<0.01*	<0.01
Model 6 – serum cotinine	referent	0.9 (0.4-2.3)	1.0 (0.4-2.5)	1.9 (0.8-4.5)	2.1 (0.9-5.0)	0.02*	0.54
Model 7 – alcohol consumption	referent	4.0 (0.4-39.2)	7.1 (0.7-68.8)	8.2 (0.9-76.1)	15.4 (1.8-135.2)	0.06*	0.35
Model 8 – leisure physical activity	referent	0.9 (0.3-2.2)	1.0 (0.4-2.4)	1.9 (0.8-4.5)	1.9 (0.8-4.4)	0.02*	0.02
Model 9 - BMI	referent	0.7 (0.3-1.8)	0.8 (0.3-1.9)	1.4 (0.6-3.4)	1.5 (0.6-3.5)	0.01	<0.01

*quadratic trend

The demographic and behavioral characteristics thought to be involved in the etiology of metabolic syndrome were examined above. Carotenoids were not considered in this analysis. The p-trend for all models was found to be significant, indicating the trend across quartiles for PCB 126 and covariate showed significant associations in the probability of metabolic syndrome. However, only BMI had a linear trend. All other models showed a quadratic trend. Alcohol consumption revealed much larger adjusted odds ratios and confidence intervals, suggesting an interaction. P-covariate modeling, which considered the effect of the covariate alone, indicated that cigarette smoking, leisure time physical activity, and BMI showed significant associations with metabolic syndrome. Age was found to be borderline significant (p = 0.05); however, age has shown a strong independent association with metabolic syndrome in cross-sectional studies. Notably, the PCB 126 model had a below LOD reference group, which allowed for adjusted odds ratio comparisons across four quartiles, unlike PCB 118 and PCB 153.

Table 4.6 Analysis of PCB 126 and alcohol consumption

Table of PCB126_p by alcohol				
PCB126_p(Values of PCB126_p Were Replaced by Ranks)	Alcohol			
Frequency, Percent Row Pct, Col Pct	.	non-drinker	yes	Total
0	9 2.67 39.13 11.11	12 3.56 52.17 5.31	2 0.59 8.70 6.67	23 6.82
1	24 7.12 32.88 29.63	42 12.46 57.53 18.58	7 2.08 9.59 23.33	73 21.66
2	10 2.97 14.49 12.35	58 17.21 84.06 25.66	1 0.30 1.45 3.33	69 20.47
3	16 4.75 19.51 19.75	63 18.69 76.83 27.88	3 0.89 3.66 10.00	82 24.33
4	22 6.53 24.44 27.16	51 15.13 56.67 22.57	17 5.04 18.89 56.67	90 26.71
Total	81 24.04	226 67.06	30 8.90	337 100.00
Frequency Missing = 3279				

An additional analysis of PCB 126 and alcohol consumption was done to further understand any possible effect between these variables. The overall number of participants responding to the questions related to alcohol consumption, including the reference group, was 30. The high adjusted odds ratios and confidence intervals found in Table 4.7 were related to small sample sizes within the PCB 126 quartiles and an excessively high frequency of missing responses (n = 3279), rather than any meaningful interaction between these variables.

Table 4.7 PCB 126 concentration as a continuous variable in the probability of metabolic syndrome

Model	PCB 126	$P_{covariate}$
Model 0	0.02	
Model 1 – age	0.24	0.02
Model 2 – gender	0.01	0.14
Model 3 – race/ethnicity	0.01	0.20
Model 4 – PIR	0.02	0.46
Model 5 – cigarette smoking	0.01	<0.01
Model 6 – serum cotinine	0.02	0.4
Model 7 – alcohol consumption	0.19	0.26
Model 8 – leisure-time physical activity	0.09	0.03
Model 9 - BMI	0.03	<0.01

PCB 126 was examined as a continuous variable rather than as a categorical variable in quartile analysis. Significance was observed for several covariates in modeling with PCB 126 in the probability of developing metabolic syndrome. Model 0 indicated that PCB 126 had a significant association with metabolic syndrome without consideration of other covariate influence. The CDC's Fourth National Report on Human Exposure to Environmental Chemicals 2009 has recommended quartile analysis for studies of environmental chemical contamination.

C. PCB 153 – 2,2',4,4',5,5'-Hexachlorobiphenyl

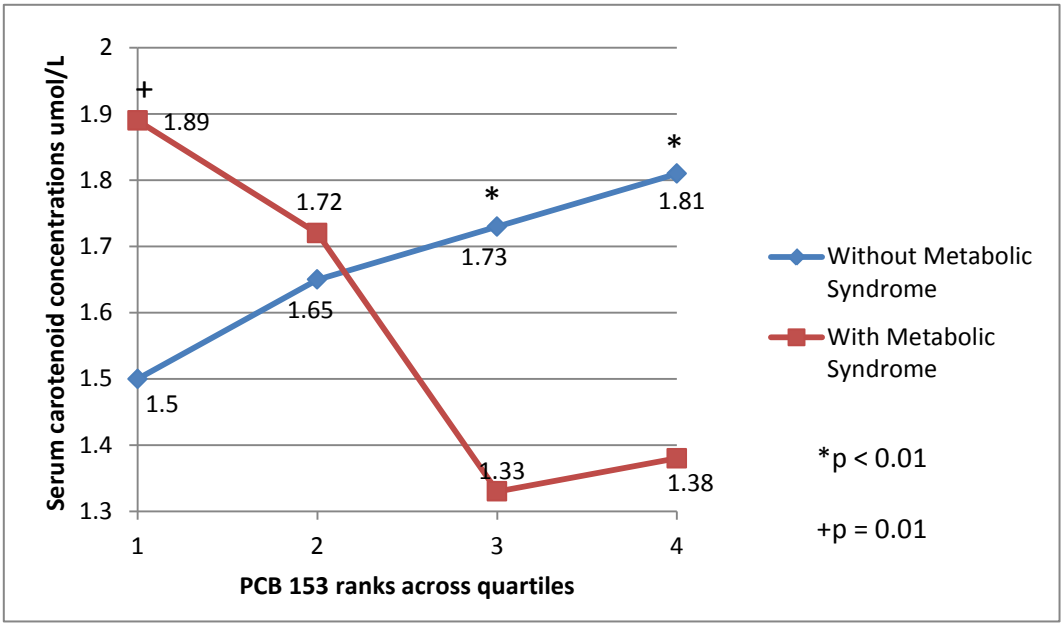
PCB 153 status was categorized by rank; n = 921. No observations were found below the LOD for PCB 153 in the 2003-2004 data release.

Table 4.8 PCB 153 rank analysis by quartile

Analysis Variable : LBX153LA PCB153 Lipid Adj (ng/g)						
Values of PCB153_p Were Replaced by Ranks	N Obs	N	Mean	Std Dev	Minimum	Maximum
1	230	230	5.6653913	1.5173859	1.0500000	7.8600000
2	230	230	11.2623913	2.2049157	7.9000000	15.3000000
3	231	231	23.7300433	6.2078795	15.3600000	36.4000000
4	230	230	77.7783913	57.7951485	36.5000000	546.0000000

The number of observations in each quartile was nearly identical. The mean, minimum, and maximum do not reflect actual pollutant concentrations. A ranking system, as used in similar studies (Ha, et al. 2007; Cave, 2010), was applied. The mean and maximum in the highest quartile was indicative of outliers, a common occurrence with environmental pollutant measures.

Figure 4.3 Mean serum carotenoid concentrations across PCB 153 quartiles



The above graph tracks the mean serum carotenoid concentrations of participants with metabolic syndrome (red) and participants without metabolic syndrome (blue) across increasing exposure quartiles of PCB 153 by rank. Significant associations were seen at the third and fourth quartiles ($p < 0.01$) for participants with higher serum carotenoid concentrations and a reduced probability of metabolic syndrome. A reverse trend was seen at the first quartile with higher serum carotenoid concentrations being associated with metabolic syndrome at significant levels ($p = 0.01$). Carotenoids appear to be dramatically protective at higher levels of serum PCB 153.

Table 4.9 Multivariate Regression: PCB153 and the probability of metabolic syndrome

Analyte	<25th percentile	25th-<50th percentile	50th-<75th percentile	>75th percentile	P_{trend}	$P_{covariate}$
Concentration (ng/g of lipid)	5.67/1.52	11.26/2.20	23.73/6.21	77.78/57.80		
Cases / n	32/230	31/230	34/231	56/230		
Prevalence (%)	12.21	11.88	12.83	19.58		
Model 0	referent	0.8 (0.5-1.5)	0.6 (0.3-1.2)	1.6 (0.9-2.7)	0.03	
Model 1 – age	referent	0.7 (0.4-1.3)	0.4 (0.2-0.8)	0.7 (0.4-1.5)	0.56	<0.01
Model 2 – gender	referent	0.8 (0.4-1.5)	0.6 (0.4-1.2)	1.5 (0.9-2.6)	0.03	0.38
Model 3 – race/ethnicity	referent	0.8 (0.4-1.5)	0.6 (0.3-1.2)	1.6 (0.9-2.9)	0.02	0.12
Model 4 – PIR	referent	0.8 (0.4-1.5)	0.7 (0.4-1.2)	1.7 (1.0-3.0)	0.02	0.90
Model 5 – cigarette smoking	referent	0.8 (0.5-1.5)	0.6 (0.3-1.2)	1.6 (0.9-2.7)	0.03	<0.01
Model 6 – serum cotinine	referent	0.8 (0.4-1.5)	0.6 (0.3-1.2)	1.5 (0.9-2.6)	0.03	0.59
Model 7 – alcohol consumption	referent	1.0 (0.3-3.6)	0.8 (0.2-2.5)	2.1 (0.7-6.2)	0.26	0.47
Model 8 – leisure physical activity	referent	0.8 (0.5-1.5)	0.6 (0.3-1.1)	1.4 (0.8-2.4)	0.07	0.06
Model 9 - BMI	referent	0.8 (0.4-1.5)	0.7 (0.4-1.3)	1.6 (1.0-2.8)	0.02	<0.01

The demographic and behavioral characteristics thought to be involved in the etiology of metabolic syndrome were examined above. Carotenoids were not considered in this analysis. The first quartile of PCB 153 was used as reference group as no observations were detected below the LOD. The p-trend for most models was significant, indicating the trend across quartiles for PCB 153 and covariate showed significant associations in the probability of developing metabolic syndrome. The lack of a reference group below the LOD in this analysis may be expected to impact findings as trends were compared to the first quartile, ultimately allowing only for tertile analysis of the adjusted odds ratios. P-covariate, which considers only the covariate's influence (in absence of PCB 153), indicated that age, cigarette smoking, and BMI, had a significant effect on the probability of developing metabolic syndrome.

Table 4.10 PCB 153 concentration as a continuous variable in the probability of metabolic syndrome

Model	PCB 153	$P_{covariate}$
Model 0	0.03	
Model 1 – age	0.67	0.02
Model 2 – gender	0.03	0.26
Model 3 – race/ethnicity	0.02	0.17
Model 4 – PIR	0.02	0.99
Model 5 – cigarette smoking	0.02	<0.01
Model 6 – serum cotinine	0.03	0.49
Model 7 – alcohol consumption	0.05	0.42
Model 8 – leisure-time physical activity	0.11	0.05
Model 9 - BMI	0.01	<0.01

When examined as a continuous variable, PCB 153 was significant in several covariate models in the probability of developing metabolic syndrome. Age, which is known to have a strong independent association with metabolic syndrome, was not indicated as being significant in modeling with PCB 153. When considered in absence of PCB 153, age did show a significant association ($p = 0.02$). Cigarette smoking and BMI were significantly associated with the probability of developing metabolic syndrome ($p < 0.01$). When evaluated as a continuous variable, PCB 153 was shown to have a significant association with the probability of metabolic syndrome. Continuous and categorical/quartile analysis showed similar findings. Quartile analysis has been recommended by the CDC Fourth National Report on Human Exposure to Environmental Chemicals 2009 (CDC National Report).

Project II. PCB 118, PCB 126, PCB 153, and fruit consumption

The purpose of this project was to correlate dietary intake patterns with serum carotenoid findings in Project I. NHANES examines food and fluid intakes individually in the Individual Foods File, and cumulatively for the day, in the Total Nutrients File. Carotenoids obtained from foods and fluids can be accessed from both files. Carotenoids are found in the colorful pigments of fruits and vegetables. The objective of this project was to determine total fruit consumption using the U.S.D.A. interfaced databases for analysis (1)MyPyramid Equivalents Database 2.0 (MPED 2.0) (Bowman, Friday, Moshfegh 2008), and/or(2) Food and Nutrient Database for Dietary Studies 2.0 (FNDDS 2.0) (USDA FNDDS 2.0 2006).

The MPED 2.0 converts the dietary intake data from NHANES 2003-2004 into "cup equivalents" or "ounce equivalents" per 100 grams, as based on the U.S.D.A. National Nutrient Database for Standard Reference, Release 18. The resulting portions were often both counter-intuitive and exceeded nutritional recommendations for a serving. The MPED 2.0 fruit group was divided into two subgroups; the vegetable group was divided into six. Subgroups were relevant, in particular for vegetables, as they included starchy vegetables, not especially rich in carotenoids.

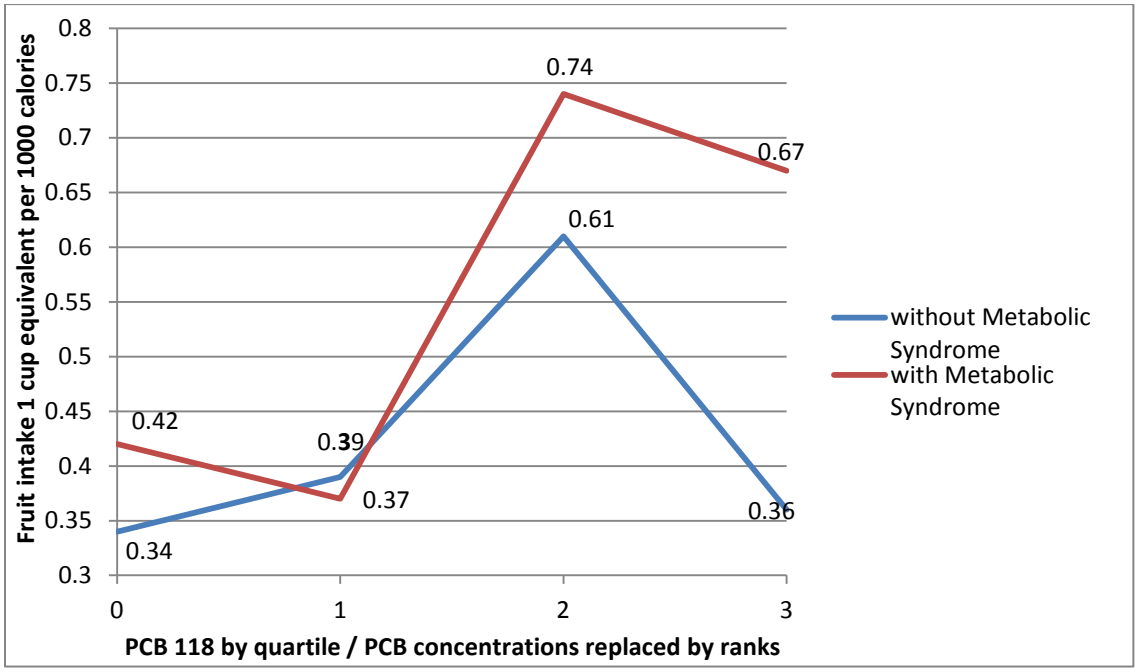
MPED 2.0 was utilized for fruit consumption. Data was extracted from NHANES 2003-2004 Individual Foods File, Day 1 and Day 2 diet recalls. Total fruit consumption was determined by summing intakes (cup equivalents) over two days and factoring per 1000 kcals. This disengaged the analysis from the participants' overall (or recommended) calorie intake, i.e. a ratio of nutrient density per total calories, to analysis of the nutrient density of diet based on fruit consumption per participant per 1000 calories.

PCBs 118, 126, and 153 were assessed individually as in Project 1, utilizing subsample C weight and adhering to all other NHANES tutorial instructions. The response variable was metabolic syndrome, and a positive finding of metabolic syndrome was met by three or more ATPIII criteria.

A. PCB 118 - 2,3',4,4',5-Pentachlorobiphenyl

PCB 118 status was categorized by rank across quartiles; n = 917. No observations were found below the LOD for PCB 118 in the 2003-2004 data release.

Figure 4.4 Mean fruit intake per 1000 calories across PCB 118 quartiles and the probability of metabolic syndrome



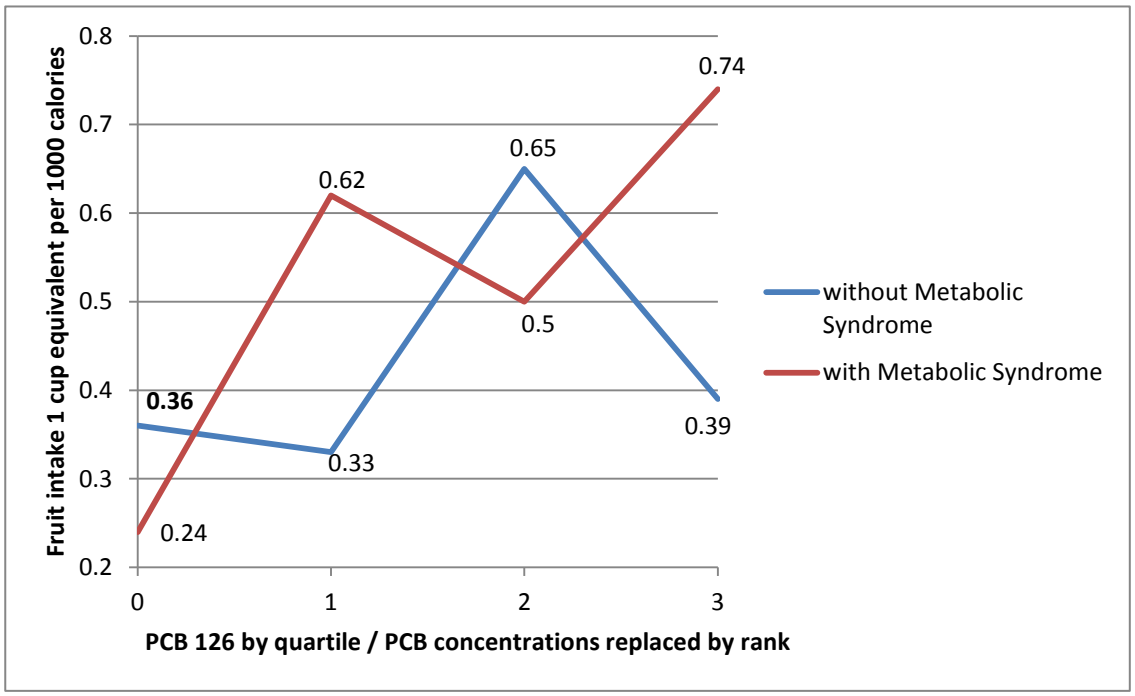
No associations were observed, no significance found. The mean fruit intake of participants with metabolic syndrome was shown to be higher at the first, third, and fourth quartiles. Consideration was given to the balance of the diet, which was unknown at this time, as well as the MPED 2.0 method of disaggregating foods. Hence, a 10% fruit juice product would be considered as one-tenth of a fruit cup equivalent.

The remaining 90% of the product, which may be sugar, water, alcohol, or other additions, would not be considered in the analysis at all. Last, two days of dietary recalls may not reflect usual intakes. Occasional or acute intakes would not accurately reflect cardiometabolic risks related to diet.

B. PCB 126 - 3,3',4,4',5-Pentachlorobiphenyl

PCB 126 status was categorized by rank across quartiles; n = 996.

Figure 4.5 Mean fruit intake per 1000 calories across PCB 126 quartiles and the probability of metabolic syndrome

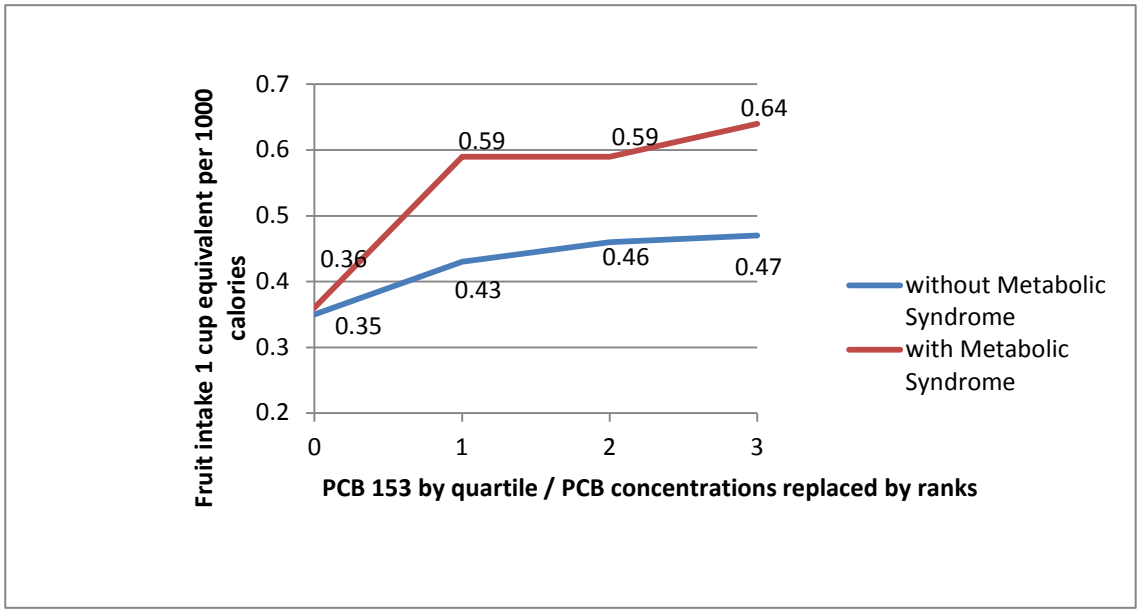


No associations were observed; no significance found. The lack of any meaningful pattern, while troubling, may be attributed to many factors yet unknown about the entirety of the diet, or other relevant demographic or behavioral factors.

C. PCB 153 – 2,2',4,4',5,5'-Hexachlorobiphenyl

PCB 153 status was categorized by rank; n = 921.

Figure 4.6 Mean fruit intake per 1000 calories across PCB 153 quartiles and the probability of metabolic syndrome



No significant associations were seen in analysis of PCB 153, fruit intake based on MPED 2.0 cup equivalents per 1000 calories, and the likelihood of developing metabolic syndrome. However, for this non-dioxin-like PCB, participants with higher fruit intake were consistently shown to have a greater likelihood of having metabolic syndrome.

D. Log transformed regression analysis. Associations between independent variables: PCB 118, PCB 126, PCB 153, and serum carotenoids, with carotenoids sourced from the Individual Foods Files (IFF) and Total Nutrients Files (TNF)

Table 4.11 Correlations between serum variables and food-sourced carotenoids

Model	Food-sourced log(food+0.01)	p-value		Nutrient-sourced Log(nutr+0.01)	p-value	
PCB 118 (log)	α-carotene	0.25	No correlation	α-carotene	0.15	No correlation
	β-carotene	0.31	No correlation	β-carotene	0.25	No correlation
	β-cryptoxanthin	0.78	No correlation	β-cryptoxanthin	0.56	No correlation
	Lycopene	0.40	No correlation	Lycopene	0.51	No correlation
	Lutein/zeaxanthin	0.54	No correlation	Lutein/zeaxanthin	0.40	No correlation
	Total carotenoid	0.38	No correlation	Total carotenoid	0.63	No correlation
PCB 126 (log)	α-carotene	0.18	No correlation	α-carotene	0.19	No correlation
	β-carotene	0.22	No correlation	β-carotene	0.28	No correlation
	β-cryptoxanthin	0.85	No correlation	β-cryptoxanthin	0.73	No correlation
	Lycopene	0.38	No correlation	Lycopene	0.42	No correlation
	Lutein/zeaxanthin	0.51	No correlation	Lutein/zeaxanthin	0.59	No correlation
	Total carotenoid	0.94	No correlation	Total carotenoid	0.99	No correlation
PCB 153 (log)	α-carotene	0.21	No correlation	α-carotene	0.09	No correlation
	β-carotene	0.64	No correlation	β-carotene	0.37	No correlation
	β-cryptoxanthin	0.87	No correlation	β-cryptoxanthin	0.61	No correlation
	Lycopene	0.30	No correlation	Lycopene	0.37	No correlation
	Lutein/zeaxanthin	0.83	No correlation	Lutein/zeaxanthin	0.70	No correlation
	Total carotenoid	0.21	No correlation	Total carotenoid	0.49	No correlation
Total serum carotenoids	α-carotene	<0.01	Ec=0.027	α-carotene	<0.01	Ec=0.026
	β-carotene	<0.01	Ec=0.092	β-carotene	<0.01	Ec=0.090
	β-cryptoxanthin	<0.01	Ec=0.039	β-cryptoxanthin	<0.01	Ec=0.036
	Lycopene	<0.01	Ec=0.034	Lycopene	<0.01	Ec=0.028
	Lutein/zeaxanthin	<0.01	Ec=0.122	Lutein/zeaxanthin	<0.01	Ec=0.114
	Total carotenoid	<0.01	Ec=0.116	Total carotenoid	<0.01	Ec=0.117

Ec = Estimated coefficient

In models of PCB 118, PCB 126, and 153, no correlation was found between the PCBs and carotenoids from either NHANES dietary data file. Carotenoids sourced from foods can be accessed from: (1) the Individual Foods File (IFF) ("food-sourced"), or (2) the Total Nutrients File (TNF) ("nutrient-sourced").

A significant correlation was found in modeling of total serum carotenoids and carotenoids from the individual foods files and the total nutrients files. The dietary recall portion of NHANES represents two days of intake at approximately seven to ten days apart. Only participants with two days of complete and reliable dietary recalls were included in the dataset. The values were summed and divided by "2" to represent average intake over two days. Serum carotenoid concentrations were assessed once at the MEC physical examination. A positive correlation was shown for all significant models and provided internal validation of subjective dietary intake data with the serum carotenoid concentrations.

Project III. Diet Quality Analysis for Toxin Exposure, DQATE

The purpose of this project was to create an instrument that could be used to score food and fluid intakes of participants with environmental chemical contamination exposures. Comparing the dietary intakes of participants with similar PCB concentrations, but very different clinical outcomes, may provide an important first step in the development of nutritional recommendations for individuals exposed to PCBs, as well as the other halogenated organics and POPs. The primary objective of this project was to develop an instrument to score diet quality, DQATE. Ancillary objectives were to (1) review existing diet quality indices; (2) review current dietary recommendations for adequacy and maintenance of health, as well as those in place for modulation of specific disease; and (3) to determine the best method to access the full diet of NHANES 2003-2004 subsample C participants (those evaluated for PCB concentrations) for analysis using DQATE.

Dietary quality may be based on several criteria. Have the dietary reference intakes been met? Have the prevailing recommendations of an oversight body been met? Has the instrument been validated, the findings reproduced, are they related to risk reduction? Dietary recommendations for the public generally reflect the consensus of an expert panel and are based on their conclusions about the relationship of diet to specific health parameters with regard to risk reduction (Cronin, et al. 1987) or with the intent to address population requirements for nutrient adequacy (IOM 2000) by determining the needs of 50% of the healthy population where possible, plus two standard deviations.

The diet indices reviewed included Healthy Eating Index (HEI) 1995 (Bowman, et al., 1998), Healthy Eating Index-Revised (HEI-R) 2005 (Guenther, et al., 2005), Diet Quality Index (Seymour, et al. 2003), and Diet Quality Index Revised (DQI-R) (Haines, et al. 1999). Five modified diet indices were examined in relation to plasma markers of

inflammation and endothelial dysfunction (Fung, et al. 2005). In addition to the HEI and DQI-R, these indices included an Alternate HEI, Alternate Mediterranean Diet Index (aMED), and the Recommended Food Score (RFS). Similar intake patterns were associated with reduced concentrations of inflammation and endothelial dysfunction.

The health maintenance/adequacy dietary recommendations reviewed included Dietary Guidelines 2005 and 2010, DASH, My Pyramid (Britton, et al. 2006; Marcoe, et al. 2006), and the Mediterranean Diet (Gavrilla, et al. 2011). A cursory categorization of the prevailing nutritional recommendations by system involvement, i.e. cardiac, vascular, metabolic, immune, and for the reduction of persistent organic pollutant body burden follows.

Table 4.12 Current nutritional recommendations by system involvement

System Involvement	Program/Governing Body	Recommendations
Cardiac	NCEP ATPIII	Saturated fat < 7% PUFA≤ 10% MUFA ≤ 20% Dietary cholesterol < 200 mg/day Plant stanols/sterols 2 g/day Dietary fiber 20-30 g/day ≥ 3 vegetables/day - ≥ 1 dark green or orange ≥ 2 fruits/day Carbohydrate – 50-60% Protein ~15% Total fat – 25-35% Expend ≥ 200 kcal/day physical activity
Vascular	NHLBI, DASH Diet	Saturated fat - 6% Dietary cholesterol – 150 mg. Dietary fiber – 30 g/day ~9 fruits & vegetables (plentiful) Carbohydrate – 55% Protein – 18% Total fat – 27% Low fat dairy, nuts, seeds, legumes
Metabolic	ADA Position Statement: Standards of Care for the Prevention, Delay, & Management of Diabetes Mellitus	Saturated fat < 7% total calories 14 grams fiber/1000 calories Carbohydrate management per guidelines of ≥ 130 g/day Meet DRIs No supplementation of C, E, carotene
	ATPIII Guidelines Metabolic Syndrome	Total fat – 35% Total carbohydrate – 50% Total protein – 15%
	Gerald Reaven, MD, Insulin Resistance Diet	45% carbohydrate 15% protein 40% fat – mostly unsaturated 5-10% saturated fat 5-10 svg. Fruits & vegetables/day Calcium supplement
Immune	AICR's Food, Nutrition, Physical Activity, & the Prevention of Cancer: a Global Perspective	Eat mostly plant-based foods - Low in energy density - High in micronutrients, fiber
Minimize body burden	Undetermined	Maintenance of healthy weight* High fiber diet* Augment meals with Olestra®* Aerobic activity*

*These recommendations have not undergone randomized controlled clinical trials.

Table 4.13 The Dietary Approaches to Stop Hypertension (DASH) Eating Plan—Number of Food Servings by Calorie Recommendation & per 1000 calories

Food Group	1,200 Cal.	1,400 Cal.	1,600 Cal.	1,800 Cal.	2,000 Cal.	2,600 Cal.	3,100 Cal.
Per 1000 kcal	**1,200/1.2**	**1,400/1.4**	**1,600/1.6**	**1,800/1.8**	**2,000/2.0**	**2,600/2.6**	**3,100/3.1**
Grains[a]	4–5	5–6	6	6	6–8	10–11	12–13
Grains: servings per 1000 kcal (1/2 whole grain)	3.33-4.17 (1.67-2.08)	3.57-4.29 (1.79-2.14)	3.75 (1.88)	3.33 (1.67)	3.0-4.0 (1.5-2.0)	3.85-4.23 (1.93-2.12)	3.87-4.19 (1.94-2.1)
Vegetables	3–4	3–4	3–4	4–5	4–5	5–6	6
Vegetables: servings per 1000 kcal e	2.5-3.33	2.14-2.86	1.88-2.5	2.22-2.78	2.0-2.5	1.92-2.31	1.94
Fruits f	3–4	4	4	4–5	4–5	5–6	6
Fruits: servings per 1000 kcal (1/2 whole fruit)	2.5-3.33 (1.25-1.67)	2.86 (1.43)	2.5 (1.25)	2.22-2.78 (1.11-1.39)	2.0-2.5 (1.0-1.25)	1.92-2.31 (0.96-1.16)	1.94 (0.97)
Fat-free or low-fat milk and milk products[b]	2–3	2–3	2–3	2–3	2–3	3	3–4
Fat-free/low-fat dairy: servings per 1000 kcal	1.67-2.5	1.43-2.14	1.25-1.88	1.11-1.67	1.0-1.5	1.15	0.97-1.29
Lean meats, poultry, and fish	3 or less	3–4 or less	3–4 or less	6 or less	6 or less	6 or less	6–9
Lean meats, poultry, & fish: servings per 1000 calories	≤2.5	≤2.14-2.86	≤1.88-2.5	≤3.33	≤3.0	≤2.31	1.94-2.90
Nuts, seeds, and legumes	3 per week	3 per week	3–4 per week	4 per week	4–5 per week	1	1
Nuts, seeds, & legumes, servings per 1000 calories	2.5 (0.36)	2.14 (0.31)	1.88-2.5 (0.27-0.36)	2.22 (0.32)	2.0-2.5 (0.29-0.36)	(0.38)	(0.32)
Fats and oils[c]	1	1	2	2–3	2–3	3	4
Fats and oils, servings per 1000 calories	0.83	0.71	1.25	1.11-1.67	1.0-1.5	1.15	1.29
Sweets and added sugars	3 or less per week	3 or less per week	3 or less per week	5 or less per week	5 or less per week	≤2	≤2
Maximum sodium limit[d]	2,300 mg/day	2,300 mg/day	2,300 mg/day	2,300 mg/day	2,300 mg/day	2,300 mg/day	2,300 mg/day

A Whole grains are recommended for most grain servings as a good source of fiber and nutrients.

B For lactose intolerance, try either lactase enzyme pills with milk products or lactose-free or lactose-reduced milk.

C Fat content changes the serving amount for fats and oils. For example, 1 Tbsp regular salad dressing = one serving; 1 Tbsp low- fat dressing = one-half serving; 1 Tbsp fat-free dressing = zero servings.

D The DASH eating plan consists of patterns with a sodium limit of 2,300 mg and 1,500 mg per day.

E No starchy vegetables included as vegetable servings – potatoes, corn, lima beans, legumes.

F No more than ½ fruit servings from juice.

Table 4.14 Diet Quality Analysis for Toxin Exposure (DQATE)

Dietary Recommendation	Score	Cutpoint
Total fat intake at ≤ 30% total calories	2 1 0	≤ 30% 30-40% >40%
Saturated fat intake at ≤ 7% total calories	2 1 0	≤ 7% 7.01 – 10% ≥10%
Dietary cholesterol intake at < 200 mg/day	2 1 0	<200 mg. 200-300 mg. >300 mg.
Dietary fiber intake at 14 grams/1000 calories	2 1 0	≥14 grams 10 < 14 grams < 10 grams
Grain, cereal servings ≥ 3.0 ounce equivalents per 1000 calories	2 1 0	≥3.0 per 1000 kcal 1.5-2.9 per 1000 kcal <1.5 per 1000 kcal
Grain, cereal servings *from whole grain* ≥ 1.5 ounce equivalents per 1000 calories	2 1 0	≥ 1.5 per 1000 kcal 0.75-<1.5 per 1000 kcal <0.75 per 1000 kcal
Vegetable servings at 1.9 cup equivalents per 1000 calories	2 1 0	≥1.9 per 1000 kcal 0.95-1.9 per 1000 kcal <0.95 kcal per 1000 kcal
Dark green vegetable servings at 0.15 cup equivalents per 1000 calories	2 1 0	≥0.15 per 1000 kcal 0.07-<0.15 per 1000 kcal <0.07 per 1000 kcal
Orange & red vegetable servings at 0.6 cup equivalents per 1000 calories	2 1 0	≥0.6 per 1000 kcal 0.3-<0.6 per 1000 kcal <0.3 per 1000 kcal
Fruit servings at 1.9 cup equivalents per 1000 calories	2 1 0	≥1.9 per 1000 kcal 0.95-1.9 per 1000 kcal <0.95 kcal per 1000 kcal
Milk & milk products at 1.0 cup equivalents per 1000 calories	2 1 0	≥1.0 per 1000 kcal 0.1-0.99 per 1000 kcal 0 cup equivalents
Lean meats, poultry, & fish at 2.0 ounce equivalents per 1000 calories	2 1 0	≥2.0 per 1000 kcal 1.0-1.99 per 1000 kcal <1.0 oz. per 1000 kcal
Nuts, seeds, & legumes at 0.27 ounce equivalents per 1000 calories	2 1 0	≥0.27 per 1000 kcal 0.1-0.26 per 1000 kcal 0 ounce equivalents
Maximum sodium limit 1,500 mg/day	2 1 0	≤1,500 mg/day 1,501-3,600 mg/day >3,600 mg/day
Added sugars & alcohol, ≤ 20% total calories	2 1 0	≤20% total calories >29-35% total calories >35% total calories

Project IV. PCBs, Serum Carotenoids, and the Probability of Metabolic Syndrome: Covariate Modeling

A. Baseline and Lifestyle Characteristics

The study subpopulation of 1058 men and women was taken from the MEC subsample C NHANES 2003-2004 population, who were individuals tested for subsample C pollutants. These pollutants included, but were not limited to, PCBs. Participants were 20 years of age or older, did not have type 1 or 2 diabetes mellitus, completed two days of reliable and complete dietary data collection, and provided sufficient clinical parameters for a determination of metabolic syndrome. Demographic and lifestyle characteristics are categorized below. As shown below, prevalence of MetS was 22.1%.

Table 4.15 Baseline Characteristics

Gender	Frequency	Percentage
male	516	48.77
female	542	51.23

Race	Frequency	Percentage
Mexican American	197	18.62
Other Hispanic	37	3.50
Non-Hispanic White	605	57.18
Non-Hispanic Black	176	16.64
Other Race – including multi-racial	43	4.06

Age	Frequency	Percentage
20 – 34	303	28.64
35 – 49	279	26.37
50 – 64	218	20.60
65 – 79	182	17.20
>= 80	76	7.18

PIR	Frequency	Percentage
Above (>= 1)	857	84.27
Below (< 1)	160	15.73

BMI	Frequency	Percentage
underweight	18	1.71
healthy	331	31.37
overweight	366	34.69
obese	340	32.22

MetS	Frequency	Percentage
MetS+	234	22.12
MetS-	824	77.88

Table 4.16 Characteristics by Metabolic Syndrome Status

Baseline & Lifestyle Characteristics by Metabolic Syndrome Status		MetS+ N=234	MetS- N=824
Gender	Female	106	436
	Male	128	388
Race/ethnicity	Mexican American	45	152
	Other Hispanic	9	28
	Non-Hispanic White	137	468
	Non-Hispanic Black	32	144
	Other Race – Including Multi-Racial	11	32
Cigarette smoking	Never	109	436
	Former	46	186
	Current	79	201
Serum Cotinine	≥ 0.015 ng/mL	187	649
	<0.015 ng/ml	47	173
Alcohol consumption	Non-drinker	63	139
	Non-excessive drinker	81	321
	Excessive drinker	50	221
Physical activity	Sedentary (no physical activity)	61	233
	Low activity	28	100
	Moderately to vigorously active	34	184
BMI	<18.5	0	18
	18.5-24.9	32	299
	25.0-29.9	91	275
	≥30	110	230
Dietary supplement	yes	121	436
	no	113	387

Table 4.17 Mean Analysis of Serum Nutrients by Metabolic Syndrome Status

Nutrient Analyte umol/L	Without metabolic syndrome Mean	With metabolic syndrome Mean
Age at Screening	46.866	55.641
Family Poverty Income Ratio	2.716	2.602
Alpha-carotene	0.0831683	0.0634927
Alpha-cryptoxanthin	0.0492606	0.0405013
Beta-carotene	0.3776834	0.3105232
Beta-cryptoxanthin	0.1764506	0.1552558
Lycopene	0.7738627	0.6761464
Lutein/zeaxanthin	0.2936455	0.2688734
Alpha-tocopherol	31.5914209	36.4841017
Delta-tocopherol	0.1530406	0.2054309
Gamma-tocopherol	5.1014411	6.4090137
Retinyl palmitate	0.0413989	0.0478064
Retinyl stearate	0.0132151	0.0145120
Retinol	2.0141357	2.1117940
Vitamin C	57.9829448	52.6510823
Vitamin D (ng/mL)	23.4453883	21.7435897
Vitamin B6 (nmol/L)	69.9550617	51.6043103
Vitamin B12 (pmol/L	446.8441677	372.110042
Folate, serum (nmol/L)	32.3224787	30.5752137

B. Mean associations between serum carotenoids, PCBs, and metabolic syndrome: covariate modeling

NHANES dietary recall data have a dedicated sample weight that is substantially larger than the environmental pollutant subsample C weight or the fasting blood work weight. While tutorials instructed to use the smallest sample weight, they also instructed to use the dietary recall weight preferentially. Further, they stated that using too large a sample weight, especially with data having outliers (common with both environmental pollutants and dietary intake data), would likely distort results. This dissertation project utilizes several NHANES 2003-2004 subpopulations with dedicated weights, as well as some similar and disparate characteristics. A review of the relevant literature informed of a procedure for applying an unweighted estimation using the same covariates that had been used to construct the sample weights (Graubard & Korn 1999).

A comparison of the mean serum carotenoid concentrations was executed to seek associations between participants with and without metabolic syndrome. While similar to earlier data runs, these differed in two significant ways. First, three covariate modeling was used in lieu of subsample C weight. Second, pooled PCB subclasses and total PCBs were assessed rather than three individual congeners, PCBs 118, 126, and 153.

Table 4.18 Non-dioxin-like PCBs, serum carotenoids, & the probability of metabolic syndrome

Non_dioxin-like PCBs		1st quartile	2nd quartile	3rd quartile	4th quartile
n = (without/with MetS)		119/21	195/40	265/73	256/88
Prevalence		15.0%	17.0%	21.6%	25.6%
p-value		0.2009	0.1345	0.0007	0.0141
mean	Metabolic syndrome (0 – 1)	-0.27	0.18	0.35	0.25
	without metabolic syndrome	1.80	1.92	1.93	1.89
	With metabolic syndrome	2.08	1.74	1.58	1.63
median	Metabolic syndrome (0 – 1)	-0.10	0.14	0.38	0.12
	without metabolic syndrome	1.85	1.85	1.85	1.68
	With metabolic syndrome	1.95	1.71	1.47	1.56

Mean analysis of serum carotenoid concentrations within the four exposure quartiles of non-dioxin-like PCBs highlight differences between individuals with and without metabolic syndrome. Persons with higher concentrations of serum carotenoids were less likely to have MetS and this was significant at higher exposures in the third and four quartiles. Higher concentrations of serum carotenoids, i.e. higher fruit and vegetable intake, were significantly associated with a lower probability of metabolic syndrome in the third and fourth quartiles of non-dioxin-like PCBs. The first exposure quartile was associated with an increased risk of metabolic syndrome, a finding that was predominant throughout the study.

Figure 4.7 Probability of metabolic syndrome across non-dioxin-like PCB quartiles in relation to serum carotenoid concentrations in umol/L

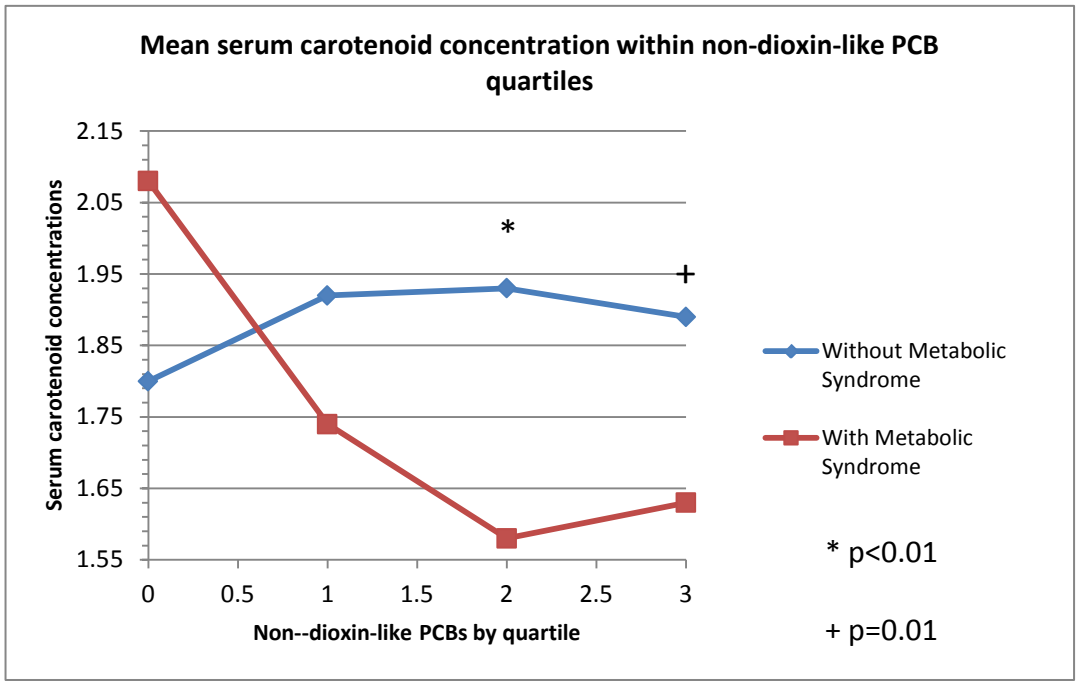


Table 4.19 Dioxin-like PCBs, serum carotenoids and probability of metabolic syndrome

Dioxin-like PCBs		1st quartile	2nd quartile	3rd quartile	4th quartile
n = without/with MetS		118/20	202/37	287/69	246/100
Prevalence		14.5%	15.5%	19.4%	28.9%
mean	p-value	0.0746	0.0816	0.0159	0.0010
	Metabolic syndrome (0 – 1)	-0.30	0.23	0.27	0.32
	without metabolic syndrome	1.74	1.86	1.93	1.94
	With metabolic syndrome	2.04	1.63	1.67	1.62
median	Metabolic syndrome (0 – 1)	-0.20	0.41	0.29	0.15
	without metabolic syndrome	1.65	1.84	1.82	1.72
	With metabolic syndrome	1.85	1.43	1.53	1.57

Mean analysis of serum carotenoid concentrations within the four exposure quartiles of dioxin-like PCBs reveal differences between individuals with and without metabolic syndrome. Persons with higher concentrations of serum carotenoids were less likely to have metabolic syndrome and this was significant at higher exposures in the third and four quartiles. Higher concentrations of serum carotenoids, i.e. higher fruit and vegetable intake, were significantly associated with a lower probability of metabolic syndrome in the third and fourth quartiles of dioxin-like PCBs.

Figure 4.8 Probability of metabolic syndrome across dioxin-like PCB quartiles in relation to serum carotenoid concentrations in umol/L

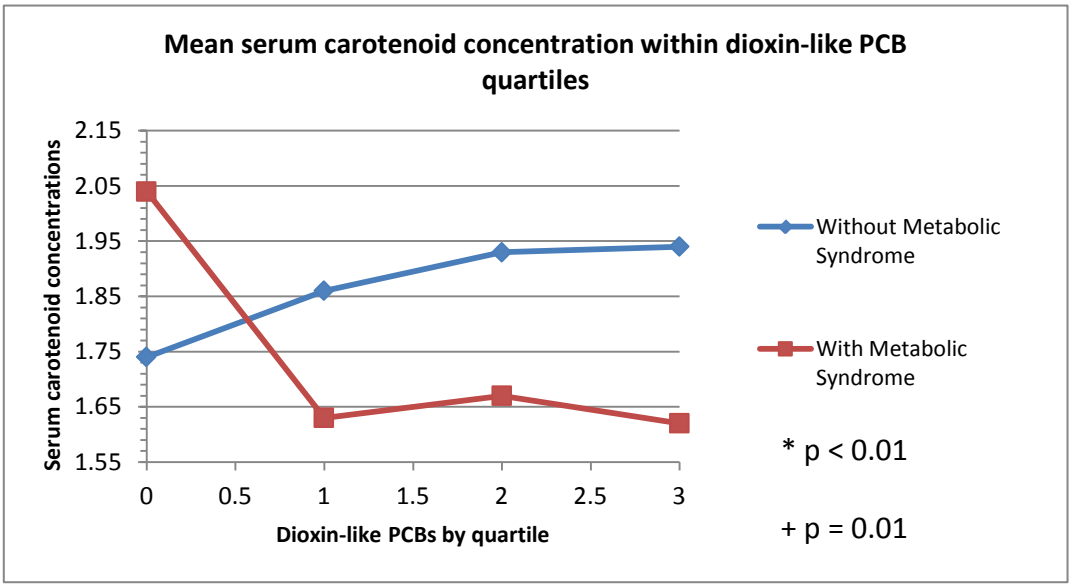
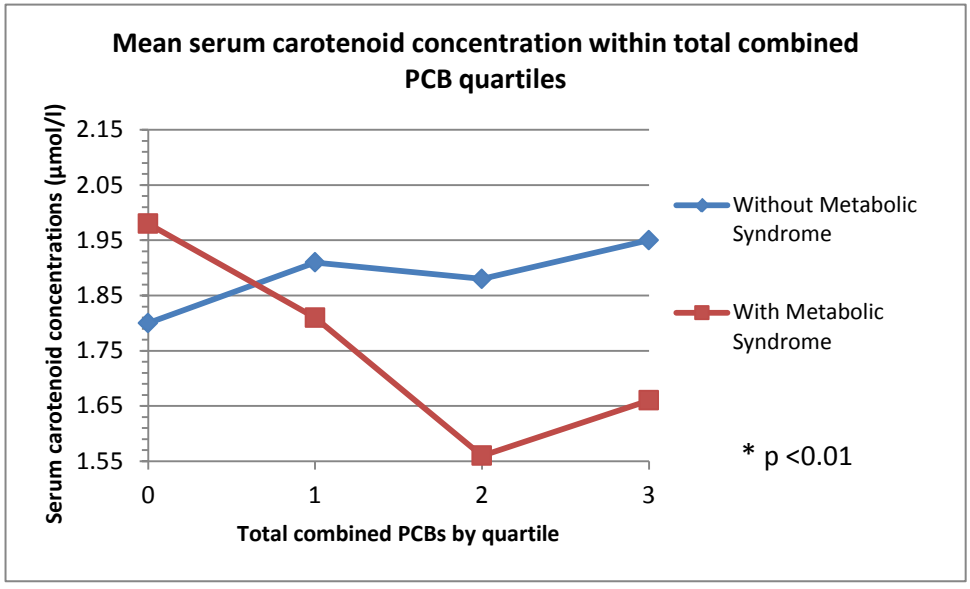


Table 4.20 Combined total PCBs, serum carotenoids and probability metabolic syndrome

	Combined total PCBs	1st quartile	2nd quartile	3rd quartile	4th quartile
	n = without/with MetS	108/18	204/37	269/81	254/86
	Prevalence	14.3%	15.4%	23.1%	25.3%
mean	p-value	0.4579	0.4438	0.0012	0.0066
	Metabolic syndrome (0 – 1)	-0.18	0.10	0.33	0.28
	without metabolic syndrome	1.80	1.91	1.88	1.95
	With metabolic syndrome	1.98	1.81	1.56	1.66
median	Metabolic syndrome (0 – 1)	0.08	0.07	0.38	0.14
	without metabolic syndrome	1.84	1.81	1.81	1.72
	With metabolic syndrome	1.76	1.74	1.43	1.58

Mean analysis of serum carotenoid concentrations within the four exposure quartiles of total pooled PCBs reveal differences between individuals with and without metabolic syndrome. Persons with higher concentrations of serum carotenoids were less likely to have metabolic syndrome and this was significant at higher exposures in the third and four quartiles ($p < 0.01$) of combined total PCBs. The first exposure quartile was associated with an increased risk of metabolic syndrome.

Figure 4.9 Probability of metabolic syndrome across combined total PCB quartiles in relation to serum carotenoid concentrations in umol/L



C. Logistical Regression: Serum Nutrients, Polychlorinated Biphenyls

Individual associations were sought between various serum nutrients and metabolic syndrome. Variables were adjusted for age, race, and poverty income ratio as a surrogate to NHANES sample weighting.

Table 4.21 Logistical regression of serum nutrients on the probability of metabolic syndrome

Nutrients	Coefficient	p-value
Serum carotenoids	0.3540	0.0004
Vitamin E (α, δ, γ-tocopherols)	-0.0159	0.0009
Vitamin A (retinol,retinyl-esters)	-0.1008	0.4411
Vitamin C	0.00833	0.0016
Vitamin D	0.0286	0.0036
Vitamin B6	0.00538	0.0002
Pooled carotenoids, Vitamins C & E	0.00260	0.2047
Pooled carotenoids, A, C, E, D, B6	0.00368	0.0002

Combined serum carotenoids were strongly associated with a reduced risk of metabolic syndrome at $p = 0.0004$. Regression coefficients for pooled Vitamins A and E indicate that greater concentrations of these two vitamins were associated with an increased risk of developing metabolic syndrome, although only the tocopherols were significant at $p < 0.05$. Significant associations were shown for other serum nutrients and a reduced probability of metabolic syndrome, i.e. Vitamin C, Vitamin D, and Vitamin B6. Carotenoids, Vitamins C and E have been thought to work synergistically as antioxidants. Neither association nor significance was observed in this analysis with metabolic syndrome as the response variable. When all of the above nutrients were pooled, however, significance was shown at $p = 0.0002$. The strongest coefficient overall was indicated with pooled serum carotenoids.

Individual associations were sought between serum levels of PCBs and metabolic syndrome. Variables were adjusted for age, race, and poverty income ratio as a surrogate to NHANES sample weighting.

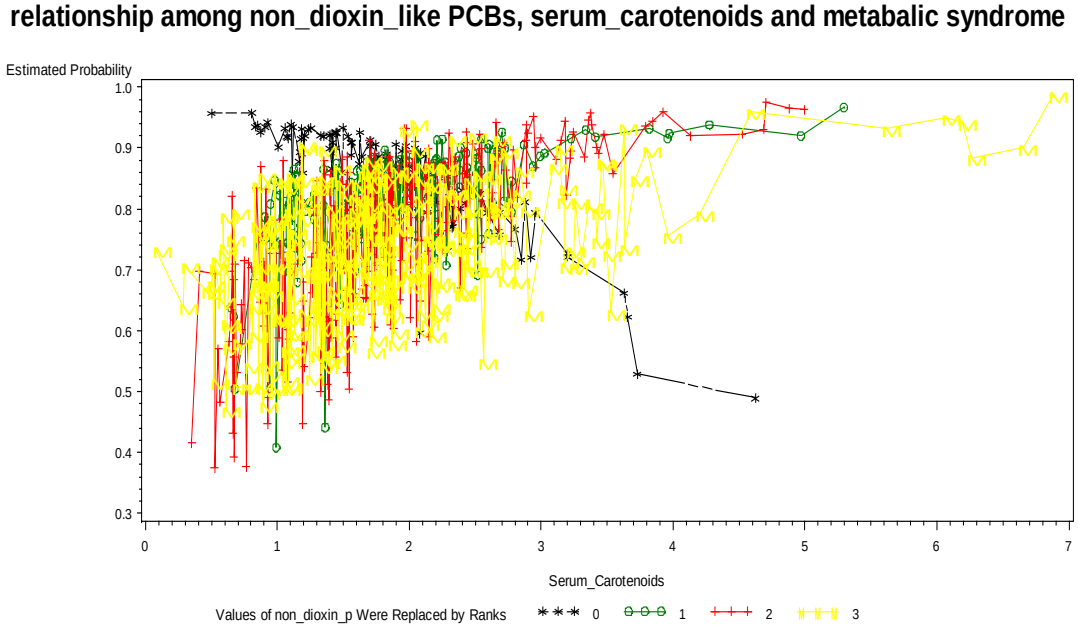
Table 4.22 Logistical regression of serum PCBs on the probability of metabolic syndrome

Serum PCBs by Subclass	p-value
Non-dioxin-like PCBs	0.5597
Dioxin-like PCBs	0.9932
Combined total PCBs	0.2377

Individual PCB congeners were not evaluated in this data set and may well have yielded different results. Coplanar and mono-ortho-substituted PCBs, which exhibit partial coplanarity, were combined into "Dioxin-like PCBs". Neither PCB subclass, nor pooled total PCBs, were significantly associated with metabolic syndrome in this analysis. The toxicology of PCBs is well documented (ATSDR, Toxicological Profile for PCBs). Earlier studies have documented the damaging effects of PCBs in relation to cardiac, vascular, and metabolic diseases (Chen, et al. 2008; Codru, et al. 2007; Ha, et al. 2007 & 2009; Hennig, et al. 2001 & 2005; Lee, et al. 2006, 2007a,b,c, 2010, 2001a,b; Lind, et al. 2004; Rignell-Hydbom, et al. 2007; Ruzzin, et al. 2010; Rylander, et al. 2005; Turyk, et al. 2006 & 2009; Uemura, et al. 2009; & Vasiliu, et al. 2006). Some of these studies utilized earlier NHANES releases in which smaller blood volumes were drawn, yielding more observations below the LOD, and a reference group important for multivariate regression analysis across quartiles. Other factors may account for this lack of statistical significance that could not be identified from logistical regression. For these reasons, a statistical interaction between serum PCBs and serum carotenoids was sought for significance and any other relevant factors.

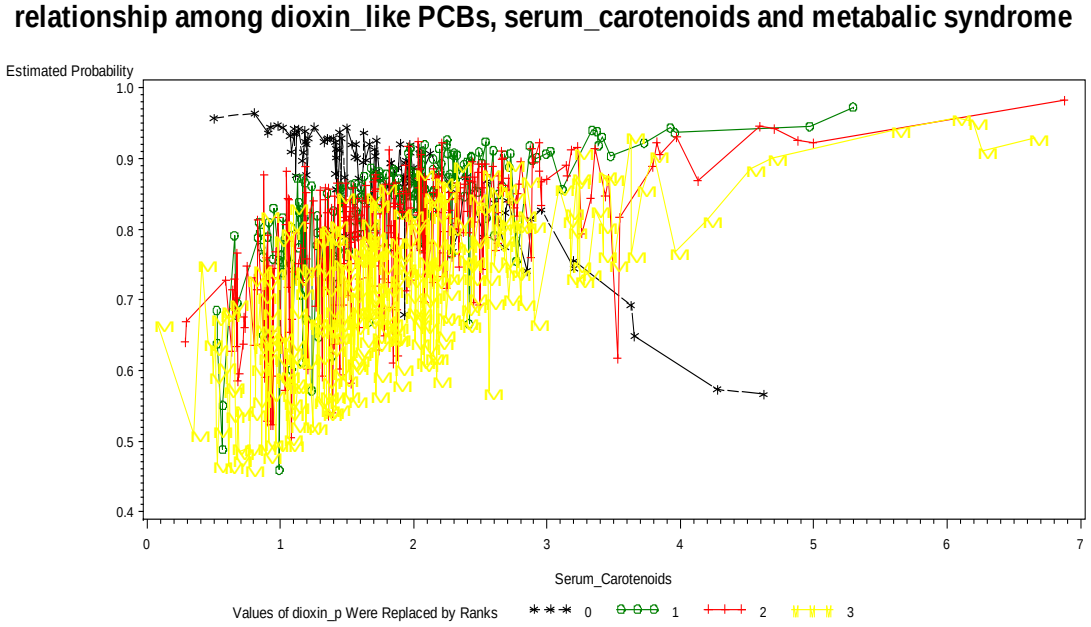
D. Interaction Plots: PCBs, serum carotenoids, and their combined associations with metabolic syndrome

Figure 4.10 Non-dioxin-like PCBs, serum carotenoids, and the probability of metabolic syndrome



The y-axis above represents the probability of *not* developing metabolic syndrome. The x-axis represents increasing serum carotenoid concentrations as a continuous variable. It should be clear that only the first PCB quartile, represented by a black line, characterizes a somewhat dramatic increase in the probability of metabolic syndrome. The second, third, and fourth quartiles, shown in green, red, and yellow, respectively, clearly demonstrate a reduced probability of metabolic syndrome as serum concentrations of carotenoids increase. Serum carotenoids were associated with a reduction in the probability of metabolic syndrome at higher PCB concentrations in this analysis.

Figure 4.11 Dioxin-like PCBs, serum carotenoids, and the probability of metabolic syndrome



As observed with non-dioxin-like PCBs above, the first quartile of dioxin-like PCBs was associated with an increased probability of developing metabolic syndrome. The second, third, and fourth exposure quartiles were associated with a reduced probability of metabolic syndrome as serum carotenoid concentrations increased. Notably, this occurred at higher PCB quartiles, which relate to higher serum PCB concentrations. The first PCB quartile represented the lowest exposure category above the 60% LOD. In this analysis, carotenoids were associated with a reduced likelihood of developing metabolic syndrome at higher PCB concentrations.

E. Mean analysis: Individual Carotenoids / PCB Subclasses

In this analysis, PCBs were evaluated by subclass: non-dioxin-like, dioxin-like, and total, combined PCBs; and each quartile was evaluated individually. Each serum carotenoid was analyzed individually. Differences between the means of those with and without metabolic syndrome were compared across quartiles for each carotenoid. An increase in the probability of metabolic syndrome was observed in the first PCB quartile in twelve of eighteen cases, although those findings were significant only once, in the first quartile of lycopene for dioxin-like PCBs (p = 0.03). A significant benefit was seen most often in the third and/or fourth exposure quartiles and in the "overall" row, which represented the sum of all quartiles for combined PCB congeners, suggesting a protective influence at higher exposures.

Table 4.23 Mean distinctions between Alpha-carotene and PCBs

Alpha-Carotene		mean without MetS	mean with MetS	difference (0 – 1)	p-value
Overall		0.08	0.06	0.02	0.0004
non dioxin like	1st quartile	0.06	0.07	-0.01	0.7448
	2nd quartile	0.08	0.07	0.01	0.5775
	3rd quartile	0.08	0.06	0.02	0.0436
	4th quartile	0.10	0.06	0.04	0.0008
dioxin like	1st quartile	0.06	0.07	-0.01	0.6822
	2nd quartile	0.07	0.05	0.02	0.1720
	3rd quartile	0.09	0.07	0.02	0.1397
	4th quartile	0.09	0.06	0.03	<0.0001
combined	1st quartile	0.06	0.06	0	0.9422
	2nd quartile	0.07	0.07	0	0.9370
	3rd quartile	0.08	0.06	0.02	0.0588
	4th quartile	0.10	0.06	0.04	0.0002

Table 4.24 Mean distinctions between Alpha-cryptoxanthin and PCBs

Alpha-Cryptoxanthin		mean without MetS	mean with MetS	difference (0 – 1)	p-value
Overall		0.05	0.04	0.01	<0.0001
non dioxin like	1st quartile	0.06	0.05	0.01	0.2270
	2nd quartile	0.05	0.04	0.01	0.0001
	3rd quartile	0.05	0.04	0.01	0.0005
	4th quartile	0.05	0.04	0.01	0.0009
dioxin like	1st quartile	0.05	0.04	0.01	0.0641
	2nd quartile	0.05	0.04	0.01	<0.0001
	3rd quartile	0.05	0.04	0.01	0.0352
	4th quartile	0.05	0.04	0.01	<0.0001
combined	1st quartile	0.05	0.04	0.01	0.0555
	2nd quartile	0.05	0.04	0.01	0.0056
	3rd quartile	0.05	0.04	0.01	0.0015
	4th quartile	0.05	0.04	0.01	0.0005

Table 4.25 Mean distinctions between total beta-carotene and PCBs

Total beta-carotene		mean without MetS	mean with MetS	difference (0 – 1)	p-value
Overall		0.37	0.31	0.01	0.0099
Non dioxin like	1st quartile	0.28	0.33	-0.05	0.6157
	2nd quartile	0.30	0.23	0.07	0.0397
	3rd quartile	0.35	0.27	0.08	0.0093
	4th quartile	0.46	0.38	0.08	0.1132
Dioxin like	1st quartile	0.25	0.33	-0.08	0.4303
	2nd quartile	0.29	0.21	0.08	0.1037
	3rd quartile	0.36	0.29	0.07	0.0457
	4th quartile	0.46	0.35	0.11	0.0171
Combined PCBs	1st quartile	0.28	0.30	-0.02	0.7795
	2nd quartile	0.29	0.24	0.05	0.1765
	3rd quartile	0.34	0.27	0.07	0.0145
	4th quartile	0.48	0.38	0.10	0.0671

Table 4.26 Mean distinctions between beta-cryptoxanthin and PCBs

Beta-cryptoxanthin		mean without MetS	mean with MetS	difference (0 – 1)	p-value
Overall		0.18	0.15	0.03	0.0108
Non dioxin like	1st quartile	0.19	0.22	-0.03	0.6059
	2nd quartile	0.19	0.16	0.03	0.1433
	3rd quartile	0.17	0.14	0.03	0.0084
	4th quartile	0.16	0.15	0.01	0.1942
Dioxin like	1st quartile	0.17	0.18	-0.01	0.8412
	2nd quartile	0.18	0.14	0.04	0.0840
	3rd quartile	0.18	0.16	0.02	0.3469
	4th quartile	0.18	0.14	0.04	0.0263
Combined PCBs	1st quartile	0.19	0.16	-0.03	0.3733
	2nd quartile	0.19	0.19	0	0.9266
	3rd quartile	0.17	0.14	0.03	0.0067
	4th quartile	0.17	0.15	0.02	0.1560

Table 4.27 Mean distinctions between Lycopene and PCBs

Total Lycopene		mean without MetS	mean with MetS	difference (0 – 1)	p-value
Overall		0.76	0.68	0.08	0.0006
Non dioxin like	1st quartile	0.79	0.91	-0.13	0.1073
	2nd quartile	0.84	0.80	0.04	0.5861
	3rd quartile	0.80	0.67	0.13	0.0119
	4th quartile	0.65	0.60	0.05	0.1753
Dioxin like	1st quartile	0.79	0.96	-0.17	0.0362
	2nd quartile	0.81	0.77	0.04	0.6186
	3rd quartile	0.79	0.66	0.13	0.0064
	4th quartile	0.68	0.63	0.05	0.1798
Combined PCBs	1st quartile	0.79	0.94	-0.15	0.0841
	2nd quartile	0.84	0.82	0.02	0.7303
	3rd quartile	0.78	0.66	0.12	0.0107
	4th quartile	0.67	0.61	0.06	0.1619

Table 4.28 Mean distinctions between lutein/zeaxanthin and PCBs

Combined lutein/zeaxanthin		mean without MetS	mean with MetS	difference (0 – 1)	p-value
Overall		0.29	0.27	0.02	0.0171
Non dioxin like	1st quartile	0.26	0.29	-0.03	0.4861
	2nd quartile	0.28	0.26	0.02	0.2164
	3rd quartile	0.30	0.26	0.04	0.0073
	4th quartile	0.32	0.27	0.05	0.0323
Dioxin like	1st quartile	0.26	0.25	0.01	0.7522
	2nd quartile	0.28	0.25	0.03	0.1390
	3rd quartile	0.29	0.29	0	0.8655
	4th quartile	0.33	0.26	0.07	0.0008
Combined PCBs	1st quartile	0.26	0.27	-0.01	0.8224
	2nd quartile	0.28	0.26	0.02	0.2556
	3rd quartile	0.29	0.26	0.03	0.0294
	4th quartile	0.32	0.28	0.04	0.0188

F. First Quartile Interactions: Individual carotenoids / PCB subclasses

The analysis below examines three models: non-dioxin-like PCBs, dioxin-like PCBs, and combined total PCBs. Within each model, regression analysis assesses the effect of an individual carotenoid, individual PCB subclass, and the interaction between individual serum carotenoids and PCB subclasses. A significant interaction between serum carotenoid and PCB concentrations implies a significant PCB effect. Relationships involving PCBs are complex and analysis of their effects may be more clearly determined by evaluating several models for continuity. The negative coefficient indicates an inverse relationship between serum carotenoids and the probability of developing metabolic syndrome.

Table 4.29 First quartile analysis of alpha-carotene : PCB interactions

Variable	p-value
Non-dioxin-like PCBs	
Non-dioxin-like PCBs	0.9644
Alpha-Carotene (coefficient = -2.5856)	0.0436
Alpha-Carotene *Non-dioxin-like PCBs	0.0862
Dioxin-like PCBs	

Dioxin-like PCBs	0.3830
Alpha-Carotene (coefficient = -2.7521)	0.0341
Alpha-Carotene *Dioxin-like PCBs	0.0332
Combined PCBs	
Combined PCBs	0.9922
Alpha-Carotene (coefficient = 2.6563)	0.0423
Alpha-Carotene *Combined PCBs	0.0499

variable	p-value
Non-dioxin-like PCBs	
Non-dioxin-like PCBs	0.4979
Alpha-Cryptoxanthin (coefficient = -20.1596)	<0.0001
Alpha-Cryptoxanthin *Non-dioxin-like PCBs	0.6442
Dioxin-like PCBs	
Dioxin-like PCBx	0.1650
Alpha-Cryptoxanthin (coefficient = -22.0729)	0.0001
Alpha-Cryptoxanthin *Dioxin-like PCBs	0.1743
Combined PCBs	
Combined PCBs	0.7444
Alpha-Cryptoxanthin (coefficient = -21.3296)	0.0001
Alpha-Cryptoxanthin *CombinedPCBs	0.9171

variable	p-value
Non-dioxin-like PCBs	
Non-dioxin-like PCBs	0.1299
Total beta-carotene (coefficient = -0.9229)	0.0189
Total beta-carotene *Non-dioxin-like PCBs	0.0421
Dioxin-like PCBs	
Dioxin-like PCBs	0.2778
Total beta-carotene (coefficient = -0.6808)	0.0910
Total beta-carotene *Dioxin-like PCBs	0.0666
Combined PCBs	
Combined PCBs	0.2183
Total beta-carotene (coefficient = -0.7139)	0.0540
Total beta-carotene *Combined PCBs	0.2283

Significant interactions were seen between alpha-carotene and dioxin-like PCBs ($p = 0.03$), alpha-carotene and combined PCBs ($p = 0.049$), beta-carotene and non-dioxin-like PCBs ($p = 0.04$). Near significant interactions were seen between beta-carotene and dioxin-like PCBs ($p = 0.06$) and alpha-carotene and non-dioxin-like PCBs ($p = 0.08$). Beta-cryptoxanthin, lycopene, and lutein/zeaxanthin lacked significance and results were not included in this section.

G. PCB & carotenoid interactions: Modeling of serum variables

PCBs were examined by subclass with pooled and individual serum carotenoids to seek significant interactions using three models:

1) PCBs in four quartiles and in interaction with serum carotenoids;
2) PCBs as summed ranks on a continuous scale in interaction with serum carotenoids;
3) Quadratic (squared) PCBs on a continuous scale in interaction with serum carotenoids.

Table 4.30 Statistical Interactions: Pooled serum carotenoids

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * SC	PCB (continuous) * SC	PCB (continuous) * SC	PCB(continuous) *PCB(continuous) *SC
Non-dioxin-like PCBs	0.6402	0.8270	0.1936	0.1803
Dioxin-like PCBs	0.9105	0.9402	0.0131	0.0133
Combined PCBs	0.3401	0.9242	0.0846	0.0800

Table 4.31 Statistical Interactions: Alpha-carotene

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * AC	PCB (continuous) * AC	PCB (continuous) * AC	PCB(continuous) *PCB(continuous) *AC
Non-dioxin-like PCBs	0.5090	0.2559	0.8517	0.9766
Dioxin-like PCBs	0.0771	0.0689	0.2994	0.4715
Combined PCBs	0.2728	0.0417	0.2571	0.3969

Table 4.32 Statistical Interactions: Beta-carotene

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * BC	PCB (continuous) * BC	PCB (continuous) * BC	PCB(continuous) *PCB(continuous) *BC
Non-dioxin-like PCBs	0.3546	0.1933	0.5650	0.6692
Dioxin-like PCBs	0.3439	0.1265	0.4155	0.2873
Combined PCBs	0.4743	0.3124	0.5193	0.4269

Table 4.33 Statistical Interactions: Lycopene

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * Lycopene	PCB (continuous) * Lycopene	PCB (continuous) * Lycopene	PCB(continuous) *PCB(continuous) *Lycopene
Non-dioxin-like PCBs	0.2486	0.6802	0.0559	0.0491
Dioxin-like PCBs	0.6793	0.4742	0.1309	0.1071
Combined PCBs	0.5843	0.3873	0.1919	0.1573

H. Five covariate modeling: Carotenoids

A five covariate model was applied to evaluate the additional effects of covariates BMI and total cholesterol. PCBs and carotenoids concentrate to lipids, including serum lipids and adipose tissue. BMI is independently associated with metabolic syndrome. Several individual PCBs lacked observations below a 60% LOD, which eliminated a reference group. The prevalent first quartile trend yielded a non-monotonic curve that suggested low dose toxicity, yet resisted a linear dose-response finding in the p-values. The first quartile was eliminated in this analysis due to a confounding effect on understanding overall relationships.

Table 4.34 Five covariate modeling of PCB subclasses and serum carotenoids for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * SC	PCB (continuous) * SC	PCB (continuous) * SC	PCB(continuous) *PCB(continuous) *SC
Non-dioxin-like PCBs	0.5190	0.7166	0.1793	0.1600
Dioxin-like PCBs	0.9588	0.8372	0.0171	0.0154
Combined PCBs	0.2979	0.7544	0.0992	0.0880

Table 4.35 Five covariate modeling of PCB subclasses and alpha-cryptoxanthin for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * α-cryptoxanthin	PCB (continuous) * α-cryptoxanthin	PCB (continuous) * α-cryptoxanthin	PCB(continuous) *PCB(continuous) * α-cryptoxanthin
Non-dioxin-like PCBs	0.1220	0.5916	0.3720	0.4070
Dioxin-like PCBs	0.4398	0.2175	0.0281	0.0447
Combined PCBs	0.1706	0.4949	0.3243	0.3739

Table 4.36 Five Covariate modeling of PCB subclasses and beta-cryptoxanthin for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Models 3 & 4	
	PCB (quartile) * B-cryptoxanthin	PCB (continuous) * B-cryptoxanthin	PCB (continuous) * B-cryptoxanthin	PCB(continuous) *PCB(continuous) *B-cryptoxanthin
Non-dioxin-like PCBs	0.2649	0.8078	0.0851	0.0781
Dioxin-like PCBs	0.2200	0.4888	0.0015	0.0018
Combined PCBs	0.1191	0.9946	0.0300	0.0295

Table 4.37 Five Covariate modeling of PCB subclasses and total lycopene for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Models 3 & 4	
	PCB (quartile) * Lycopene	PCB (continuous) * Lycopene	PCB (continuous) * Lycopene	PCB(continuous) *PCB(continuous) *Lycopene
Non-dioxin-like PCBs	0.1282	0.4926	0.0477	0.0364
Dioxin-like PCBs	0.6549	0.3690	0.0938	0.0698
Combined PCBs	0.3712	0.2580	0.1670	0.1215

Significance in the above models was revealed when PCBs were observed in all three models – quartile, continuous, and quadratic, with the first quartile removed. The dioxin-like-PCB subclass showed significant interactions with pooled serum carotenoids, alpha-cryptoxanthin, and beta-cryptoxanthin. Non-dioxin-like PCBs showed significant interactions with lycopene. The two classes combined showed significant interactions with beta-cryptoxanthin. The remaining carotenoids analyzed, alpha-carotene, beta-carotene, and lutein/zeaxanthin lacked significance overall in this set and were not included above.

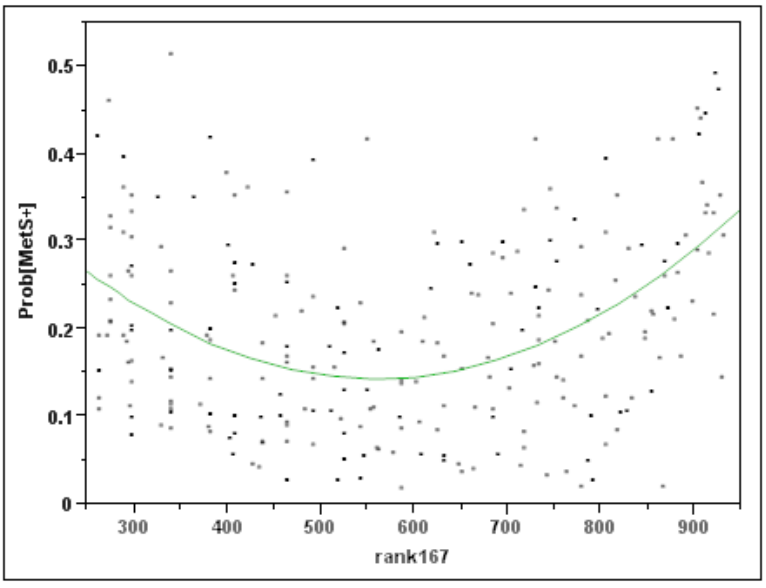
I. Expanded interaction analysis: dioxin-like PCBs & serum carotenoids

Table 4.38 Expanded Interaction Analysis: Continuous quadratic modeling of dioxin-like PCBs and serum carotenoids

PCB	Model 3	
	PCB (continuous) * SC	PCB(continuous) *PCB(continuous) *SC
PCB 28	0.0372	0.0396
PCB 66	0.9063	0.8322
PCB 74	0.0116	0.0128
PCB 126	0.4830	0.5253
PCB 169	0.0778	0.0802
PCB 105	0.2030	0.1844
PCB 118	0.1111	0.1052
PCB 156	0.3719	0.3359
PCB157	0.1425	0.1337
PCB 167	0.0049	0.0071

J. Serum carotenoids ≥ 2.0 µmol/L are associated with a reduced probability of metabolic syndrome at median rank 600 PCB 167

Figure 4.12 Bivariate fit of the probability of metabolic syndrome by rank of PCB 167 and serum carotenoids at or above the mean concentration of 2.0 µmol/L.



Polynomial Fit Degree=2

Summary of Fit

RSquare	0.188419
RSquare Adj	0.181926
Root Mean Square Error	0.099474
Mean of Response	0.197141
Observations (or Sum Wgts)	253

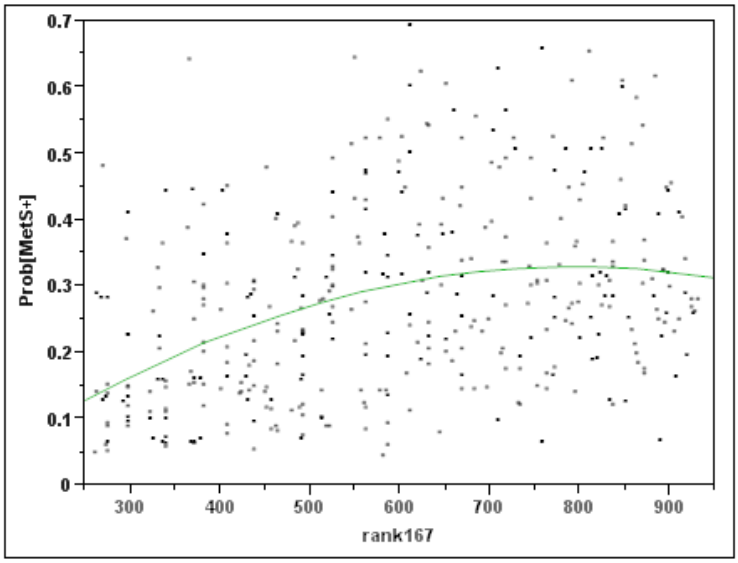
Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	0.5743135	0.287157	29.0203
Error	250	2.4737549	0.009895	**Prob > F**
C. Total	252	3.0480684		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.1096053	0.019986	5.48	<.0001*
rank167	5.9163e-5	3.073e-5	1.92	0.0554
(rank167-582.692)^2	1.278e-6	1.76e-7	7.26	<.0001*

Figure 4.13 Bivariate fit of the probability of metabolic syndrome by rank of PCB 167 and serum carotenoids below the mean concentration of 2.0 µmol/L.



Polynomial Fit Degree=2

Polynomial Fit Degree=2
Prob[MetS+] = 0.1429183 + 0.0002679*rank167 - 6.8297e-7*(rank167-596.14)^2

Summary of Fit

RSquare	0.150928
RSquare Adj	0.146574
Root Mean Square Error	0.132847
Mean of Response	0.277668
Observations (or Sum Wgts)	393

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Ratio
Model	2	1.2234711	0.611736	34.6625
Error	390	6.8828611	0.017648	**Prob > F**
C. Total	392	8.1063323		<.0001*

Parameter Estimates

Term	Estimate	Std Error	t Ratio	Prob>|t|
Intercept	0.1429183	0.023096	6.19	<.0001*
rank167	0.0002679	0.000035	7.65	<.0001*
(rank167-596.14)^2	-6.83e-7	2.027e-7	-3.37	0.0008*

The first figure above (Figure 4.12) shows high serum carotenoid concentrations, above the median of 2.0 umol/L. At approximately rank = 600 for PCB 167, the probability of metabolic syndrome diminishes to about 15% then rises to about 30% by rank 900. The second graph represents low serum carotenoid concentrations, below the median concentration of 2.0 umol/L. At rank 600, the probability of metabolic syndrome rises to 30% and stays there until rank = 900. Comparing the two graphs, it is apparent that a participant in the median of the graph (rank = 600, ≈ 700) has two times the risk of metabolic syndrome with low serum carotenoid concentrations than the participant with high serum carotenoid concentrations. Further, this elevated risk was observed nearly to rank 900, where it began to taper off and the benefit of carotenoids appeared less pronounced. The trajectory of above-median and below-median carotenoid curves were entirely counter to each other, suggesting a pronounced influence of the carotenoids.

K. Five Covariate modeling: Tocopherols and Vitamin C

Table 4.39 Five Covariate modeling of PCB subclasses and serum tocopherols for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * Vitamin E	PCB (continuous) * Vitamin E	PCB (continuous) * Vitamin E	PCB(continuous) *PCB(continuous) *Vitamin E
Non-dioxin-like PCBs	0.1866	0.0767	0.6789	0.5017
Dioxin-like PCBs	0.0644	0.0251	0.1970	0.3180
Combined PCBs	0.2805	0.0427	0.9480	0.7235

Table 4.40 Five Covariate modeling of PCB subclasses and serum Vitamin C for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * Vitamin C	PCB (continuous) * Vitamin C	PCB (continuous) * Vitamin C	PCB(continuous) *PCB(continuous) *Vitamin C
Non-dioxin-like PCBs	0.0336	0.0288	0.7587	0.9988
Dioxin-like PCBs	0.0077	0.0086	0.1031	0.2046
Combined PCBs	0.1326	0.0178	0.7042	0.9705

Table 4.41 Five Covariate modeling of PCB subclasses and combined serum carotenoids, Vitamin C, and tocopherols for significant interactions: 1st quartile eliminated

PCB subclasses	Model 1	Model 2	Model 3	
	PCB (quartile) * SC+VC+VE	PCB (continuous) * SC+VC+VE	PCB (continuous) * SC+VC+VE	PCB(continuous) *PCB(continuous) * SC+VC+VE
Non-dioxin-like PCBs	0.0514	0.0388	0.9283	0.7042
Dioxin-like PCBs	0.0090	0.0135	0.1088	0.2017
Combined PCBs	0.2234	0.0265	0.8946	0.8546

Dioxin-like PCBs were shown to have significant interactions with vitamin E, vitamin C, and combined carotenoids + vitamin C + vitamin E, but not consistently. Non-dioxin-like PCBs had a significant interaction with vitamin C in quartile modeling. Combined total PCBs were observed to have some significant interactions with vitamin E and with combined carotenoids + vitamin C + vitamin E in continuous modeling. Importantly, these interactions do not indicate whether there was a direct or inverse association with probability of metabolic syndrome. Vitamin E was shown to typically be associated with an increased probability of metabolic syndrome in earlier analyses.

L. Moving Quartiles

The first quartile was replaced and quartile analysis (rather than continuous) resumed. PCBs and serum carotenoids have demonstrated complicated relationships, which encouraged quartile analysis, even though significance was more often observed in continuous linear and quadratic modeling. To refine the graphs further, each quartile was divided into four additional graphs, each representing a 5% shift. In the graphs below, the first plot represented quartile 1 (0-25%). Subsequent plots occurred at 5% shifts: 5-30%, 10-35%, 15-40%...75-100%. This yielded a more detailed illustration of the PCB: serum carotenoid interaction in relation to the probability of metabolic syndrome. Of particular interest was the prevalent first quartile effect of increased probability of metabolic syndrome, suggestive of endocrine disruption, and the positive, protective influence of serum carotenoids at higher PCB concentrations.

Figure 4.14 Moving quartiles 1-6 of non-dioxin-like PCBs and serum carotenoids on the probability of metabolic syndrome

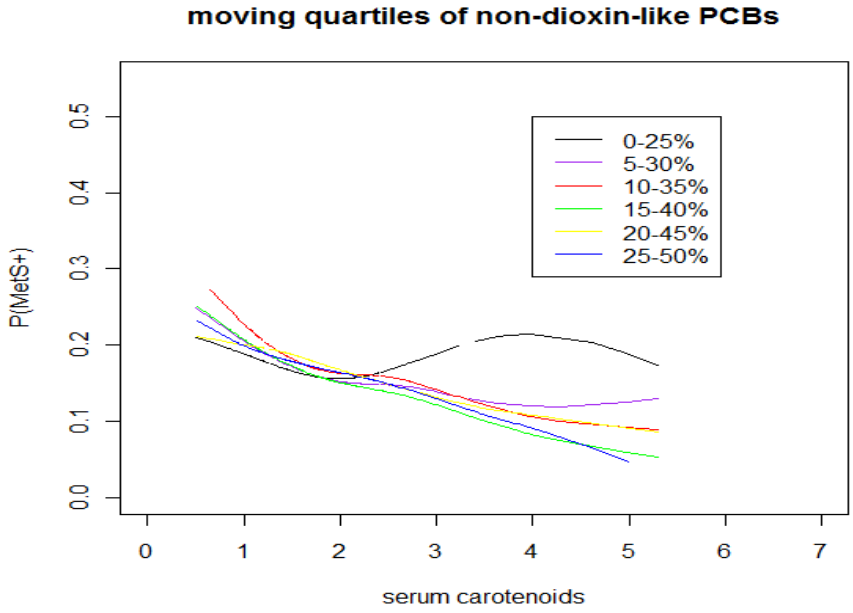


Figure 4.15 Moving quartiles 6-11 of non-dioxin-like PCBs and serum carotenoids on the probability of metabolic syndrome

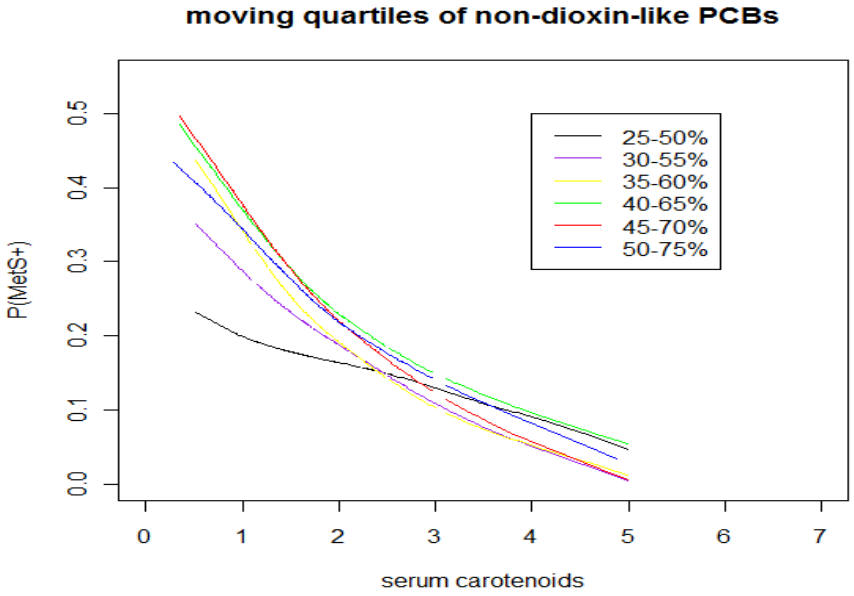
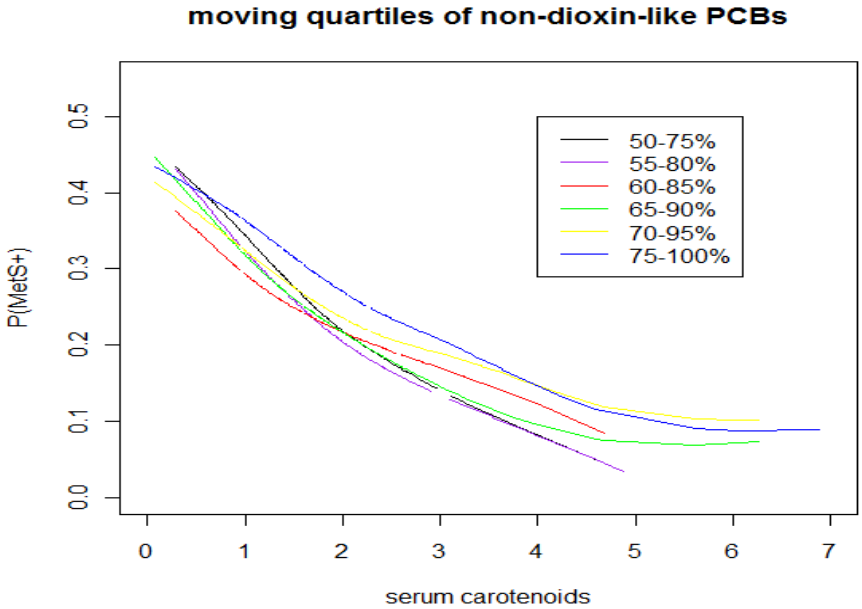
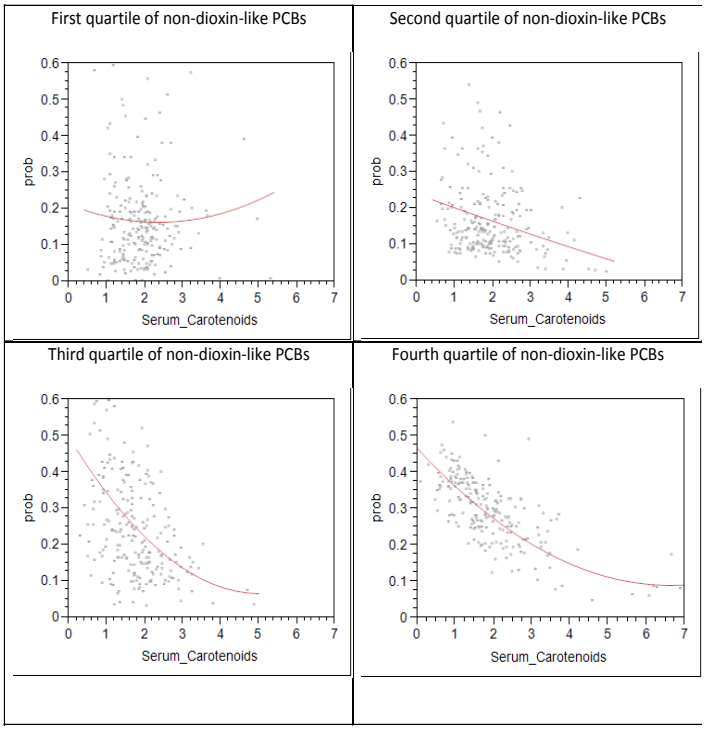


Figure 4.16 Moving quartiles 11-16 of non-dioxin-like PCBs and serum carotenoids on the probability of metabolic syndrome



The slope can be observed gradually increasing across increasing PCB quartiles, which indicates that serum carotenoids have a bigger positive effect at higher concentrations of PCBs. The highest set of quartile exposures has similar slopes for each moving quartile. Overall, serum carotenoids & the variables associated with them have demonstrated a strong protective effect on the probability of metabolic syndrome.

Figure 4.17 Non-dioxin-like PCBs, serum carotenoids, & the probability of metabolic syndrome: Four Quartile Scatterplot



M. Statistical Interactions: Congener PCBs and Total, Serum Carotenoids

Table 4.42 Statistical interactions between PCB 118, PCB 126, and PCB 153, & serum carotenoids: 3 covariate modeling

Interaction p-values between PBCs & SC	
Analyte	3 covariate model
PCB118	0.09
PCB126	0.86
PCB153	0.04

PCB 118, PCB 126, and PCB 153 are congeners from three different subclasses of PCBs and are known to be damaging from animal models and human studies. The PCB 153 and serum carotenoid interaction reached significance above (p = 0.04). PCB 118 and carotenoids reached near significance (p = 0.09), considered comment worthy when using small sample sizes in non-linear studies (Lee, et al. 2011b).

Figure 4.18 PCB 118 Best Fit

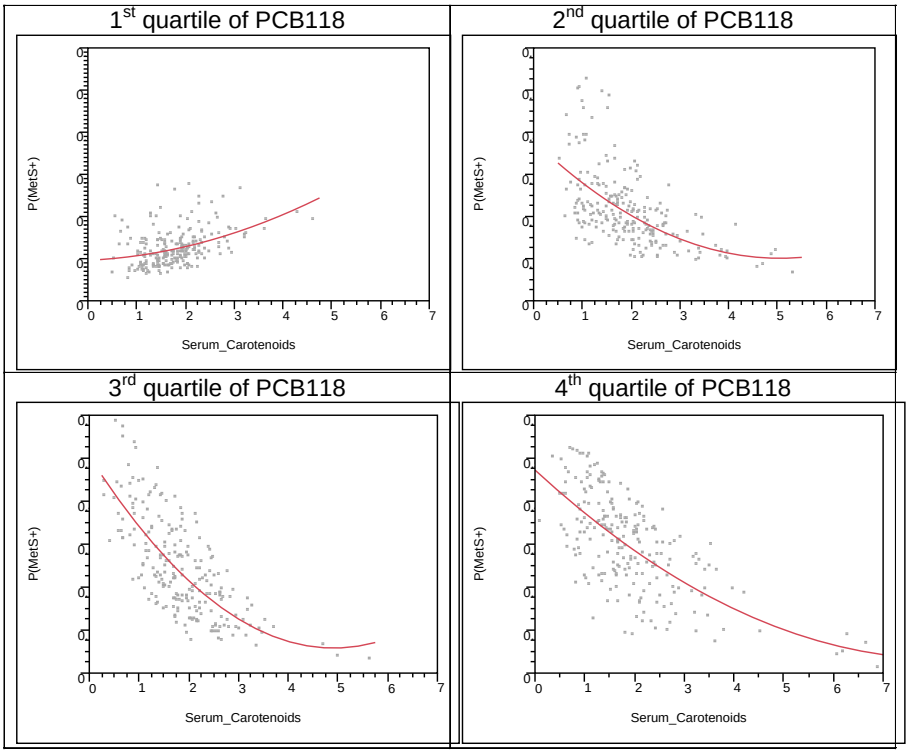


Table 4.43 PCB 118 Baseline and Lifestyle Characteristics

PCB118 Baseline & Lifestyle Characteristics		MetS+ N=234	MetS- N=824
Gender	Female	97	386
	Male	109	344
Poverty Income Ratio	average	2.58	2.67
Race/ethnicity	Mexican American	36	136
	Non-Hispanic White	126	416
	Non-Hispanic Black	27	127
	Other Race – Including Multi-Racial	17	51
Cigarette smoking	Never	95	384
	Former	68	177
	Current	43	169
Serum Cotinine	≥ 0.015 ng/mL	168	579
	<0.015 ng/ml	38	149
Alcohol consumption	Non-drinker	50	123
	Non-excessive drinker	77	281
	Excessive drinker	42	201
Physical activity	Sedentary (no physical activity)	56	213
	Low activity	22	88
	Moderately to vigorously active	32	157
BMI	<18.5	0	15
	18.5-24.9	25	267
	25.0-29.9	83	247
	≥30	98	200
Dietary supplement use	yes	107	393
	no	99	336

Table 4.44 Adjusted Odds Ratios and 95% Confidence Intervals for the Probability of Metabolic Syndrome across quartiles of PCB 118

PCB118	<25th	25th-<50th	50th-<75th	>75th	P_{trend}	$P_{covariate}$
Concentration (ng/g of lipid)	2.45/0.62	4.59/0.70	8.62/1.81	30.51/24.32		
Cases/n	30/233	50/235	56/234	70/234		
Prevalence (%)	12.88	21.28	23.93	29.91		
Model 0	referent	1.6 (1.0 – 2.7)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.6)	0.34	
Model 1 (age)	referent	1.6 (1.0 – 2.7)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.6)	0.34	<0.01
Model 2 (gender)	referent	1.6 (1.0 – 2.8)	1.5 (0.8 – 2.5)	1.5 (0.8 – 2.7)	0.32	0.19
Model 3 (race/ethnicity)	referent	1.6 (1.0 – 2.7)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.6)	0.34	0.68
Model 4 (PIR)	referent	1.6 (1.0 – 2.7)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.6)	0.34	0.29
Model 5 (cigarette smoking)	referent	1.7 (1.0 – 2.8)	1.5 (0.8 – 2.5)	1.5 (0.8 – 2.8)	0.29	0.39
Model 6 (serum cotinine)	referent	1.7 (1.0 – 2.8)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.7)	0.30	0.16
Model 7 (alcohol consumption)	referent	1.6 (1.0 – 2.7)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.6)	0.33	0.91
Model 8 (leisure-time physical activity)	referent	1.7 (0.8 – 3.3)	1.3 (0.6 – 2.8)	1.2 (0.5 – 2.9)	0.53	0.91
Model 9 (BMI)	referent	1.7 (1.0 – 2.9)	1.3 (0.7 – 2.3)	1.2 (0.6 – 2.3)	0.26	<0.01
Model 10 (total calories)	referent	1.6 (1.0 – 2.7)	1.4 (0.8 – 2.5)	1.4 (0.8 – 2.6)	0.34	0.96
Model 11 (dietary supplement use)	referent	1.6 (1.0 – 2.7)	1.5 (0.8 – 2.5)	1.4 (0.8 – 2.7)	0.33	0.03
Model 12 (total cholesterol)	referent	1.7 (1.0 – 2.8)	1.5 (0.8 – 2.5)	1.4 (0.8 – 2.7)	0.29	0.12
Model 13 (Non-HDL cholesterol)	referent	1.8 (1.1 – 3.1)	1.6 (1.0 – 2.9)	1.7 (1.0 – 3.2)	0.16	<0.01

Figure 4.19 PCB 126 Best Fit

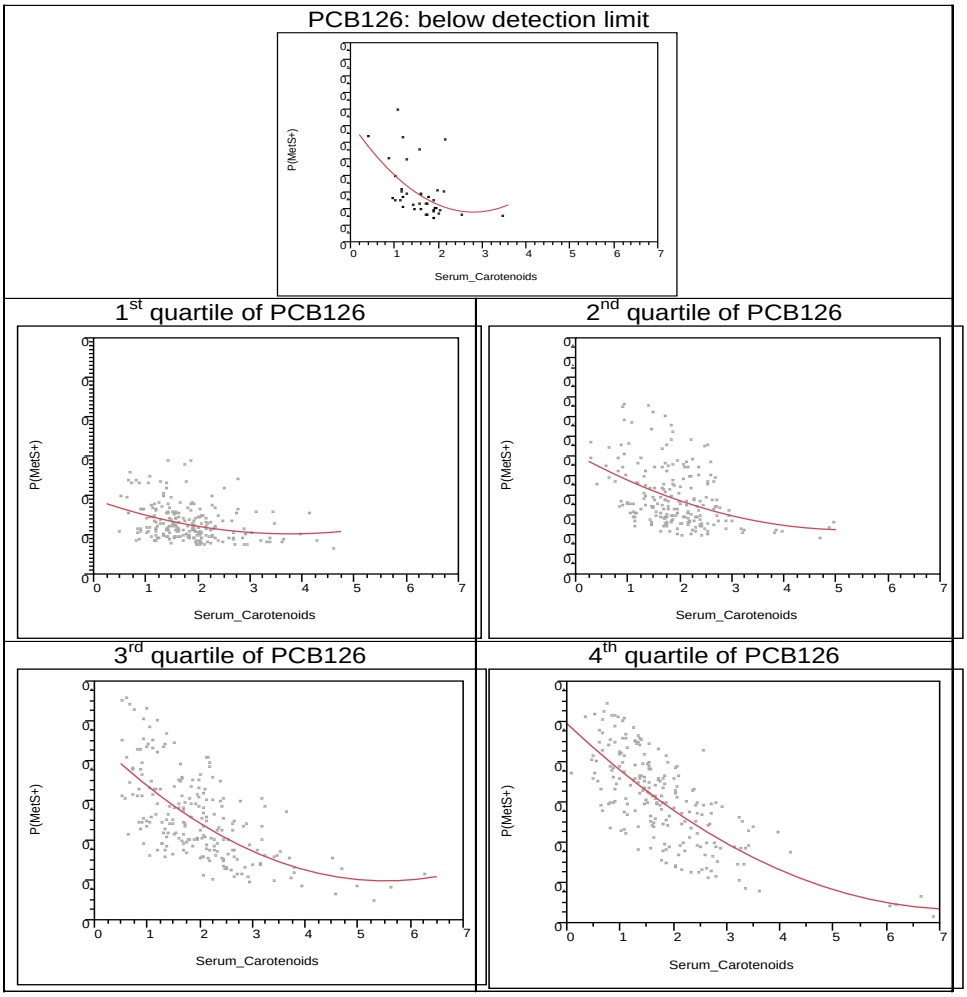


Table 4.45 PCB 126 Baseline and Lifestyle Characteristics

PCB126 Baseline & Lifestyle Characteristics		MetS+ N=234	MetS- N=824
Gender	Female	96	385
	Male	106	343
Poverty Income Ratio	average	2.61	2.68
Race/ethnicity	Mexican American	35	135
	Non-Hispanic White	122	412
	Non-Hispanic Black	27	129
	Other Race – Including Multi-Racial	18	52
Cigarette smoking	Never	95	382
	Former	68	177
	Current	39	169
Serum Cotinine	≥ 0.015 ng/mL	165	579
	<0.015 ng/ml	37	147
Alcohol consumption	Non-drinker	52	120
	Non-excessive drinker	74	286
	Excessive drinker	38	197
Physical activity	Sedentary (no physical activity)	53	212
	Low activity	23	88
	Moderately to vigorously active	29	159
BMI	<18.5	0	14
	18.5-24.9	27	265
	25.0-29.9	76	247
	≥30	98	201
Dietary supplement use	yes	104	388
	no	98	339

Table 4.46 Adjusted Odds Ratios and 95% Confidence Intervals for the Probability of Metabolic Syndrome across quartiles of PCB 126

PCB126	Not detectble	<25th	25th-<50th	50th-<75th	>75th	P_{trend}	$P_{covariate}$
Concentration (ng/g of lipid)	4.89/1.60	8.80/1.78	14.74/1.94	23.19/3.17	65.68/56.99		
Cases/n	7/44	30/233	41/219	56/222	68/222		
Prevalence(%)	15.91	13.45	18.72	25.23	30.63		
Model 0	referent	0.9 (0.3 – 2.4)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.8)	1.4 (0.5 – 3.7)	0.47	
Model 1 (age)	referent	0.9 (0.3 – 2.4)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.8)	1.4 (0.5 – 3.7)	0.47	<0.01
Model 2 (gender)	referent	0.9 (0.3 – 2.5)	1.4 (0.5 – 3.5)	1.5 (0.6 – 4.0)	1.5 (0.6 – 3.9)	0.40	0.18
Model 3 (race/ethnicity)	referent	0.9 (0.3 – 2.4)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.8)	1.4 (0.5 – 3.7)	0.47	0.64
Model 4 (PIR)	referent	0.9 (0.3 – 2.4)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.8)	1.4 (0.5 – 3.7)	0.47	0.38
Model 5 (cigarette smoking)	referent	0.9 (0.3 – 2.4)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.9)	1.5 (0.6 – 3.9)	0.43	0.43
Model 6 (serum cotinine)	referent	0.9 (0.4 – 2.5)	1.4 (0.5 – 3.6)	1.6 (0.6 – 4.1)	1.5 (0.6 – 4.0)	0.40	0.12
Model 7 (alcohol consumption)	referent	0.9 (0.3 – 2.5)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.9)	1.5 (0.6 – 3.8)	0.44	0.53
Model 8 (leisure-time physical activity)	referent	1.4 (0.4 – 5.1)	1.4 (0.4 – 5.1)	1.5 (0.4 – 5.5)	1.6 (0.4 – 5.9)	0.98	0.78
Model 9 (BMI)	referent	0.8 (0.3 – 2.3)	1.1 (0.4 – 3.1)	1.3 (0.5 – 3.5)	1.1 (0.4 – 3.0)	0.64	<0.01
Model 10 (total calories)	referent	0.9 (0.3 – 2.4)	1.3 (0.5 – 3.4)	1.5 (0.6 – 3.8)	1.4 (0.5 – 3.7)	0.47	0.99
Model 11 (dietary supplement use)	referent	0.9 (0.3 – 2.3)	1.3 (0.5 – 3.4)	1.5 (0.6 – 4.0)	1.4 (0.6 – 3.8)	0.35	0.02
Model 12 (total cholesterol)	referent	0.9 (0.3 – 2.3)	1.3 (0.5 – 3.3)	1.4 (0.6 – 3.7)	1.4 (0.5 – 3.6)	0.45	0.46
Model 13 (Non-HDL cholesterol)	referent	0.7 (0.3 – 1.9)	1.1 (0.4 – 2.8)	1.3 (0.5 – 3.3)	1.2 (0.5 – 3.3)	0.26	<0.01

Figure 4.20 PCB 153 Best Fit

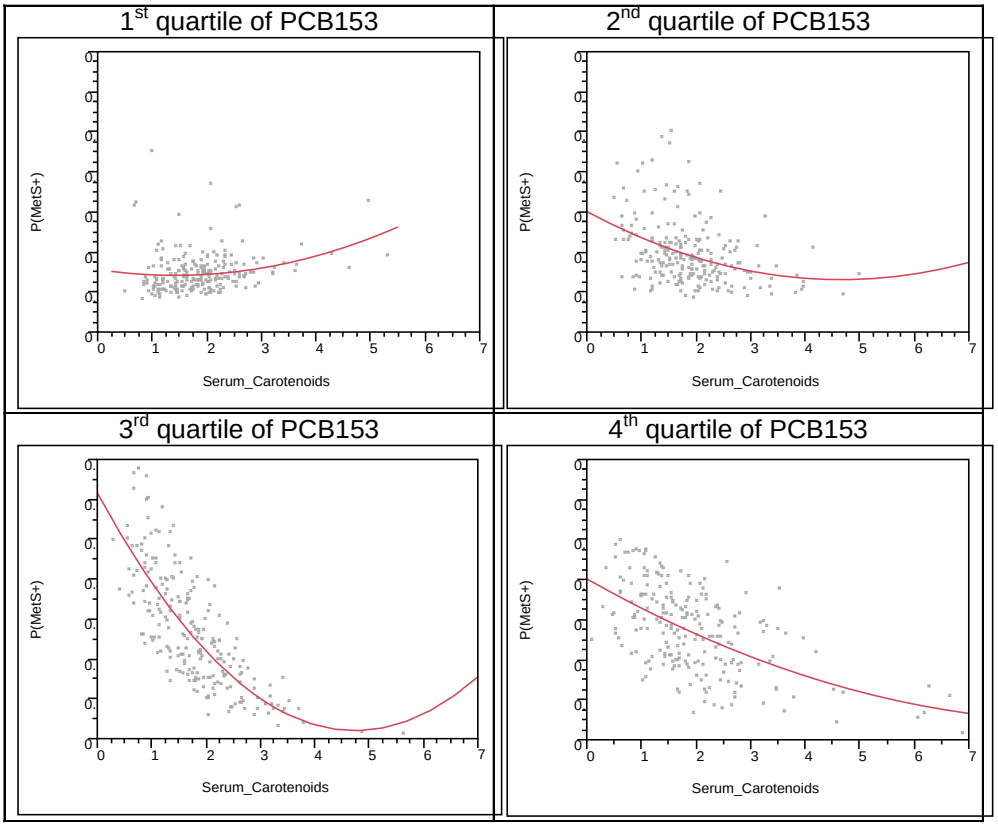


Table 4.47 PCB 153 Baseline and Lifestyle Characteristics

PCB153 Baseline & Lifestyle Characteristics		MetS+ N=234	MetS- N=824
Gender	Female	98	387
	Male	110	348
Poverty Income Ratio	average	2.58	2.67
Race/ethnicity	Mexican American	36	136
	Non-Hispanic White	127	420
	Non-Hispanic Black	27	128
	Other Race – Including Multi-Racial	18	51
Cigarette smoking	Never	96	384
	Former	69	180
	Current	43	171
Serum Cotinine	≥ 0.015 ng/mL	170	584
	<0.015 ng/ml	38	149
Alcohol consumption	Non-drinker	52	123
	Non-excessive drinker	77	285
	Excessive drinker	42	202
Physical activity	Sedentary (no physical activity)	56	213
	Low activity	22	89
	Moderately to vigorously active	32	158
BMI	<18.5	0	15
	18.5-24.9	26	267
	25.0-29.9	83	249
	≥30	99	203
Dietary supplement use	yes	108	395
	no	100	339

Table 4.48 Adjusted Odds Ratios and 95% Confidence Intervals for the Probability of Metabolic Syndrome across quartiles of PCB 153

PCB153	<25th	25th-<50th	50th-<75th	>75th	P_{trend}	$P_{covariate}$
Concentration (ng/g of lipid)	6.91/2.40	17.75/4.11	36.58/6.80	97.07/81.62		
Cases/n	36/235	45/236	62.236	65/236		
Prevalence(%)	15.32	19.07	26.27	27.54		
Model 0	referent	1.0 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.52	
Model 1 (age)	referent	1.0 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.52	<0.01
Model 2 (gender)	referent	0.9 (0.5 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.3)	0.51	0.24
Model 3 (race/ethnicity)	referent	1.0 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.52	0.54
Model 4 (PIR)	referent	1.0 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.52	0.33
Model 5 (cigarette smoking)	referent	1.0 (0.6 – 1.7)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.57	0.58
Model 6 (serum cotinine)	referent	0.9 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.52	0.19
Model 7 (alcohol consumption)	referent	1.0 (0.6 – 1.7)	1.0 (0.5 – 1.8)	0.7 (0.3 – 1.4)	0.56	0.85
Model 8 (leisure-time physical activity)	referent	1.0 (0.5 – 2.1)	1.0 (0.4 – 2.3)	0.8 (0.3 – 2.2)	0.89	0.94
Model 9 (BMI)	referent	1.1 (0.6 – 1.9)	1.0 (0.5 – 1.9)	0.8 (0.4 – 1.7)	0.79	<0.01
Model 10 (total calories)	referent	1.0 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.53	0.80
Model 11 (dietary supplement use)	referent	1.0 (0.6 – 1.7)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.56	0.04
Model 12 (total cholesterol)	referent	1.0 (0.6 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.3 – 1.4)	0.59	0.20
Model 13 (Non-HDL cholesterol)	referent	0.9 (0.5 – 1.6)	0.9 (0.5 – 1.7)	0.7 (0.4 – 1.5)	0.81	<0.01

The p-trend looks for significance across each of the four quartiles in relation to the reference group. There were no significant p-trend values for PCB 118, PCB 126, or PCB 153 in the multi-regression covariate analyses. These data were obfuscated by the lack of a true reference group and possibly too small of a sample size. P-covariate considers only the effect of the covariate to see what effect it has on the probability of metabolic syndrome. All three PCBs showed significance for the same covariates: age, BMI, dietary supplement use, and non-HDL cholesterol. Adjusted odds-ratio tables

seek a larger number for a greater association. It is noteworthy that the higher ORs are found in the 2nd and 3rd quartiles in most cases, suggestive of a low dose effect. PCB 126 was the only PCB of the three with observations below the 60% LOD, providing a reference group and allowing for p-trend comparisons across four quartiles. PCB 126 also did not reveal the first quartile increase in the probability of metabolic syndrome, as was seen with PCB 118 and PCB 153.

Model 0 shows the significance of the PCB on the probability of developing metabolic syndrome across quartile exposures without covariate influence. None of the three PCBs had significant p-trends: PCB 118-0.34; PCB 126-0.47; and PCB 153-0.52. Again, this may be a reflection of the complex relationships involving PCBs and the lack of a traditional dose-response relationship.

N. Statistical Interactions: Four Covariate Modeling / All Known Relevant, "Full" Covariate Modeling

Table 4.49 Dioxin-like PCBs and Serum Carotenoids: Four Covariate / Full Covariate Modeling

Dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	SC	PCB*SC (interaction)	PCB	SC	PCB*SC (interaction)
3,4,4'5-Tetrachlorobiphenyl (PCB 81)	0.93	0.99	0.62	0.29	0.29	0.32
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	0.11	<0.01	0.26	0.64	0.27	0.55
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	0.01	<0.01	0.05	0.36	0.05	0.14
2,4,4'-Trichlorobiphenyl (PCB 28)	0.90	0.25	0.94	0.14	0.05	0.07
2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	0.06	<0.01	0.21	0.45	0.31	0.33
2,4,4',5-Tetrachlorobiphenyl (PCB 74)	0.16	<0.01	0.36	0.63	0.22	0.40
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	0.06	<0.01	0.13	0.38	0.10	0.34
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	0.05	<0.01	0.17	0.43	0.14	0.44
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	0.28	<0.01	0.41	0.93	0.42	0.92
2,3,3'4,4',5'-Hexachlorobiphenyl (PCB 157)	0.16	0.01	0.32	0.94	0.24	0.84
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	0.03	<0.01	0.09	0.54	0.26	0.750.03
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	0.02	<0.01	0.02	0.40	0.08	0.48
pooled	0.02	<0.01	0.06	0.96	0.56	0.82

Table 4.50 Non-dioxin-like PCBs and Serum Carotenoids: Four Covariate / Full Covariate Modeling

Non-dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	SC	PCB*SC (interaction)	PCB	SC	PCB*SC (interaction)
2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	0.26	0.05	0.50	0.04	0.05	0.09
2,2',4,5'-Tetrachlorobiphenyl (PCB 49)	0.57	0.09	0.86	0.14	0.15	0.33
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	0.63	0.13	0.93	0.09	0.04	0.08
2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	0.57	0.26	0.84	0.37	0.69	0.93
2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	0.18	<0.01	0.26	0.66	0.29	0.79
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	0.22	0.05	0.72	0.18	0.49	0.59
2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	0.33	0.11	0.99	0.25	0.47	0.76
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	0.23	0.93	0.27	0.31	0.94	0.73
(PCB 138 & PCB 158)	0.10	<0.01	0.18	0.95	0.33	0.72
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	0.16	0.01	0.39	0.67	0.54	0.94
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	0.30	0.65	0.03	0.28	0.11	0.03
2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	0.45	0.10	0.88	0.68	0.61	0.24
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	0.08	<0.01	0.21	0.97	0.30	0.64
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	0.07	<0.01	0.16	0.27	0.08	0.14
2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172)	0.11	0.01	0.26	0.30	0.10	0.16
2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	0.26	<0.01	0.31	0.56	0.06	0.10

Table 4.50 (continued)

2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178)	0.16	0.01	0.41	0.46	0.11	0.20
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	0.06	0.01	0.35	0.33	0.15	0.20
2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	0.12	<0.01	0.25	0.65	0.10	0.16
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	0.15	<0.01	0.29	0.62	0.13	0.18
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	0.06	0.01	0.30	0.26	0.13	0.12
2,2',3,3',4,4',5,6-Octachlorobipenyl (PCB 195)	0.06	<0.01	0.09	0.31	0.13	0.18
(PCB 196 & PCB 203)	0.06	<0.01	0.22	0.40	0.03	0.03
2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)	0.03	<0.01	0.10	0.18	0.01	0.01
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	0.16	<0.01	0.23	0.56	0.08	0.08
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (PCB 209)	0.17	<0.01	0.19	0.63	0.08	0.12
pooled	0.05	<0.01	0.18	0.77	0.41	0.57

The first column applies a four covariate analysis, which was used in place of the sample weights used by NHANES. The four covariates were age, gender, race/ethnicity, and poverty income ratio. The second column augmented the four covariates with all additional demographic and lifestyle covariates known to be significant to the variables of interest – metabolic syndrome, PCB concentrations, and carotenoid concentrations. These include the four previously mentioned, as well as cigarette smoking, serum cotinine, leisure time physical activity, alcohol consumption, BMI, dietary supplement use, total cholesterol, and non-HDL cholesterol. The PCBs were analyzed as concentrations, not as ranks. The first quartile was removed for this analysis to remove any possible confounding within the first quartile that could obfuscate a true significant relationship. Most significance was lost in the full covariate analysis (second column).

Table 4.51 Dioxin-like PCBs and Serum Vitamin C: Four Covariate / Full Covariate Modeling

Dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	Vit C	PCB*VC (interaction)	PCB	Vit C	PCB*VC (interaction)
3,4,4'5-Tetrachlorobiphenyl (PCB 81)	0.12	0.12	0.16	0.88	0.60	0.33
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	0.27	<0.01	0.61	0.67	0.61	0.66
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	0.45	0.37	0.56	0.96	0.56	0.73
2,4,4'-Trichlorobiphenyl (PCB 28)	0.89	0.13	0.95	0.40	0.41	0.26
2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	0.49	0.04	0.73	0.77	0.87	0.86
2,4,4',5-Tetrachlorobiphenyl (PCB 74)	0.73	0.05	0.60	0.48	0.90	0.55
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	0.64	0.01	0.70	0.81	0.19	0.61
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	0.58	0.01	0.71	0.78	0.44	0.53
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	0.47	0.65	0.17	0.66	0.64	0.61
2,3,3'4,4',5'-Hexachlorobiphenyl (PCB 157)	0.64	0.56	0.20	0.85	0.91	0.75
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	0.37	0.01	0.91	0.80	0.70	0.89
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	0.32	0.02	0.37	0.76	0.10	0.96
pooled	0.52	0.18	0.62	0.22	0.05	0.21

Table 4.52 Non-dioxin-like PCBs and Serum Vitamin C: Four Covariate / Full Covariate Modeling

Non-dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	Vit C	PCB*VC (interaction)	PCB	Vit C	PCB*VC (interaction)
2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	0.31	0.07	0.62	0.15	0.33	0.31
2,2',4,5'-Tetrachlorobiphenyl (PCB 49)	0.68	0.19	0.99	0.24	0.52	0.56
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	0.80	0.24	0.86	0.36	0.43	0.27
2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	0.57	0.21	0.70	0.17	0.55	0.51
2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	0.96	0.01	0.72	0.93	0.69	0.79
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	0.27	0.09	0.99	0.12	0.42	0.39
2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	0.17	0.04	0.86	0.06	0.14	0.19
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	0.34	0.86	0.42	0.24	0.77	0.75
(PCB 138 & PCB 158)	0.77	0.23	0.33	0.34	0.56	0.42
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	0.97	0.43	0.24	0.25	0.40	0.32
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	0.77	0.64	0.19	0.85	0.52	0.38
2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	0.74	0.13	0.44	0.76	0.21	0.12
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	0.93	0.32	0.29	0.28	0.43	0.32
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	0.63	0.18	0.63	0.60	0.66	0.59
2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172)	0.62	0.26	0.58	0.91	0.91	0.88

Table 4.52 (continued)

2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	0.97	0.07	0.62	0.42	0.98	0.91
2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178)	0.60	0.23	0.56	0.37	0.47	0.48
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	0.34	0.25	0.46	0.55	0.51	0.49
2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	0.67	0.07	0.77	0.39	0.94	0.76
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	0.56	0.05	0.71	0.32	0.95	0.77
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	0.29	0.15	0.64	0.49	0.47	0.48
2,2',3,3',4,4',5,6-Octachlorobipenyl (PCB 195)	0.38	0.01	0.71	0.67	0.73	0.61
(PCB 196 & PCB 203)	0.53	0.19	0.44	0.32	0.82	0.61
2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)	0.37	0.09	0.76	0.25	0.72	0.52
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	0.74	0.11	0.38	0.02	0.15	0.08
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (PCB 209)	0.82	0.12	0.36	0.04	0.42	0.20
pooled	0.57	0.14	0.43	0.16	0.10	0.11

No significant interactions were observed between PCBs and Vitamin C in either the four covariate model or the full covariate model. Carotenoids and Vitamin C have been demonstrated to interact in vitro as antioxidants. However, this would not be reflected in the above analysis.

Table 4.53 Dioxin-like PCBs and Serum Alpha-Tocopherol: Four Covariate / Full Covariate Modeling

Dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	A-toc	PCB*αt (interaction)	PCB	A-toc	PCB*αt (interaction)
3,4,4'5-Tetrachlorobiphenyl (PCB 81)	0.81	0.20	0.54	0.69	0.29	0.73
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	0.56	0.96	0.95	0.34	0.29	0.33
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	0.29	0.83	0.96	0.82	0.87	0.51
2,4,4'-Trichlorobiphenyl (PCB 28)	0.44	0.19	0.39	0.31	0.18	0.36
2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	0.49	0.15	0.14	0.19	0.17	0.20
2,4,4',5-Tetrachlorobiphenyl (PCB 74)	0.91	0.29	0.59	0.23	0.30	0.28
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	0.94	0.22	0.48	0.58	0.33	0.45
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	0.94	0.34	0.50	0.60	0.38	0.41
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	0.85	0.33	0.61	0.96	0.70	0.99
2,3,3'4,4',5'-Hexachlorobiphenyl (PCB 157)	0.69	0.17	0.39	0.39	0.19	0.39
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	0.87	0.27	0.34	0.50	0.17	0.29
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	0.88	0.48	0.91	0.66	0.50	0.46
pooled	0.50	0.57	0.80	0.34	0.28	0.38

Table 4.54 Non-dioxin-like PCBs and Serum Alpha-Tocopherol: Four Covariate / Full Covariate Modeling

Non-dioxin-like PCBs	4 covariates			All known covariates		
	PCB	α-toc	PCB*αt (interaction)	PCB	α-toc	PCB*αt (interaction)
2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	0.11	0.57	0.15	0.34	0.71	0.54
2,2',4,5'-Tetrachlorobiphenyl (PCB 49)	0.35	0.94	0.36	0.76	0.28	0.87
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	0.43	0.97	0.53	0.63	0.77	0.63
2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	0.86	0.13	0.39	0.96	0.11	0.41
2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	0.53	0.30	0.30	0.57	0.52	0.37
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	0.38	0.48	0.91	0.91	0.14	0.62
2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	0.51	0.40	0.87	0.64	0.25	0.86
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	0.11	0.27	0.12	0.40	0.59	0.75
(PCB 138 & PCB 158)	0.60	0.21	0.31	0.20	0.18	0.26
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	0.59	0.09	0.21	0.17	0.12	0.26
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	0.05	<0.01	0.01	0.11	<0.01	0.02
2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	0.42	0.10	0.12	0.54	0.12	0.19
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	0.68	0.15	0.25	0.25	0.20	0.32
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	0.87	0.43	0.60	0.79	0.54	0.85
2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172)	0.80	0.27	0.37	0.66	0.37	0.66

Table 4.54 (continued)

2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	0.67	0.42	0.49	0.42	0.45	0.80
2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178)	0.65	0.14	0.25	0.23	0.15	0.34
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	0.98	0.16	0.24	0.79	0.49	0.82
2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	0.35	0.08	0.11	0.12	0.11	0.22
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	0.24	0.03	0.08	0.11	0.09	0.26
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	0.81	0.23	0.37	0.79	0.48	0.85
2,2',3,3',4,4',5,6-Octachlorobipenyl (PCB 195)	0.69	0.84	0.93	0.84	0.86	0.82
(PCB 196 & PCB 203)	0.33	0.03	0.05	0.35	0.32	0.67
2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)	0.99	0.34	0.39	0.88	0.91	0.56
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	0.51	0.15	0.24	0.73	0.91	0.50
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (PCB 209)	0.73	0.25	0.49	0.53	0.93	0.51
pooled	0.75	0.19	0.22	0.26	0.18	0.28

Table 4.55 Dioxin-like PCBs and Serum Delta-Tocopherol: Four Covariate / Full Covariate Modeling

Dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	δ-toc	PCB*δt (interaction)	PCB	δ-toc	PCB*δt (interaction)
3,4,4'5-Tetrachlorobiphenyl (PCB 81)	0.77	0.19	0.26	0.87	0.66	0.69
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	0.62	0.02	0.52	0.81	0.59	0.94
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	0.07	0.25	0.58	0.89	0.31	0.33
2,4,4'-Trichlorobiphenyl (PCB 28)	0.27	<0.01	0.15	0.66	0.83	0.73
2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	0.99	<0.01	0.18	0.48	0.57	0.54
2,4,4',5-Tetrachlorobiphenyl (PCB 74)	0.74	<0.01	0.38	0.75	0.84	0.78
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	0.61	<0.01	0.49	0.83	0.34	0.58
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	0.45	<0.01	0.49	0.74	0.94	0.96
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	0.42	0.09	0.59	0.47	0.24	0.19
2,3,3'4,4',5'-Hexachlorobiphenyl (PCB 157)	0.30	0.19	0.56	0.45	0.31	0.22
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	0.53	<0.01	0.33	0.32	0.54	0.47
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	0.53	0.96	0.67	0.25	0.31	0.22
pooled	0.24	0.06	0.80	0.98	0.72	0.59

Table 4.56 Non-dioxin-like PCBs and Serum Delta-Tocopherol: Four Covariate / Full Covariate Modeling

Non-dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	δ-toc	PCB*δt (interaction)	PCB	δ-toc	PCB*δt (interaction)
2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	0.83	0.01	0.37	0.44	0.97	0.99
2,2',4,5'-Tetrachlorobiphenyl (PCB 49)	0.80	0.02	0.53	0.39	0.89	0.93
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	0.60	0.01	0.34	0.90	0.94	0.98
2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	0.21	0.11	0.61	0.16	0.64	0.56
2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	0.95	<0.01	0.49	0.61	0.98	0.80
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	0.26	0.05	0.99	0.17	0.62	0.74
2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	0.25	0.01	0.89	0.18	0.95	0.85
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	0.88	0.29	0.91	0.43	0.05	0.43
(PCB 138 & PCB 158)	0.39	0.01	0.99	0.94	0.41	0.39
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	0.15	0.06	0.51	0.99	0.46	0.40
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	0.11	0.19	0.29	0.07	0.42	0.17
2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	0.20	0.03	0.83	0.19	0.89	0.48
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	0.24	0.02	0.80	0.77	0.34	0.27
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	0.34	0.02	0.96	0.82	0.55	0.58
2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172)	0.45	0.01	0.67	0.81	0.90	0.82

Table 4.56 (continued)

2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	0.62	0.02	0.93	0.30	0.65	0.88
2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178)	0.36	0.01	0.86	0.63	0.90	0.97
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	0.11	0.03	0.90	0.76	0.48	0.55
2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	0.48	0.01	0.88	0.50	0.71	0.95
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	0.46	0.01	0.91	0.22	0.80	0.80
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	0.14	0.06	0.84	0.96	0.76	0.77
2,2',3,3',4,4',5,6-Octachlorobipenyl (PCB 195)	0.81	0.04	0.50	0.89	0.85	0.85
(PCB 196 & PCB 203)	0.22	0.01	0.90	0.43	0.66	0.94
2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)	0.21	0.02	0.94	0.29	0.61	0.98
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	0.45	0.02	0.88	0.82	0.12	0.13
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (PCB 209)	0.50	0.01	0.82	0.18	0.81	0.77
pooled	0.27	0.01	0.90	0.99	0.89	0.83

Table 4.57 Dioxin-like PCBs and Serum Gamma-Tocopherol: Four Covariate / Full Covariate Modeling

Dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	γ-toc	PCB*γt (interaction)	PCB	γ-toc	PCB*γt (interaction)
3,4,4'5-Tetrachlorobiphenyl (PCB 81)	0.45	0.06	0.17	0.97	0.94	0.88
3,3',4,4',5-Pentachlorobiphenyl (PCB 126)	0.63	<0.01	0.50	0.87	0.68	0.65
3,3',4,4',5,5'-Hexachlorobiphenyl (PCB 169)	0.23	0.03	0.77	0.62	0.29	0.21
2,4,4'-Trichlorobiphenyl (PCB 28)	0.49	0.01	0.41	0.45	0.59	0.33
2,3',4,4'-Tetrachlorobiphenyl (PCB 66)	0.81	<0.01	0.35	0.81	0.34	0.86
2,4,4',5-Tetrachlorobiphenyl (PCB 74)	0.78	<0.01	0.15	0.71	0.75	0.24
2,3,3',4,4'-Pentachlorobiphenyl (PCB 105)	0.54	<0.01	0.55	0.97	0.17	0.78
2,3',4,4',5-Pentachlorobiphenyl (PCB 118)	0.46	<0.01	0.53	0.55	0.60	0.66
2,3,3',4,4',5-Hexachlorobiphenyl (PCB 156)	0.70	0.01	0.80	0.39	0.39	0.19
2,3,3'4,4',5'-Hexachlorobiphenyl (PCB 157)	0.76	0.01	0.55	0.42	0.64	0.27
2,3',4,4',5,5'-Hexachlorobiphenyl (PCB 167)	0.57	<0.01	0.32	0.26	0.66	0.38
2,3,3',4,4',5,5'-Heptachlorobiphenyl (PCB 189)	0.92	0.22	0.76	0.56	0.82	0.80
pooled	0.32	0.01	0.67	0.68	0.92	0.34

Table 4.58 Non-Dioxin-like PCBs and Serum Gamma-Tocopherol: Four Covariate / Full Covariate Modeling

Non-dioxin-like PCBs	4 covariates			All relevant covariates		
	PCB	γ-toc	PCB*γt (interaction)	PCB	γ-toc	PCB*γt (interaction)
2,2',3,5'-Tetrachlorobiphenyl (PCB 44)	0.99	0.04	0.69	0.61	0.74	0.98
2,2',4,5'-Tetrachlorobiphenyl (PCB 49)	0.74	0.11	0.88	0.36	0.99	0.68
2,2',5,5'-Tetrachlorobiphenyl (PCB 52)	0.96	0.02	0.76	0.96	0.58	0.97
2,2',3,4,5'-Pentachlorobiphenyl (PCB 87)	0.64	<0.01	0.56	0.19	0.74	0.59
2,2',4,4',5-Pentachlorobiphenyl (PCB 99)	0.79	<0.01	0.27	0.63	0.39	0.85
2,2',4,5,5'-Pentachlorobiphenyl (PCB 101)	0.54	0.01	0.64	0.35	0.73	0.94
2,3,3',4',6-Pentachlorobiphenyl (PCB 110)	0.41	<0.01	0.82	0.33	0.41	0.99
2,2',3,3',4,4'-Hexachlorobiphenyl (PCB 128)	0.49	0.05	0.52	0.52	0.56	0.97
(PCB 138 & PCB 158)	0.73	<0.01	0.44	0.89	0.93	0.50
2,2',3,4',5,5'-Hexachlorobiphenyl (PCB 146)	0.48	<0.01	0.63	0.89	0.80	0.65
2,2',3,4',5',6-Hexachlorobiphenyl (PCB 149)	0.21	0.05	0.51	0.08	0.76	0.17
2,2',3,5,5',6-Hexachlorobiphenyl (PCB 151)	0.37	<0.01	0.73	0.23	0.87	0.55
2,2',4,4',5,5'-Hexachlorobiphenyl (PCB 153)	0.55	<0.01	0.49	0.70	0.69	0.33
2,2',3,3',4,4',5-Heptachlorobiphenyl (PCB 170)	0.73	<0.01	0.31	0.99	0.77	0.94
2,2',3,3',4,5,5'-Heptachlorobiphenyl (PCB 172)	0.64	<0.01	0.48	0.84	0.80	0.84

Table 4.58 (continued)

2,2',3,3',4,5',6'-Heptachlorobiphenyl (PCB 177)	0.84	<0.01	0.41	0.22	0.65	0.58
2,2',3,3',5,5',6-Heptachlorobiphenyl (PCB 178)	0.64	<0.01	0.48	0.56	0.50	0.80
2,2',3,4,4',5,5'-Heptachlorobiphenyl (PCB 180)	0.32	<0.01	0.54	0.98	0.69	0.99
2,2',3,4,4',5',6-Heptachlorobiphenyl (PCB 183)	0.65	<0.01	0.56	0.57	0.93	0.99
2,2',3,4',5,5',6-Heptachlorobiphenyl (PCB 187)	0.87	<0.01	0.35	0.27	0.54	0.79
2,2',3,3',4,4',5,5'-Octachlorobiphenyl (PCB 194)	0.19	0.02	0.74	0.82	0.47	0.83
2,2',3,3',4,4',5,6-Octachlorobipenyl (PCB 195)	0.66	0.04	0.59	0.91	0.90	0.89
(PCB 196 & PCB 203)	0.22	0.01	0.94	0.60	0.98	0.84
2,2',3,3',4,5,5',6-Octachlorobiphenyl (PCB 199)	0.37	<0.01	0.60	0.37	0.94	0.96
2,2',3,3',4,4',5,5',6-Nonachlorobiphenyl (PCB 206)	0.37	0.01	0.86	0.90	0.20	0.11
2,2',3,3',4,4',5,5',6,6'-Decachlorobiphenyl (PCB 209)	0.41	0.01	0.83	0.38	0.80	0.57
pooled	0.62	<0.01	0.32	0.90	0.33	0.92

Alpha-, delta-, and gamma-tocopherols are naturally occurring forms of vitamin E, but are neither inter-convertible nor metabolically interchangeable (IOM DRIs 2000). All three forms are found in foods, but the EAR is determined from alpha-tocopherol alone. Significant interactions were observed above for alpha-tocopherol and PCB 149 and PCB196/203. Direction is not indicated by this data run. The tocopherols have been associated with an increased probability of metabolic syndrome in earlier portions of this project, a compelling question not yet considered.

O. PCB & Nutrient Interactions from the 24-hour Dietary Recalls (Total Nutrients File)

Significant interactions were sought between nutrients obtained from NHANES 2003-2004 Total Nutrients Files, Days 1 & 2, which represent the nutrients obtained from foods recorded in day 1 and day 2 dietary recalls. These data were statistically combined with PCB subclasses, total PCBs, dioxins, furans, and dioxin-like chemicals, to seek significance across various modeling: quartile, continuous, and continuous quadratic. The purpose was to assess their combined effect on the probability of metabolic syndrome.

Table 4.59 Lycopene, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, dioxins, and furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * L	PCB (continuous) * L	PCB (continuous) * L	PCB(continuous) *PCB(continuous) *L
Non-dioxin-like PCBs	0.5522	0.4514	0.2506	0.2047
Dioxins, furans, dioxin-like PCBs	0.2038	0.0244	0.7108	0.4797
Dioxins	0.1433	0.2111	0.0354	0.0220
Furans	0.0378	0.0255	0.9822	0.7208
Dioxin-like PCBs	0.1904	0.1474	0.2120	0.1474
Combined PCBs	0.7151	0.1734	0.6049	0.4784

Table 4.60 Vitamin C, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, dioxins, and furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * VC	PCB (continuous) * VC	PCB (continuous) * VC	PCB(continuous) *PCB(continuous) * VC
Non-dioxin-like PCBs	0.6514	0.2883	0.2101	0.1650
Dioxins, furans, dioxin-like PCBs	0.0096	0.0019	0.6003	0.9620
Dioxins	0.0043	0.0009	0.9560	0.6048
Furans	0.8416	0.5280	0.9446	0.8620
Dioxin-like PCBs	0.0223	0.0047	0.9728	0.6424
Combined PCBs	0.6867	0.0824	0.2538	0.1688

Table 4.61 Vitamin K, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * VK	PCB (continuous) * VK	PCB (continuous) * VK	PCB(continuous) *PCB(continuous) * VK
Non-dioxin-like PCBs	0.8905	0.9361	0.1220	0.1207
Dioxins, furans, dioxin-like PCBs	0.9656	0.9344	0.8030	0.7902
Dioxins	0.2591	0.3576	0.2677	0.3300
Furans	0.4112	0.2122	0.6268	0.7608
Dioxin-like PCBs	0.4337	0.7176	0.9838	0.9400
Combined PCBs	0.8729	0.8529	0.1512	0.1550

Table 4.62 Magnesium, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * M	PCB (continuous) * M	PCB (continuous) * M	PCB(continuous) *PCB(continuous) * M
Non-dioxin-like PCBs	0.5157	0.1137	0.5184	0.6643
Dioxins, furans, dioxin-like PCBs	0.9889	0.2892	0.3587	0.2770
Dioxins	0.3749	0.9114	0.0838	0.0803
Furans	0.1088	0.0525	0.4151	0.5944
Dioxin-like PCBs	0.2719	0.4729	0.2997	0.2478
Combined PCBs	0.4718	0.1305	0.7391	0.9069

Table 4.63 Potassium, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * P	PCB (continuous) * P	PCB (continuous) * P	PCB(continuous) *PCB(continuous) * P
Non-dioxin-like PCBs	0.3404	0.0684	0.7945	0.9944
Dioxins, furans, dioxin-like PCBs	0.9845	0.2935	0.2804	0.2119
Dioxins	0.0689	0.4989	0.0206	0.0240
Furans	0.0648	0.0762	0.7142	0.5198
Dioxin-like PCBs	0.3573	0.2896	0.3030	0.2304
Combined PCBs	0.2004	0.0550	0.8128	0.9671

Table 4.64 Calcium, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * C	PCB (continuous) * C	PCB (continuous) * C	PCB(continuous) *PCB(continuous) * C
Non-dioxin-like PCBs	0.5361	0.0616	0.5141	0.3550
Dioxins, furans, dioxin-like PCBs	0.6325	0.6721	0.0945	0.0819
Dioxins	0.8430	0.7803	0.1171	0.1209
Furans	0.1961	0.2298	0.5701	0.4332
Dioxin-like PCBs	0.8483	0.8073	0.0698	0.0663
Combined PCBs	0.4230	0.0877	0.4863	0.3405

Table 4.65 Magnesium + Potassium + Calcium, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * MPC	PCB (continuous) * MPC	PCB (continuous) * MPC	PCB(continuous) *PCB(continuous) * MPC
Non-dioxin-like PCBs	0.3934	0.0505	0.9918	0.7859
Dioxins, furans, dioxin-like PCBs	0.9842	0.3447	0.1840	0.1382
Dioxins	0.1709	0.5744	0.0272	0.0306
Furans	0.0717	0.0792	0.7536	0.5502
Dioxin-like PCBs	0.4380	0.3984	0.1468	0.1128
Combined PCBs	0.2233	0.0473	0.9859	0.7539

Table 4.66 Monounsaturated Fatty Acid (MUFA) to Polyunsaturated Fatty Acid (PUFA) ratio, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxin, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * MtP	PCB (continuous) * MtP	PCB (continuous) * MtP	PCB(continuous) *PCB(continuous) * MtP
Non-dioxin-like PCBs	0.3172	0.1977	0.7931	0.6609
Dioxins, furans, dioxin-like PCBs	0.1799	0.2418	0.3799	0.4755
Dioxins	0.2708	0.3385	0.2127	0.2659
Furans	0.2983	0.0675	0.6891	0.9069
Dioxin-like PCBs	0.9949	0.8415	0.2472	0.2325
Combined PCBs	0.4749	0.2778	0.7562	0.6412

Table 4.67 Polyunsaturated Fatty Acid (PUFA) to Saturated Fatty Acid (SFA) ratio, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * PtS	PCB (continuous) * PtS	PCB (continuous) * PtS	PCB(continuous) *PCB(continuous) * PtS
Non-dioxin-like PCBs	0.9240	0.1767	0.5856	0.5222
Dioxins, furans, dioxin-like PCBs	0.9757	0.1348	0.9238	0.8989
Dioxins	0.9673	0.2045	0.5863	0.5199
Furans	0.9985	0.2559	0.3347	0.3336
Dioxin-like PCBs	0.9987	0.4559	0.3148	0.3175
Combined PCBs	0.9982	0.1408	0.9267	0.9302

Table 4.68 Dietary Fiber, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * F	PCB (continuous) * F	PCB (continuous) * F	PCB(continuous) *PCB(continuous) * F
Non-dioxin-like PCBs	0.3935	0.3490	0.3870	0.3258
Dioxins, furans, dioxin-like PCBs	0.4433	0.6081	0.1787	0.1527
Dioxins	0.4627	0.4880	0.3208	0.2650
Furans	0.2463	0.1457	0.2756	0.3544
Dioxin-like PCBs	0.9538	0.9872	0.1489	0.1453
Combined PCBs	0.3683	0.7281	0.1017	0.0917

Table 4.69 Selenium, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * S	PCB (continuous) * S	PCB (continuous) * S	PCB(continuous) *PCB(continuous) * S
Non-dioxin-like PCBs	0.1582	0.0419	0.2834	0.4148
Dioxins, furans, dioxin-like PCBs	0.5052	0.0397	0.2935	0.1747
Dioxins	0.0249	0.1749	0.0044	0.0021
Furans	0.1437	0.0741	0.6284	0.4415
Dioxin-like PCBs	0.2395	0.1103	0.5168	0.3728
Combined PCBs	0.1992	0.0248	0.3538	0.5286

P. PCB and nutrient interactions from food: Modeling of fruit and vegetable servings

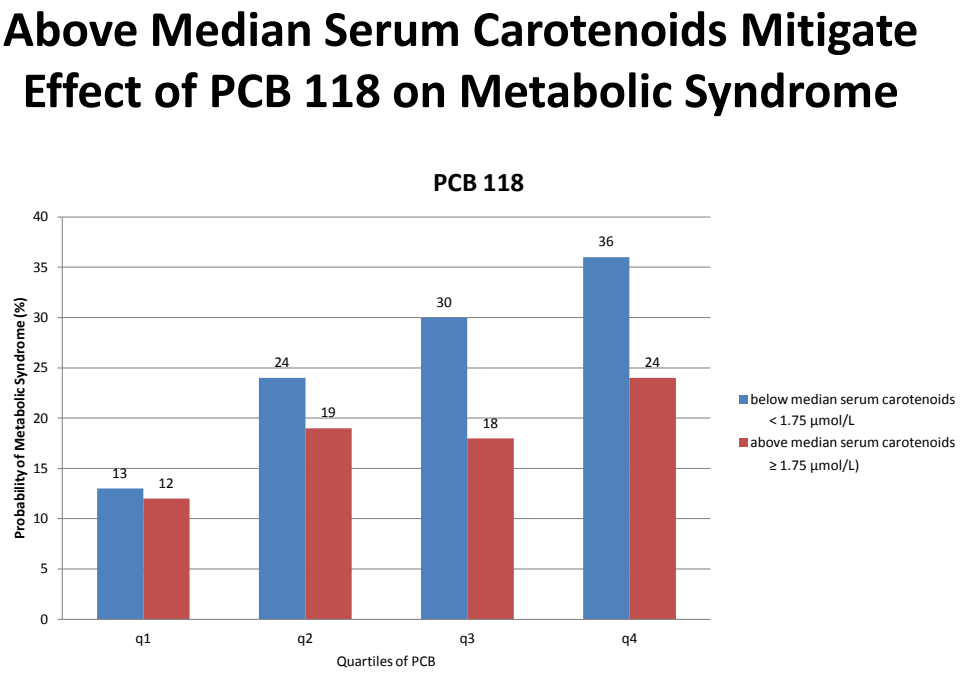
Significant interactions were sought between fruit and vegetable servings obtained from NHANES 2003-2004 Individual Foods Files, Days 1 & 2, which represent the foods and fluids obtained from the diet as recorded in day 1 and day 2 dietary recalls. These data were statistically combined with PCB subclasses, total PCBs, dioxins, furans, and dioxin-like chemicals, to seek significance across various modeling: quartile, continuous, and continuous quadratic. The purpose was to assess their combined effect on the probability of developing metabolic syndrome. NHANES Individual Food Files offer raw data in gram weight. The U.S.D.A Food and Nutrient Database 2.0 (FNDDS 2.0) facilitates extraction of data, however, still in gram weight. A review of relevant literature revealed a 1990 article, in which fruits and vegetables from NHANES II were converted into servings (Patterson, et al. 1990). A modified version of this method was adopted.

Table 4.70 Fruit and Vegetable Servings, sourced from 24-hour dietary recalls, in statistical interaction with PCBs, Dioxins, and Furans

PCB subclasses	model 1	model 2	model 3	
	PCB (quartile) * FVS	PCB (continuous) * FVS	PCB (continuous) * FVS	PCB(continuous) *PCB(continuous) * FVS
Non-dioxin-like PCBs	0.3886	0.1821	0.6725	0.5504
Dioxins, furans, dioxin-like PCBs	0.6634	0.2977	0.9955	0.8697
Dioxins	0.1914	0.0466	0.7540	0.5512
Furans	0.3424	0.3734	0.1701	0.2032
Dioxin-like PCBs	0.8225	0.6197	0.9332	0.8774
Combined PCBs	0.7036	0.1915	0.8895	0.7490

Q. Above Median Serum Carotenoids Mitigate the Effects of PCBs on the Probability of Metabolic Syndrome

Figure 4.21 At or above median 1.75 µmol/L serum carotenoids mitigates the effects of PCB 118 on the probability of metabolic syndrome across all quartiles

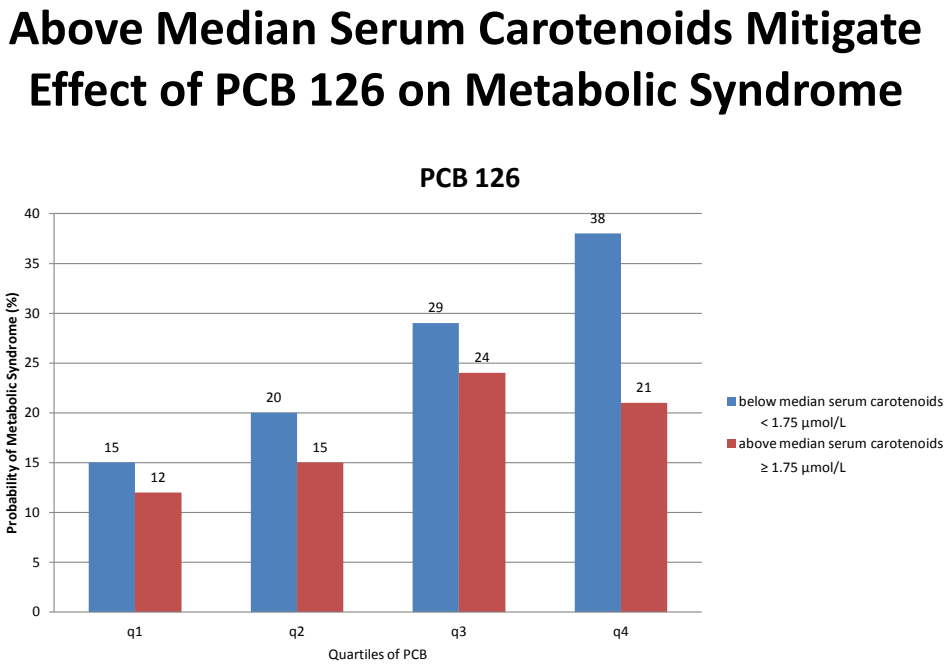


Only participants with metabolic syndrome were represented on this figure. The serum carotenoid concentrations of participants with metabolic syndrome were examined in order to extrapolate risk ratios to the general population based on serum carotenoid concentrations, i.e. fruit and vegetable consumption (IOM, 2000).

This graph analyzed PCB 118 concentrations across four increasing exposure quartiles. Serum carotenoid concentrations were divided at a median cutpoint of 1.75 umol/L. Above median observations were represented by the red bars; below median observations were represented by the blue bar. There was a significant increasing trend in the probability of metabolic syndrome with below median serum carotenoid

concentrations. While the trend did increase in the above median carotenoid group, it lacked significance.

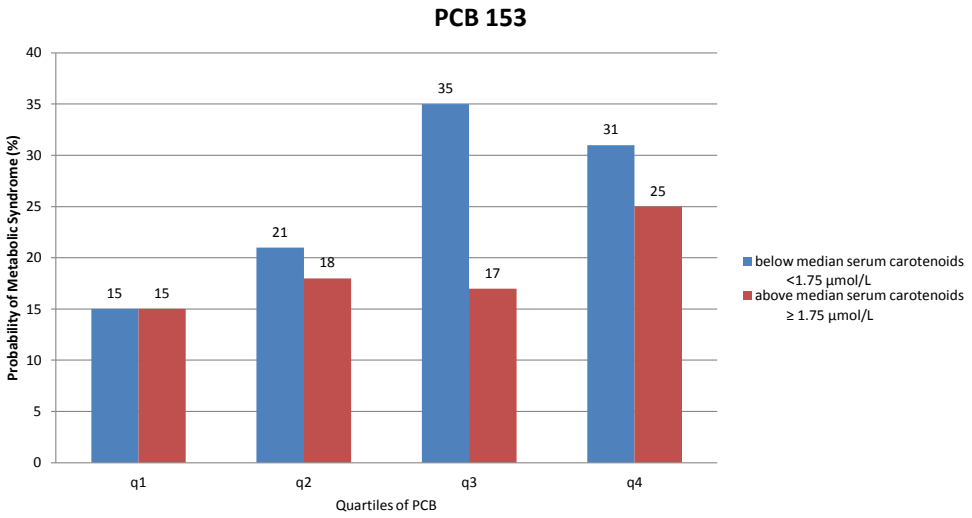
Figure 4.22 At or above median 1.75 µmol/L serum carotenoids mitigates the effects of PCB 126 on the probability of metabolic syndrome across all quartiles



Only participants with metabolic syndrome were represented on this figure with the intent of comparing risk ratios of those with high versus low carotenoid concentrations; therefore, high versus low fruit and vegetable consumption (IOM, 2000).

There was a significant increasing trend in the probability of metabolic syndrome with below median serum carotenoid concentrations (blue bar) for PCB 126. For above median carotenoid concentrations (red bar), the trend increased across quartiles 1, 2, and 3, but then slightly decreased in quartile. Overall, the trend for above median carotenoid concentrations lacked significance.

Figure 4.23 At or above median 1.75 µmol/L serum carotenoids mitigates the effects of PCB 153 on the probability of metabolic syndrome across three quartiles



Only participants with metabolic syndrome were represented on this figure. The serum carotenoid concentrations of participants with metabolic syndrome were examined in order to extrapolate risk ratios to the general population based on serum carotenoid concentrations, i.e. fruit and vegetable consumption (IOM, 2000).

Participants with below median serum carotenoid concentrations, represented by the blue bar, had a significant increasing trend for metabolic syndrome. The above median serum carotenoid group, represented by the red bar, also demonstrated an increasing trend for metabolic syndrome, but this trend lacked significance overall.

CHAPTER FIVE

DISCUSSION

Research Question 1. The prevalence of metabolic syndrome was 22.1% in this NHANES 2003-2004 subpopulation of 1058 participants. The most recent National Center for Health Statistics (NCHS) report on metabolic syndrome among adults found a prevalence of 34.0%, using both crude and age-adjusted data (Ervin, 2009). The NCHS dataset utilized the NHANES release years 2003-2006, n = 3,423. The current study was taken from two of the same release years, but no reason for the disparity was easily apparent beyond those created by different data sets. The present study assessed participants who had been evaluated for serum PCB concentrations, i.e. MEC subsample C, a random subsample of approximately 1/3 mobile examination center participants.

Research Question 2. PCBs were associated with the probability of metabolic syndrome, although not consistently. Logistical regression modeling of serum PCBs on the probability of metabolic syndrome (Table 4.22) failed to reach significance. Only three subclasses were examined in this analysis, dioxin-like PCBs (p=0.99), non-dioxin-like PCBs (p=0.56), and combined PCBs (p=0.24). Individual PCBs were not examined. While disappointing, this was not a deterrent to proceeding. Cross-sectional studies have shown damaging effects of PCBs with metabolic and cardiovascular clinical endpoints, and the relationships involving PCBs are complex. While some of these studies used NHANES datasets, they were from NHANES 1999-2002. Analytical samples from NHANES 1999-2002 were based on a 1-2 ml blood sample. NHANES 2003-2004 analytical samples were based on 5-10 ml. The larger blood sample yielded fewer observations below the LOD. While this was a desirable effect on one hand, it also diminished the likelihood of a reference group, which was provided by observations below the 60% LOD.

It is also noteworthy that no true reference group exists with environmental chemical contamination human studies. Everyone has a body burden. There are no zero values. Demographic and lifestyle covariates known to act as confounders in PCB data analysis are relevant as well. Age, BMI, and diet-related lipids, in particular, are known to be independently associated with PCBs. Some PCB congeners demonstrate a non-linear, dose-response relationship. In cross-sectional analysis of non-dioxin-like PCBs and metabolic syndrome (Lee, et al. 2007b), a non-monotonic inverted U-shaped curve was prevalent with non-dioxin-like PCBs. This curve is suggestive of a greater effect at lower doses, as might be seen with background exposures through food, breast milk, and placental transfer. While this is certainly worthy of further consideration, it may not be reflected in a small p-value. The lack of a reference group for some PCB congeners and non-monotonic curves may dramatically affect the p-value. The logical regression results for PCBs were noted.

In a separate analysis, multivariate logical regression of three individual PCBs – 118, 126, and 153, found both 126 and 153 to be significant for metabolic syndrome. PCB 126, a coplanar PCB with AhR receptor affinity, was significant with $p_{trend} < 0.02$ (Table 4.5). Several demographic and behavioral covariates were significant in modeling with PCB 126; however, all except BMI had a quadratic trend. In modeling of PCB 153 (Table 4.9), a significant $p_{trend} < 0.03$ was observed for metabolic syndrome. Covariates associated with the etiology of metabolic syndrome were significant for several PCB 153 models. However, these all showed a linear trend.

Neither PCB 118 nor PCB 153 had observations below the 60% LOD. For logistical regression modeling, the 1st quartile substituted as the reference group, allowing for tertile analysis. Further complicating matters, there often was observed a rise in the first quartile in the probability of metabolic syndrome. These two factors may obfuscate statistical outcomes concerning the influence of PCB exposure on cardiometabolic health.

PCB 126 did have observations below the 60% LOD allowing for quartile multivariate regression analysis. However, the prevalent first quartile increase in the probability of metabolic syndrome may have contributed to the non-linear trend and use of polynomial regression for PCB 126 and all covariates, except BMI.

The first quartile trend in increasing probability of metabolic syndrome was clearly demonstrated in modeling of PCB subclasses (Figures 4.10 & 4.11). While distinctions between the 2nd, 3rd, and 4th quartiles were unclear in this analysis, the first quartile increase in probability of metabolic syndrome in both the dioxin-like and non-dioxin-like PCB subclasses was distinct and dramatic. While there are extensive data on the low-dose endocrine effects of PCBs, much of it to date has focused on the thyroid, estrogen and androgen hormone systems, and neurodevelopment (Brouwer, et al. 1999). Cross-sectional and case-control studies have linked PCBs to T2DM and metabolic syndrome, showing a quadratic trend for non-dioxin-like PCBs and metabolic syndrome, and both quadratic and cubic trends for T2DM and various PCBs (Lee, et al. 2010 & 2011b).

Research Question 3. Serum carotenoids were shown to be associated with a reduced probability of metabolic syndrome. Logistical regression modeling (Table 4.21) indicated a strong inverse association between pooled total carotenoids and the probability of developing metabolic syndrome at $p = 0.0004$ (coefficient 0.3540). When combined with other serum nutrients – Vitamins A, C, E, D, and B6 – significance was observed at $p = 0.0002$.

In comparisons of the mean differences between carotenoid concentrations of individuals with and without metabolic syndrome, a significant benefit was shown for individuals with greater carotenoid concentrations at higher PCB exposures. In Table 4.18, non-dioxin-like PCBs were assessed across four quartiles of increasing PCB concentrations. Although prevalence of metabolic syndrome was seen to increase with

increasing PCB concentrations (15.0%, 17.0%, 21.6%, 25.6%), at the highest, i.e. third and fourth, exposure quartiles, the mean serum carotenoid concentrations of participants without metabolic syndrome were significantly higher at $p < 0.01$ and $p = 0.01$, respectively. This demonstrated that carotenoids were protective at higher serum PCB concentrations.

In modeling of dioxin-like PCBs and total combined PCBs (Tables 4.19 & 4.20), similar protection was observed. The prevalence of metabolic syndrome in the fourth quartile of dioxin-like PCBs was 28.9%. However, the mean carotenoid concentrations of those without metabolic syndrome in the 4th quartile was also found to be higher, with significance observed at $p = 0.001$ (3rd quartile, $p = 0.02$). For the combined PCB group, significance was observed at $p < 0.01$ for both the 3rd and 4th quartiles. Despite higher levels of PCBs, serum carotenoids were protective.

Later evaluations of the effects of above and below median serum carotenoid concentrations on individuals with metabolic syndrome and individual PCB congener concentrations (Figures 4.21-4.23) were interesting when considered in relation to the above analyses. A significant increase in the probability of metabolic syndrome was observed for participants with below median concentrations of serum carotenoids (<1.75 μmol/L) at all quartiles of PCBs 118, 126, and 153. For participants with above-median serum carotenoid concentrations, however, while an increasing trend was observed in the probability of metabolic syndrome, it lacked statistical significance overall. Participants with below median serum carotenoid concentrations in the third quartile of PCB 153 were observed to have twice the probability of metabolic syndrome than those above the median. No evaluation was executed for individuals without metabolic syndrome in this analysis.

In mean analysis of the individual carotenoids (Tables 4.23 – 4.28), significant protections were observed. Most impressive were the findings of the “overall” row,

which represented the sum of all quartiles for all PCBs. For differences between the mean for overall PCBs, an inverse association was seen between the probability of metabolic syndrome and alpha-carotene ($p = 0.0004$), alpha-cryptoxanthin ($p < 0.0001$), beta-carotene ($p = 0.01$), beta-cryptoxanthin ($p = 0.01$), lycopene ($p = 0.0006$), and lutein/zeaxanthin ($p = 0.02$).

Significance was most often observed in the third and fourth quartiles of non-dioxin-like PCBs, dioxin-like PCBs, and combined PCBs, indicating that the individual carotenoids were also protective at higher PCB concentrations against metabolic syndrome. Of the eighteen first quartile observations in this analysis, twelve revealed an increase in the probability of metabolic syndrome. This trend was provocative and, again, may represent a low-dose, endocrine-related effect. It is quite possible that down-regulation or saturation of hormone receptors occur at the second quartile, at which time a distinctly different linear trend occurs.

A new modeling was applied (Table 4.29) moving forward in order to first seek a statistical interaction between PCBs and serum carotenoids, and then to observe their combined effect on the probability of metabolic syndrome. PCBs and carotenoids share some characteristics. They both concentrate to lipids in serum, are both stored primarily in adipose tissue, and some function as ligands for nuclear receptors. PCBs, however, are known to be damaging. Carotenoids are beneficial. They have opposing effects on the probability of metabolic syndrome. Statistical significance for a PCB carotenoid interaction would also infer significance overall where significance for the PCB alone may have been lacking. While several cross-sectional and prospective studies have examined the effects of two factors, i.e. one dependent and one independent variable, such as the effect of PCBs on diabetes, no studies were found in these areas that considered three factors from the outset. This complicated data analysis greatly. Seeking a statistical interaction between PCBs, either one congener or an entire subclass, and carotenoids, either pooled or individual, made intuitive and deductive sense. For

interactions, significance was observed for alpha-carotene: dioxin-like PCBs (p = 0.03) & combined PCBs (p = 0.05); and for beta-carotene & non-dioxin-like PCBs (p = 0.04). A near significant interaction was seen for beta-carotene and dioxin-like PCBs (p = 0.06). Analysis of individual PCB congeners may have yielded additional significance.

An expanded interaction analysis of dioxin-like PCBs and total serum carotenoids found significant interactions for PCBs 28, 74, and 167 using rank analysis of the PCBs in continuous, quadratic modeling (Table 4.38). PCB 167, a mono-ortho-substituted PCB, was found to have significant interactions with total carotenoids at p = 0.007. Both are considered to have an effect on the probability of metabolic syndrome, but an opposing effect. Figures 4.12 & 4.13 illustrate their combined effect. Persons ≥ median 2.0 µmol/L carotenoid concentrations (Figure 4.12) were found to have a 15% probability of developing metabolic syndrome at rank 600 PCB 167. By contrast, persons with below median 2.0 µmol/L carotenoid concentrations (Figure 4.13), demonstrated a 30% probability of developing metabolic syndrome at rank 600 PCB 167, and this trend stayed elevated at 30% until nearly rank 900. Persons with low serum carotenoid concentrations were observed to have twice the probability of developing metabolic syndrome at rank 600 PCB 167. Serum carotenoids provided clear protective benefits in this analysis, most dramatically at the median PCB level.

Various modeling techniques have been used to better understand the serum PCB-carotenoid relationship. Models were run with and without the first quartile to determine the effect of the first quartile rise on statistical outcomes. PCBs were assessed using chemical concentration and using rank. PCBs and carotenoids have been assessed individually and as pooled subclasses. PCBs have been assessed as linear terms, continuous and categorical (quartile); and as quadratic continuous terms. Significant interactions were most often shown using the continuous quadratic term; only alpha-carotene demonstrated significant interactions with total combined PCBs using

continuous linear modeling ($p < 0.05$). Even so, quartile analysis of the interaction between non-dioxin-like PCB subclass and pooled carotenoids was executed next.

Environmental chemical exposure studies usually lack normality. Quartile analysis may be expected to provide information that could be missed using continuous analysis and measurements of central tendency and distribution. To better understand the effects of the PCB:carotenoid interaction, the four quartiles were further divided into four additional plots each, yielding sixteen plots. These moving quartiles shifted by 5% increments: 0-25%, 5-30%, 10-35%...75-100% (Figures 4.14 – 4.17). The resulting sixteen plots of pooled non-dioxin-like PCBs and serum carotenoids showed some familiar trends. The first quartile (0-25%) was distinctly different from the remaining fifteen, with a shape similar to a horizontal "s". This trend disappeared at the first 5% shift, however, with a flattening and slight increase in slope. A noticeable trend in increasing slope could also be observed by the second 5% shift, and continued across increasing PCB quartiles, with the most dramatic slopes observed at the third quartile (45-70% & 50-75%) plots. While a slight leveling of slope was observed in the fourth quartile, this analysis clearly demonstrated that higher serum carotenoids were associated with a lower probability of developing metabolic syndrome. Further, serum carotenoids had a bigger positive effect at higher concentrations of PCBs.

Serum carotenoids, singly and combined, were associated with the reduced probability of metabolic syndrome. Carotenoids provided protection despite the presence of higher PCB concentrations. In some analyses, above-median carotenoid concentrations assigned one-half the probability of developing metabolic syndrome.

Research Question 4. Logistical regression of Vitamins C, D, and B6 indicated a strong inverse association with the probability of developing metabolic syndrome, at $p < 0.01$ for all three (Table 4.21). This was neither unexpected nor surprising. Good nutrition has been long associated with good health. The regression coefficients were

larger for serum carotenoids. When Vitamins C, D, and B6 were combined with serum carotenoids, significance overall was observed at $p < 0.0002$.

Mean nutrient analysis of participants with and without metabolic syndrome revealed clear differences between the two groups (Table 4.17). Persons without metabolic syndrome were consistently found to have higher means of all carotenoids, and Vitamins C, D, B6, B12, and serum Folate. Conversely, participants with metabolic syndrome were shown to have higher mean concentrations of alpha-, delta-, and gamma-tocopherol, and retinol, retinyl-palmitate, and retinyl-stearate. It is unclear why Vitamins E and A would be higher in persons with metabolic syndrome. However, they were consistently observed to be higher.

A five covariate model was applied with PCB subclasses to seek statistical interactions in the probability of metabolic syndrome. Table 4.39 examined pooled tocopherols in quartile, continuous linear, and continuous quadratic modeling. Using a continuous linear model, significance was shown for all PCB subclasses and Vitamin E. While this analysis did not indicate direction, all tocopherols in this project, as well as similar cross-sectional studies (Beydoun, et al. 2011), observed direct associations between Vitamin E and probability of metabolic syndrome.

The same five covariate modeling was executed for Vitamin C and PCB subclasses (Table 4.40). Significance was observed for all PCB subclasses with continuous linear modeling, as above. Significance was also seen for non-dioxin-like PCBs ($p < 0.05$) and dioxin-like PCBs ($p < 0.01$) with quartile analysis. Combined PCBs lacked significance.

Combined carotenoids, tocopherols, and Vitamin C were significant (Table 4.41) for all PCB subclasses with continuous linear modeling ($p < 0.05$). Significance was also seen for non-dioxin-like and dioxin-like PCBs using quartile modeling ($p < 0.05$).

Importantly, these last three analyses (Tables 4.39 – 4.41) were an early attempt to seek interactions between serum nutrients and serum PCBs. Several questions remain, among them direction of the association and influence of relevant covariates. The relationships between PCBs and serum nutrients have proven challenging, but useful.

Four covariate modeling and full covariate modeling was executed for alpha-tocopherol, delta-tocopherol, and gamma-tocopherol with each individual PCB congener to seek significant interactions (Tables 4.53 – 4.58). The four covariate model adjusted for age, gender, race/ethnicity, and PIR. The full covariate model adjusted for all covariates known to be relevant to the etiology of metabolic syndrome. Only alpha-tocopherol was observed to be significant for PCB 149, a non-dioxin-like PCB, at $p = 0.01$, 4 covariate model, and $p = 0.02$, full covariate model. Near significance was shown for PCB 196/203 at $p = 0.05$. PCB 196 and PCB 203 are reported together in NHANES 2003-2004. Again, direction of the association was not indicated in this analysis.

The same four covariate and full covariate modeling was executed for Vitamin C and individual PCB congeners. No significant interactions were observed. Near significance was noted for PCB 206, a non-dioxin-like PCB, at $p = 0.08$, and for overall, pooled non-dioxin-like PCBs at $p = 0.11$ in the full covariate model. Carotenoids and Vitamin C provide antioxidant protection and may work synergistically. The lack of a significant statistical interaction between Vitamin C and PCBs does not negate the benefit of Vitamin C as an antioxidant in biological systems.

The four covariate and full covariate modeling was undertaken in part to examine the PCB : carotenoid, PCB : Vitamin C, and PCB : Vitamin E interactions from new perspectives. These models evaluated PCBs as categorical terms, in quartiles; and as continuous terms, linear and quadratic (squared). Neither PCBs nor carotenoids are likely to yield normal curves and outliers are common with both. These data runs were

executed to survey any three dimensional characteristics of the data. Although significance was somewhat more often seen with continuous linear and continuous quadratic modeling, it was determined that analysis of PCBs would proceed as quartiles, and that a broader interpretation of the data could be accomplished with this format.

Research Question 5. Fruit and vegetable servings were evaluated from two 24-hour dietary recalls (Table 4.70). Foods and fluids were extracted from the NHANES database in gram weight and converted to servings using a modified version of a method developed to measure fruit and vegetable servings in an NHANES II study (Patterson, et al. 1990). Fruits and vegetables were extracted in one of four groups: cooked or raw fruit, cooked or raw vegetable, juice, and salad/raw greens. An interesting observation was that of twelve individuals who consumed between 5-10 servings of juice, four met the criteria for metabolic syndrome. This intake level of juice throughout the day would be expected to contribute to hyperglycemia in vulnerable populations, but these individuals would still score higher in overall fruit servings using this method. Notably, this method was developed to gauge population adherence to Five-A-Day recommendations rather than risk reduction related to metabolic disease.

Significant interactions were sought between fruit and vegetables from the diet and PCB subclasses using quartile modeling, continuous linear modeling, and continuous quadratic modeling. No significance was observed until the last group of 120 salad servings was entered. Significant interactions were seen for dioxins (not dioxin-like PCBs), but polychlorinated dibenzo-*p*-dioxins, and fruit and vegetable servings, using continuous linear modeling ($p < 0.05$). Dioxins are most often produced as a byproduct of incineration and have no commercial utility. Dioxin-like PCBs act through similar mechanisms as the dioxins to produce toxic effects; both are ligands for the AhR and induce gene expression of the cytochrome P450 enzyme, CYP1A1, CYP1A2, and CYP1B1, families (Williams, et al. 2005). The most potent dioxin, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), has been assigned a TEF = 1.0. The coplanar and mono-ortho-substituted

PCBs have also been assigned a TEF, determined by the product of the individual congener concentration and potency relative to TCDD. Despite these similarities, no PCBs were found to be significant in this analysis.

In Project II, a preliminary analysis of PCBs 118, 126, and 153 was executed with fruit servings (Figures 4.4 – 4.6) in the probability of metabolic syndrome. The fruit servings were based on two 24-hour dietary recalls, but the data were converted by the MPED 2.0 to "cup equivalents". The cup equivalents were based on MyPyramid servings. For this data run, cup equivalents were assessed "per 1000" calories to disengage from calorie intake. This permitted assessment of nutrient density per 1000 calories without regard for calorie recommendations or total calorie intake. No associations were observed, however, between fruit intake and metabolic syndrome in any PCB model. The MPED2.0 database presented some challenges. "Cup equivalents" were based on amounts per 100 gram weight as extracted from Release 18 of the U.S.D.A. National Nutrient Database for Standard Reference. Resulting amounts did not necessarily represent a "serving" or make intuitive sense when analyzing intakes. Further, the MPED 2.0 method of disaggregating foods would, for instance, count only the strawberries on a cheesecake. Too much of the diet remained unknown, but may have been a factor in disease risk. No associations were observed between fruit intake and reduced probability of metabolic syndrome using the MyPyramid Equivalent Database 2.0 in models of PCB 118, PCB 126, and PCB 153. Further, this analysis occurred prior to the application of covariate modeling as a surrogate for sample weights. The use of MEC subsample C weight in this analysis across several samples with disparate characteristics was structured according to NHANES tutorials to use the smallest sample weight. Applying the covariate model may well yield different results, although the same challenges would exist with MPED 2.0.

A stated objective of Project III was to determine the best method to access the full diet of NHANES 2003-2004 subsample C participants (those evaluated for PCB

concentrations) for analysis using DQATE. Analysis of the broader, overall diet was not a task easily accomplished with NHANES. The database more easily accommodated extraction and analysis of specific data points related to diet.

Research Question 6. Nutrient analysis was based on two 24-hour dietary recalls (Tables 4.59 – 4.69). These nutrients were analyzed in quartile, continuous linear, and continuous quadratric models for all PCB subclasses, but also dioxins, furans, and combined dioxin-like chemicals (dioxin-like PCBs + dioxins + furans). Statistical significance spanned all models for various nutrients, but the recall-sourced nutrients with significance included lycopene, Vitamin C, potassium, magnesium + potassium + calcium, and selenium. Dietary carotenoids and food-sourced nutrients are associated with lower prevalence of several chronic diseases.

The mean nutrient intake of participants was assessed in Appendix I across fruit and vegetable servings. Participants were classified into one of five groups ranging from zero to ≥ 9 servings per day. Magnesium, potassium, and dietary fiber increased across the five groups. These nutrients are plentiful in fruits and vegetables. Total sugars and total carbohydrate were also higher in the ≥ 9 servings category, although participants consuming no fruits and vegetables had higher carbohydrate and total sugar intakes than participants in either of the next two groups (>0<3 & 3<6). Total sugar intake represents not only the natural sugars (glucose, fructose, & galactose) found within carbohydrate-containing foods, but also the added sugars (sucrose, high-fructose corn sweetener) found in refined, sweetened foods, desserts, and soft drinks. Several nutrients were found at the highest mean concentrations in the fourth intake level, 6 up to 9 servings per day, and then tapering off at ≥ 9 servings per day. Only nineteen participants comprised this highest intake category, twelve of whom consumed between 5-10 servings of juice. While these participants were more likely to have met the recommended fruit and vegetable intake level, questions remain as to the quality of their overall diet and lifestyle.

General Discussion. The mean age for participants with metabolic syndrome in this study was 55.6 years as compared to 46.9 years for those without metabolic syndrome. The subpopulation was fairly equally distributed by gender, with females making up 51.2% of the dataset. Among women, 19.6% met the criteria for metabolic syndrome. Among men, 24.8% met the criteria.

Of the overall dataset, non-Hispanic whites made up 57.2%, Mexican Americans made up 18.6%, and non-Hispanic blacks comprised 16.6%. The "other" category included Hispanics other than Mexican heritage, multi-racial, American Indian, and other races that may have been mentioned. NHANES oversampled Hispanic and black participants to increase validity of responses, but corrected for oversampling by applying sample weights to ensure the responses were a reflection of the U.S. Census for that timeframe. As mentioned in Chapter 3, the application of NHANES sample weights was not an option due to the use of different subsamples with disparate characteristics and dedicated subsample weights. Instead, the variables used by NHANES to establish their weighting methodology were used, one of which was "race/ethnicity" (Graubard & Korn, 1999). Among Mexican Americans, 22.8% met the criteria for metabolic syndrome. 22.6% of non-Hispanic whites and 18.2% of non-Hispanic blacks met the criteria of metabolic syndrome.

Demographic and lifestyle characteristics examined by metabolic syndrome status revealed some interesting distinctions. Among participants with metabolic syndrome, only 33.8% reported being current cigarette smokers. Yet, 79.9% of participants with metabolic syndrome tested positive for serum cotinine (≥0.015 ng/mL), considered a marker for passive and/or active smoking. Similar trends were seen for participants without metabolic syndrome (24.4% current smokers, 79.0% positive serum cotinine). This either illustrates one challenge encountered with subjective data collection or this dataset possessed a great many passive smokers.

Alcohol consumption was evaluated categorically as non-drinker, moderate drinker, and excessive drinker. Of the 875 participants that responded to this part of the questionnaire, moderate drinkers made up the largest category without regard for their metabolic syndrome status. Of those not meeting the criteria for metabolic syndrome, 47.1% were moderate drinkers, whereas 41.8% of moderate drinkers did meet the criteria for metabolic syndrome.

Epidemiological studies have categorized PCBs in various ways to better understand their complicated relationships and health effects. Different congener profiles evoke different biological responses. PCBs have often been classified by whether they possess one or more chlorine atoms in the ortho- position. Non-ortho-substituted PCBs are dioxin-like and are given a TEF rating based on their toxicity in relation to tetrachlorodibenzo-*p*-dioxin (TCDD), which has the highest toxicity rating. Dioxin-like PCBs are ligands for the aryl hydrocarbon receptor (AhR), a nuclear receptor that regulates transcription of the CYP 1A1 and 1B1 families. The mono-ortho-substituted PCBs have one chlorine atom in the ortho- position, exhibit partial dioxin-like activity, and are also given a TEF rating, although weaker. Finally, the di-ortho-substituted PCBs are non-dioxin-like and follow different pathways. New evidence suggests they are ligands for the constitutive androstane receptor (CAR) transcription factor. Researchers have also categorized PCBs by their degree of chlorination without regard for subclass or receptor affinity, finding that dose response curves differed with degree of chlorination. A recent prospective study found that with moderately chlorinated PCBs, such as 87, 99, and 118, insulin resistance (determined by elevated HOMA-IR) occurred above the 3rd quartile, but with the higher chlorinated PCBs 178, 194, and 199, HOMA-IR increased from the 1st to the 2nd quartile, then decreased through the 4th quartile, forming the inverted U-shape (Lee, et al. 2011b). Yet, other researchers have considered analyzing PCB effects based on underlying hormonal effects, i.e. estrogenic, neurotoxic, anti-estrogenic (Wolff M, Camann D, Gammon M, Stellman S, 1997). The majority of studies have classified PCBs according to their

classification based on the ortho- positioning of chlorine atoms, and NHANES classified PCBs in this manner and placed non-ortho- and mono-ortho-substituted PCBs with dioxins and furans exhibiting similar characteristics. This study followed the NHANES classification method. PCB categorization was but one of the methodological issues that could influence results and had to be considered before nutrition could be factored.

It became clear early on that interpreting the effect of two variables, diet and PCBs, on the probability of metabolic syndrome would have to be approached with caution. Both PCBs and dietary intake data lack normality and outliers are common. Analyzing the broader diet beyond extracting specific data points became untenable in conjunction with serum PCB concentrations. Earlier NHANES data releases have facilitated analysis of the diet using the Healthy Eating Index 2005 (HEI-2005) for evaluation. NHANES 2003-2004 does not allow for dietary analysis using HEI-2005 at this time. The HEI-2005 may be a better measure of intakes when assessing nutrient adequacy rather than as a measure of risk reduction for individuals exposed to PCBs or at risk of developing metabolic syndrome. However, it could establish a baseline.

One type of measurement error that can arise with 24-hour dietary intake recalls in the assessment of diet-disease relationships concerns foods that are episodically consumed. These are foods that may be a usual part of the diet, but are not consumed daily, such as salad greens. Two 24-hour dietary recalls can miss consumption of these foods entirely. Assessing overall diet in this manner and then relating to a health outcome could lead to error, as well as oversight of the true dietary patterns relevant to disease risk. The NHANES 2003-2004 food frequency questionnaire (FFQ) measured usual dietary intake for the preceding twelve month period. While FFQs are known to be susceptible to reporting bias, combining a FFQ with 24-hour dietary intake recalls has been shown to substantially increase the measurement of true intakes of episodically consumed foods (Kipnis, et al. 2009). These episodically consumed foods would include not only salad greens, but most colorful vegetables. While combining two 24-hour

recalls and the FFQ may provide an increased ability to predict true intake, the interaction of any and all dietary constituents/food patterns with PCBs must be factored first.

The demographic and behavioral covariates relevant to metabolic syndrome, PCB concentrations, and nutrient concentrations, have been previously defined and categorized. Adjustment for these variables after PCB : nutrient statistical interaction modeling has proven challenging. In particular, age has been shown to have a strong independent influence on the probability of metabolic syndrome. Age-adjusted analysis of the prevalence of metabolic syndrome for significant PCB : carotenoid interactions has been ongoing.

A significant inverse association has been demonstrated in this study between (1) serum carotenoids and (2) food-sourced (dietary recall-sourced) nutrients in modeling with PCBs, and (3) fruit and vegetable servings in modeling with polychlorinated dibenzo-*p*-dioxins, in the probability of developing metabolic syndrome. While several of these analyses may be expanded, these results are encouraging. In particular, serum carotenoids have been shown to be strikingly protective in the probability of metabolic syndrome, despite even the presence of higher PCB concentrations.

CONCLUSIONS

PCBs have neither been manufactured nor distributed since the late 1970s, yet they persist in the environment and will take decades or longer to degrade. Evidence of their harmful effects has been well documented in animal and human studies. Results from animal models have suggested nutrient therapies, but these studies often analyze one PCB and one nutrient at levels that do not represent actual environmental risk ratios. Importantly, individuals and communities have lived with environmental chemical contamination for decades and continue to do so.

The various modeling that has been developed and executed in this work has been undertaken to evaluate the effects of PCBs and serum carotenoids on the probability of developing metabolic syndrome. Further, it was based on the actual serum concentrations and clinical parameters of individuals residing in the United States in 2003-2004. Metabolic syndrome is a precondition of CVD and T2DM, which are leading causes of morbidity and mortality in Kentucky and the United States. This work represents an initial step toward understanding the complex relationships that determine the fate of PCB exposure on cardiovascular and metabolic health. It is noteworthy that these data represent actual pollutant and nutrient concentrations in human beings undergoing a comprehensive clinical medical examination to assess health status.

Nutrition is the ideal mode for mitigating the damage caused by PCBs. On the molecular level, a growing number of phytochemicals have been identified as natural ligands for nuclear receptors influencing various pathways related to homeostasis. The biochemical and physiological functions of vitamins, minerals, and fiber to health have been long established. As a behavioral modality, improved nutrition may be best gauged by fruit and vegetable consumption. Nine servings per day seldom occur by chance, and the commitment to good nutrition often yields benefits beyond an improved nutritional status to other healthy behaviors across the lifestyle. And, yet, the primary route of exposure to PCBs is also through food. Importantly, the foods

suggested in this work to effectively decrease the probability of developing metabolic syndrome are the foods less likely to concentrate with PCBs and they are plentiful in vitamins, minerals, phytochemicals, and fiber. Mechanistic studies may undoubtedly reveal additional benefits.

Nutrition professionals daily provide recommendations based on the current evidence with confidence that these recommendations will improve the health and wellbeing of their clientele. The current state of evidence recommends a normal weight BMI of 18.5-24.9 to prevent the development of chronic disease, in particular the cardiometabolic abnormalities addressed in this dissertation. If the excess adipose tissue of a BMI ≥ 25.0 is concentrated with PCBs and other lipophilic contaminants, it should be considered that weight loss may not be an entirely safe and recommended therapy. Many questions remain. However, where weight loss may be neither advisable nor achievable, the practice of consistent, sound nutrition may be expected to yield positive and dynamic benefits.

APPENDIX A

ACRONYMS

AACE	American Academy of Clinical Endocrinologists
AHA	American Heart Association
AhR	aryl hydrocarbon receptor
AICR	American Institute for Cancer Research
AMI	acute myocardial infarction
ARMD	age-related macular degeneration
ATBC	alpha-tocopherol beta-carotene
ATPIII	Adult Treatment Panel III
ATSDR	Agency for Toxic Substances and Disease Registry
BRFSS	Behavioral Risk Factor Surveillance System
BMI	body mass index
CARET	Beta Carotene and Retinol Efficacy Trial
CBC	complete blood count
CDC	Centers for Disease Control and Prevention
CEC	Community Engagement Core
CHD	coronary heart disease
cm	centimeters
C-RP	c-reactive protein
CYP1A1	cytochrome P450 1A1
CVD	cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
DBP	diastolic blood pressure
DDE	dichlorodiphenyldichloroethylene
DDT	1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, - commonly dichlorodiphenyltrichloroethane
DHA	dehydroascorbic acid
DNA	deoxyribonucleic acid
DQATE	Diet Quality Index for Toxin Exposure
DRIs	Dietary Reference Intakes
EDCs	endocrine disrupting chemicals
ERB	Ethics Review Board
ETS	environmental tobacco smoke
FOS	Framingham Offspring Study
FNDDS	Food and Nutrient Database for Dietary Studies
HCB	hexachlorobenzene
HDL	high-density lipoprotein
Hg	mercury
HOMA-IR	homeostatic model assessment of insulin resistance

IARC	International Agency for Research on Cancer
IFF	Individual Foods File
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IL-10	interleukin 10
IOM	Institute of Medicine of the National Academies
IRB	Institutional Review Board
kcals	kilocalories
kg	kilogram
LDL	low-density lipoprotein
LOD	limit of detection
m^2	per squared meter
MEC	Mobile Examination Center
MET	metabolic equivalent
MetS	metabolic syndrome
µg/dL	micrograms per deciliter
µmol/L	micromoles per liter
mg/dL	milligrams per deciliter
MI	myocardial infarction
mmol/L	millimoles per liter
MPED	My Pyramid Equivalents Database
mV	millivolt
MUFA	monounsaturated fatty acid
NADPH	nicotinamide adenine dinucleotide phosphate
NALFD	non-alcoholic fatty liver disease
NCEP	National Cholesterol Education Program
NCHS	National Center for Health Statistics
NF-kB	nuclear factor kappa-B
ng/g	nanograms per gram
ng/mL	nanograms per milliliters
NGT	normal glucose tolerance
NHANES	National Health and Nutrition Examination Survey
NHLBI	National Heart Lung and Blood Institute
NHSR	National Health Statistics Reports
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
nm	nanometers
NTP	National Toxicology Program
OR	odds ratio
PBBs	polybrominated biphenyls
PCDDs	polychlorinated dibenzodioxins
PCDFs	polychlorinated dibenzofurans
PCBs	polychlorinated biphenyls
pg/g	picograms per gram

POPs	persistent organic pollutants
ppm	parts per million
ppt	parts per trillion
PSU	primary sampling unit
PUFA	polyunsaturated fatty acid
RDA	Recommended Daily Allowance
ROS	reactive oxygen species
RR	relative risk
SBP	systolic blood pressure
SC	serum carotenoids
SFA	saturated fatty acid
T1DM	Type 1 diabetes mellitus
T2DM	Type 2 diabetes mellitus
TCDD	2,3,7,8-tetrachlorodibenzo-*p*-dioxin
TEQ	toxicity equivalent
TLC	therapeutic lifestyle change
TNF	Total Nutrients File
UK-SRP	University of Kentucky Superfund Research Program
USDA	United States Department of Agriculture
USDHHS	United States Department of Health and Human Services
UV	ultraviolet
WDP	western dietary pattern

APPENDIX B

CONCEPTUAL DEFINITIONS

Bioaccumulation. The progressive increase in the amount of a substance in an organism or part of an organism, which occurs because the rate of intake exceeds the organism's ability to remove the substance from the body. (International Union of Pure & Applied Chemistry, 1993)

Bioconcentration. A process leading to a higher concentration of a substance in an organism than in the environmental media to which it is exposed (Barron, 1990; Toxic Substances Hydrology Program, United States Geological Survey)

Biomagnification. Biomagnification is the sequence of processes in an ecosystem by which higher concentrations of a particular chemical, such as the pesticide DDT, are reached in organisms higher up the food chain, generally through a series of prey-predator relationships (Oxford University, 2008)

Biotransformation. Biochemical mechanism employed by target to breakdown the agent; enzymatic pathways. Metabolic activation or detoxification reactions that increase hydrophilicity and promote excretion by changing an agent into its metabolite which may be more or less toxic to target (Eaton, 2005)

Body burden. The total amount of a chemical, metal, or radioactive substance present at any time after absorption in the body of a human or animal (biology-online.org).

Dose-response relationship. A relationship in which a change in the amount, intensity or duration of exposure is associated with a change, either increase or decrease, in risk of a specified outcome (International Programme on Chemical Safety 2001)

Exposure. Contact between an agent and a target with contact taking place at an exposure surface over an exposure period by an exposure route (International Programme on Chemical Safety, 2000)

Mixture. Any combination of two or more agents regardless of source or of special or temporal proximity (Agency for Toxic Substances and Disease Registry, 2001)

Persistent organic pollutant. Persistent organic pollutants (POPs) are chemical substances that persist in the environment, bioaccumulate through the food web, and pose a risk of causing adverse effects to human health and the environment. (United Nations Environmental Programme)

APPENDIX C

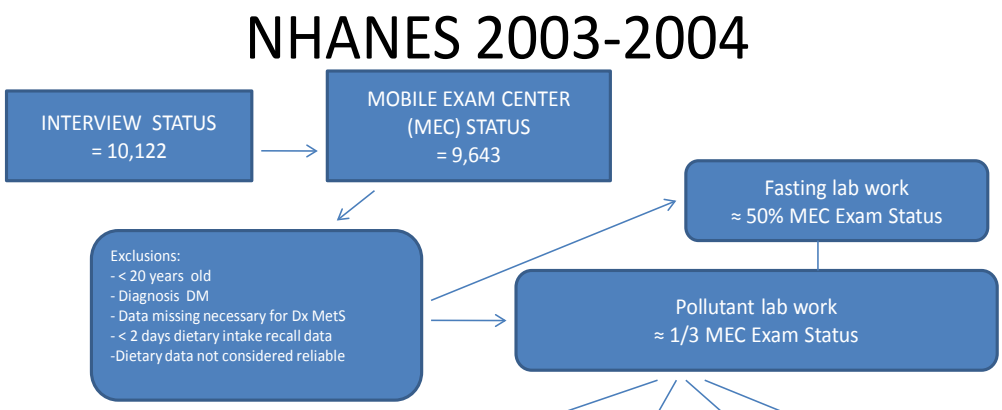
HISTORY OF NHANES

YEAR	EVENT
1902	U.S. Bureau of the Census established to collect vital statistics
1949	U.S. National Committee on Vital and Health Statistics
1951	Subcommittee on National Morbidity Surveys Report
1953	Merged into U.S. Department of Health, Education, and Welfare
1953	Proposal for Collection of Data on Illness and Impairment
1956	National Health Survey Act (Public Law 652)
	National Center for Health Statistics (NCHS)
1956-1960	Public Health Services Report to the Surgeon General
	Public Health Conference on Records & Statistics
1960-1962	First National Health Examination Survey NHES I
1963-1965	NHES II
1966-1970	NHES III
1969	White House Task Force Report on **Nutrition**
1971-1975	National Health and Nutrition Examination Survey
	NHANES I
1976-1980	NHANES II
1982-1984	Hispanic NHANES
1988-1994	NHANES III
1999-present	Continuous NHANES

U.S. Vital Statistics System, Major Activities and Developments, 1950-1995; by the CDC, NCHS 1997.

APPENDIX D

NHANES SAMPLING STATUS



SERUM POLLUTANT DATA

SUBSAMPLE C			SUBSAMPLE B		SUBSAMPLE A	VOC WEIGHT
Dioxins, Furans, Coplanar PCBs N ≈ 1850	Non-dioxin-like PCBs N ≈ 1850	Total combined PCBs *(Class created by Cave)*	Organochlorine pesticides N ≈ 1950	Polybrominated diphenyl ethers N ≈ 2000	Polyfluorinated compounds* N ≈ 2000	Volatile organic compounds* (whole blood)
Other Subsample C pollutants:			**Other Subsample B pollutants:**		**Other Subsample A pollutants:**	
Urinary OrganoPhosphate Insecticides, Urinary Iodine*, Urinary Environmental Phenols*, Urinary Current Use Pesticides, Urinary Environmental Pesticides*			Urinary Phthalates*, Urinary PAHS		Total & Speciated Arsenics*, Heavy metals*, Urinary Mercury*	

* Also available in 2005-2006

APPENDIX E

FRUIT AND VEGETABLE SERVINGS

3RD & 4TH PCB QUARTILES

PARTICIPANTS NOT MEETING CRITERIA OF METABOLIC SYNDROME

Fruit and vegetable servings of participants within the 3rd and 4th PCB exposure quartiles who do not meet the criteria of MetS

			MEAN	MINIMUM	MAXIMUM
Non-dioxin-like PCBs	3rd quartile	Fruit	1.0848	0	6.0490
		Vegetable	1.6890	0	6.3335
	4th quartile	Fruit	1.1953	0	7.3985
		Vegetable	1.5442	0	6.4715
Dioxin-like PCBs	3rd quartile	Fruit	1.1244	0	7.3985
		Vegetable	1.7132	0	8.8725
	4th quartile	Fruit	1.2488	0	8.2535
		Vegetable	1.6139	0	6.4715
Combined PCBs	3rd quartile	Fruit	1.0980	0	8.2535
		Vegetable	1.7564	0	8.8725
	4th quartile	Fruit	1.2570	0	7.3985
		Vegetable	1.5464	0.0090	6.4715

APPENDIX F

FRUIT AND VEGETABLE SUBCATEGORY SERVINGS

3RD & 4TH PCB QUARTILES

PARTICIPANTS NOT MEETING CRITERIA OF METABOLIC SYNDROME

Fruit and vegetable subcategory servings of participants within the 3rd and 4th PCB exposure quartiles who do not meet the criteria of MetS

			MEAN	MINIMUM	MAXIMUM
Non-dioxin-like PCBs	3rd quartile	fruit	1.0848	0	6.0490
		F_CITMLB	0.4954	0	4.9555
		F_OTHER	0.5893	0	4.9555
		vegetable	1.6890	0	6.3335
		V_POTATO	0.3801	0	2.1800
		V_TOMATO	0.3971	0	3.1595
		V_DRKGR	0.1166	0	2.3320
		V_ORANGE	0.0911	0	2.0130
		V_STARCHY	0.0759	0	1.6580
		V_OTHER	0.6280	0	2.4835
	4th quartile	Fruit	1.1953	0	7.3985
		F_CITMLB	0.4908	0	3.9595
		F_OTHER	0.7044	0	6.5385
		Vegetable	1.5442	0	6.4715
		V_POTATO	0.3958	0	3.1140
		V_TOMATO	0.3117	0	2.2895
		V_DRKGR	0.1245	0	1.5500
		V_ORANGE	0.0936	0	1.4685
		V_STARCHY	0.0936	0	2.0410
		V_OTHER	0.5253	0	2.8335
Dioxin-like PCBs	3rd quartile	Fruit	1.1244	0	7.3985
		F_CITMLB	0.5213	0	4.9555
		F_OTHER	0.6031	0	5.0735
		Vegetable	1.7132	0	8.8725
		V_POTATO	0.4155	0	4.9875
		V_TOMATO	0.4056	0	2.7560
		V_DRKGR	0.1148	0	1.4680
		V_ORANGE	0.0941	0	2.0130
		V_STARCHY	0.0835	0	1.6580
		V_OTHER	0.5996	0	2.6105
	4th quartile	Fruit	1.2488	0	8.2535
		F_CITMLB	0.5147	0	6.8275
		F_OTHER	0.7341	0	6.5385
		Vegetable	1.6139	0	6.4715
		V_POTATO	0.3795	0	3.1140
		V_TOMATO	0.3521	0	3.1595
		V_DRKGR	0.1266	0	2.3320
		V_ORANGE	0.0877	0	1.4685
		V_STARCHY	0.0893	0	2.0410
		V_OTHER	0.5786	0	2.8335

APPENDIX F (CONTINUED)

	3rd quartile	Fruit	1.0980	0	8.2535
		F_CITMLB	0.5314	0	6.8275
		F_OTHER	0.5665	0	4.9555
		Vegetable	1.7564	0	8.8725
		V_POTATO	0.4292	0	4.9875
		V_TOMATO	0.4133	0	3.1595
		V_DRKGR	0.1228	0	2.3320
		V_ORANGE	0.0843	0	1.0755
		V_STARCHY	0.0816	0	1.6580
		V_OTHER	0.6249	0	2.4835
	4th quartile	Fruit	1.2570	0	7.3985
		F_CITMLB	0.4948	0	3.9595
		F_OTHER	0.7621	0	6.5385
		Vegetable	1.5464	0.0090	6.4715
		V_POTATO	0.3668	0	3.1140
		V_TOMATO	0.3170	0	2.2895
		V_DRKGR	0.1211	0	1.5500
		V_ORANGE	0.1047	0	2.0130
		V_STARCHY	0.0936	0	2.0410
		V_OTHER	0.5431	0	2.8335

APPENDIX G

GRAMS TO SERVINGS CONVERSION TABLE

Food and Nutrient Database for Dietary Studies 2.0

The following considerations were used to convert gram weight to servings:

Fruit:	average piece of whole fruit
	6 ounces juice
Vegetables:	½ cup cooked or raw

Lower limits:	30 grams fruit or vegetable
	62 grams juice
	20 grams salad/raw greens

A large single serving of fruit or vegetable could not exceed 2 servings.
Juice consumed on a single eating occasion could not exceed 3 servings.

1 serving:	Vegetables – many ½ cup portions weight ~ 75 grams
	Fruits – many whole fruits weigh ~ 120 grams

2 servings:	any portion of vegetables (except salad) weighing ≥ 150 grams
	Any portion of fruit (except fruit juice) weighing ≥ 240 grams
	Fruit juice: 372-557 grams

3 servings:	Fruit juice: ≥ 558 grams

Salad:	Coded as one serving per eating occasion up to a maximum of 4 per day
	(1 cup Romaine = 45 grams)

Extended as follows:

Juice:	6 ounces < 12 ounces (186–371 g)
	12 ounces < 18 ounces (372-557 g)
	18 ounces < 24 ounces (558-743 g)
	24 ounces < 30 ounces (744-930 g)

Fruit:	1 serving = 30-239 g	Vegetable:	1 serving = 30-149 g
	2 servings = 240-449 g		2 servings = 150-269 g
	3 servings = 450-659 g		3 servings = 270-389 g
	4 servings = 660-869 g		4 servings = 390-509 g
	5 servings = 870-1079 g		5 servings = 510-629 g
	6 servings = 1080-1289 g		6 servings = 630-749 g

APPENDIX G (CONTINUED)

Eliminate white potatoes and legumes. White potatoes were considered as a starchy vegetable in this analysis. Legumes were considered a plant protein or starchy vegetable.

	VEGETABLE	FRUIT	JUICE	SALAD/RAW GREENS
Lower limit	30 grams	30 grams	62 grams	20 grams
1 serving	30-149 grams	30-239 grams	62-371 grams	1 serving per eating occasion
2 servings	150-269 grams	240-449 grams	372-557 grams	1 serving per eating occasion
3 servings	270-389 grams	450-659 grams	558-743 grams	1 serving per eating occasion
4 servings	390-509 grams	660-869 grams	744-929 grams	1 serving per eating occasion-maximum of 4
5 servings	510-629 grams	870-1079 grams	930-1115 grams	
6 servings	630-749 grams	1080-1289 grams	1116-1301 grams	
7 servings	750-869 grams	1290-1499 grams	1302-1487 grams	
8 servings	870-989 grams	1500-1709 grams	1488-1673 grams	
9 servings	990-1109 grams	1710-1919 grams	1674-1859 grams	
10 servings	1110-1229 grams	1920-2129 grams	1860-2045 grams	

Modified from Patterson, Block, Rosenberger, Pee, Kahle, 1990.

APPENDIX H

BASELINE CHARACTERISTICS BY FRUIT AND VEGETABLE INTAKE

Fruit and Vegetable Intake based on 2 day dietary recalls
FNDDS 2.0 & NHANES Individual Food Files, Days 1 & 2.
FNDDS2.0 analyzes portion size per gram weight.
Conversion to servings per day based Patterson, et al. 1990

		0 servings/day	> 0 < 3.0 servings	3.0 < 6.0 servings	6.0 < 9.0 servings	≥ 9.0 servings/day
Number participants		29	427	448	100	19
Fruit/vegetable intake, mean (servings)		0	1.56	3.71	6.81	10.05
Age at screening		39.31	46.07	52.37	48.82	49.74
Gender	Male	18	215	205	45	13
	Female	11	212	243	55	6
Race/ethnicity	Mexican American	6	90	79	14	3
	Other Hispanic	0	11	23	3	0
	Non-Hispanic White	15	229	266	62	12
	Non-Hispanic Black	8	81	63	12	4
	Other Race – Uncluding Multi-Racial	0	16	17	9	0
Poverty income ratio		2.22	2.53	2.86	2.92	3.44
Total cholesterol		197.60	202.24	202.24	203.02	202.63
Cigarette smoking status	never	15	211	239	54	11
	current	13	122	68	14	2
	former	1	94	142	32	6
Serum cotinine	<0.015	2	75	114	21	5
	>=0.015	27	352	332	79	14
Physical activity status	<=390 mins	4	80	75	12	2
	>390 mins	10	151	222	57	12
Alcohol status	drinker	4	15	21	0	0
	non-drinker	19	284	298	70	16
BMI	underweight	1	8	5	2	0
	normal	12	131	134	38	5
	overweight	8	129	171	37	9
	obese	8	158	136	23	5
Dietary supplement use	yes	8	198	260	66	12
	no	21	229	188	34	7
C-reactive protein	<0.02	3	6	10	2	0
	>=0.02	26	421	438	98	19

APPENDIX I

MEAN NUTRIENT INTAKES
PER SERVINGS FRUITS & VEGETABLES

	0 servings/day	> 0 < 3.0 servings	3.0 < 6.0 servings	6.0 < 9.0 servings	≥ 9.0 servings/day
Number of participants	29	427	448	100	19
Fat grams	82.44	82.03	78.68	86.27	83.91
Carbohydrate grams	280.43	253.59	265.36	328.20	330.77
Protein grams	77.23	80.45	82.08	98.23	92.42
Dietary fiber (grams)	11.92	13.56	17.60	23.01	26.53
Total sugars (grams)	137.85	115.61	124.59	160.31	160.62
Magnesium (mg)	220.60	255.04	295.22	382.54	400.47
Potassium (mg)	2131.69	2418.01	2883.88	3878.33	4361.66
Calcium (mg)	703.66	819.03	878.28	1180.17	1002.26
Zinc (mg)	10.94	11.99	12.09	14.63	12.59
Iron (mg)	14.00	15.59	16.07	20.92	17.30
Selenium (mcg)	102.95	106.74	110.34	128.44	122.18
Vitamin B6 (mg)	1.48	1.74	2.02	2.61	2.39
Total Folate (mcg)	332.28	369.98	427.70	562.14	505.92
Vitamin B12 (mcg)	4.22	5.89	5.31	6.06	4.09

APPENDIX J

BASELINE & LIFESTYLE CHARACTERISTICS BY METABOLIC SYNDROME STATUS

Baseline & Lifestyle Characteristics by Metabolic Syndrome Status		MetS+ N=234	MetS- N=824
Gender	Female	106	436
	Male	128	388
Race/ethnicity	Mexican American	45	152
	Other Hispanic	9	28
	Non-Hispanic White	137	468
	Non-Hispanic Black	32	144
	Other Race – Including Multi-Racial	11	32
Cigarette smoking	Never	109	436
	Former	46	186
	Current	79	201
Serum Cotinine	≥ 0.015 ng/mL	187	649
	<0.015 ng/ml	47	173
Alcohol consumption	Non-drinker	63	139
	Non-excessive drinker	81	321
	Excessive drinker	50	221
Physical activity	Sedentary (no physical activity)	61	233
	Low activity	28	100
	Moderately to vigorously active	34	184
BMI	<18.5	0	18
	18.5-24.9	32	299
	25.0-29.9	91	275
	≥30	110	230
Dietary supplement	yes	121	436
	no	113	387

APPENDIX K

MEAN SERUM NUTRIENT ANALYSIS BY METABOLIC SYNDROME STATUS

Nutrient Analyte umol/L	Without metabolic syndrome Mean	With metabolic syndrome Mean
Age at Screening	46.866	55.641
Family Poverty Income Ratio	2.716	2.602
Alpha-carotene	0.0831683	0.0634927
Alpha-cryptoxanthin	0.0492606	0.0405013
Beta-carotene	0.3776834	0.3105232
Beta-cryptoxanthin	0.1764506	0.1552558
Lycopene	0.7738627	0.6761464
Lutein/zeaxanthin	0.2936455	0.2688734
Alpha-tocopherol	31.5914209	36.4841017
Delta-tocopherol	0.1530406	0.2054309
Gamma-tocopherol	5.1014411	6.4090137
Retinyl palmitate	0.0413989	0.0478064
Retinyl stearate	0.0132151	0.0145120
Retinol	2.0141357	2.1117940
Vitamin C	57.9829448	52.6510823
Vitamin D (ng/mL)	23.4453883	21.7435897
Vitamin B6 (nmol/L)	69.9550617	51.6043103
Vitamin B12 (pmol/L	446.8441677	372.110042
Folate, serum (nmol/L)	32.3224787	30.5752137

APPENDIX L

ANALYTE, NUMBER OF OBSERVATIONS, LOD, MEAN, MEDIAN, GEOMETRIC MEAN
for
NON-DIOXIN-LIKE PCBS

Chemical Compound	Variable	N=	LLOD value	% above LOD	Mean	Median	Geometric mean
Non-dioxin-like PCBs							
PCB 44	LBX044LA	939	-	100	2.45	2.00	2.01
PCB49	LBX049LA	933	0.11	99.46	1.54	1.30	1.26
PCB52	LBX052LA	943	-	100	3.22	2.70	2.62
PCB87	LBX087LA	939	0.19	83.39	1.07	0.90	0.67
PCB99	LBX099LA	935	0.10	99.89	6.95	4.20	4.56
PCB101	LBX101LA	942	0.26	97.03	2.20	1.65	1.62
PCB110	LBX110LA	933	0.33	99.25	1.62	1.20	1.20
PCB128	LBX128LA	934	0.22	27.19	0.20	0.08	0.11
PCB138 & 158	LBX138LA	942	-	100	29.58	18.62	18.07
PCB146	LBX146LA	943	0.08	99.58	4.67	2.79	2.69
PCB149	LBX149LA	932	0.11	96.24	0.79	0.60	0.59
PCB151	LBX151LA	928	0.16	80.60	0.41	0.30	0.27
PCB153	LBX153LA	943	-	100	39.61	25.80	24.10
PCB170	LBX170LA	942	0.22	99.58	11.66	8.21	6.95
PCB172	LBX172LA	938	0.22	82.84	1.60	1.11	0.82
PCB177	LBX177LA	941	0.22	92.14	2.76	1.65	1.48
PCB178	LBX178LA	940	0.22	90.74	2.28	1.50	1.21
PCB180	LBX180LA	942	0.16	99.89	33.24	22.50	19.15
PCB183	LBX183LA	939	0.16	96.17	3.12	2.00	1.85
PCB187	LBX187LA	939	0.16	99.47	9.43	5.90	5.36
PCB194	LBX194LA	919	0.22	92.71	7.53	5.04	3.59
PCB195	LBX195LA	911	0.42	70.36	1.63	1.10	0.82
PCB196 & 203	LBX196LA	938	0.16	96.80	6.21	4.20	3.41
PCB199	LBX199LA	932	0.16	95.82	7.97	4.71	3.82
PCB206	LBX206LA	933	0.31	98.61	5.21	2.82	2.66
PCB209	LBX209LA	930	0.31	98.17	3.95	1.58	1.80

LLOD is the value below which the chemical was not detectable.

APPENDIX M

ANALYTE, NUMBER OF OBSERVATIONS, LOD, MEAN, MEDIAN, GEOMETRIC MEAN
for
DIOXIN-LIKE PCBS

Chemical Compound	Variable	N=	LLOD value	% above LOD	Mean	Median	Geometric mean
Dioxin-like PCBs							
PCB28	LBX028LA	923	-	100	5.59	4.98	4.89
PCB66	LBX066LA	943	0.38	99.15	1.80	1.37	1.43
PCB74	LBX074LA	943	-	100	8.58	4.90	5.44
PCB105	LBX105LA	933	0.13	98.71	2.29	1.20	1.36
PCB118	LBX118LA	936	-	100	11.54	6.00	6.89
PCB156	LBX156LA	941	0.16	94.79	6.35	4.03	3.26
PCB157	LBX157LA	929	0.22	80.94	1.52	0.98	0.73
PCB167	LBX167LA	931	0.22	72.18	1.52	0.90	0.62
PCB189	LBX189LA	912	0.22	31.80	0.38	0.09	0.16
3,3',4,4',5-pncb PCB126	LBXPCBLA	930	8.90	95.27	27.03	17.60	18.61
3,4,4',5-tcb, PCB81	LBXTC2LA	930	9.10	39.46	5.86	4.50	5.00
3,3',4,4',5,5'-hxcb PCB169	LBXHXCLA	931	10.20	76.48	17.24	12.10	11.65

LLOD is the value below which the chemical was not detectable.

APPENDIX N

ANALYTE, NUMBER OF OBSERVATIONS, LOD, MEAN, MEDIAN, GEOMETRIC MEAN
for
SERUM CAROTENOIDS, TOCOPHEROLS, VITAMIN C

Chemical Compound	Variable	N=	Fill Value/ Below LOD	% above LOD	mean	Median	Geometric mean
Serum Carotenoids							
Alpha-carotene	LBDALCSI	1050	0.0039	98.67	0.08	0.05	0.05
Alpha-cryptoxanthin	LBDACYSI	1050	0.0025	99.33	0.05	0.04	0.04
Total beta-carotene	LBDBCCSI	1050	-	100	0.36	0.24	0.25
Beta-cryptoxanthin	LBDCRYSI	1050	0.0025	99.81	0.17	0.13	0.13
Total lycopene	LBDLCCSI	1050	0.0039	100	0.75	0.73	0.66
Lutein/zeaxanthin	LBDLUZSI	1050	0.0025	100	0.29	0.26	0.26
Vitamin E							
Alpha-tocopherol	LBDATCSI	1050	0.1625	100	32.68	28.33	29.89
Delta-tocopherol	LBDDTCSI	1050	0.0497	67.62	0.16	0.12	0.12
Gamma-tocopherol	LBDGTCSI	1050	0.1681	99.90	5.39	4.83	4.48
Vitamin C							
Vitamin C	LBDVICSI	1046	0.01	99.62	56.81	57.30	46.13

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Vita

Carolyn Hofe, Ph.D., R.D., L.D.

South Charleston, West Virginia
May 14, 1952

Education:

July 2008, concurrent MS degrees

MS in Nutritional Sciences, Graduate Center for Nutritional Sciences
Emphasis in Clinical Nutrition
MS in Hospitality and Dietetic Administration, College of Agriculture
Thesis: "Challenges and opportunities to rural nutrition education programs in Kentucky's Superfund communities"
University of Kentucky, Lexington, KY 40506
Cumulative GPA: 3.73

March 2007, Nutrition Data System for Research (NDSR)
Nutrition Coordinating Center, School of Public Health
University of Minnesota 55454-1087

August 2004, BS in Dietetics
Department of Nutrition and Food Science, College of Agriculture
University of Kentucky, Lexington, KY 40506
Honors: Summa cum Laude
Cumulative GPA: 3.95

Experience:

Research Assistant, Superfund Research Program, Community Engagement Core, 2006-2012.

Invited guest lecturer, NS/CNU/AS 602, Mineral Metabolism, Integrated Nutritional Sciences II, spring 2011 & 2012.

Teaching Assistant, Department of Nutrition and Food Science, College of Agriculture. 2005-2006.

Consultant Registered Dietitian: Counseling, randomized controlled trials; 2005-2010.

Consultant Registered Dietitian: Clinical, long-term care consulting; 2004-2007.

Published Abstracts:

Willett Elizabeth, **Carolyn Hofe**, Limin Feng, Lisa Gaetke: Improved risk communication through assessment of Kentucky citizens' perception of environmental pollutants, health, and

nutrition behavior. Superfund Research Program Annual Meeting, Poster presentation, Portland, OR, November 2010.

Gaetke Lisa, Elizabeth Willett, **Carolyn Hofe**, Limin Feng. Knowledge of nutrition, chronic diseases, and environmental health hazards influence actions taken toward consuming fish. Society of Environmental Toxicology and Chemistry (SETAC) Annual Meeting, Poster presentation, Portland, OR, November 7-10, 2010.

Gaetke Lisa, **Carolyn Hofe**, Elizabeth Willett. Translating sensitive environmental pollutant research and nutrition through trusted members of Superfund communities. Emerging Issues, Emerging Progress, 2009 Annual Meeting of the NIEHS Superfund Research Program, poster presentation, New York, NY.

Finnie Megan, **Carolyn Hofe**, Lisa Gaetke. Nutrition outreach to Kentucky Superfund sites. Kentucky Dietetic Association Annual Meeting, poster presentation, April 2008.

Hofe Carolyn, Megan Finnie, Lisa Gaetke. A holistic approach to effective nutrition education programs for affected Superfund communities. For the NIEHS Superfund Basic Research Program Annual Meeting, Durham, NC, December, 2007.

Ormsbee Lindell, Lisa Gaetke, Anna Hoover, Stephanie Jenkins, **Carolyn Hofe**. Leveraging partnerships for improved research translation. NIEHS Superfund Basic Research Program Annual Meeting, San Diego, CA, December, 2006.

Poster Abstracts:

Hofe Carolyn, Limin Feng, Lisa Gaetke. Relationship between serum concentrations of polychlorinated biphenyls, markers of nutritional status, and metabolic syndrome in the NHANES 2003-2004, University of Kentucky Barnstable Brown Obesity and Diabetes Research Day, Lexington, KY, May 17, 2011.

Gaetke Lisa, **Carolyn Hofe**, Elizabeth Willett. Translating sensitive environmental pollutant research through trusted members of Superfund communities, Linda H. Chen Symposium on Oxidative Stress and Nutrition, Lexington, KY, May 2010.

Willett Elizabeth, **Carolyn Hofe**, Megan Finnie, Lisa Gaetke. Superfund Nutrition Education: Opportunities for partnership, HES conference, Lexington, KY, November 2009.

Hofe Carolyn, Lisa Gaetke. Challenges and opportunities to rural nutrition education programs in Kentucky's Superfund Communities, Linda H. Chen Symposium on Oxidative Stress and Nutrition, Lexington, KY, May 2009.

Hofe Carolyn, Megan Finnie, Lisa Gaetke. Nutrition education provides outreach to improve health-related behaviors for Superfund communities. Kentucky Research Consortium for Energy and the Environment Technical Symposium, Lexington, KY, October 30-31, 2007.

Extension Publications:

1) Gaetke, Lisa, Elizabeth Willett, **Carolyn Hofe**, Megan Finnie: FCS3-545, Environmental Pollutants and Nutrition: Nuts and Seeds. Additional materials and writing for FCS publication includes: Background for Extension Educators, Facilitators Guide, Nuts and Seeds Powerpoint slides, Nuts and Seeds Evaluation materials, and Nuts and Seeds Puzzle, 2010.
    - Communications Educational Technology, 1st place. National Extension Association of Family and Consumer Sciences, Kentucky Affiliate, 2010.
    - Communications Educational Curriculum Package, 2nd place. National Extension Association of Family and Consumer Sciences, Kentucky Affiliate 2010.

2) Gaetke, Lisa, **Carolyn Hofe,** Anna Goodman-Hoover, Stephanie Jenkins, Lindell Ormsbee: Hazardous Chemicals and Your Body: Eating Right for a Healthier You. (I-76) and (I-77), 2008.

Awards:

School of Human Environmental Sciences, Student of Distinction, 2008
Future 500, University of Kentucky, School of Human Environmental Sciences, 2007
Outstanding Dietetic Student, Area V, American Dietetic Association, 2004
Outstanding Dietetic Student, Coordinated Program, Kentucky Dietetic Association, 2004
Summa cum laude, University of Kentucky, 2004

Professional Affiliations:

Academy of Nutrition and Dietetics (formerly American Dietetic Association)
American Association of Diabetes Educators
Bluegrass District Dietetic Association
Kentucky Association of Diabetes Educators
Kentucky Dietetic Association
Gamma Sigma Delta Honor Society, College of Agriculture