POLYCHLORINATED BIPHENYL EXPOSURE AND NEUROBEHAVIORAL FUNCTION IN OLDER ADULTS

by

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Abstract

Polychlorinated biphenyls (PCBs) are a group of lipophilic compounds that are ubiquitous in the environment. Although banned from production in the United States in 1977, their persistent nature has resulted in bioaccumulation in the food chain. Several epidemiologic studies have investigated potential predictors of PCB concentrations or examined adverse health effects associated with PCB exposure. However, there have been few population-based studies of older adults.

Using data from a longitudinal study, this cross-sectional analysis examined: 1) the predictors of polychlorinated biphenyls in a sample from the Baltimore Memory Study population; and 2) the relationship between PCB levels and neurobehavioral function. An additional objective was to determine whether an endothelial nitric oxide synthase (eNOS) polymorphism modified the relationship between PCB exposure and outcome. Our sample, drawn from a population-based longitudinal study, consisted of 198 individuals between age 50 and 70 years who were racially diverse and living in an urban setting.

Lipid-adjusted serum PCB concentrations ranged from 0.04 to 1.57 μg/g lipid with a mean value of 0.30 μg/g lipid. Demographic factors, health history, medication use and dietary information were evaluated as potential predictors of serum PCB concentrations. Nonwhite, older age and seafood consumption were positively associated with lipid-adjusted serum PCB concentrations. More specifically, within the seafood categories, measures for total seafood as well as shellfish consumption were strongly associated with serum PCB concentrations.

The relationship between serum PCB concentrations and neurobehavioral test

scores was evaluated while controlling for several covariates. All models demonstrated trends of lower neurobehavioral test scores with increasing lipid-adjusted PCB concentrations. Race/ethnicity, a complex variable, was strongly associated with serum lipid-adjusted PCB levels and neurobehavioral test scores, thus raising concern regarding the inclusion of race/ethnicity in the models. Exclusion of race/ethnicity revealed stronger associations between PCB concentrations and neurobehavioral test scores, suggesting that serum PCB concentrations may impair language, verbal memory and complex motor function domains. No evidence was found for an interaction effect between the eNOS polymorphism and PCB exposure on neurobehavioral outcomes.

The degree to which serum PCB concentrations represent total body burden is not well understood, although these results suggest a link between PCB exposure and neurobehavioral function.

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List of Acronyms

ANOVA	Analysis of variance
BMI	Body mass index
BMS	Baltimore Memory Study
eNOS	Endothelial nitric oxide synthase
EPA	Environmental Protection Agency
FDA	Food and Drug Administration
GI	Gastrointestinal
IQR	Inter-quartile range
LOD	Limit of detection
LTP	Long-term potentiation
MDE	Maryland Department of the Environment
NHANES	National Health and Nutrition Examination Survey
NIOSH	National Institute of Occupational Safety and Health
NO	Nitric oxide
NOS	Nitric oxide synthase
PCB	Polychlorinated biphenyl
RAVLT	Rey Auditory Verbal Learning Test

Chapter 1: INTRODUCTION

1.0 Introduction

Polychlorinated biphenyls (PCBs) are a group of lipophilic compounds that are ubiquitous in the environment and are associated with several health effects. Because PCBs tend to bioaccumulate in the food chain, diet is thought to be the main source of exposure for the general population (1;2). Several studies have been conducted to identify predictors of PCBs in humans (3-13); however, few have focused on populations of males and females without known risk factors. Consequently, little is known about the predictors of PCB concentrations or health effects in adult populations with presumably low lifetime exposures. These questions were explored in the work described here.

Animal studies have shown PCBs to affect the nervous system (14-17). In children, *in utero* PCB exposure has been linked to neurodevelopmental disorders as well as neurobehavioral outcomes (18-29). Relatively few studies have examined neurobehavioral effects of PCB exposure for adults (30-34). This is especially true for older populations, who are generally thought to be exposed to relatively low-levels and who may be susceptible to neurotoxins (35). The only paper identified on this topic suggested a relationship between low PCB serum concentrations and a range of neurobehavioral outcomes, including learning and memory deficits in older adults (30). We further explored this potential relationship in a more diverse, urban population.

Although the exact mechanism by which PCBs cause neurotoxic effects is not clearly understood, some animal studies suggest PCBs decrease long-term potentiation (LTP) in the hippocampus region of the brain, possibly through inhibition of nitric oxide (NO) production (15;36). In turn, reduction in LTP may affect neurobehavioral function (15;37). Nitric oxide production may also be affected by factors responsible for variation

in its key regulator, endothelial nitric oxide synthase (eNOS), also found in the hippocampus. In this regard, differences in the G^{894}-T^{894} exon 7 polymorphism of the eNOS gene may inhibit NO production (38-41). As an additional goal, this study considered potential links between eNOS genotype, serum PCB concentrations and neurobehavioral function.

1.1 Purpose of the Research

The main goals of this study were: 1) to determine predictors of serum PCB concentrations within a population-based study of older urban adults; 2) to examine the association between serum PCB concentration and scores on tests of neurobehavioral function in the same population; and 3) to determine whether the G^{894}-T^{894} exon 7 polymorphism in the endothelial nitric oxide synthase (eNOS) gene modifies the association between PCB concentration and neurobehavioral functioning. In order to accomplish these goals, the following specific aims were proposed:

1. Characterize total serum PCB concentrations within an elderly urban population.
2. Examine the relationships between demographic, health history, medication use, dietary information and PCB concentrations.
3. Examine the association between measures of serum PCB concentrations and neurobehavioral test scores for a battery of tests covering six different cognitive domains.
4. Determine whether the G^{894}-T^{894} exon 7 polymorphism acts as an effect modifier in the relationship between serum PCB concentrations and neurobehavioral test performance.

This research tested the following hypotheses:

1. Increased seafood consumption and age as well as nonwhite race/ethnicity and female sex are significant predictors of higher serum PCB concentrations.
2. Increasing serum PCB concentrations are associated with lower neurobehavioral test scores, with the strongest association existing for tests of learning and memory.
3. Endothelial nitric oxide synthase genotype modifies the association between PCB concentration and neurobehavioral performance. With increasing PCB exposure, individuals who are TT homozygous and TG heterozygous score even lower than expected on neurobehavioral tests than those individuals who are GG homozygous.

1.2 Organization of Research

This dissertation is composed of six sections. Following the introduction, the background section presents the rationale for this study based on a literature review of PCBs, long-term potentiation, nitric oxide, the eNOS polymorphism and neurobehavioral testing. Research methods, in Chapter 3, briefly describe the study design, variables and analyses. Chapters 4 and 5 describe two main sets of findings, formatted as articles to be submitted for publication. The first describes information about serum PCB concentrations for the sample population and examines the role of demographic variables, health conditions and diet as potential predictors. The second article examines the association between serum PCB concentrations and neurobehavioral test scores while controlling for several covariates. The influence of race/ethnicity on observed associations is also discussed. Conclusions, in Chapter 6, review the overall results and

findings with regard to the role of the eNOS polymorphism (specified above). The final chapter also discusses the strengths, limitations and public health significance of this study.

Chapter 2: BACKGROUND

2.0 Background

2.1 Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are a group of lipophilic compounds that were first produced in the United States in the 1920s and were used in a variety of industrial settings due to their flame retardant properties. Their main use was in transformers and capacitors as hydraulic, capacitor, and transformer fluids. They were also used in carbonless copying paper, paint plasticizers, television sets, air conditioners, and fluorescent light fixtures (42). Monsanto was the sole producer of PCBs in the United States, but stopped production in 1977, due to concerns that these chemicals were accumulating in the environment and had the potential to affect human health. However, even though the production of PCBs has stopped in the United States, other countries continued to produce these compounds (2). In fact, in 1984, more than 1.1 million tons of PCBs were produced throughout the world (43). As a result of the continued production by other countries, as well as the previous U.S. production, the U.S. population is still being exposed to PCBs, which persist in the environment for long periods of time.

Once in the environment, the characteristics of PCBs allow them to move easily between different media, such as land, air, and water (1;2). In the air, PCBs condense on aerosol particles and can travel long distances before being deposited into water or land, which explains how small amounts of PCBs can still be detected almost everywhere, including in outdoor air, indoor air, surface water, and soil (1).

While individuals who live near hazardous waste sites or illegal dump sites, or who still use PCB-containing products (such as old electrical appliances and equipment) have the potential to be exposed to higher levels of PCBs than the rest of the population

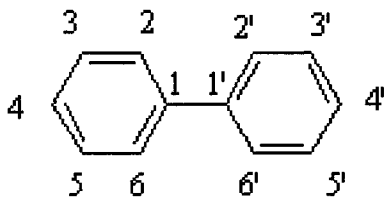
(1;44;45), occupational exposure to PCBs is still the greatest source of high exposure. It has been shown that individuals who maintain and repair old electrical appliances and equipment (such as transformers) are exposed to relatively high levels of PCBs (31;46-48). Firefighters who respond to fires where PCB-containing equipment is present also have the potential to be exposed to relatively high levels (49-51).

However, the main source of PCB exposure for the general population is diet (1;2). Due to their characteristics and properties, PCBs tend to bioaccumulate and, therefore, they can be found in several types of fatty foods, including fish (especially sports fish), meats, and dairy products. In fact, PCB levels in fish have sometimes been found to be a thousand times higher than the PCB levels in surrounding water (1).

2.1.1 Characteristics of PCBs

There are 209 different chlorinated biphenyls. Each congener consists of two benzene rings and one to ten chlorine atoms. Congeners differ according to the number and location of chlorine atoms. Figure 2.1 shows the basic chemical structure of PCBs.

Figure 2.1. Basic structure of PCBs



The physical properties of PCBs, such as vapor pressure and solubility, differ according to each congener.

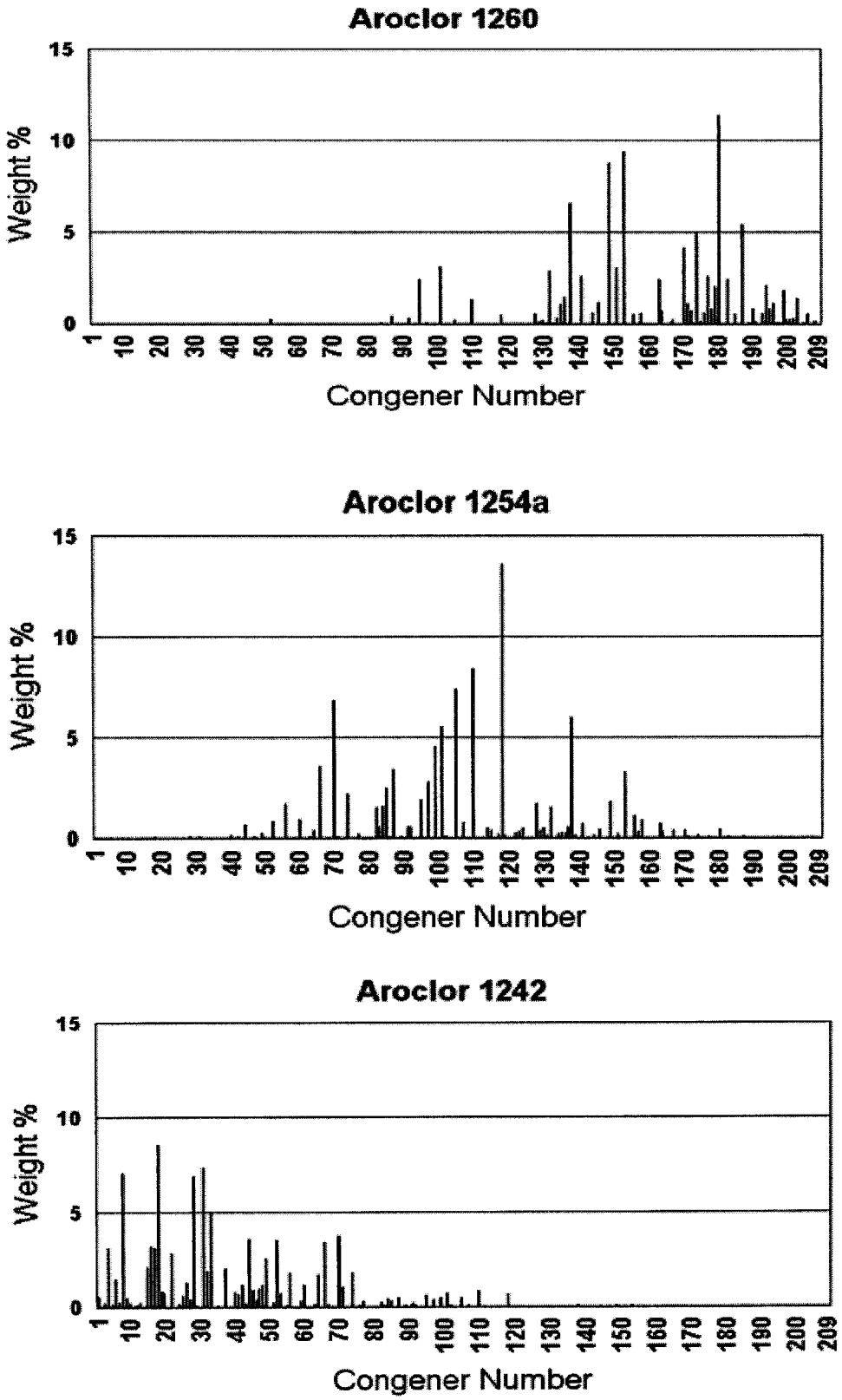
Based on their structure, PCBs can be divided into coplanar PCBs, which

typically have chlorines substituted in both para, at least two meta, and no ortho positions, and noncoplanar PCBs, which have two or more chlorine substitutions in the ortho positions. Within each category, PCBs can be further classified into homologues groups, based on the number of chlorine substitutions (e.g., monochlorobiphenyls, dichlorophenyls, trichlorobiphenyls, etc).

The significance of the different groups is their role in determining the potential health effects. Neurotoxic effects are associated with the position of the chlorine atoms on the biphenyl rings as well as the number of chlorine substitutions (52). The coplanar PCBs have toxicological effects similar to dioxin, as they bind with high affinity to the aryl hydrocarbon (Ah) receptor and, therefore, may be potentially less neurotoxic than noncoplanar PCBs (2;53-55). However, it is often difficult to determine the health effect of each congener, because 98% of all PCBs have been sold as mixtures containing between 50 and 90 different congeners (2).

Most PCBs were produced and sold in the United States under the trade name Aroclor (1). As there were many different mixtures, the name Aroclor was often followed by four digits (e.g., Aroclor 1260, Aroclor 1254), signifying the composition of the mixture. The first two digits represented the number of carbon atoms the molecule contained and the last two digits represented the approximate percent of chlorine by weight (56). Although there was variability in many of the mixtures produced, the following graphs illustrate the percentage of congener by weight for three commonly used mixtures.

Figure 2.2. PCB congeners by percentage of weight for different Aroclor compounds.



Source: Environmental Protection Agency (57)

The above figures indicate typical distributions of congeners frequently found in Aroclor mixtures; however, these distributions do not necessarily correspond to the distribution of congeners found in human biological samples. Specific congeners, depending on their degree of chlorination and location of chlorine atoms, degrade at different rates in the environment (1;43;58). In addition, rates of metabolism of specific congeners vary (e.g., those which are lower in chlorination are generally excreted more rapidly compared to those that are more highly chlorinated). Tables 2.1 and 2.2 compare congeners measured in the National Nutrition and Health Examination Survey (NHANES) 2001-2002 to the amount (percent by weight) found in typical Aroclor mixtures. These tables further illustrate that congeners marketed for distribution in Aroclor mixtures are not necessarily the same congeners found in human samples.

Table 2.1. Congeners detected in NHANES population (2001-2002) and their percent contribution to Aroclor mixtures.

Congener detected in at least 10% of the NHANES population (Chlorine substitution pattern)[†]	**Type of Congener (Coplanar or Noncoplanar)**	**% of Aroclor 1260 mix by weight**[††]	**% of Aroclor 1254 mix by weight**[††]	**% of Aroclor 1242 mix by weight**[††]
74 (2,4,4',5)	Noncoplanar	0.05	2.19	1.81
99 (2,2',4,4',5)	Noncoplanar	0.04	4.53	0.46
118 (2,3',4,4',5)	Noncoplanar	0.48	13.59	0.66
126 (3,3',4,4',5)	Coplanar	-	0.02	-
138* (2,2',3,4,4',5')	Noncoplanar	6.54	5.95	0.10
144 (2,2',4,4',5)	Noncoplanar	0.61	0.12	-
146 (2,2',3,4',5,5')	Noncoplanar	1.15	0.45	-
153* (2,2',4,4',5,5')	Noncoplanar	9.39	3.29	0.06
156 (2,3,3',4,4',5)	Noncoplanar	0.52	1.13	0.01
158 (2,3,3',4,4',6)	Noncoplanar	0.58	0.90	0.01
169 (3,3',4,4',5,5')	Coplanar	-	-	-
170 (2,2',3,3',4,4',5)	Noncoplanar	4.11	0.35	-
180* (2,2',3,4,4',5,5')	Noncoplanar	11.38	5.95	-
187 (2,2',3,4',5,5',6)	Noncoplanar	5.4	0.09	-
194 (2,2',3,3',4,4',5,5')	Noncoplanar	2.07	-	-
196 (2,,2',3,3',4,4',5,6')	Noncoplanar	1.09	-	-
203 (2,2',3,4,4',5,5',6)	Noncoplanar	1.40	-	-

*Most frequently found congeners in biological medium.

[†] Information from Third National Report of Human Exposure to Environmental Chemicals (59)

[††] Information from Environmental Protection Agency (57)

Table 2.2. Congeners below the limit of detection (LOD) in the NHANES population (2001-2002) and their percent contribution to Aroclor mixtures.

Congeners below the LOD in the NHANES population (Chlorine substitution pattern)[†]	**Type of Congener (Coplanar or Noncoplanar)**	**% of Aroclor 1260 mix by weight**[††]	**% of Aroclor 1254 mix by weight**[††]	**% of Aroclor 1242 mix by weight**[††]
28 (2,4,4')	Noncoplanar	0.03	0.06	8.50
52 (2,2',5,5')	Noncoplanar	0.24	0.83	3.53
66 (2,3',4,4')	Noncoplanar	0.02	3.56	3.39
81 (3,4,4',5)	Coplanar	-	trace	0.01
87 (2,2',3,4,5')	Noncoplanar	0.41	3.41	0.46
101 (2,2',4,5,5')	Noncoplanar	3.13	5.49	0.69
105 (2,3,3',4,4')	Noncoplanar	0.22	7.37	0.47
110 (2,3,3',4',6)	Noncoplanar	1.83	8.42	0.83
128 (2,2',3,3',4,4')	Noncoplanar	0.53	1.71	0.12
149 (2,2',3,4',5',6)	Noncoplanar	8.75	1.82	0.06
151 (2,2',3,5,5',6)	Noncoplanar	3.04	0.22	-
157 (2,3,3',4,4',5')	Noncoplanar	0.02	0.30	-
167 (2,3',4,4',5,5')	Noncoplanar	0.19	0.35	-
172 (2,2',3,3',4,5,5')	Noncoplanar	0.70	0.03	-
177 (2,2',3,3',4,5',6')	Noncoplanar	2.57	0.08	-
178 (2,2',3,3',5,5',6)	Noncoplanar	0.83	-	-
183 (2,2',3,4,4',5',6)	Noncoplanar	2.41	0.09	-
189 (2,3,3',4,4',5,5')	Noncoplanar	0.10	0.01	-
195 (2,2',3,3',4,4',5,6)	Noncoplanar	0.84	-	-

[†] Information from Third National Report of Human Exposure to Environmental Chemicals (59)
[††] Information from Environmental Protection Agency (57)

2.1.2 Environmental Fate and Transport of PCBs

Once in the environment, PCBs tend to exist for several years, which is why they are referred to as persistent organic pollutants. They move easily from the air to water and soil, and they have been found in regions far from where they were originally used (60;61). The level of chlorination as well as the pattern of chlorination plays an important role in the environmental degradation of each congener. In general, the higher the degree of chlorination and the more uniform the chlorination, the more persistent the congener is in the environment (58).

2.1.3 Toxicokinetics of PCBs

2.1.3.1 Absorption and distribution of PCBs in animal studies

Although inhalation is often the main route of PCB exposure in occupational settings, relatively little is known about the absorption and distribution of PCBs via this route. Studies of rats exposed to PCBs through inhalation have shown that levels of PCBs peaked in the liver two hours after exposure (62); whereas, a study conducted in ferrets found that the highest levels of PCBs were found in the olfactory bulbs of the animal (1;63).

More information is known regarding the absorption of PCBs via ingestion. In animal studies, PCBs are rapidly absorbed through the gastrointestinal (GI) tract, primarily by passive diffusion (1). The rate of absorption through the GI tract varies between 66% and 96%, depending on the individual congener (43).

Dermal absorption of PCBs has been studied in several different types of animals. Overall, the studies suggest that PCBs are not as readily absorbed through the skin as compared to absorption in the GI tract. Depending on the type of animal and the PCB congener, the absorption rate through the skin can range from 20% to 60% (43), with the ability of the congener to penetrate the skin being inversely related to the degree of chlorination (1;64).

Because PCBs are lipophilic, they are typically found in higher concentrations in the liver, adipose tissue, the skin, the nervous system, and breast milk. PCB congeners that are highly chlorinated tend to be found in even higher concentrations in lipid-rich tissue (65;66).

2.1.3.2 Metabolism of PCBs in Animals

The metabolism of PCBs has been frequently discussed (1;65;66). Once absorbed, PCBs are biotransformed at various rates and via different pathways, depending on the specific congener. In general, the higher the number of chlorine substitutions, the slower the metabolism. Rates of metabolism also depend on concentrations of relevant enzymes at the target organ.

In the liver, PCBs have been shown to induce levels of cytochrome P-450 enzymes. Coplanar PCBs tend to induce levels of CYP1A enzymes, whereas noncoplanar PCBs tend to induce levels of CYP2B enzymes (67). Some specific congeners, such as the mono-ortho PCBs, induce levels of both enzymes (68).

The major PCB metabolites are hydroxylated products. The hydroxylation usually occurs at the para or meta positions of the congener (43). PCBs can also be oxidized into highly reactive arene oxides intermediates that can rearrange to form phenolic metabolites, or that can become conjugated, by glutathione S-transferases, into other metabolites that can be excreted. Some studies suggest that PCBs may also be oxidized via pathways that do not involve the creation of arene oxide intermediates (66). Regardless of the metabolic pathway, it is believed that metabolism is an important process in the detoxification of PCBs (1).

2.1.3.3 Excretion

Prior to excretion, most PCBs undergo biotransformation. PCB metabolites are excreted mainly in the feces, but they can also be found at lower concentrations in the urine. The major PCB metabolites are phenolic products. In addition, sulfur-containing metabolites and methyl ether derivatives have also been identified (1). PCBs that are not

excreted tend to be stored in the liver, adipose tissue, and skin (58), and in lesser amounts in the brain (2;63).

2.1.3.4 Available Human Toxicokinetic Information

Most of the information about the toxicokinetics of PCBs in humans comes from studies in which PCB-contaminated food was ingested, and from occupational studies of humans who had inhaled high doses of PCBs (1). In the general population, ingestion is often the main route of exposure to PCBs. Studies conducted on human volunteers who ingested PCB-contaminated food found that PCBs were readily absorbed by the gastrointestinal tract and that the amount absorbed varied according to the specific congener. These studies have also suggested that PCBs avoid the first-pass metabolism in the liver because they are most likely absorbed through the gut via the lymphatic circulatory system (1).

Relatively little is known about the absorption of PCBs through inhalation or via dermal exposure in humans. However, studies have shown that there is a correlation between air concentrations of PCBs and levels of PCBs in blood serum (33).

In humans, the distribution of PCBs is not well defined. Although, based on animal data and the characteristics of PCBs, it can be assumed that PCBs will concentrate in lipid-rich tissues. As in animals, relatively high concentrations of PCBs have been found in the human liver, skin, adipose tissue, and breast milk (1;43). One study that examined human tissues from autopsies also found PCBs in the brain, albeit at lower concentrations (69). The higher chlorinated PCBs, such as PCB 153 and PCB 180, are more likely to persist in these tissues, while congeners with less than three chlorine atoms are often not detected (69).

Metabolism of PCBs varies according to congener, and it is estimated that some PCBs can remain in the body for months to years (1). Occupational studies have found that lower chlorinated congeners have half-lives of one to six years, while higher chlorinated congeners have half-lives that range from eight to 24 years (70). Metabolites of PCBs have been found to be excreted mainly in the urine, whereas PCBs that are not metabolized are frequently found in feces. In females, lactation is also considered an important route of excretion, as it can decrease overall body burden by 20 percent (71).

2.1.4 Neurotoxicity in Animals

In animal studies, small doses of PCBs have been shown to affect several different systems, including the nervous system (14). Among different species, including mice, rats, and monkeys, studies indicate that exposure to PCBs, especially noncoplanar PCBs, can result in altered motor activity (e.g., decreased spontaneous motor activity; "spinning syndrome," consisting of swift circling movements sustained in one direction, restlessness, and hyperkinesia) and in learning and memory deficits (1;15;72). Many of these studies were conducted only on young animals or animals exposed prenatally to PCBs. However, a study conducted on adult rats found that spatial learning behavior was altered due to PCB exposure (73), thereby indicating that PCBs can alter the nervous system in adult animals as well.

2.1.5 Neurotoxicity in Humans

A wide range of human health effects associated with PCB exposure has been documented in the literature, including skin conditions (such as chloracne), hepatic effects (such as increased liver enzymes and hepatomegaly), peripheral neuropathies, ocular disorders, and reproductive effects. A few studies have also associated PCB

exposure with certain types of cancer; however, these results are inconclusive (68;74). The current study focused on the neurobehavioral effects (reflecting central nervous system changes) related to PCB exposure.

The first evidence that high-level PCB exposure can have neurobehavioral effects was discovered in 1968 when rice oil contaminated with PCBs was ingested in Japan (75). Individuals who ate the rice reported several health effects, including headache, memory loss, neuralgia of the limbs, and numbness within a year after exposure (76). In a similar occurrence in Taiwan, infants born to mothers who ingested rice oil contaminated with PCBs developed neurodevelopmental problems, including behavioral problems and lower intelligence quotient (IQ) scores. Since these findings, several studies have been conducted to examine the neurobehavioral effects of PCB exposure on humans. Most of these studies have examined the effects on children who were exposed prenatally (Appendix A, Table 1.)

Lipophilic compounds, such as PCBs, are easily transferred through the placenta to the developing fetus and through breast milk to infants and young children. Because the blood-brain barrier is not fully developed and the brain is undergoing further development (e.g., myelination and expansion of nerve fibers and development of hormone and neurotransmitter receptors), infants and young children are often identified as a susceptible group for adverse effects associated with neurotoxicant exposure (77).

However, declines in neurological function in the aging brain, such as age-dependent changes in neurotransmitters or neuroreceptors (78), may also place older adults at increased risk of adverse effects associated with neurotoxicants. Despite this fact, other than the reports of neurocognitive effects associated with ingesting PCB-

contaminated rice oil, relatively few studies have been published that examine the effects of PCB exposure on the nervous system of adults. (Appendix A, Table 2.) One study conducted in an occupational setting found that almost half of the workers exposed to PCBs at a capacitor manufacturing plant reported a variety of symptoms associated with neurotoxicity, including headaches, dizziness, fatigue, memory loss, nervousness, and sleeplessness (32). Other researchers have found similar results among occupational cohorts exposed to PCBs (32;49;79). While these studies suggest involvement of the central nervous system, a study conducted by Smith and others found an association between serum PCB levels and altered peripheral sensation (80).

Only one has examined the neurobehavioral effects of PCB exposure on older adults. Schantz and colleagues conducted a cross-sectional study on older adults, ages 49-86 (median age 64.3 years), to examine potential neurobehavioral effects of PCB exposure (30). This study involved a group of "fish eaters" and a group of "non-fish eaters" in the Lake Michigan area that were randomly selected from a larger cohort. "Fish eaters" were defined as individuals who ate fish from the Great Lakes at least once a week and "non-fish eaters" ate fish less than once a week. Blood samples were analyzed for total PCB levels and a battery of neurobehavioral tests were administered to participants. Total PCB levels (based on the sum of 25 congeners) ranged from below the limit of detection (which was 3ng/mL) to 75ng/mL. Even after controlling for other covariates, such as mercury and lead, the researchers concluded that PCB exposure was associated with impairments in memory and learning. Schantz and colleagues also suggest that older adults may be more susceptible to neurobehavioral effects because body burdens of lipophilic compounds, such as PCBs, increase with age, while

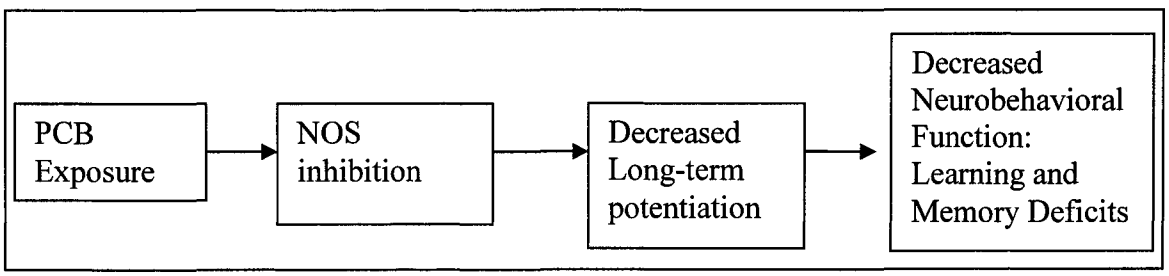
neurological function declines (30).

2.1.6 Mode of Action

The mechanism of neurotoxicity related to PCB exposure is unknown. Based on animal studies, some researchers have suggested that PCBs can decrease the neurotransmitter dopamine by inhibiting the enzyme tyrosine hydroxylase (81;82), while others have found that PCBs can kill cerebellar granular cell neurons (52;83;84) and induce apoptosis in neurons (85).

While trying to explain the learning and memory deficits, Niemi and colleagues concluded that PCBs have the potential to impair the process of long-term potentiation (LTP) in the CA1 neurons of the hippocampus (15). (LTP is described below.) Several other studies also provide evidence for the potential of PCBs to inhibit LTP (86-91). One possible way in which PCBs may impair the process of LTP is by inhibiting nitric oxide (NO). This idea has been supported by work done in *in vitro* models. For example, Sharma and Kodavanti suggested that impairments in learning and memory processes associated with PCB exposure might be a result of PCBs inhibiting nitric oxide synthase (NOS) production (36). While this mechanism is discussed in greater detail below, the following diagram (Figure 2.3) illustrates a possible relationship between PCBs, NOS, LTP, and neurobehavioral function based on evidence from the studies described.

Figure 2.3. Potential pathway for PCB neurotoxicity



2.2 Long-term Potentiation

Long-term potentiation (LTP), a form of synaptic plasticity, is a complex phenomenon that can be defined as a long-lasting increase in sensitivity of neurons following a brief, high-frequency patterned stimulus (87;92). LTP is measured by placing a stimulating electrode in the fiber pathway leading into the hippocampus and a recording electrode on the hippocampus (93). A baseline response is identified by administering a single stimulus, and the response following a patterned stimulus is recorded. LTP is quantified as the percent increase in amplitude or slope of the response following a patterned stimulus, compared to the baseline. It has been suggested that LTP results from increases in both the numbers of neurons spiking threshold and in synaptic activity (92).

While it is not possible to directly measure LTP in humans, increased LTP in animals has been correlated with the ability to learn (15). Most of these studies have been conducted on young animals (37;86;94-97). Studies conducted on older animals are limited but do suggest that such a relationship exists (98;99). Based on the available animal data, it has been hypothesized that, in humans, a decrease in long-term potentiation may be associated with problems in learning and memory (37). One possible mechanism by which LTP can be decreased is by nitric oxide inhibition (36;100).

2.3. Nitric Oxide Synthase

2.3.1 Nitric Oxide Synthase and Nervous System Effects

Nitric oxide (NO) is a gas at standard temperature and pressure that is dissolved in biological fluids in vivo. Consisting of one atom of nitrogen and one atom of oxygen, NO is not stored in the body, but it is produced from arginine, on-demand, by nitric oxide synthase (NOS) (101). Currently, NO is the only known gaseous molecule that is

believed to act as a neurotransmitter in both the peripheral and the central nervous system (102). Because it can move freely across membranes, it is considered to be ideal for nonsynaptic interactions and the transport of messages over long-distances (102).

In the peripheral nervous system, NO is believed to play a role in smooth muscle relaxation in arteriole walls. The release of NO, combined with the effects of acetylcholine and bradykinin, triggers the release of cyclic guanosine monophosphate, which is essential for muscle relaxation and decreased vascular resistance (101). Several studies have been conducted that examine the role of NO in blood pressure regulation (103) and vascular disease (such as atherosclerosis). However, data from these studies have often been inconclusive, as functional data are lacking (104).

In the central nervous system, NO plays a role as an intracellular second messenger and a diffusible retrograde messenger, which means it produces a signal in the postsynaptic cell that results in an increase in presynaptic neurotransmitter release (102). It is believed that when NO is synthesized in neurons, several physiological and pathophysiological processes are modulated, including cerebrovascular perfusion and wakefulness (102). As a retrograde messenger, NO is believed to play an important role in long-term potentiation, although the exact mechanism of how nitric oxide alters long-term potentiation is controversial (36). At least one study has found that NO inhibition has no effect on LTP (105); however, several more recent studies suggest that decreased NO production results in decreased long-term potentiation in the hippocampus (92;106;107). If this is true, a decrease in NO levels may ultimately result in alterations in neurobehavioral outcomes, such as memory and learning processes.

Because NOS produces nitric oxide, NOS is believed to play a key role in motor

activity, hormonal regulation, and long-term potentiation (92). There are three forms of NOS that have been identified: neuronal NOS (nNOS), endothelial NOS (eNOS), and an inducible form (iNOS). According to Dinerman and colleagues (1994), nNOS is primarily found in neurons throughout the central and peripheral nervous system, whereas eNOS is concentrated in hippocampal pyramidal cells and in endothelial cells that line the lumen of blood vessels (108). The inducible form is usually only detected if there is trauma. This research study focused on eNOS because it is believed to play an important role in LTP and because it is concentrated in the hippocampus, the area of the brain believed to be responsible for learning and memory.

2.3.2 NOS production and Genotype

Endothelial NOS (eNOS) is a key regulator of nitric oxide production and has been recently found to play a role in the development and disruption of LTP (36). The eNOS gene, which is located on the 7q35-36 chromosome, consists of 26 exons and spans 21 kb DNA (104). This gene has several polymorphisms that have been identified. One polymorphism is referred to as the $G^{894} - T^{894}$ nucleotide substitution, which results in a guanine to thymine switch in the nucleotide sequence of the eNOS gene at nucleotide 894, and in a glutamic acid (Glu) to aspartic acid (Asp) switch in the eNOS protein at amino acid 298 (38). When referring to populations who have this polymorphism, an individual can be classified as being a GG homozygote (wild type), a TG heterozygote, or a TT homozygote. Several studies have determined the prevalence of this polymorphism within populations. (Table 2.3.) The relative proportions of genotypes are fairly consistent among different white populations.

Table 2.3. Prevalence of the G^{894}-T^{894} exon 7 polymorphism in the eNOS gene

Author (Year) (Reference)	Location of Population	Sample Size	% GG Homozygous	% TG Heterozygous	% TT Homozygous
Chrysohoou et al (2004) (109)	Athens, Greece	595	50%	41%	11%
Czarnecka et al (2005) (110)	Poland	294	55%	40%	5%
Fatini et al (2005) (111)	Florence, Italy	1287	43%	46%	12%
Granath (2001) (112)	Australia	1194	44%	45%	11%
Guzik et al (2001) (104)	United Kingdom	104	46%	43%	11%
Jeerooburkhan et al (2001) (113)	United Kingdom	2584	44%	44%	11%
Naber et al (2001) (114)	Germany	97	40%	45%	14%
Persu et al (2002) (115)	Belgium	173	47%	45%	9%
Veldman et al (2002) (116)	Netherlands	41	46%	46%	7%
Wang et al (2000) (41)	Wales	32	53%	41%	6%

Until recently, only a few studies have examined the proportions of genotypes across different racial backgrounds (40;117-123). (Table 2.4.) Findings from these studies indicate that the GG genotype tends to be more common among African Americans and Asians than among whites.

Table 2.4. Prevalence of the G^{894}-T^{894} exon 7 polymorphism in the eNOS gene among various races/ethnicities

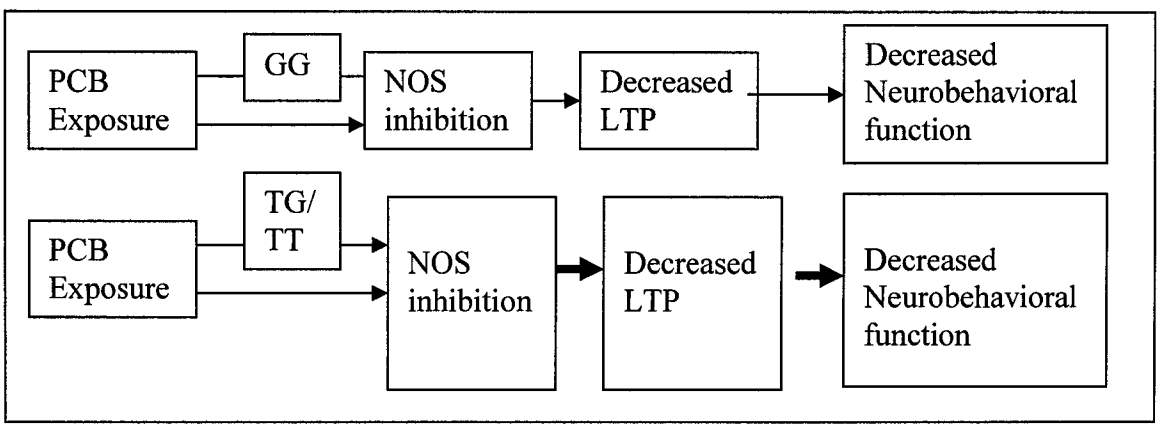
Author (Year) (reference)	**Race/ Ethnicity**	**Sample Size**	**% GG Homozygous**	**% TG Heterozygous**	**% TT Homozygous**
Cam et al (2005) (122)	Turkish	198	51%	31%	18%
Chen et al (2000) (120)	African Americans	300	80%	18%	2%
	Whites	721	45%	45%	10%
Hoffman et al (2005) (119)	Hispanics	126	51%	42%	6.6%
Li et al (2004)(118)	African Americans	60	70%	24%	6%
Lustberg et al (2004) (117)	Korean	803	85%	14%	1%
Marroni et al (2005) (121)	Whites	154	42%	50%	8%
	Blacks	136	71%	27%	2%
Ogimoto et al (2005) (123)	Japanese	150	83%	15%	3%
Tanus-Santos (2001) (40)	African American	100	70%	29%	1%
	Whites	100	42%	47%	11%
	Asians	105	88%	16%	1%

There is some evidence of an association between genotype and NO production. However, this issue has not been well studied and therefore is not clearly understood. The reason for this may be that it is often difficult to measure NO, especially within humans. In humans, plasma levels of NO are often used to detect differences of NO levels among various genotypes. However, this measurement may not be sensitive enough to detect genetic differences, as other factors (such as diet) can greatly influence results (113). Despite this limitation in measurement, some studies have found no association between genotype and NO levels (104;113), while several studies have suggested that individuals who are TT homozygous (or homozygous for the Asp 298 allele) have significantly lower NO production when compared to GG homozygotes (38;39;115;124). Currently, only one

study, conducted in a Korean population, found contrasting results (125). In this study, Yoon and colleagues (2000) found that, only in their control group of 128 individuals who did not have coronary artery disease, plasma NO levels were significantly increased in individuals who were TG homozygous or TT homozygous as compared to GG homozygous individuals (125).

Little is known regarding the association between this polymorphism and neurobehavioral function. However, as discussed previously, there is sufficient evidence in animals that NOS inhibition can contribute to problems in memory and learning by affecting long-term potentiation. Exposures that further suppress NO levels might be expected to lead to even greater deficits in learning and memory in those who already have decreased NOS production. Therefore, assuming that PCBs have the potential to decrease LTP through NOS inhibition, effects on learning and memory may differ by genotype. This would suggest that exposure to PCBs would not only result in impaired learning and memory, but would have the greatest effect in the TG and TT genotypes. Figure 2.4 illustrates this concept; larger boxes and larger arrows depict a hypothesized greater effect.

Figure 2.4. Proposed model of PCB neurotoxicity



2.4 Neurobehavioral testing

More than 750 workplace chemicals have been identified as neurotoxicants (126). For many of these chemicals, the information obtained about the neurotoxic effects is often limited, and is based on animal data or a collection of case reports of individuals with relatively high levels of exposure (126). As a result, clinically obvious neurological changes are often the first identified health effects. However, subtle neurobehavioral changes, as measured by a thorough neurobehavioral assessment, have recently been viewed as more sensitive indicators of CNS function and may provide a better opportunity for earlier intervention strategies (77).

As many neurotoxic substances do not produce any clinical morphological change, one way to detect early effects is through the measurement of functional indices (127). This is done by administering tests that assess functional domains and that have been well validated. Functional domains of interest include attention, language, memory, visuoconstruction, executive function, and motor and psychomotor speed. Batteries of neurobehavioral tests have been used in epidemiological studies to evaluate potential effects of exposures on the central nervous system when clinical changes are not yet apparent (126;128). Often, the central nervous system exhibits neurotoxic effects at levels of exposure lower than the peripheral nervous system (129). (Details regarding the neurobehavioral tests selected for use in this study are discussed in the Methods section.)

While neurobehavioral tests are often considered valid and reliable tools when administered in a prescribed format (130), several potential factors - including age, level of education, sex, and certain medical diagnoses (i.e., diabetes, brain injury, substance

abuse, and depression) - may influence the outcome of neurobehavioral tests (128). For example, differences by sex have been noted in tests of reaction time and verbal learning (131-133). It is therefore advisable to control for these factors in studies of neurobehavioral function. (Factors considered in this study are listed in the Methods section.)

2.5 Biomarkers

Although the main source of PCB exposure for this study sample was presumably through diet, other potential routes of exposure may also be significant. Biomarkers are therefore used to account for all routes of exposure. In humans, there are several biological media that can be tested for PCB exposure, including blood, body fat, and breast milk.

In general, serum PCB concentrations are most commonly used to assess recent exposure (1). However, the half-life of PCBs in serum varies greatly depending on the specific congener. In humans, studies have estimated the half-life of serum PCBs to range from a few months to several years (134-136). In general, highly chlorinated PCBs tend to remain in the body longer than PCBs that are less chlorinated. A study, conducted by Orloff and colleagues, estimated that six highly chlorinated congeners (PCB congeners 118, 138, 153, 158, 180, and 187) account for 60% of all congeners found in biological samples (45). Serum measurements are highly correlated with PCB levels found in adipose tissue (137). However, it remains unclear how well either of these measurements reflect lifetime cumulative dose (137). For this study, PCB levels in the serum were measured, as serum was readily available.

The two most common methods used to measure PCB levels in serum involve the

analyses of serum samples for specific congeners or Aroclor mixtures (138). Methods used for analyzing serum samples based on Aroclor mixtures involve gas chromatography, using a packed column and an electron capture detector. The Webb-McCall method is used to record chromatographic elution peaks, based on Aroclor standards, which can represent either a single PCB congener or several congeners that have similar retention time peaks (138-140). The sum of retention time peaks is then used to quantify total PCBs. Although this method does not identify specific congeners, it has been used frequently in earlier studies and is believed to detect peaks associated with congeners often found in biologic samples (140). (Tables 2.1 and 2.2, in section 2.1.1, compare congeners found in Aroclor mixtures to congeners examined for the NHANES population 2001-2002.) Additionally, this method is relatively insensitive to coplanar congeners (141). However, noncoplanar congeners are believed to be responsible for neurotoxic effects (58) and are, therefore, considered to be more important for this study.

In more recent studies, capillary or high resolution gas chromatography has allowed specific congeners to be measured (1;138;141). While this method is preferred due to varying biologic effects for each congener, it is quite costly - ranging from $200 a sample for identification of a few congeners to more than $700 per sample for identification of all congeners. (Cost estimates provided by two commercial laboratories.)

2.5.1. Predictors of PCB levels

Several studies have looked at determinants of serum or plasma PCB concentrations among various populations, including populations at risk for exposure or health effects, such as occupational cohorts with known PCB exposure, pregnant mothers, women with breast cancer, or fish-eating populations (5;142-144). Factors such

as occupation, age, body mass index (BMI), fish consumption, and lactation have been found to be associated with PCB levels. Population-based epidemiological studies that have determined predictors of PCB serum concentrations (12;13;145-150) have found similar associations between serum PCB concentrations and age, BMI, breast-feeding, fish consumption, and place of residence. (Appendix B, Table 1.)

The positive association between age and serum PCB concentrations is consistent across several studies (6;12;13;148;151) and is expected, since an older person is more likely to have been exposed to PCBs than a younger individual and there is age-dependent bioaccumulation (13). Sex has also been found to be associated with serum PCB levels (4;143;151), with males having higher levels of serum PCBs than females. In addition, significant differences in PCB levels have been found across different racial and ethnic groups. Studies that analyzed blood samples from the late 1960's and the 1970's have suggested that nonwhites living in urban environments have significantly higher PCB levels than whites (7;149), and more recent studies have also reported similar findings of differences by race among different populations of women (150;152).

The association between BMI and serum PCB level depends on the degree of chlorination for specific congeners. Studies examining specific PCB congener data (4;12;150) found that lower chlorinated congeners were positively associated with BMI, while higher chlorinated congeners were negatively associated with BMI. In studies examining total PCB levels, no association was found (13).

Of all of the factors examined, total seafood consumption has been identified as the strongest predictor of PCB levels in several studies (143;148). Where total seafood was broken into various seafood categories, results have suggested that the type of fish

consumed plays an important role in predicting PCB levels. For example, several studies have indicated that salmon or other fatty fish consumption is associated with increased serum PCB levels (4;5;12;13). However, their generalizability is limited because studies have largely targeted white populations living in the Great Lakes Region or in European countries, such as Norway and Sweden.

2.6 Summary

In summary, several cross-sectional studies have examined potential predictors – such as diet, age, and body mass index - of PCB serum levels in targeted populations. Studies of fish-eating populations have emphasized the link between sport caught fish consumption and serum PCB levels; whereas, other studies have considered broader dietary histories. The latter were primarily conducted in women. Little is known about dietary predictors of PCB levels within older populations of males and females whose selection was not based on high fish consumption.

Another focus in the literature examines the association of PCBs with a wide range of health effects, including disorders of the central and peripheral nervous system. Although the mechanism of PCB neurotoxicity is not fully understood, many cross-sectional and prospective studies of children exposed *in utero* or during early childhood have pointed to impaired neurobehavioral function. However, studies of adults are limited. While one cross-sectional study assessed neurobehavioral function in older adults (who may be more susceptible to low-level neurotoxicants) and PCB exposure those findings were limited to an all white population. More studies are needed to examine the relationship between neurobehavioral function and serum PCB concentrations in older adults. If a relationship between PCB exposure and

neurobehavioral function is supported, the mechanism of this action should be explored.

Chapter 3: RESEARCH DESIGN AND METHODS

3.1 Overview

Using data from a longitudinal study, this cross-sectional analysis was designed to examine: 1) the predictors of polychlorinated biphenyls (PCBs) in a sample from the Baltimore Memory Study population; and 2) the relationship between PCB levels and neurobehavioral function. An additional objective was to determine whether an endothelial nitric oxide synthase (eNOS) polymorphism modified the relationship between PCB exposure and outcome.

3.2 Parent Study

The Baltimore Memory Study (BMS) is a multi-level cohort study designed to investigate the causes of cognitive decline in Baltimore City residents living in specific neighborhoods. Residents living in identified neighborhoods were randomly contacted via telephone to determine study eligibility (i.e., those between the ages of 50 and 70 and who had lived in Baltimore City for at least five years) (153). The overall goal of the study was to examine the effect of lead absorption, specific genes, individual social and behavioral factors, and blood pressure on cognitive function, while controlling for race/ethnicity and socioeconomic status. A total of 1,140 subjects were enrolled in the study between May 2001 and October 2002. Subjects in the study were asked to participate in three visits at 14-month intervals. During these visits, information on cognitive function, blood pressure, lead levels, social and behavioral factors, and specific genotypes was collected. The current study utilized data that came primarily from the initial visit.

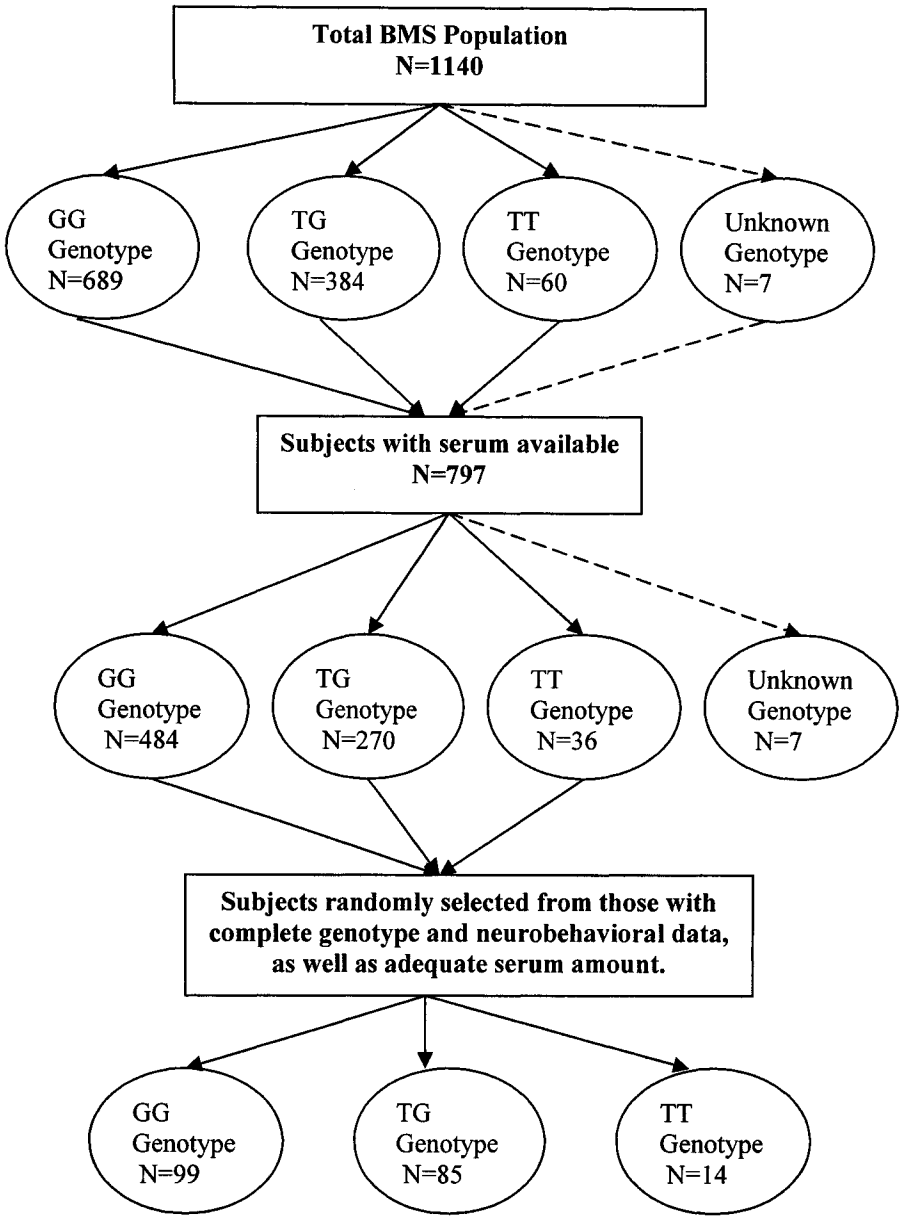
3.3 Study Sample and Subject Selection

The parent study consisted of 1,140 50-70 year olds who were randomly selected

from specific neighborhoods. The first 346 BMS participants were excluded from the study reported here because only plasma samples, not serum samples, were available for analysis. A stratified random sample based on eNOS genotype was drawn from the remaining 794 individuals, to yield 198 individuals who met specific inclusion criteria, including of a complete set of neurobehavioral testing data, eNOS genotype information, and banked serum samples of adequate volume (at least 1 mL) for PCB analysis.

The total BMS population included 689 (60.4%) GG homozygotes, 384 (33.7%) TG heterozygotes, 60 (5.3%) TT homozygotes, and 7 (0.6%) individuals for whom genotype information was not available. After excluding individuals lacking serum samples, 484 (60.7%) GG homozygotes, 270 (33.9%) TG heterozygotes, 36 (4.5%) TT homozygotes, and 7 (0.9%) unknown genotypes remained. The original sampling strategy for this study was to select 99 GG homozygotes, 45 TG heterozygotes and 45 TT homozygotes, thus allowing comparison of the GG group to others. However, only 14 TT homozygous individuals were available based on adequate volumes of serum (2 of whom had incomplete neurobehavioral test data). The final distribution, therefore, included 99 GG homozygous individuals, 85 TG heterozygous individuals and 14 TT homozygous individuals. (Refer to Figure 3.1.)

Figure 3.1. Sample selection



3.4 Data Collection

After obtaining written informed consent, trained research assistants at the clinic were responsible for collecting data from each subject. Each visit followed a structured format: neurobehavioral testing, blood pressure, weight and height measurements,

collection of a urine sample, a structured interview, and venipuncture. At the end of the visit, subjects completed a satisfaction survey and were paid $50 for their time.

3.4.1 PCB Serum Concentration Measurements

A 10 mL blood sample had been obtained during the first visit to the study clinic, and serum was frozen at -70 degrees Celsius. However, not all remaining samples met the criterion of ≥1 mL volume, as some had been used for other measurements (such as lead and mercury). Total serum PCB analyses were performed by Pacific Toxicology (Chatsworth, Calif.) by first extracting PCBs from de-proteinized serum using a 1:1 hexane/ethyl ether ratio, separating PCBs from organochlorine pesticides and biogenic material by chromatography on a silica gel using hexane as an eluent, and then determining PCB concentrations in the eluate using gas chromatography with electron capture by the Webb-McCall method, using the Aroclor standards 1242, 1254, and 1260 (140). Standards for these three Aroclors are often used because they include peaks associated with congeners found most often in biological samples (see Tables 2.1 and 2.2 in background section) and these Aroclor mixtures were also sold in large quantities in the United States (1). The Webb-McCall method records chromatographic elution peaks based on standards that contain one or more congeners (138;140). The sum of 29 different retention time peaks were used to quantify total PCBs. Due to cost considerations, concentrations of individual congeners could not be determined. The limit of detection of total serum PCBs was 0.1 ppb, and all values for the samples exceeded this level. The laboratory reported the coefficient of variation based on duplicate analysis of 23 samples to be 14.6%.

Because blood measures of lipophilic compounds vary greatly depending on

serum lipid concentration, and because blood samples were non-fasting, all PCB serum concentrations were adjusted for total serum lipids according to the method of Philips et al (154;155). Although the most precise method for adjustment relies on concentrations of total cholesterol (TC), triglycerides (TG), phospholipids, and free cholesterol (154), existing study data did not include phospholipids and free cholesterol concentrations. Therefore, adjustment was based on levels of TC and TG using the following equation described by Philips et al:

Total Lipids = 2.27(TC) + TG + 0.623.

This method of adjustment assumes that free cholesterol comprises a constant proportion of total cholesterol (27%), and it estimates phospholipids (154). Another assumption is that the distribution of PCBs between serum lipids and lipids in body tissues is a dynamic equilibrium (12). Serum cholesterol and serum triglyceride concentrations were measured using an Olympus AU5200 UV spectrophotometer with coefficients of variation ranging from 2.15% to 2.28% and 2.88% to 3.32%, respectively. For this population, serum cholesterol concentrations ranged from 112 to 348 mg/dL with a median value of 205 mg/dL, and triglyceride concentrations ranged from 40 to 763 mg/dL with a median value of 147 mg/dL.

Although this method of lipid adjustment is commonly used, some researchers argue that lipid-correction is not necessary or opt for other methods of adjustment. These methods include treating serum lipids as an independent variable in regression analyses (148) or using the residuals of serum lipids regressed on PCB concentrations as an independent variable (156;157). In this study, we employed the technique of serum lipid-adjustment suggested by Philips et al. However, each alternative method described by

Schisterman et al. was tested in the final regression models that examined the relationship between serum PCB concentrations and neurobehavioral function (157). (Appendix C.)

3.4.2 Genotyping

The single nucleotide polymorphism in exon 7 of the eNOS gene, which results in a glutamic acid to aspartic acid change at amino acid 298, was determined by the Malaria Institute's Laboratories at the Johns Hopkins Bloomberg School of Public Health, Baltimore, Md. using standard methods (114;158). The genomic sequence for eNOS was validated by automated sequencing with the primer sequences 5'- AGC TCT GCA TTC AGC GCT -3' and 3'-GCC CTT CTT GAG AGG CTC-5'. The sequences were reacted with Taq Man probes, allele G probe [Vic-labeled]: CCA GAT GAG CCC CCA, and allele T probe [6Fam-labeled]: CCC AGA TGA TCC CCC A, resulting in a 120 bp amplification product.

Results of genotyping indicated whether an individual was GG homozygous, TG heterozygous, or TT homozygous. For the purpose of analyses, the TG and TT genotypes were combined into one category.

3.4.3. Dietary Information

Prior to the second BMS visit, which occurred 14 months after the initial visit, participants were asked to complete the Block version 98.2 Food Frequency nutritional questionnaire (Berkeley Nutrition Services, Berkeley, Calif.). This questionnaire was developed using the same methodology as the well-validated Block 95, and information gathered from the National Health and Nutrition Examination Survey III (NHANES III). The current version places more emphasis on gathering information relevant to different racial/ethnic categories including whites, blacks, and Hispanics (159). The tool contains

109 food item questions and takes approximately 30 minutes to complete. The questionnaire focuses on self-reports of the participants' "usual eating habits within the past year or so." To increase the accuracy of serving size estimation, participants are shown pictures of plates and bowls with illustrations of serving sizes.

Participants were asked to complete and bring the questionnaire to the second visit. After the questionnaires were checked for completion, they were mailed to Block Dietary Data Systems, where they were summarized by food type and analyzed for nutritional consumption. Both raw data (frequency and serving size data) and calculated information (such as estimated daily meat servings per day) were obtained. Specific food items of interest in this study were the following fish and dairy categories: oysters, shellfish, tuna, fried fish, other fish, eggs, cheese, yogurt, and milk.

Additional information calculated from the Block data included daily servings of vegetables, fruits, meats, dairy products, and total fat, as well as omega-3 fatty acids. These data were based on the food pyramid definitions of food categories and serving sizes.

3.4.4 Neurobehavioral Assessment

During each initial visit, participants in the Baltimore Memory Study were given a 90-minute battery of neurobehavioral tests that addressed a range of cognitive domains. (Table 3.1.) Tests were selected based on four factors: variation by age, variation by race/ethnicity and socioeconomic status, validity and reliability among levels of socioeconomic status and in different race/ethnicity groups, and documented associations with at least one of the exposures of interest, which included lead and mercury (153). Technicians who had been trained by a neuropsychologist administered the tests in a

consistent order after reading an introduction explaining the format and purpose of testing. To ensure consistency and accuracy, the testing sessions were recorded, and the tests were scored by each tester and by another trained staff member. Results from the initial visit were used for this study.

Table 3.1. Order of test administration and cognitive domain being tested

	Name of Test	**Cognitive Domain**
1.	Boston Naming	Language
2.	Raven's Coloured Progressive Matrices	Nonverbal Reasoning/ Intelligence
3.	Rey Complex Figure copy	Visuo-construction/ Visuoperception
4.	Rey Auditory Verbal Learning Test immediate recall	Verbal Learning
5.	Purdue Pegboard dominant hand	Motor Skills
6.	Purdue Pegboard nondominant hand	Motor Skills
7.	Purdue Pegboard both hands	Motor Skills
8.	Purdue Pegboard assembly	Executive Function
9.	Stroop Test (A, B, C forms)	Executive Function
10.	Trail Making Test A	Executive Function
11.	Trail Making Test B	Executive Function
12.	Symbol Digit Paired Associate Learning	Visual Memory
13.	Rey Complex Figure delayed recall	Visual Memory
14.	Finger tapping dominant hand	Motor Skills
15.	Finger tapping nondominant hand	Motor Skills
16.	Rey Auditory Verbal Learning Test delayed recall	Verbal Memory
17.	Rey Auditory Verbal Learning Test recognition	Verbal Memory
18.	Simple Reaction Time	Motor Skills
19.	Letter Fluency	Language
20.	Category Fluency	Language

3.4.4.1 Executive Function Tests

Stroop - The Stroop test measures the ability to change one's perceptual set to meet changing demands (77). First, participants read the words shown on a card that consists of 10 rows of randomized color names (i.e., "green," "blue," "red") that are printed in black ink (Form A). Second, participants identify the colors on a card consisting of 100 colored dots (Form B). Finally, participants are asked to state the color of the print on a card on which the names of colors appear in a different color (e.g. the word "blue" is

printed in red ink) (Form C). This trial is the most sensitive, requiring inhibition of a learned response. This is a timed task, with faster times indicating better performance. The difference between Form C and Form A times, thus controlling for reading speed, was used in this study.

Purdue Pegboard Assembly (Lafayette Instrument Corporation Model 32020, Lafayette, Indiana) - This test of executive function requires the participant to quickly assemble pins, collars, and washers in a specified order. The score is the average number of items placed in three 60-second trials. A higher score indicates better performance.

Trail Making A & B - This paper and pencil test measures visual conceptual and visuomotor tracking in two parts. In Part A, the participant connects consecutively numbered circles on a worksheet, correcting any mistakes noted by the tester. This is followed by Part B, a more complex task that presents consecutively numbered and lettered circles that must be connected in an alternating pattern. For example, a participant starts the task by drawing a line from number 1 to letter A to number 2 to letter B, and so on (77). This is also a timed test for which faster times signify better performance.

3.4.4.2 Eye-Hand Coordination/Manual Dexterity Tests

Purdue Pegboard (Lafayette Instrument Corporation Model 32020, Lafayette, Indiana) – This test measures manual dexterity and eye-hand coordination. Participants remove small metal pegs from a cup in a pegboard and consecutively place them into holes vertically aligned on the board using their dominant hand. This is followed with the nondominant hand and then with both hands, and the entire process is repeated for three trials of 30 seconds. Each part of the test (dominant hand, nondominant hand, both hands)

is scored separately by averaging the numbers of pegs placed correctly within the allotted time for each trial.

Simple Reaction Time (Standard Reaction Time Tester, Software Science, Cincinnati, Ohio) - This test measures speed of processing and response to a visual stimulant. Subjects press a button as quickly as possible when an indicator light appears at frequent, varied intervals. The average reaction times in response to 64 visual stimuli comprise the score. This test is considered to be extremely sensitive to the adverse effects of toxicant exposures (160).

Finger tapping – This test, part of the Halstead-Reitan battery, is highly reliable and widely used to measure manual dexterity. After a practice trial, participants press the key of a recording device with their index finger of their dominant hand as fast as they can for 10 seconds. The process is repeated with the nondominant hand. Counts are averaged over five trials for each hand. Because this test is highly associated with age and sex (77), these must be considered as potential confounders.

3.4.4.3 Visuoconstruction/Visuoperception Tests

Rey-Osterreith Complex Figure Test copy – This test measures perceptual organization and visual memory. Participants are given as much time as needed to replicate a complex two-dimensional line drawing. A maximum of 36 points can be obtained if each section of the drawing is depicted correctly and in the proper place.

3.4.4.4 Visual Memory Tests

Rey-Osterreith Complex Figure delayed recall - Thirty minutes after copying the figure described above, participants are given as much time as needed to recreate the figure

from memory. This measures incidental memory which is defined as the amount of learning that occurs daily throughout one's life and is believed to less affected by factors such as race/ethnicity (161).

Symbol digit paired associative learning – Participants are shown seven cards, each showing a number paired with a symbol. For three trials, the participant is shown a symbol for three seconds and responds with the corresponding number. The correct number is shown after each response. The order of the cards is set for each trial and the maximum number of correct responses is 21.

3.4.4.5 Verbal Memory Tests

Rey Auditory Verbal Learning Test (RAVLT) – This test measures various types of verbal learning and memory. Participants listen to a list of 15 words and recall as many as possible in any order. The process is repeated four more times for a maximum score of up to 75 words. Approximately 30 minutes later, participants recall the words without the original list being repeated. The maximum score on this part of the test is 15. Finally, participants identify and circle the original words from a list of 50 words, for a maximum score of 15.

This test measures learning, rate of acquisition, interference, recall from short-term memory and delayed recall (160). It is considered one of the most sensitive tests for detecting neurobehavioral differences in populations exposed to toxicants (160). However, some studies indicate this test is minimally affected by emotional conditions such as depression and anxiety (162). It has been validated in several different groups of people, including patients with epilepsy, dementias, and brain injury (162).

3.4.4.6 Language Tests

Boston Naming test (30 item) – Participants give the common name of familiar objects shown in drawings. The drawings progress from simple objects, such as a "tree," to more complex objects, such as a "trellis." The total number of spontaneous correct responses within 20 seconds is recorded. To shorten testing time in the BMS study, every other item was omitted, resulting in a maximum total score of 30. This test identifies naming impairments and can detect right hemisphere damage. It is also a sensitive indicator of the presence and the degree of deterioration of dementia.

Letter Fluency and Category Fluency – These tests measure language retrieval ability. Over 60 seconds participants generate as many words as they can that begin with a particular letter ("C," "F," "L), excluding proper nouns or the same word with different endings. Final scores represent the sum of responses for each trial.

For Category Fluency, participants have 60 seconds to identify as many words as they can that are associated with a particular category (animals, food, and clothing). The number of responses for each trial is added together for a final score. For both tests, higher scores indicate better language retrieval abilities.

3.4.4.7 Non-verbal reasoning/intelligence Test

Raven's Coloured Progressive Matrices (CPM) (Psychological Corporation, San Antonio, Texas) – This test measures non-verbal reasoning and a person's capacity for observation. Participants are given three sets of 12 problems (sets A, Ab, and B). Each problem consists of a pattern that has a piece of it missing. Participants identify which of four pieces completes the pattern. Each set builds on the previous one and becomes more difficult. Set A is the simplest set and measures the ability to complete continuous patterns. Set Ab is slightly more complex and measures the ability to identify discrete

figures as spatially related wholes. Set B is the most difficult and measures the ability to think using analogy. Scores range from zero to 36, based on the number of correct problems. While this measure was first considered in this study as a means for adjusting for native intelligence (a "hold measure"), it was later excluded from the predictive models due to its association with PCB levels. (Appendix D.)

3.4.5 Other variables

As discussed in the Background section, several covariates may influence the outcome of neurobehavioral tests. Age, level of education, sex, and certain medical diagnoses (i.e., diabetes, cardiovascular disease, brain injury, substance abuse, and depression) can potentially influence the results of neurobehavioral test results and therefore must be considered (128). During the initial BMS visit, a structured computerized interview assessed several of these variables (specified below).

Demographic Information –

Self-reported age, sex, and race/ethnicity information were obtained for each subject. Subjects reported whether they were Hispanic or non-Hispanic, as well as which population groups best describes them based on the following categories: Black or African American, white, Asian, Native Hawaiian or other Pacific Islander, and Native American or Alaskan Native. Although subjects might have identified with more than group, race/ethnicity was later dichotomized into nonwhite and white due to small numbers of persons in individual categories, including White/Native American, African American/mixed, Asian or Hawaiian, and Native American.

Years of schooling, as well as other acquired credentials including trade school experience and certificates were used to classify educational attainment. Again due to

limited numbers, nine original groups of educational attainment were further collapsed into six and then four levels. (Appendix E shows the distribution of the several demographic variables compared to the BMS population.)

A detailed self-report of household income (salaries, bonuses, extra income) and assets (home value, debts, etc.) were obtained for each subject and summed for an estimate of household wealth. Subjects who did not want to provide exact dollar amounts were asked to choose from bracketed value ranges in order to help decrease the number of refused/missing answers.

Health Conditions –

Subjects reported if a doctor had ever told them they had a specific health condition including panic disorders, arthritis, hypertension, high cholesterol, depression, and diabetes. History of a condition was considered to be positive if they had ever been told they had the condition and negative if they had not been told or if a physician had told them the condition was "probable or suspected." Medication use was also evaluated by asking subjects to bring all medications they had taken within the past two weeks with them. Specific medications of interest for this study included aspirin, antidiabetics, antihypertensives, nonsteroidal anti-inflammatories, antidepressants, and thyroid medications.

During each study visit, trained technicians weighed and measured the height of each participant. Body mass index (BMI) was then calculated using the following equation: BMI= weight in kg/ (height in meters)^2. Information about smoking history and alcohol consumption was also gathered.

Smoking and alcohol history –

Subjects were asked to identify whether they currently smoked, previously smoked, or never smoked. In addition, subjects reported if they had consumed an alcoholic drink (such as beer, wine, wine coolers, or liquor) within the past month. If they responded "yes," they were asked how many days a week or in the month they drunk alcoholic beverages, and they were also asked the average amount of drinks they consumed each time.

Laboratory measurements-

Blood samples from the first study visit were used to obtain concentrations of triglycerides, total cholesterol, blood lead, mercury, and homocysteine. Tibia lead concentrations were determined during the second study visit. (Appendix E, Table 6.)

3.5 Power Considerations

According to Cohen (1988), four different parameters are key in conducting power analysis (163). These parameters include: (1) sample size (n), (2) power, (3) significance criterion, and (4) effect size (r) (or the expected correlation between an independent and dependent variable). Given any three of the parameters, the fourth can be determined.

As neurobehavioral effects related specifically to PCB exposures have not been well studied in an older population, effect sizes as identified by Cohen (1988) were used (163). He described $r = 0.1$ as a small effect size, $r = 0.3$ as a medium effect size, and $r = 0.5$ as a large effect size. As Cohen described in detail, a medium effect is one that a reasonably sensitive observer can detect with the naked eye and one that is often appropriate to use for psychological variables (163). As a result, it was reasonable to assume a medium (0.3) effect for this study.

In determining sample size, we selected 0.80 for power and 0.05 for the level of significance (alpha). Although the primary hypotheses in this research were directional, the sample size calculations were based on two-sided hypothesis testing, which is more conservative. Using the method described by Cohen (1988), a sample size of 85 individuals per genotype group were required to attain power = 0.80 with alpha = 0.05 for a two-sided test (163). (Table 3.2.)

Table 3.2. Calculation of sample size using alpha criterion = 0.05 (two-sided) and power = 0.80 at various effect sizes per Cohen (1988) (163).

	Effect Size				
	"small"		"medium"		"large"
Power	.1	.2	.3	.4	.5
0.75	692	172	75	41	25
0.80	783	194	***85***	46	28
0.85	895	221	97	52	32

Sample Sizes Appear within the body of table.
Derived from: Cohen, J. (1988). *Statistical power analysis for the behavioral sciences (2nd ed.).* (p. 102). Hillsdale, NJ: Lawrence Erlbaum Associates.

For multiple regression analysis, Cohen (1983) described methods to determine sample size that are based on significance criterion (alpha), power, the number of independent variables (k), and estimated effect size (f^2). The equation used to determine the sample size (n) is as follows (164):

$$n = L / f^2 + k + 1$$

In the above equation, L is a constant, which is derived from a table where the number of

independent variables, level of power, and alpha are specified. For values of effect size (f^2), Cohen and Cohen (1983) offered values to use when population parameters cannot be readily estimated (164). These values include small effect size = 0.02, medium = 0.15, and large = 0.35. Using the above equation, Table 3.3 identifies the sample sizes needed to detect a medium effect size (0.15) and maintain power = 0.80 and alpha = 0.05 (two-sided) for a variety different numbers of independent variables.

Table 3.3. Calculation of sample size using alpha criterion = 0.05 (two-sided) at various effect sizes and levels of power and number of independent variables, per Cohen and Cohen (1983) (164)

power	number of independent variables (k)	f^2 (effect size) "small" = 0.02	"medium" = 0.15	"large"=0.35
0.80	4	602	85	39
	5	648	92	43
	6	688	***98***	46
	8	760	109	52
	10	823	119	57
0.70	4	489	70	33
	5	529	76	36
	6	564	82	39
	8	627	91	44
	10	681	100	49

Sample size estimations appear in body of table.

Derived from: Cohen, J. & Cohen, P. (1983). *Applied multiple regression/correlation analysis for the behavioral sciences (2nd ed.).* (p. 527). Hillsdale, NJ: Lawrence Erlbaum Associates.

Given the two calculations for sample size above, the sample size for this study was 99 individuals in each group. Ninety-nine in each genotype group was used instead of 98 to compensate for two missing neurobehavioral scores. This sample size would

allow for six independent variables to be included in the regression analysis to detect a medium effect, while maintaining a power of 0.80 with an alpha level of 0.05.

3.6 Statistical Analyses

3.6.1 Univariate analyses

Descriptive statistics were used to describe measures of central tendency and measures of variability for continuous variables, such as neurobehavioral test scores and PCB levels. Data were examined for normality using the Shapiro-Wilks test, and were transformed when necessary to better approximate a normal distribution. Potential outliers were identified and frequencies of discrete variables - such as genotype information, demographic information, and health history information - were determined. Initial analyses of the data also included exploratory analyses to summarize the data and display the results.

3.6.2 Bivariate analyses

Bivariate analyses included: 1) examining the crude relationships between independent variables (such as age, sex, diet, etc.) and the dependent variable (lipid-adjusted PCB concentrations); and 2) examining the relationships between the serum PCB concentrations as an independent variable and neurobehavioral test scores as dependent variables. Correlations between variables were graphically displayed with scatter plots. Independent variables were also examined for collinearity.

Before collapsing the three genotypes into two groups (due to small numbers in the TT homozygous group), one-way ANOVA was performed to determine if the mean neurobehavioral test scores were different for at least one of the genotype groups. When the data were then collapsed into two genotype groups (TT/TG group and GG group), t-

tests were used to examine the relationship between the genotype and the neurobehavioral function test score.

3.6.3 Regression analyses

Multiple linear regression and logistic regression analyses were used to examine the relationship between independent variables and the dependent variables of interest while controlling for other covariates (i.e., age, education level, sex, health conditions, and medication use). Variables found to be significant at $p<0.10$ in bivariate analyses were included in the multiple linear regression models, along with variables that were suspected a priori as covariates. In models examining the relationship between PCB concentrations and neurobehavioral tests scores, race/ethnicity was first excluded. Race/ethnicity was then added in subsequent models to examine its influence on the relationship between PCB concentrations and neurobehavioral outcomes. Regression diagnostics included plotting residuals against fitted values, evaluating added variables with adjusted regression lines, comparing these regressions with lowess lines, and assessing variance inflation factors for collinearity.

Finally, regression analyses, examining PCB concentrations and test scores of measures of neurobehavioral function, were also performed with an interaction term for serum PCB level and genotype. Models with a significant interaction term were further explored to determine the relationship between variables.

Chapter 4: RESEARCH FINDINGS – Predictors of PCB Levels within an Elderly Urban Population

4.1 Abstract

Polychlorinated biphenyls (PCBs) are a group of compounds that are ubiquitous in the environment and have been associated with a wide range of health effects. Although production was banned in the U.S. in 1977, their persistent nature leads to bioaccumulation in the food chain and continued population exposure. This cross-sectional study examined predictors of serum PCB concentrations in a population-based sample of 50 to 70 year olds, diverse in race/ethnicity and sex and living in an urban setting. The variables of interest included seafood consumption, other dietary measures, demographic factors, health history, and medication use. Dietary information was collected using the Block version 98.2 Food Frequency Nutritional Questionnaire. Race/ethnicity was the strongest predictor of PCB concentrations. Nonwhites had median PCB concentrations 46% higher than median concentrations of whites ($p<0.001$). Within seafood categories, shellfish and total seafood consumption were positively associated with serum PCB concentrations. Results inform decisions regarding food standards and monitoring.

4.2 Background on Polychlorinated Biphenyls

Polychlorinated biphenyls (PCBs) are a group of lipophilic compounds, consisting of 209 congeners that are ubiquitous in the environment. They have been associated with a wide variety of health effects, including neurobehavioral effects, skin conditions, cancer and reproductive effects (1;43). They were first produced in the United States in the late 1920s and were often sold as mixtures under the trade name Aroclor (1). Because of their flame retardant properties, PCBs were used in a variety of industrial settings and primarily marketed for use in capacitors and transformers (42). PCBs were also utilized

in television sets, air conditioners, carbonless copying paper, paints and fluorescent light fixtures. Production of PCBs in the United States stopped in 1977, due to their persistent nature, their accumulation in the environment and their potential to affect human health (1).

However, even though the U.S. production of PCBs has been halted, numerous studies have illustrated that exposure to PCBs continues to be an issue partly due to PCB bioaccumulation in the food chain. Therefore, the main source of PCB exposure for the general population is through diet (1;2). Once in the body, PCBs can remain for months to years, depending on the specific congener, and they can be detected in several types of biological media, including blood, body fat and breast milk. Although many studies have used PCB concentrations in body fat and breast milk as biomarkers of exposure, the most commonly used method for estimating PCB exposure is serum measurement (1).

Several studies have looked at determinants of serum PCB concentrations among various populations, including populations at high risk for exposure and groups with specific health conditions; examples include occupational cohorts with known PCB exposure, pregnant mothers, women with breast cancer, and fish-eating populations (5;142-144). In studies of these populations, factors such as occupation, age, body mass index (BMI), fish consumption and lactation have been associated with serum PCB concentrations.

Population-based epidemiological studies that have determined predictors of serum PCB concentrations have found similar associations between serum PCB concentrations and age, BMI, breast-feeding, fish consumption and place of residence (13;145-150). Total seafood consumption has been repeatedly identified as a strong

predictor of PCB levels (143;148). In addition, these studies have suggested that the type of fish consumed plays an important role in predicting PCB concentrations. For example, several studies indicate that consumption of salmon or other fatty fish is associated with serum PCB concentrations (4;5;12;13). However, it is often difficult to generalize these results to other populations, as many investigations have been conducted in mostly white populations living in the Great Lakes Region or in European countries, such as Norway and Sweden.

As with many other environmental exposures, significant differences in serum PCB concentrations have been found across different racial categories. Studies that analyzed blood samples from the late 1960s and 1970s (a time when PCB distribution in the environment was likely to be drastically different from the current distribution) suggested that nonwhites living in urban environments had significantly higher PCB concentrations than whites (7;149). More recent studies also have reported differences by race among populations of women (150;152). While these studies have suggested that race/ethnicity plays an important role, no study has closely examined whether such racial differences exist in a population of males and females exposed to low levels of lipid-adjusted PCBs when other potential confounders, such as age and diet, are considered.

This study examined several potential predictors of serum PCB concentrations in a population-based sample over the age of 50 years, diverse in race/ethnicity and sex distribution, and living in an urban setting (153). Although the primary purpose of the overall study was to evaluate the association between serum PCB concentrations and neurobehavioral outcomes, the data were also used to evaluate demographic factors, health measures, fish consumption and other dietary factors as potential predictors of

serum PCB levels.

4.3 Materials and Methods

Study Design – Participants comprised a subset of the 1,140 subjects enrolled in the Baltimore Memory Study (BMS) between May 2001 and October 2002. The BMS is a multi-level population-based longitudinal cohort study designed to investigate the causes of cognitive decline in Baltimore City residents, aged 50 to 70 years, who live in specific neighborhoods. The overall goal of the study was to examine the effect of lead absorption, selected genes, individual social and behavioral factors, contextual factors, and blood pressure on cognitive function, while controlling for race/ethnicity and socioeconomic status in an older population (153). Data collection took place over three visits at 14-month intervals and included measures of cognitive function, blood pressure, blood lead concentrations, social and behavioral factors, contextual factors and specific genotypes. Additionally, serum samples were obtained and banked for future studies.

The cross-sectional analyses reported here examined the relationship between serum PCB concentrations and data collected as part of the BMS for a subset of the Baltimore cohort. PCB concentrations were determined for those in the sample and predictors of PCB concentrations were identified. An additional goal of this study (reported separately) was to determine the effect of PCB exposure and genetic factors on neurobehavioral function. Subject inclusion criteria for this study required a complete set of neurobehavioral data, endothelial nitric oxide genotyping (eNOS), and presence of banked serum samples of at least 1 mL for PCB analysis. One hundred ninety-eight individuals were identified from a random sample stratified on eNOS genotype.

Variables of Interest

During the initial BMS study visit, participants completed a detailed structured interview that assessed, among other variables, self-reported race/ethnicity, health history, medication use within the past two weeks and smoking history and alcohol consumption within the past month. This interview also determined the level of educational attainment for each subject and identified household income and assets. Education level was derived from self-reported years of schooling as well as other credentials (e.g., certificates, trade schools), resulting in a more detailed assessment of a subject's education compared to the use of years of schooling alone. Determination of economic status also went beyond the typical measure of self-reported income level with the inclusion of other sources of income (social security, welfare and supplemental security income) which created a more sensitive indicator than income level alone. Details about these variables have been published previously (153).

Prior to the second visit of the BMS, which occurred 14 months after the initial visit, participants completed the Block version 98.2 Food Frequency Nutritional Questionnaire (Berkeley Nutrition Services, Berkeley, Calif.). This questionnaire focused on the participants' "usual eating habits within the past year or so." Respondents indicated the frequency of consumption and the average serving size for a wide variety of foods. Illustrations of serving sizes were presented to assist participants in their estimations. Specific food items of interest in this study were the following fish and dairy categories: oysters, shellfish (i.e., shrimp, scallops, and crabs), tuna, fried fish, other fish (not fried), eggs, cheese, yogurt and milk.

Additional data, calculated by Block, included daily servings of vegetables, fruits, meats, dairy products and fat, as well as omega-3 fatty acids. These data were based on

the food pyramid definitions of food categories and approximate serving size definitions. Dietary information was available for 183 of the 198 subjects selected for this study. Subjects who did not complete the diet questionnaire were either lost to follow-up, deceased, or had withdrawn from the study prior to the second visit.

PCB concentrations

During the first visit to the study clinic, a 10 mL blood sample was obtained. Serum from study subjects was frozen at -70° Celsius. Total serum PCB analyses were performed by Pacific Toxicology (Chatsworth, Calif.) by gas chromatography with electron capture using the Webb-McCall method with Aroclor 1242, 1254, and 1260 as reference standards (140). These three Aroclors are often used for biological analyses because they had been sold in large quantities in the United States and they include congeners most often found in biological samples (1). The Webb-McCall method records chromatographic elution peaks that contain one or more congeners, based on standards (138;140). The sum of 29 different retention time peaks were used to quantify total PCBs. Due to budget limitations, concentrations of individual congeners could not be determined. The limit of detection of total serum PCBs was 0.1 ppb. Values for all samples exceeded this level; thus, none of the PCB data were censored. The laboratory reported a coefficient of variation of 14.6% based on repeat analyses of 23 samples.

Because measures of lipophilic compounds are known to vary greatly depending on serum lipid concentration, all PCB serum levels were adjusted for total serum lipids according to the method of Philips et al. (154). Although the most precise method for adjustment relies on values of total cholesterol, triglycerides, phospholipids and free cholesterol, available study data did not include phospholipids or free cholesterol

concentrations (154). Therefore, adjustment was based on concentrations of total cholesterol (TC) and triglycerides (TG) (154). For this population, serum cholesterol concentrations ranged from 112 to 348 mg/dL, with a median value of 205 mg/dL, and triglyceride concentrations ranged from 40 to 763 mg/dL, with a median value of 147 mg/dL. Lipid adjustment was performed using the following equation described by Philips et al. (154):

Total Lipids = 2.27(TC) + TG + 0.623.

This method of adjustment assumes that free cholesterol is a constant proportion of total cholesterol (27%), and estimates of phospholipids are based on prior studies (154).

Statistical Analyses

Statistical analyses were performed using Intercooled STATA version 8.2 (Stata Corporation, College Station, Texas). Because lipid-adjusted serum PCB concentrations were positively skewed, PCB levels were natural-log (ln) transformed to better approximate a normal distribution. A single serum PCB value of 38.1 ppb was considered to be an outlier and therefore was reassigned the next highest value in the distribution, 10.9 ppb.

Some racial/ethnic categories, such as Asian or African American/mixed, were represented by small numbers, making it necessary to collapse all nonwhite groups for further analyses. (Table 4.1.) Two main groups were thus identified: whites (62.1%) and nonwhites (37.9%).

Educational attainment was classified as: less than high school education; high school diploma plus trade school; some college or associate degree; baccalaureate degree;

some post-baccalaureate education; and post-baccalaureate degree. Individuals with a high school diploma served as the reference group. Self-reported history of each health condition was coded as positive if the subject reported that their physician told them the condition was present. For greater specificity, if a physician told them the condition was "probable or suspected," they were included in the reference group (non-diseased).

Smoking history and alcohol consumption within the past month were treated as categorical variables, with "never smoked" and "no alcoholic beverages within the past month" used as the reference groups. Household income and household asset values were natural-log transformed.

Estimated daily servings of various food groups were analyzed as continuous variables. For oysters, shellfish, tuna, fried fish, other fish, eggs, cheese, yogurt and milk, annual consumption was estimated by the product of reported frequency of consumption (in days per year) and the estimated usual portion sizes. For missing serving size data, the mean population value for that food category was used. Additionally, a total seafood category was created by summing across annual consumption categories for all seafood variables. Each of the identified food variables were first analyzed as continuous data and then divided into quartiles for analysis in multivariate regression models, with the lowest quartile (less than 25^{th} percentile) used as the reference group. Total seafood consumption amounts were also divided into two groups based on fish consumption recommendations (more than two servings or 12 ounces per week, two servings or 12 ounces per week or less).

Multiple linear regression models that included age, race/ethnicity and sex were used to identify predictors of the lipid-adjusted serum PCB concentrations. Medication

use and health history information for the same condition were initially analyzed in two separate models because of strong correlations between these two indices. Food variables were added only after a "final base" model was identified using demographic, health history and medication use data. Criteria for inclusion in the final model included all variables for which crude associations were at the $p \leq 0.10$ level in the binary analyses and that contributed to the model's ability to explain the observed variance. Regression models were evaluated for normality of residuals, linearity, homoscedasticity and influential points.

4.4 Results

Characteristics of the Study Sample

The racial/ethnic distribution of the study sample is displayed in Table 4.1. About two-thirds of the selected population was white. Table 4.2 shows select characteristics of the population. Ages ranged from 50 to 70 years, with a mean of 58.5 (SD 5.9) years. The majority of the population was female (66%), and more than half were educated at the baccalaureate level or higher. Almost two-thirds had smoked at some time during their life and had consumed alcohol within the past month. Compared to the source population, the study sample was more highly educated and contained a higher percentage of whites (Appendix E).

Unadjusted serum concentrations of total PCBs ranged from 0.2 ppb to 10.9 ppb (after recoding the outlier), with a median PCB concentration of 1.8 ppb. After adjusting for lipids, total serum PCB concentrations ranged from 0.035 μg/g lipid to 1.569 μg/g lipid, with a median value of 0.249 μg/g lipid.

Serum PCB levels by Demographic, Health History and Medication Use

For the independent variables discussed above (Methods), Table 4.3 shows the corresponding PCB concentrations. Differences by racial/ethnic category were most striking, with nonwhites having median PCB concentrations 46% higher than median concentrations of whites ($p<0.001$). Lipid-adjusted log transformed PCB values did not differ between males and females ($p = 0.23$). Of the six medical conditions examined (reported history of high cholesterol, anxiety, arthritis, hypertension, diabetes, and depression), diabetes and hypertension were associated with higher PCB concentrations, while higher levels of depression were associated with lower PCB concentrations. Additionally, reported use of antidiabetics, antihypertensives and aspirin were associated with higher PCB concentrations, but use of antidepressants was associated with lower PCB concentrations. Use of antilipemics, thyroid medications and nonsteroidal anti-inflammatories were not associated with PCB concentrations.

As mentioned above, all variables significant at the $p\leq0.10$ level listed in Table 4.3 were considered in the development of multiple linear regression models: exceptions were the medication categories for which there was corresponding health history information. For three health conditions - diabetes, hypertension and depression - both health history and corresponding medication use were independently associated with PCB concentrations. Because history and medication use were viewed as representing the same condition, only reported history of disease was included. This decision was based on two factors. Medication use addressed only use within the previous two weeks, while longer-term health experience was of greatest interest in predicting concentrations of compounds of long half-life. Further, not everyone with a history of disease takes medication; thus health history was considered a more sensitive indicator of health

conditions.

Age was also included in regression models because it was found to be positively correlated with lipid-adjusted PCB concentrations ($p \leq 0.01$). Other continuous demographic variables such as household income, household assets and BMI were not associated with PCB levels.

When dietary variables were considered individually in simple linear regression models, shellfish, fried fish and total seafood were significantly associated with lipid-adjusted PCB concentrations. Other nutrition variables were not significant. In further regression analyses, the seafood consumption values were divided into quartiles, with the lowest (less than 25th percentile) used as the reference group.

Results of the Multiple Linear Regression Analyses

The base regression model (Model 1) included age, race/ethnicity and sex as predictors of lipid-adjusted log transformed PCB concentrations. (Table 4.4.) This model accounted for only 8% of the observed variability in PCB concentrations ($R^2 = 0.083$). Race/ethnicity was the strongest predictor of serum PCB concentrations. PCB concentrations for nonwhites were significantly higher compared to whites after controlling for age and sex ($p=0.001$). Age was also positively associated with PCB concentrations. Overall, females tended to have lower PCB concentrations than males; however, sex was not a significant predictor.

In the second model, age, race/ethnicity, sex and reported history of diabetes, depression and hypertension were considered. This model accounted for approximately 10% of the observed variability in PCB levels ($R^2 = 0.099$), and race/ethnicity remained the strongest predictor. Neither history of diabetes nor history of hypertension were found

to be significant predictors (probably due to the strong association between these disorders and race/ethnicity) and therefore were dropped from the final model. Unexpectedly, history of depression was found to be negatively associated with PCB concentrations ($p = 0.03$) after controlling for all other variables.

In addition to the factors identified in Model 1 and the significant variables identified in Model 2, the final model, Model 3, incorporated recent aspirin use. This model explained almost 12% of the variability in lipid-adjusted PCB concentrations, and race/ethnicity remained the strongest predictor. This model served as the "base" model preceding the models that included dietary information ($R^2 = 0.118$).

While the models in Table 4.4 focus on personal characteristics of the study subjects, the next set of models focus on potential sources of exposure. Table 4.5 compares several models that explore seafood categories as predictors of lipid-adjusted PCB concentrations. Oyster consumption was reported by only 50% of the population and showed limited variability in amounts consumed. These factors, plus the lack of correlation with PCBs, formed the rationale for excluding oyster consumption from consideration. As noted in Table 4.5, "other fish" consumption did not ultimately predict lipid-adjusted PCBs or contribute to the model's overall ability to explain the variance of PCBs in our population. However, the "other fish" category was included in diet models because it incorporated fish such as salmon, catfish and trout. The total seafood variable in Model 6 is therefore the sum of the estimated annual consumption of shellfish, tuna, fried and other fish.

The highest percentile of total shellfish consumption significantly predicted PCB exposure after controlling for all other variables ($p = 0.013$). However, in Model 6 -

which included age, race/ethnicity, sex, history of depression and total seafood consumption – an exposure response trend was noted, with the two highest percentiles of fish consumption being stronger predictors of PCB concentrations when compared to the lowest percentile. Age, race/ethnicity, history of depression and aspirin use each remained significant after controlling for total seafood consumption. For this model, race/ethnicity was the strongest predictor of PCB concentrations. However, the greater than 50^{th} and greater than 75^{th} percentiles for total seafood consumption in Model 6 were the next strongest predictors.

Finally, seafood consumption based on the dietary recommendations (servings per week) was also found to be significant after controlling for all other variables included in the "base model" ($p = 0.02$). This model explained almost 14% of the variability in serum PCB concentrations. (Table 4.6.)

4.5 Discussion

This study found measures of seafood consumption (particularly shellfish consumption and total seafood consumption) as well as race/ethnicity and age to be associated with increased PCB concentrations within an older urban population. Although differences in methodology make comparisons difficult across studies, the average total serum PCB concentrations in this Baltimore population were similar to those reported in other population-based studies. However, concentrations were lower than those found when samples were selected on suspected risk factors, such as health conditions or diet. For example, the concentrations found here were lower compared to those of study subjects in the Great Lakes region who eat predominantly fish or in populations, studied in previous years when environmental exposure to PCBs was likely

to be higher (143;148;165-167). This study, therefore, investigated the influence of dietary and demographic factors on serum PCB concentrations similar to those of the general population between the ages of 50 and 70 years.

Because diet is thought to be the main source of PCB exposure for humans (1), this study evaluated the influence of specific dietary components using the Block version 98.2 Food Frequency Nutritional Questionnaire, a method that yields a detailed account of individuals' dietary habits by computing and summarizing nutrient intake. The detailed and comprehensive dietary assessment obtained through use of the Block 98.2 made it possible to estimate the dietary content of several food types, including dairy and seafood, the foods in which PCB concentrations have been noted to be higher (59). For example, the PCB congeners commonly found in biological samples, such as PCB 138, PCB 153, and PCB 180, are known to concentrate in seafood (2). We found no association between PCB concentrations and eggs, milk, or other dairy products, although PCB concentrations in these foods, when sold commercially, are monitored and controlled according to tolerance limits set by the Food and Drug Administration (FDA). Tolerance limits also exist for seafood; however, our analyses of seafood consumption produced more striking results.

Although the Block 98.2 Food Frequency questionnaire data do not differentiate between species of ingested seafood, this tool classifies seafood consumption according to specific categories. This feature enabled us to explore potential relationships for categories, such as oysters, shellfish, tuna, fried fish, other fish and summed total seafood measures, whereas others have considered only total seafood consumption, frequency of seafood consumption, or consumption of specific types of sport fish (3-5;8;143;143;168).

Of the specific categories that we were able to address, shellfish consumption, which included shrimp, scallops and crabs, was most strongly associated with PCB concentrations after controlling for other variables. To date, no other epidemiologic studies have identified shellfish as predictors of PCB concentrations.

One can assume that shellfish PCB content would vary according to type of shellfish as well as regional variation in water contamination. However, the current study could not identify specific types or the geographic sources of shellfish that may be responsible for the association with serum PCB levels. Nor was it possible to evaluate the historic consumption of shellfish types or sources that could affect the PCB body burden of the study participants.

Some information is available, however, on the current status of water and shellfish PCB contamination in the Chesapeake Bay, which is widely known for its crab production and contribution to shellfish in the diet of regional residents. In addition to commercial crab production, the bay is also the location of subsistence crabbing, sometimes carried out to augment the diets of those with limited income. The Chesapeake Bay is one of the most heavily sampled bodies of water in the United States for PCB levels in shellfish and fish. The Maryland Department of the Environment (MDE) estimated that for 2005, only 60% of the bay's fish and shellfish population would meet PCB concentrations specified by Environmental Protection Agency (EPA) standards for allowable PCB levels, which are based on the conservative assumption that individuals eat two recreationally caught seafood meals a month (169). This has resulted in several local advisories, including warnings to avoid eating the hepatopancreas of crabs (referred to by several common names such as "mustard") as PCBs tend to concentrate in this

organ (170). It is not known, however, whether these advisories reach those who eat crabs on a regular basis or whether they are heeded by this potentially vulnerable population.

Other studies have identified fatty fish or dark meat fish, such as herring and salmon, as the strongest seafood predictors of PCB concentrations in humans (4;12;13). Although our summary measure termed "other fish" incorporated these types of fish, it also included fish not known to be high in fat or PCB content. Thus, it was probably too non-specific to demonstrate an association with serum PCB concentrations in this study.

We also looked at the association between serum PCB concentrations according to whether individuals met or exceeded the limit of two fish servings per week, the amount recommended by the American Heart Association and American Dietetic Association based on recognized health benefits, but capped at two servings because of concerns related to mercury (171). The same limit is recommended by the EPA and FDA for pregnant women, nursing mothers, and young children (171). We found that individuals who ate more than two servings of total seafood per week had higher serum PCB concentrations.

With regard to demographic factors, race/ethnicity was the strongest predictor of serum PCB levels. Nonwhites had higher concentrations of lipid-adjusted PCBs than whites, even after adjusting for potential dietary sources. Environmental exposures, genetics, and cultural/social factors, known to differ by race/ethnicity, may account for this finding (172). Epidemiologic studies describing PCB levels in the general population have not always examined race/ethnicity. When race/ethnicity was considered in studies examining PCBs and other lipophilic compounds, results appear to be similar to ours.

Results are difficult to compare, however, because most failed to control for serum lipids, diet or factors that were not adequately represented in target populations, such as sex (7;149;152).

Consistent with findings in other studies (6;12;13;148;151), PCB concentrations increased with age after controlling for race/ethnicity, sex, history of depression, aspirin use, and total seafood consumption. To the extent that serum PCB concentration reflects total body burden in this older population, this association could be due to early life exposure when PCB use was more common. Continued lifetime environmental sources of exposure, including diet, may have further contributed to bioaccumulation of these lipophilic compounds (13). Alternatively, there may be biologic explanations for increased PCB concentrations at older ages. These theories remain speculative without availability of historical exposure data, while the significance of serum PCB concentrations as an index of PCB body burden is poorly understood (137;173). Additionally, age-related increases in PCB measures might be better elucidated in a population of wider age range.

In this population, sex was not associated with serum PCB concentrations. Some studies that have focused on fish-eating populations have found males to have a higher concentrations of PCBs than females (4;8;143;151;174). These findings have sometimes been attributed to the excretion of PCBs while breastfeeding (71), a practice that was uncommon until the late 1970's (175). We therefore had not anticipated differences by sex in this older population.

BMI was not significantly associated with serum PCB concentrations, even though lipophilic compounds tend to be stored in lipid rich tissues (1). Other studies have

found similar results when examining total PCBs (167). Studies that have found an association between BMI and PCB levels measured specific congeners allowing them to demonstrate differences by degree of chlorination (4;12;150). However, in our study, inability to distinguish specific congeners may have masked congener-specific associations.

Unexpected findings included a negative association between history of depression and PCB concentrations and a positive association with aspirin use. Due to multiple comparisons, these may be spurious findings. To our knowledge no other study has reported an effect of mental health conditions. There is also no evidence that aspirin is associated with the toxicokinetics of PCBs; however there may be an association with an underlying medical condition that warrants aspirin use.

Strengths of this study include the ability to evaluate a demographically diverse population, use of well-validated dietary measures, and control for multiple covariates. However, these findings may not hold true for the source population-based sample as this study group was different in demographic characteristics. Also, inferences regarding temporality and causality were not possible. This was particularly true for analyses of diet and PCB concentration; because diet was evaluated one year after the PCB measure was obtained. However, further analyses demonstrated that BMIs of study participants had not changed significantly over the one-year period preceding dietary assessment, suggesting their diets had remained relatively stable.

The final model explained 14% of the variance in PCB concentration, suggesting that other important predictors were not considered. For example, additional sources of exposure, such as occupational factors, were not assessed. In addition, reliance on

Aroclor standards for PCB measurement limited our ability to address relationships between specific congeners and potential predictors.

In conclusion, we found relatively low concentrations of lipid-adjusted total serum PCBs in an elderly urban population living in Baltimore City. While race/ethnicity was the strongest predictor of PCB concentrations, total seafood consumption and shellfish consumption were also positively associated with PCB concentrations. This reinforces the need for sound policies directed at: setting acceptable PCB concentrations in seafood; conducting monitoring of PCB levels in seafood according to standardized practices; and effectively communicating findings to the public. With respect to locally obtained seafood, efforts should be made to facilitate understanding and compliance with advisories. Further research is needed to examine the relationship between regional availability and dietary practices that can influence PCB concentrations.

Table 4.1. Racial/Ethnic distribution of study sample.

	PCB Population	
Race/Ethnicity	**N**	**%**
White	123	62.1
White/Native American	5	2.5
Black/African American	62	31.3
African American/mixed	3	1.5
Asian or Hawaiian	1	0.5
Native American	4	2.0
Total	198	100.0

Table 4.2. Select characteristics of the population.

Characteristic	**Number (% of Selected Population)**
Race/Ethnicity	
Whites	123 (62.1%)
Nonwhites	75 (37.9%)
Educational Attainment	
Less than High School Education	15 (7.5%)
High School Diploma	23 (11.6%)
High School Diploma plus Trade school, Some college or Associate Degree	54 (25.3%)
Baccalaureate Degree	40 (20.2%)
Some post-Baccalaureate Education	21 (10.6%)
Post-Baccalaureate Degree	45 (22.7%)
Sex	
Male	68 (34.3%)
Female	130 (65.7%)
Smoking History	
Never Smoked	80 (40.4%)
Previous Smoker	82 (41.4%)
Current Smoker	36 (18.2%)
Alcohol Use with the Past Month	
0 Drinks	70 (35.4%)
4 or less	44 (22.2%)
More than 4, less than 20	46 (23.2%)
20 or more	38 (19.2%)
Other Characteristics	**Mean (SD)**
Age (years)	58.5 (5.9)
BMI (kg/m^2)	28.9 (6.5)

Table 4.3. Crude associations between lipid-adjusted serum PCB levels and demographic, health history and medication use (n=198).

	Lipid-adjusted Serum PCB levels (μg of PCB/g of lipid)			
Predictor	**N**	**Median**	**Range**	**P-value***
Race/Ethnicity				
Whites	123	0.233	0.035 – 1.569	0.001
Nonwhites	75	0.341	0.065 – 1.194	
Sex				
Male	68	0.272	0.050 – 1.569	0.23
Female	130	0.240	0.035 – 1.194	
History of diabetes				
No	169	0.240	0.035 – 1.569	0.03
Yes	29	0.342	0.087 – 0.901	
Use of antidiabetic medications				
No	178	0.241	0.035 – 1.569	0.04
Yes	20	0.340	0.087 – 0.900	
History of high blood pressure				
No	118	0.236	0.035 – 1.569	0.06
Yes	80	0.269	0.065 – 0.969	
Use of antihypertensives				
No	122	0.226	0.035 – 1.569	0.03
Yes	76	0.286	0.065 – 0.969	
History of depression or bipolar disease				
No	169	0.268	0.045 – 1.569	0.003
Yes	29	0.156	0.035 – 0.477	
Use of antidepressants				
No	169	0.252	0.045 – 1.569	0.09
Yes	29	0.217	0.035 – 1.000	
Use of aspirin				
No	142	0.233	0.035 – 1.569	0.05
Yes	56	0.302	0.087 – 1.194	
History of high cholesterol				
No	113	0.236	0.035 – 1.569	0.14
Yes	85	0.275	0.050 – 1.194	
Use of antilipemic medications				
No	160	0.239	0.035 – 1.569	0.23
Yes	38	0.298	0.050 – 0.932	
History of arthritis				
No	111	0.251	0.045 – 1.569	0.42
Yes	87	0.246	0.035 – 1.194	
Use of non-steroidal anti-inflammatory medications	158	0.245	0.045 – 1.569	0.15
No	40	0.266	0.035 – 0.648	
Yes				
History of anxiety				
No	165	0.254	0.045 – 1.194	0.47
Yes	32	0.208	0.035 – 1.569	
Use of thyroid medications				
No	180	0.249	0.035 – 1.569	0.46
Yes	18	0.210	0.078 – 1.194	

* Based on t-tests comparing the ln transformed lipid-adjusted PCB levels. All other values in the table reported as de-transformed lipid-adjusted PCB levels.

Table 4.4. Multiple linear regression models examining demographic factors, health history, and medication use as predictors of lipid-adjusted ln transformed PCB levels (n=198).

	Model 1	**Model 2**	**Final "Base" Model**
Predictor	β coefficient (95% CI)	β coefficient (95% CI)	β coefficient (95% CI)
Age	0.018* (0.004, 0.033)	0.014 (-0.002, 0.029)	0.014 (-0.001, 0.028)
Nonwhite	0.306*** (0.129, 0.483)	0.258** (0.076, 0.441)	0.303*** (0.127, 0.478)
Female	-0.137 (-0.317, 0.044)	-0.113 (-0.294, 0.068)	-0.086 (-0.266, 0.094)
History of Depression	---	-0.272* (-0.519, -0.024)	-0.289* (-0.534, -0.044)
History of diabetes	---	0.131 (-0.124, 0.386)	---
History of High Blood Pressure	---	0.042 (-0.145, 0.228)	---
Aspirin Use	---	---	0.209* (0.019, 0.398)
Adjusted R-square	0.083	0.099	0.118

* $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

Table 4.5. Regression models examining dietary predictors of lipid-adjusted ln transformed PCB levels (n=183).

	Model 1 (Base model)[1]	**Model 2 (Shellfish)[2]**	**Model 3 (Tuna)[3]**	**Model 4 (Fried fish)[4]**	**Model 5 (Other fish)[5]**	**Model 6 (Total Seafood)[6]**
Predictor	β coefficient (95% CI)	β coefficient (95% CI)	β coefficient (95% CI)	β coefficient (95% CI)	β coefficient (95% CI)	β coefficient (95% CI)
Age	0.014 (-0.001, 0.028)	0.018* (0.002, 0.033)	0.016* (0.001, 0.030)	0.014 (-0.001, 0.028)	0.015 (-0.001, 0.030)	0.017* (0.002, 0.032)
Nonwhite	0.303*** (0.127, 0.478)	0.325*** (0.143, 0.508)	0.271** (0.092, 0.451)	0.166 (-0.037, 0.369)	0.288** (0.105, 0.471)	0.278** (0.097, 0.454)
Female	-0.086 (-0.266, 0.094)	-0.064 (-0.250, 0.0121)	-0.063 (-0.247, 0.122)	-0.054 (-0.242, 0.134)	-0.102 (-0.290, 0.087)	-0.092 (-0.277, 0.093)
History of Depression	-0.289* (-0.534, -0.044)	-0.303* (-0.545, -0.061)	-0.281* (-0.521, -0.040)	-0.244 (-0.491, 0.003)	-0.264 (-0.512, -0.017)	-0.278* (-0.520, -0.036)
Aspirin Use	0.209* (0.019, 0.398)	0.192* (0.001, 0.382)	0.218* (0.030, 0.407)	0.180 (-0.011, 0.370)	0.198* (0.003, 0.393)	0.190* (0.000, 0.382)
Consumed >25th and ≤50th percentile of specified food	----	0.021 (-0.222, 0.263)	-0.229 (-0.465, 0.006)	-0.081 (-0.319, 0.156)	0.051 (-0.186, 0.289)	0.080 (-0.164, 0.324)
Consumed >50th and ≤ 75th percentile of specified food	----	-0.009 (-0.247, 0.229)	0.040 (-0.192, 0.272)	0.185 (-0.064, 0.434)	0.063 (-0.168, 0.293)	0.263* (0.025, 0.500)
Consumed >75th percentile of specified food	----	0.306* (0.065, 0.547)	0.156 (-0.090, 0.403)	0.247 (-0.026, 0.519)	0.112 (-0.149, 0.372)	0.269* (0.026, 0.512)
Adjusted R-squared	0.118	0.143	0.150	0.138	0.105	0.137

1- "Base" model identified from Table 4.4; **2**- Adds estimated annual consumption of shellfish (shrimp, crabs, scallops) to the base model; **3**- Adds estimated annual consumption of tuna fish (canned and fresh) to the base model; **4**- Adds estimated annual consumption of fried fish (type of fish not specified) to the base model; **5**- Adds estimated annual consumption of other fish to the base model; **6**- Adds estimated annual consumption of seafood (shellfish, tuna, fried fish, and other fish) to the base model.

* p≤0.05; ** p≤0.01; *** p≤0.001

Table 4.6. Regression model examining total seafood consumption based on dietary recommendations (servings per week) as predictor of lipid-adjusted log transformed PCB levels (n=183).

Predictor	**Beta coefficient (95% CI)**
Age	0.018 (0.003, 0.033)*
Nonwhite	0.286 (0.108,0.465)**
Female	-0.084 (-0.268, 0.101)
History of Depression	-0.247 (-0.490, -0.005)*
Aspirin Use	0.200 (0.011, 0.389)*
Total Seafood Consumption >2 servings per week	0.223 (0.036, 0.411)*
Adjusted R-squared	0.138

* $p \leq 0.05$; ** $p \leq 0.01$

Chapter 5 : RESEARCH FINDINGS – PCB Exposure and Neurobehavioral Function in Older Adults

5.1 Abstract

Context: Polychlorinated biphenyls (PCBs) are a group of compounds, ubiquitous in the environment, that have been found to affect the central and peripheral nervous systems in animal studies. Several studies have also suggested an association with PCB concentrations and neurobehavioral function in humans; however, information for older adults, a potentially vulnerable population, is limited. Additionally, few studies have considered the effects of low PCB concentrations.

Objective: To examine the association between low serum PCB concentrations and neurobehavioral function in a racially diverse group of older adults.

Design, Setting, Participants: This cross-sectional study assessed the relationship between lipid-adjusted serum PCB levels and neurobehavioral function in 198 older adults enrolled in a longitudinal study of cognitive decline.

Main Outcome Measures: Nineteen neurobehavioral test outcomes assessed six cognitive domains.

Results: The median value for lipid-adjusted serum PCB concentrations was 0.249 μg/g lipid. Trends across all tests indicated that higher lipid-adjusted serum PCB concentrations were associated with lower neurobehavioral test scores. Exclusion of race/ethnicity from the models resulted in stronger associations between PCB concentrations and neurobehavioral function, particularly in the visual memory and language domains, as well as on more complex motor function tests.

Conclusions: Our data suggest a possible relationship between low-level PCB exposure and neurobehavioral impairment in older adults. In addition, inclusion of race/ethnicity into regression models may result in an underestimation of the true association between

serum PCB concentrations and neurobehavioral test scores.

5.2 Introduction

Polychlorinated biphenyls (PCBs) are a group of compounds that are ubiquitous in the environment and accumulate in adipose tissues because of their lipophilic properties. Human health outcomes associated with PCB exposure include skin conditions (chloracne), hepatic effects, ocular disorders, cancer and reproductive effects (1). Considerable attention has focused on possible nervous system effects since PCBs - especially those noncoplanar in structure – have been shown to concentrate in regions of the brain such as the hippocampus, frontal cortex and cerebellum (55;176;177). In animals, associated effects include altered motor activity and learning and memory deficits (1;15;178). Studies of humans have also reported findings of neurodevelopmental and neurobehavioral impairments. While many studies have been conducted in children, most studies in adult populations have been limited to occupational settings, episodes of accidental contamination of food, or populations at risk of eating fish from PCB-contaminated waters (18;25;26;30;179;180). General adult populations without increased risk of PCB exposure have not typically been examined for health effects.

Early evidence of an association between PCB exposure and human neurobehavioral function comes from episodes in which individuals had ingested relatively high concentrations of PCBs in contaminated rice oil (19;76). Japanese individuals who ate contaminated rice oil reported headache, memory loss, neuralgia of the limbs, and numbness within a year after exposure (76). Infants born to mothers who ingested contaminated rice oil in Taiwan developed behavioral problems and had lower IQ scores than matched controls (19). Further studies of the association between PCBs

and neurobehavioral functioning have focused on exposures *in utero*, addressing concerns that infants, whose brains and blood-brain barriers are not yet fully developed, represent the most susceptible population. For example, numerous cohort studies that have examined the relationship between concentrations of PCBs in cord blood and neurobehavioral function at various ages, ranging from a few hours after birth until age 11, have found decrements in verbal and memory function as well as declines in overall IQ scores (18;19;21;22;25;27;29;180-184).

There are a few occupational studies that show similar associations between relatively high exposure to PCBs and neurobehavioral function in adults (32;49;79). While some of these studies have focused on clinically obvious symptoms such as headaches, dizziness, fatigue, memory loss, nervousness and sleeplessness, others have detected less obvious changes in the central nervous system by using well-validated batteries of neurobehavioral tests that assess a wide range of functional domains (32;33;79;185). Although these sensitive tests can be useful tools in assessing the relationship between low-level exposure of a toxicant and neurobehavioral functioning, information regarding low-level PCB exposure and neurobehavioral function is limited, as it has not been well studied in adults.

While we know that infants and children are particularly vulnerable to the central nervous system (CNS) effects of PCB exposure, it has been suggested that older adults may comprise another susceptible population (35). Three reasons may account for this. First, early life exposure to PCBs may result in lifelong effects that continue to be evident in one's older years. Alternatively, processes associated with aging may unmask previous CNS insult caused by earlier neurotoxin exposure. Finally, the brain may become more

susceptible to later life neurotoxin effects because of early exposure to neurotoxins or because of age-related changes (35).

However, to date, only one study has examined the neurobehavioral effects of PCB dose on older adults not exposed in an occupational setting. Schantz and colleagues used neurobehavioral assessment techniques to study a population of adults over age 49 who were categorized according to level of fish consumption (30). Even after controlling for potential covariates, such as mercury and lead exposures measured in blood, total PCB concentrations were found to be associated with impairments in memory and learning. However, this study was limited to an all white population and serum PCB conentrations were not lipid-adjusted.

Our study is the first population-based study to examine the association between neurobehavioral function and PCB exposure in a racially diverse older urban population (between the ages of 50 and 70). Lipid-adjusted total serum PCB concentrations were used to assign exposure, and subjects were not selected for the study based on risk factors associated with high exposure to PCBs (e.g., fish consumption or occupational exposure).

5.3 Methods

Study Sample and Design – The Baltimore Memory Study (BMS) is a multi-level cohort study designed to investigate the causes of cognitive decline in Baltimore City residents living in specific neighborhoods. The overall goal of the study is to examine the effect of lead absorption, specific genes, individual social and behavioral factors and blood pressure on cognitive function while controlling for race/ethnicity and socioeconomic status (153). A total of 1,140 subjects between the ages of 50 and 70 years, and who have lived in Baltimore City for at least five years, were enrolled in the BMS between May

2001 and October 2002. Over the course of three visits, 14 months apart, information on cognitive function, blood pressure, lead levels, social and behavioral factors and specific genotypes as well as serum samples were collected.

A subset of the BMS population was selected for this cross-sectional study to determine the relationship between PCB concentrations and neurobehavioral function. Individuals were eligible for inclusion if they had a complete set of neurobehavioral data, eNOS genotype information and banked serum samples of adequate volume (at least 1.0 mL) for PCB analysis collected during the first visit. Eligible subjects, stratified on genotype, were randomly sampled to yield 198 in the study sample. (Results of separate analyses by genotype appear elsewhere.)

Data Collection - During the initial BMS visit, trained technicians administered a battery of 19 neurobehavioral tests that covered six cognitive domains. Details regarding criteria for test selection and quality control measures have been discussed previously (153). The specific tests included: the Boston Naming test (shortened to 30 items), Category Fluency, Letter Fluency, Rey Auditory Verbal Learning Test (RAVLT) immediate recall, RAVLT recognition, RAVLT delayed recall, Rey Complex Figure delayed recall, Symbol Digit Paired Associative Learning, Rey Complex Figure copy, Purdue Pegboard (dominant hand, nondominant hand, both hands, and assembly), finger tapping (dominant hand, nondominant hand), simple reaction time, Stroop Test (C-A form), and Trail Making Test A and B.

Participants also completed a detailed structured interview that assessed, among other variables, self-reported race/ethnicity, health history, medication use within the past two weeks and smoking history and alcohol consumption within the past month. Level of

educational attainment and household income and assets were also determined. Self-reported years of schooling as well as other credentials (e.g., certificates, trade schools) were used to determine level of educational attainment. In addition, economic status was assessed using a variety of measures, including self-reported income level, social security, welfare and supplemental security income. Details about these variables have been published previously (153). The height and weight of participants were also measured by trained technicians for calculation of body mass index (BMI). Additional factors collected included measures of homocysteine, blood mercury concentrations, blood lead concentrations and tibia lead concentrations. All are known to be associated with neurobehavioral function (186;187).

Serum PCB concentrations - Serum from blood samples collected during the first visit had been frozen at -70° Celsius for future analyses. For this study, only persons with samples with $\geq$ 1.0 mL volume remaining were considered for inclusion. Total serum PCB concentrations were determined by gas chromatography/electron capture using the Webb-McCall method with Aroclor 1242, 1254 and 1260 as reference standards (Pacific Toxicology, Chatsworth, Calif.). These Aroclors were widely used in the United States and contain congeners frequently found in biological samples (1). The Webb-McCall method records chromatographic elution peaks, each representing one or more congeners, based on the standards (140). The sum of 29 different retention time peaks was used to quantify total PCBs. Budget limitations prohibited determining concentrations of individual congeners. All total serum PCB concentrations exceeded the limit of detection of 0.1 ppb. The laboratory reported a coefficient of variation of 14.6% based on 23 duplicate analyses. One serum PCB concentration (38.1 ppb) was more than triple the

second highest concentration in the distribution. That data point was an outlier and was therefore assigned the value of the next highest data point, 10.9 ppb.

Measures of lipophilic compounds can vary greatly depending on serum lipid concentrations (154;155). Therefore, all PCB serum concentrations were adjusted for total serum lipid concentration using the following equation proposed by Philips et al. (154):

$$\text{Total Lipids (g/L)} = 2.27(\text{Total cholesterol}) + \text{Triglycerides} + 0.623.$$

This method of adjustment estimates phospholipids and assumes that free cholesterol is a constant proportion of total cholesterol (27%) (154). Although Philips et al. proposed a more precise method of lipid adjustment using actual free cholesterol and phospholipids concentrations, these data were not readily available. Serum cholesterol and serum triglyceride concentrations were measured using an Olympus AU5200 UV spectrophotometer and had coefficients of variation ranging from 2.15% to 2.28% and 2.88% to 3.32%, respectively. Serum cholesterol concentrations for this population ranged from 112 to 348 mg/dL with a median value of 205 mg/dL and triglyceride concentrations ranged from 40 to 763 mg/dL with a median value of 147 mg/dL.

Statistical Analyses

Multiple linear and logistic regression models were developed to examine the relationship between serum total PCB concentrations and neurobehavioral test scores while controlling for several covariates. Variables such as race/ethnicity, testing technician, smoking history, alcohol consumption, health history information and medication use were dichotomized. Educational attainment was classified into four levels. Household wealth, body mass index (BMI), blood lead concentration, tibia lead

concentrations, homocysteine and lipid-adjusted PCB concentrations were evaluated as continuous variables.

Exploratory analyses indicated that the majority of the population sampled received the highest possible score on several tests, indicating that a "ceiling effect" was present, and the data did not follow a normal distribution when log transformed. In these circumstances, the test scores were divided at the 25^{th} percentile. All other outcome variables were treated as continuous and were Z-transformed. Some outcomes, such as Trails A and B scores, were log transformed to better approximate a normal distribution and/or were reverse scored to link negative values with worse performance.

Bivariate analyses examined the crude association between independent variables of interest (listed above) and neurobehavioral test scores. Inclusion criteria for the final model included crude associations between the independent variables and the majority of the neurobehavioral outcomes at the $p \leq 0.10$ level, or a documented effect on neurobehavioral function in the larger BMS population. When medication use and its corresponding health condition (e.g., antihypertensive medication use and history of hypertension) both met inclusion criteria for the final model, only the more strongly associated variable according to the bivariate analyses was used.

In the first set of models, lipid-adjusted PCB concentrations were regressed on neurobehavioral test score, controlling for age, sex, testing technician, history of diabetes, antihypertensive medication use and household wealth. Race/ethnicity was not included in the first set of models because of the strong association with serum PCB concentrations (188). However, in order to further investigate the role of race/ethnicity, it was added to the second set of models in which lipid-adjusted PCB concentrations were

regressed on neurobehavioral test score, adjusting for all other covariates. Additional regression analyses were modeled using PCB concentrations expressed in quartiles (Appendix F.)

Because regression diagnostics revealed lack of homoscedasticity for the Letter Fluency model, a model which accounted for unequal variances was used for this test. Additionally, one subject with a lipid-adjusted serum PCB concentration of 0.889 μg/g lipid (approximately three standard deviations above the mean) and a Letter Fluency score of 82 (also approximately three standard deviations above the mean) was, according to regression diagnostics, highly influential in the model for that test. This subject was consequently excluded from the analyses for this test.

We compared the magnitude of decline in neurobehavioral test scores associated with lipid-adjusted PCB concentrations to those associated with age. These comparisons were conducted for tests for which both PCB concentration and age were associated at the $p \leq 0.10$ level. Intercooled Stata 8.2 (Stata Corp, College Station, Texas) software was used all analyses.

5.4 Results

Table 5.1 shows selected characteristics of the population (n=198), which was approximately two-thirds white, two-thirds female and relatively highly educated. Lipid-adjusted total serum PCB concentrations ranged from 0.035 μg/g lipid to 1.569 μg/g lipid with a median value of 0.249 μg/g lipid. In addition, median levels of lipid-adjusted serum PCB concentrations were significantly higher for nonwhites than whites (0.341 μg/g lipid compared to 0.233 μg/g lipid); thus raising the concern that higher PCB concentrations may be due to multiple factors associated with race/ethnicity.

Crude associations between neurobehavioral test scores and lipid-adjusted PCB concentrations are shown in Tables 5.2 and 5.3. Tests are grouped according to their respective cognitive domain. All associations were significant at the $p \leq 0.10$ level with the exception of those measuring simple motor function (e.g., Simple Reaction Time and finger tapping scores).

Table 5.4 shows the results of models developed to assess the association between lipid-adjusted serum PCB concentrations and individual neurobehavioral test scores while excluding race/ethnicity from the model and adjusting for other covariates. Results for all tests indicate that higher lipid-adjusted serum PCB concentrations were associated with lower neurobehavioral test scores. On average, an increase from the 25^{th} to the 75^{th} percentile of lipid-adjusted serum PCB concentration was associated with a decline of 0.10 standard deviations in neurobehavioral test scores. Lipid-adjusted serum PCB concentrations were associated with 5 out of the 15 (33%) neurobehavioral outcomes at the level of $p \leq 0.10$. Three of these (Category Fluency, Letter Fluency, and Purdue Pegboard both hands) were associated at p-values less than or equal to 0.05. For the same 5 tests which were significant at the $p \leq 0.10$ level, on average, an increase from the 25^{th} to the 75^{th} percentile in PCB concentrations was equivalent to a decline in neurobehavioral test scores associated with an increase of 5.5 years of age.

Table 5.4 also shows the results of models that assessed the association between lipid-adjusted serum PCB concentrations and individual neurobehavioral test scores while adjusting for other covariates and, in contrast to the above models, also including race/ethnicity in the model. In this approach, only one test, Letter Fluency, was significantly associated with serum PCB concentrations. An inter-quartile range (IQR)

increase in PCB concentration resulted in a decrease of 0.13 standard deviations on the Letter Fluency test. Results for other tests continued to show overall trends, indicating that higher lipid-adjusted serum PCB concentrations were associated with lower neurobehavioral test scores. On average, an IQR increase in lipid-adjusted PCBs concentration resulted in a decrease of 0.06 standard deviations in neurobehavioral test scores. The largest and most consistent associations were in the domains of language and visual memory. Overall, models including race/ethnicity accounted for 9-39% of the variance in neurobehavioral test scores. In terms of magnitude, on average, an increase in PCB concentration from the 25^{th} to the 75^{th} percentile (0.16 μg/g lipid to 0.37 μg/g lipid) was comparable to an effect on test scores associated with an increase of 2.6 years in age.

The results of logistic regression that excluded race/ethnicity from the models indicate that PCB concentrations are consistently associated with a decrease in neurobehavioral test scores (Table 5.5). In these models, three of out of four associations achieved statistical significance at the $p \leq 0.10$ level and the average odds ratio of scoring in the less than 25^{th} percentile on neurobehavioral tests was 4.5 times higher with a unit increase PCB concentration. Table 5.5 also shows the results of logistic regression that included race/ethnicity. Similar to findings for the neurobehavioral tests previously discussed, there was a consistent negative association between lipid-adjusted serum PCB concentrations and neurobehavioral function, but none were significant. On average, when race/ethnicity was included in the model, the odds of scoring in the less than 25^{th} percentile on neurobehavioral tests were 2.8 times higher with a unit increase in PCB concentration. The greatest effect was seen in the language domain.

5.5 Discussion/Conclusion

To our knowledge, this is the first study to examine the relationship between low-level serum PCB concentrations and neurobehavioral function in a racially diverse, older urban population. In this study, regression models were used to examine the association between total lipid-adjusted serum PCB concentrations and scores on neurobehavioral tests, while controlling for health conditions, medication use and demographic variables.

A negative association between serum PCB concentrations and the outcomes of tests measuring the language domain was suggested when race/ethnicity was included in the model. However, after excluding race/ethnicity from the model (for reasons discussed below), a stronger association between serum PCB concentrations and language domain was demonstrated for these older adults. Furthermore, the relationship between PCB concentrations and results of all tests shifted in the direction of a stronger association; this was particularly true for tests of visual memory. (See Appendix G for an example.) This trend was evident even in results of logistic regression models for which the power to detect differences may have been more limited due to the use of arbitrary cutoff values at the 25^{th} percentile. In total, with race/ethnicity excluded from the model, 8 out of 19 tests administered were significantly associated at the $p \leq 0.10$ level, in contrast to 3 out of 19 in models that controlled for race/ethnicity. The functional domains assessed by each of these tests are fairly consistent and include visual memory, language and the more complex motor function tests.

Use of a cross-sectional design did not allow the temporal relationship between PCB concentrations and neurobehavioral function to be determined. However, a prospective design, which is often used to clarify exposure-response relationships, could improve our understanding of the causal effects. Along with this, a better understanding

of the significance of serum PCB concentrations as a biomarker of exposure or total body burden would benefit the design and interpretation of a prospective study.

The decision to exclude race/ethnicity from the regression models was based on the fact that race/ethnicity was associated with the serum PCB concentrations and neurobehavioral tests scores. It has been suggested that inclusion of race/ethnicity in regression models may not be appropriate if race/ethnicity is associated with both the toxicant and the outcome of interest (188). Our findings indicated that nonwhites had average lipid-adjusted serum PCB concentrations that were 33% higher than those of whites (0.36 versus 0.27 μg/g lipid). This is consistent with the concept of environmental justice which recognizes that disadvantaged individuals are disproportionately exposed to higher levels of environmental contaminants (189). In addition, results from the larger BMS population indicate that race/ethnicity is strongly associated with outcomes on neurobehavioral tests (153).

Martin and colleagues propose three causal structures that could help explain how inclusion of race/ethnicity would underestimate the associations between PCB concentrations and neurobehavioral function. One model, referred to as the "reciprocal effects model," describes the case in which complex factors related to race/ethnicity, such as socioeconomic status, can influence toxicant levels and can also be influenced by the toxicant (188). Because exposure to PCBs has been shown to vary by race/ethnicity (7;152;190), and some studies have shown an association between childhood or *in utero* PCB exposure and decreased cognitive function later in life (18;19;25;27;191), educational achievement, reflected by later success on neurobehavioral tests, could be influenced by early life PCB exposure. It might also be argued that lower educational

achievement or other indices of lower socioeconomic status places one at risk for higher exposure to PCBs.

In the second proposed model, which Martin et al. refer to as the "measurement error model," the observed association between PCB concentrations and neurobehavioral function may decrease when accounting for race/ethnicity, because race/ethnicity may represent a better surrogate for cumulative lifetime PCB dose than does total serum PCB concentration (188). Although the average half-life of PCBs in the body is reported to be several years, and serum measurements are strongly correlated with levels found in adipose tissue (137;173), neither of these measurements may accurately represent total lifetime dose.

The final model proposed by Martin and colleagues, known as the "effect modification model," suggests that unmeasured factors associated with race – such as diet, co-morbidities and exposure to other environmental factors – can potentially modify the relationship between serum PCB concentrations and neurobehavioral function (188). The influence of such factors could mask the effects of PCB exposure and neurobehavioral function.

While our data did not allow us to determine which, if any, of the above models might be operating, the exclusion of race/ethnicity from models may more accurately reveal the association between serum PCB concentrations and neurobehavioral test scores. The validity of these models in assessing PCB exposure and neurobehavioral function must be further explored.

To date, only Schantz and colleagues have examined the relationship between PCB exposure and neurobehavioral function in older adults. While they identified

associations between serum PCB concentrations and memory and learning functions (30), they found no association with motor function after adjusting for other potential covariates (192). However, their focus on an all-white population precludes generalization to other racial/ethnic groups. Also, as with many other studies, a comparison of the results is difficult because different neurobehavioral testing techniques were used.

The design of this study also differed from that of Schantz in terms of the serum PCB measure used. In the Schantz study, the sum of known congeners (unadjusted for lipids) was reported as "total PCBs." We used a measure of total lipid-adjusted serum PCBs based on Aroclor standards, thus yielding estimates of exposure that were difficult to compare. Although different congeners have been shown to have varying biological effects (1), we were unable to identify those which were specific to our samples. However, the analysis technique used in our study likely captured most congeners found in biological samples as well as many noncoplanar PCBs (141), believed to be more neurotoxic than coplanar PCBs (2;54;193).

Comparison is further complicated by the fact that we adjusted PCB values for serum lipid concentrations, while Schantz and colleagues did not. Although there is a strong rationale for lipid adjustment, several methods have been proposed (157). When adjustment by various techniques did not vary our results, we used that which has been recommended and commonly used by others.

A strength of our study was the ability to evaluate the relationship between PCB concentrations and neurobehavioral evaluation while controlling for tibia lead levels. However, inclusion of tibia lead measure in the final model did not appreciably alter

associations between PCB concentrations and neurobehavioral function. (See Appendix H in dissertation). Blood mercury concentrations, available for less than half of the individuals, also were not associated with neurobehavioral test scores. In addition, results from a previous study conducted on 474 individuals from the BMS failed to show convincing evidence of a relationship between mercury concentrations and neurobehavioral function (194).

Our study design offered several strengths, including the ability to evaluate the potential effects of PCB exposure in a demographically diverse population. In addition, this study allowed us to focus on older adults, a potentially vulnerable population. Although our modest sample size was sufficient in size to demonstrate associations between serum PCB concentrations and neurobehavioral function, greater power offered by a larger sample size would increase the likelihood of identifying more subtle changes. This should be a consideration in future studies.

Our data revealed a consistent trend toward decrements in neurobehavioral test performance with increasing PCB concentrations among 50 to 70 year olds. Results suggest the most sensitive functions affected by PCB exposure are memory, language and the more complex motor function tests; however, the explanations for this are unknown. As suggested by Weiss, neurobehavioral deficits caused by age-related health conditions – such as diabetes or heart disease – may be intensified by exposure to neurotoxicants, thus further contributing to a decrease in function (35). Even small declines in neurobehavioral function in an aging population can have a large public health and financial impact.

Table 5.1. Select Characteristics of the Population

Characteristic	**Number (%)**
Race/Ethnicity	
Whites	123 (62.1%)
Nonwhites	75 (37.9%)
Educational Attainment	
Less than High School Education	15 (7.5%)
High School Diploma, High School Diploma plus Trade school, Some college or Associate Degree	77 (36.9%)
Baccalaureate Degree	40 (20.2%)
Some post-Baccalaureate or Post-Baccalaureate Degree	66 (33.1%)
Sex	
Female	130 (65.7%)
Male	68 (34.3%)
Smoking History	
Never Smoked	80 (40.4%)
Previous or Current Smoker	118 (59.6%)
Alcohol Use with the Past Month	
No alcohol	70 (35.4%)
At least one alcoholic drink	128 (64.6%)
Self-Reported History of Diabetes*	
Yes	29 (14.6%)
No	169 (85.4%)
Self-Reported use of antihypertensive medications within the past 2 weeks**	
Yes	76 (38.4%)
No	122 (61.6%)
Other Characteristics	**Mean (SD)**
Age (years)	58.5 (5.9)
BMI (kg/m^2)	28.9 (6.5)
Homocysteine (umol/L)	9.4 (3.1)
Tibia lead levels (ppm)	16.6 (11.0)

*History of depression, high cholesterol, hypertension, arthritis, and panic disorders were evaluated. Data not shown. **Use of diabetic, depression, non-steroidal anti-inflammatory, and thyroid medications, as well as aspirin use were evaluated. Data not shown.

Table 5.2. Crude associations showing standardized neurobehavioral test scores (z-scores) per unit increase in lipid-adjusted serum PCB levels.

	Bivariate Results (n=198)		
Cognitive Domain Test	β Coefficients for lipid-adjusted serum PCB (95% CI)	P-value	Adjusted R-squared
Language			
Category Fluency	-0.99 (-1.65, -0.34)	0.003	0.04
Letter Fluency[a]	-1.12 (-1.77, -0.47)	0.001	0.05
Visual Memory			
Rey Complex Figure delayed recall	-0.67 (-1.33, -0.01)	0.05	0.01
Verbal Memory			
Rey Auditory Verbal Learning			
Immediate Recall	-0.81 (-1.47, -0.15)	0.02	0.02
Visuoconstruction and Visuoperception			
Rey Complex Figure copy	-0.61 (-1.27, 0.06)	0.07	0.01
Motor and Manual Dexterity			
Finger Tapping			
Dominant Hand[b]	-0.23 (-0.90, 0.44)	0.49	0.00
Nondominant Hand[b]	-0.34 (-1.01, 0.33)	0.32	0.00
Purdue Pegboard			
Dominant Hand	-0.76 (-1.42, -0.10)	0.03	0.02
Nondominant Hand[b]	-0.88 (-1.53, -0.22)	0.009	0.03
Both Hands[b]	-1.01 (-1.66, -0.36)	0.003	0.04
Simple Reaction Time (negated)	-0.17 (-0.83, 0.50)	0.63	0.00
Executive Function			
Purdue Pegboard Assembly[b]	-0.73 (-1.39, -0.06)	0.03	0.02
Stroop Test (negated)	-0.61 (-1.27, 0.05)	0.07	0.01
Trail Making			
Test A (log transformed/negated)	-0.68 (-1.34, -0.01)	0.05	0.02
Test B (log transformed/negated)[b]	-0.78 (-1.44, -0.12)	0.02	0.02

[a] One influential point removed from analysis

[b] One individual did not complete the test. Value treated as missing.

Table 5.3. Bivariate Results of logistic regression analyses showing the odds of scoring in the lowest 25% for neurobehavioral tests per unit increase in lipid-adjusted serum PCBs.

	Bivariate Results (n=198)		
Cognitive Domain **Test**	Odds Ratio for lipid-adjusted serum PCB (95% CI)	P-value	Pseudo R-square
Language			
Boston Naming	6.26 (1.45, 26.96)	0.01	0.03
Verbal Memory			
Rey Auditory Verbal Learning			
Recognition	5.48 (1.28, 23.41)	0.02	0.02
Delayed Recall	4.18 (1.01, 17.22)	0.05	0.02
Visual Memory			
Symbol Digit	4.04 (0.97, 16.85)	0.06	0.02

Table 5.4. Results of multiple linear regression analyses showing standardized neurobehavioral test scores (z-scores) per unit increase in lipid-adjusted serum PCB levels

	Model 1 excluding race/ethnicity (n=198)*			**Model 2 including race/ethnicity (n=198)****		
Cognitive Domain Test	β Coefficients for lipid-adjusted serum PCB (95% CI)	P-value	Adjusted R-squared	β Coefficients for lipid-adjusted serum PCB (95% CI)	P-value	Adjusted R-squared
Language						
Category Fluency	-0.73 (-1.29, -0.16)	0.01	0.32	-0.46 (-1.01, 0.09)	0.10	0.39
Letter Fluency[a]	-0.79 (-1.26, -0.33)	0.001	0.35[†]	-0.63 (-1.11, -0.14)	0.01	0.38[†]
Visual Memory						
Rey Complex Figure delayed recall	-0.57 (-1.18, 0.03)	0.06	0.22	-0.44 (-1.05, 0.18)	0.16	0.23
Verbal Memory						
Rey Auditory Verbal Learning Immediate Recall	-0.48 (-1.08, 0.13)	0.12	0.21	-0.24 (-0.84, 0.36)	0.43	0.26
Visuoconstruction and Visuoperception						
Rey Complex Figure copy	-0.45 (-1.01, 0.11)	0.12	0.33	-0.26 (-0.82, 0.30)	0.36	0.36
Motor and Manual Dexterity						
Finger Tapping						
Dominant Hand[b]	-0.14 (-0.74, 0.46)	0.65	0.24	-0.01 (-0.62, 0.59)	0.97	0.25
Nondominant Hand[b]	-0.32 (-0.91, 0.26)	0.28	0.27	-0.21 (-0.81, 0.38)	0.48	0.28
Purdue Pegboard						
Dominant Hand	-0.33 (-0.91, 0.26)	0.27	0.27	-0.21 (-0.80, 0.39)	0.49	0.28
Nondominant Hand[b]	-0.56 (-1.17, 0.06)	0.08	0.20	-0.41 (-1.03, 0.21)	0.20	0.22
Both Hands[b]	-0.67 (-1.25, -0.09)	0.02	0.29	-0.49 (-1.06, 0.09)	0.10	0.32
Simple Reaction Time (negated)	-0.22 (-0.88, 0.44)	0.51	0.07	-0.07 (-0.74, 0.60)	0.84	0.09
Executive Function						
Purdue Pegboard Assembly[b]	-0.41 (-1.02, 0.20)	0.18	0.21	-0.21 (-0.82, 0.39)	0.49	0.25
Stroop Test (negated)	-0.31 (-0.94, 0.32)	0.33	0.16	-0.10 (-0.73, 0.53)	0.76	0.20
Trail Making						
Test A (log transformed/negated)	-0.40 (-1.04, 0.24)	0.22	0.13	-0.22 (-0.86, 0.42)	0.50	0.16
Test B (log transformed/negated)[b]	-0.46 (-1.03, 0.11)	0.12	0.31	-0.24 (-0.81, 0.32)	0.40	0.35

*All coefficients standardized; adjusted for age, sex, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication, lipid-adjusted total serum PCBs; **Includes all variables from Model 1 plus race/ethnicity. [a] One influential point removed; [b] One missing data point. [†]Value indicates R-squared unadjusted.

Table 5.5. Results of logistic regression analyses showing the odds of scoring in the lowest 25% for neurobehavioral tests per unit increase in lipid-adjusted serum PCB levels

	Model 1 excluding race/ethnicity* (n=198)			**Model 2 including race/ethnicity** (n=198)**		
Cognitive Domain Test	Odds Ratio for lipid-adjusted serum PCB (95% CI)	P-value	Pseudo R-square	Odds Ratio for lipid-adjusted serum PCB (95% CI)	P-value	Pseudo R-square
Language						
Boston Naming	7.35 (1.37, 39.62)	0.02	0.27	3.89 (0.56, 26.93)	0.17	0.35
Verbal Memory						
Rey Auditory Verbal Learning						
Recognition	4.05 (0.87, 18.93)	0.08	0.12	2.56 (0.49, 13.23)	0.26	0.17
Delayed Recall	2.95 (0.63, 13.84)	0.17	0.18	1.97 (0.38, 10.14)	0.42	0.21
Visual Memory						
Symbol Digit	3.70 (0.81, 16.94)	0.09	0.09	2.69 (0.56, 12.87)	0.22	0.11

*All coefficients have been standardized; adjusted for age, sex, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication, lipid-adjusted total serum PCBs; ** Adjusted for all variables in Model 1 plus race/ethnicity.

Chapter 6: DISCUSSION

6.1 Summary

The first phase of this study determined potential predictors of serum PCB concentrations within a population-based sample of an older urban population. This was followed by an examination of the association between low-level PCB exposure and neurobehavioral function in the same sample of older adults. Additionally, in an attempt to identify a possible gene-environment link, the G^{894}-T^{894} exon 7 polymorphism in the endothelial nitric oxide synthase gene (eNOS) was evaluated as a potential modifier of the association between PCB exposure and neurobehavioral outcome. Overall hypotheses included:

1. Increased seafood consumption and age as well as nonwhite race/ethnicity and female sex are significant predictors of higher serum PCB concentrations.
2. Increasing serum PCB concentrations are associated with lower neurobehavioral test scores, with the strongest association existing for tests of learning and memory.
3. Endothelial nitric oxide synthase genotype modifies the association between PCB concentration and neurobehavioral performance. With increasing PCB exposure, individuals who are TT homozygous and TG heterozygous score even lower than expected on neurobehavioral tests than those individuals who are GG homozygous.

The results of our study are discussed below according to the specific aims that address the above hypotheses.

Specific Aim 1: Characterize total serum PCB concentrations within an elderly urban population.

Specific Aim 2: Determine potential predictors of PCB concentrations for the same population.

The first part of this study, which examined potential predictors of serum PCB concentrations in an older population, addressed both of the specific aims listed above. The PCB concentrations of this population ranged from 0.2 ppb to 10.9 ppb (after exclusion of an outlier) with a mean of 2.1 ppb. No values were below 0.1 ppb, the limit of detection. After lipid-adjustment, PCB concentrations ranged from 0.04 to 1.57 μg/g lipid with a mean value of 0.30 μg/g lipid. Comparison of these results to those found in other studies was difficult, because many recent studies report the sum of various congeners as "total PCBs," whereas the total PCB measurements in this study were based on Aroclor standards. Also, lipid adjustment techniques vary and were not carried out in all studies. Despite these differences, it is estimated that mean background serum PCB concentrations found in other studies of adults range from 1.2 to 6.8 ppb (45). Our results fall within this range, and tend to be lower than those found in populations with known exposure or in populations known for their fish consumption.

While several studies have examined potential predictors of PCB concentrations in fish-eating populations, populations of women, or populations occupationally-exposed to PCBs, our study examined predictors of PCBs in an older, diverse urban population with representation of both sexes and with no known specific risk factors associated with PCB exposure. Numerous factors were assessed, including demographic information, health history, medication use and dietary factors. Although race/ethnicity was found to be the strongest factor associated with lipid-adjusted serum PCB concentrations, total seafood consumption was the next strongest predictor. Because dietary information was

collected using the Block 98.2 Food Frequency Nutritional Questionnaire (a dietary assessment tool developed using well-validated methods) specific categories of seafood (shellfish, tuna, fried fish and other fish) were examined; however, only high consumption of shellfish was significantly associated with serum PCB concentrations. This finding was unexpected and emphasizes that regional differences in dietary practices and seafood availability should be further investigated.

Specific Aim 3: Examine the association between low-level PCB exposure and neurobehavioral test scores.

The second part of this study described the relationship between low-level PCB exposure and neurobehavioral function in the study sample of racially diverse, older adults. Older adults are an important population in which to study neurotoxins, as they are potentially more susceptible to adverse effects (35). While numerous studies have examined the impact of PCB exposure on neurobehavioral function in children and occupationally-exposed adults, only one previous study has examined effects in a population of older adults exposed to relatively low levels of PCBs. Schantz and colleagues found deficits in memory and learning associated with PCB exposure in an older population lacking racial diversity (30).

In our older population of mixed race/ethnicity, findings suggest a potential association between lipid-adjusted serum PCB concentrations and neurobehavioral function after controlling for several factors, including demographic information, health history and medication use. In all models, higher lipid-adjusted serum PCBs concentrations were associated with decrements in neurobehavioral function. In models that assessed the verbal memory, language and complex motor speed domains, significant

associations at the $p \leq 0.10$ level were revealed after exclusion of race/ethnicity from the model. Three possible causal models, proposed by Martin and colleagues, explain how inclusion of race/ethnicity into models could result in an underestimation of the relationship between neurotoxicants, such as PCBs, and neurobehavioral function (188). Although more information is needed to validate the causal models proposed by Martin and colleagues, even a small effect of PCB exposure on neurobehavioral function in older adults could have significant public health implications.

Specific Aim 4: Determine whether the eNOS polymorphism acts as an effect modifier in the relationship between PCB exposure and neurobehavioral test scores.

In our population, an association between eNOS genotype and neurobehavioral function was found when analysis of variance (ANOVA) was used to examine crude differences in scores among all three genotypes. (Appendix I.) Differences remained when the TT and TG genotypes were combined, because of small number in the TT group. However, these differences were not in the expected direction as predicted by our hypotheses. We had expected that those individuals with TG and TT genotypes would score lower on the neurobehavioral tests than those with GG genotype. However, once combined into two groups (due to the small number of TT genotypes) and adjusted for other factors (including demographic information, health history, medication use and PCB levels), the relationships between genotype and neurobehavioral function were no longer apparent. The only significant associations observed were for dominant and non-dominant hand finger tapping tests, in which GG genotypes did more poorly when compared to TG and TT genotypes. (Appendix J.) Although these findings may be spurious, several studies suggest that nitric oxide may be an important neurotransmitter in

the peripheral nervous system (102).

An interaction term (PCB concentration*genotype) added to regression models for each of the neurobehavioral outcomes was found to be significant for the Boston Naming test, Purdue Pegboard (dominant hand) and Trail Making Test A. With the exception of the Boston Naming test, the affected tests differed from those found to be associated with neurobehavioral performance when genotype information was excluded (Chapter 4). The interpretation of these findings was difficult, because there was no consistency in the testing domains, and addition of the interaction terms did not appreciably increase the models' ability to explain the variance in the outcomes. The associated R-squared values changed only minimally. (Appendix J.) These results neither supported nor refuted the proposed link between PCB exposure, the eNOS polymorphism and neurobehavioral functions. As detailed in Chapter 2, although data from animal and *in vitro* studies suggest that such relationships may exist, the role of the eNOS polymorphism in nitric oxide production and the mechanism by which PCBs cause neurotoxicity is not clearly understood.

6.2 Strengths

One strength of this study is that it allowed characterization of lipid-adjusted total serum PCB concentrations for an older urban population that was racially diverse, whereas many other studies have focused mainly on white populations of women, children, or special populations at risk for increased PCB exposure. The sensitivity of serum PCB analyses was sufficient to detect PCBs in all serum samples; therefore no laboratory data were censored. Serum cholesterol and triglyceride concentrations were also available for all participants, which allowed serum PCB concentrations to be lipid-

adjusted. Comprehensive questionnaires were used to gather information on numerous factors, including demographic information, health history and medication use. In addition, detailed dietary information was collected using the Block 98.2 Food Frequency nutritional questionnaire, which is a well-validated assessment tool that allowed several food categories to be considered in the analyses.

Another major strength is that this study was one of the first to examine the association between PCB concentrations and neurobehavioral function in a population-based sample of older adults, while adjusting for several covariates (listed above). Unlike the study conducted by Schantz and colleagues, we were able to examine the relationship between race/ethnicity, serum PCB concentrations and neurobehavioral function. Neurobehavioral outcomes were measured using a wide range of well-validated neurobehavioral tests that assessed several cognitive domains. Other covariates – such as tibia lead concentration – were available for evaluation as potential confounders. Additionally, this study allowed for examination of specific genotypes as markers of susceptibility, which has not been done before in exploring the relationship between PCB exposure and neurobehavioral function.

6.3 Limitations

The cross-sectional design of this study could not determine temporal, or therefore, causal relationships between the independent variables and dependent variables. This limitation held for both the question of the predictors of PCB concentration and for exploring the relationship of serum PCB concentration with neurobehavioral function. Baseline data were lacking and the stability of some measures, such as dietary habits, were unknown. It is possible that neurobehavioral tests results may be more strongly

associated with previous PCB exposure not captured by our biomarker of current serum PCB. For example, if an individual was exposed to PCBs in the early years of life, especially during developmental stages, resultant neurobehavioral effects may have persisted throughout life, possibly worsening with age. The link with PCB as a causative agent, however, would not be detected unless the current serum PCB measure adequately represents past exposures. This has not been established.

The sample size, governed by budgetary constraints, may have limited our power to detect differences in the outcomes by variables of interest. The issue of small sample size played a particular role in the ability to assess the influence of eNOS genotype on neurobehavioral function. The need to collapse the three different genotype groups into two groups may have impeded the ability to detect an effect. For example, if TT homozygotes had significantly less nitric oxide production than TG heterozygotes, then combining the two groups may have masked an effect that is specific to the TT homozygote group.

Budgetary constraints also contributed to the inability to identify specific PCB congeners. If only a specific congener or a handful of congeners are responsible for neurobehavioral outcomes, these may have been diluted or unmeasured, thus resulting in misclassification of exposure and results that are biased toward the null. Similar misclassification would occur if the measured PCB congeners do not represent those found in the foods that were explored as predictors of PCB concentrations. Additionally, the inability to identify or quantify specific congeners precludes inferences that would differentiate the most neurotoxic PCB exposures.

Although participation rates were relatively high in the parent study, as with many

studies, there is a potential for selection bias since those who choose not to participate may in fact be different than those who chose to participate. The selection criteria for our sample population – which included a stratified random sample based on genotype, adequate serum volume and complete neurobehavioral test information – may have also influenced our results. There is some evidence that the education level of this group was somewhat higher than that of the overall study.

Additionally, although our study may have internal validity, because the population in our study ranged from 50 to 70 years of age, results may not be able to be generalized to other age ranges.

6.4 Public Health Significance

Although concentrations of PCBs have been declining in the environment, it is important to understand the risk factors associated with PCB exposure, past and present, and to identify subgroups of the population that may be at increased risk for adverse health effects. It is especially important to recognize the implications of relatively low-level PCB exposure, because a large proportion of the general public may be affected. Our findings linking seafood consumption (especially shellfish and total seafood consumption) to serum PCB concentrations reinforces the need for sound policies directed at setting acceptable PCB concentrations in seafood, standardizing monitoring practices and effectively communicating findings to the public. Efforts should also be made to facilitate understanding and compliance with advisories for locally caught seafood. In addition, more research is needed to determine how regional availability and dietary practices can influence PCB exposures.

Although a better understanding of the role that race/ethnicity plays in the

relationship between PCB exposure and neurobehavioral function needs to be further elucidated, our results do suggest that older adults may be at risk for neurobehavioral effects associated with low-level exposure to PCBs. While decrements in neurobehavioral function related to PCB exposure are likely to be small, one must think of the impact that small shifts in cognitive function can have on a population. Due to the significant increase in aging populations over the past few decades, even small declines can have a large public health and financial impact.

6.5 Further Research

Design and methodological differences make it difficult to compare studies that address the relationship between PCB exposure and neurobehavioral function. A standardized set of neurobehavioral tests, as well as uniform methods for PCB analysis, would improve cross-study comparisons. Additionally, PCB analytical methods that allow identification and quantification of specific congeners are essential to determining which specific congeners are responsible.

Several additional avenues of research should be pursued. Findings from the first part of the study indicate that shellfish consumption is an important predictor of serum PCBs within our population. Further investigation to elucidate the source of the shellfish, average PCB concentrations in the shellfish consumed, common dietary practices and the distribution of specific congeners within these foods (as mentioned above) would assist in understanding the true exposures of populations. A better understanding of the factors that influence diet and dietary change would inform the development of effective interventions, including policies, aimed at reducing exposure. The relative effectiveness

of such interventions, particularly as they affect vulnerable populations such as the elderly, is another area of concern.

Results from the second part of the study suggest that low-level PCB exposure is associated with neurobehavioral function in older adults when race/ethnicity is excluded from models. Studies that validate the proposed causal models examining the role of race/ethnicity, presented by Martin and colleagues, need to be conducted. Understanding the role of race/ethnicity is crucial so that appropriate modeling techniques can be identified to determine the relationship between toxicants and outcomes. Another area for further research involves gaining a better understanding of the biological effects of different congeners. Findings from mechanistic studies that show the relationship of specific congeners with neurobehavioral function may allow a more precise/appropriate PCB measurement to be used in epidemiologic studies. Also, the relationship between PCBs and serum lipids also needs to be further investigated to ensure that the least amount of bias is introduced when lipid adjustment is performed. More toxicological data are needed to determine the mechanism of PCB neurotoxicity in humans and the functional relevance of the eNOS polymorphism.

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APPENDICES

APPENDIX A: Literature review for studies examining PCB exposure and neurobehavioral function

Table A1. Cohort studies that examine neurotoxic effects of PCBs in children

Author/ Year (reference)	Sample Size and population	Age Range	Source of exposure	Main outcomes Measured	Main dose measures	Main conclusions
Boersma and Lanting/ 2000 (18)	418 children in the Netherlands	Tested at 18, 42, and 76 months of age	Exposed *in utero*	Bayley Scales of Infant Development done at 18 months; Kaufman Assessment Battery for Children at 42 months; and McCarthy Scales used at 76 months	PCBs measured in maternal and cord blood	Association found between PCB levels and poorer cognitive development at 42 and 76 months of age.
Chen et al/ 1992 (19)	118 children in Taiwan whose mothers were exposed and matched controls	Tested at ages 4-7 years.	Prenatal exposure – mother consumed contaminated rice oil	Stanford-Binet test and Weschler Intelligence Scale for Children	Exposed to PCBs *in utero* versus exposure to background levels *in utero*	Children who were exposed had poorer cognitive development than matched controls; the effect continued until age 7.
Daniels et al/ 2002 (20)	1,207 children enrolled in the Collaborative Perinatal Project across 12 US sites 1959-1965	Tested at 8 months of age	Exposed *in utero*	Bayley Scales of Infant Development (assesses cognitive, language and social development)	Maternal prenatal PCB level	No relationship was found between PCB level and children's scores on the Bayley Scales of Infant development.
Darvill et al/ 2000 (181)	216 children from the Great Lakes Region	Tested at 67 weeks and 92 weeks	Mothers breast milk – mother exposed environmentally	Fagan Test of Infant Intelligence (FTII)	Total PCBs in breast milk; Cord total PCBs; Cord Lead; Maternal hair mercury	Dose-dependent relationship found between cord PCB levels and FTII scores at both ages; no other significant relationships found.
Fein et al/ 1984 (195)	242 children whose mothers ate Great Lakes fish and	Newborns - 3 days old	Exposed *in utero* – mothers consumed contaminated	Ballard Examination for fetal maturity, neonatal behavioral Assessment Scale	Mothers' cord blood	Exposed newborns had decreased neuromuscular maturity – but not once cord blood was controlled.

Table A1 (continued). Cohort studies that examine neurotoxic effects of PCBs in children

Author/ Year (reference)	Sample Size and population	Age Range	Source of exposure	Main outcomes Measured	Main dose measures	Main conclusions
	71 children whose mothers did not eat Great Lakes fish		fish			
Gladen et al/ 1988 (21)	802 children living in a general community	6 or 12 months of age	Exposed *in utero* and through breast milk	Bayley scales of Infant Development at 6 or 12 months of age	Cord blood and breast milk	Higher PCB cord blood exposure was associated with lower psychomotor scores for both ages. Breast feeding was not associated with scores.
Grandjean et al/ 2001 (22)	435 children from the Faroese Birth cohort	Tested at 7 years of age	Exposed *in utero*	Neurobehavioral Evaluation System (NES2), finger tapping test, hand-eye coordination test and continuous performance test; Weschler Digit Span; WISC-R Similarities; block designs; Bender visual motor gestalt test, California Verbal Learning test, Boston Naming test; neurophysiological and sensory tests	PCBs measured in cord blood	Cord PCB levels found to be associated with deficits on the Boston Naming test, the Continuous Performance Test reaction time, and on the long-term recall on the California Verbal Learning test.
Gray et al/ 2005 (196)	894 Children born to women in 12 U.S. study centers	Tested at age 7	Exposed *in utero*	Seven components of the Weschler Intelligence Scale for Children at age 7 (information, comprehension, vocabulary, digit span, picture arrangement, block design and coding.)	Serum from mothers during the 3rd trimester (11 congeners measured)	Results showed that exposure *in utero* to "background" levels of PCBs was not associated with lower IQ scores at age 7.
Huisman et al/ 1995 (182)	418 Dutch newborns	Tested between the 10th and 21st	Exposed *in utero*	Comprehensive neurological exam designed by Prechtl	Cord blood and maternal plasma; breast milk at 2 weeks after birth	Higher PCB levels in breast milk were associated with higher incidence of hypotonia.

Table A1 (continued). Cohort studies that examine neurotoxic effects of PCBs in children

Author/ Year (reference)	Sample Size and population	Age Range	Source of exposure	Main outcomes Measured	Main dose measures	Main conclusions
		day after delivery				
Jacobson et al/1985, 1990,1996 (25;27;180)	212 children in Lake Michigan cohort	Tested at 4 years, 4 years 3 months, 7 years, and 11 years of age	Exposed in *utero* – Mothers exposed by eating contaminated fish	At age 11- Weschler Intelligent Scales for children; Wide Range Achievement Test, Woodcock Reading Mastery Test	Umbilical cord blood and mothers' PCB levels	Cord PCB levels associated with lower mean visual recognition memory. At age 4, cord PCB associated with poorer performance on verbal and memory scales of McCarthy scales. At four years and three months of age, declines noted on McCarthy memory scales and the general cognitive index associated with cord PCB levels. At age seven, children with higher cord PCB levels were found to perform poorly on word comprehension and overall reading comprehension. At age 11, prenatal exposure associated with lower full-scale and verbal IQ scores (especially memory and attention).
Koopman-Esseboom et al/ 1996 (197)	207 children living in a community in the Netherlands	3, 7, and 18 months of age	Exposed *in utero*	Bayley Scales of Infant Development	Mothers cord blood	Exposure to PCBs was not associated with development at ages 7 or 18, but small effect noted on the psychomotor score at 3 months.
Lai et al/ 2002 (191)	118 exposed children and 118 non-exposed in Taiwanese population	Range 10-14 years	Exposed *in utero* – mothers ate contaminated cooking oil	Tested yearly – Weschler Intelligence Scale for Children; Achenbach Child Behavior Checklist and Rutter Child Behavior Scale.	PCB levels not used in statistical analyses	Exposed children scored significantly lower on IQ tests, and higher on both the Child Behavior Checklist and Rutter Scale.
Rogan et al /1986 (184)	912 children from a North Carolina cohort	Tested at 6 -12 months	Exposed *in utero*	Brazelton Neonatal Behavioral Assessment Scales	Cord blood samples	Higher PCB levels were associated with hypotonicity and hyporeflexia.

Table A1 (continued). Cohort studies that examine neurotoxic effects of PCBs in children

Author/ Year (reference)	**Sample Size and population**	**Age Range**	**Source of exposure**	**Main outcomes Measured**	**Main dose measures**	**Main conclusions**
Stewart et al / 2000 (198)	141 children whose mothers ate Lake Ontario fish and 152 "un-exposed"	Tested twice within 48 hours of birth	Mothers exposed by eating contaminated fish	Neonatal Behavioral Assessment Scale (NBAS)	69 PCB congeners from cord blood	Significant relationship between the congeners with seven or more chlorine substitutions, and performance impairments on the Habituation and Autonomic clusters of the NBAS.
Stewart et al/ 2003 (183)	212 children enrolled in the Oswego Infant and Development Project	Tested at 38 months and 54 months of age	Mothers exposed by eating contaminated fish	McCarthy Scales of Children's abilities	69 PCB congeners from cord blood	Cord PCB levels were significant predictors of measurable deficits on the McCarthy performance test at 38 months of age. No relationship was noted between PCBs and McCarthy performance at 54 months.
Winneke et al/ 1998 (29)	171 from a German cohort	7 months of age	Exposed *in utero*	Bayley II mental (MDI); psychomotor development index (PDI); Fagan Test of Intelligence	PCBs measured in mothers' cord plasma and material milk at two and four weeks post-partum	Breast milk samples had a significant negative association with the Bayley II mental test, which assesses cognitive development, language development and personal/social development.

Table A2. Studies that examine neurotoxic effects of PCBs in adults

Author/ Year (Reference)	Sample Size and population	Mean Age (Range)	Source of exposure	Main outcomes Measured	Main dose measures	Main conclusions
Emmett et al/ 1988 (79)	55 exposed transformer repair workers and 56 non-exposed	38 years (24-60)	Occupational – transformer repair workers	Questionnaires; medical exam; delayed hypersensitivity testing	Serum PCB levels; Adipose PCB levels	Age, years of employment were significant for serum PCB. Exposed group reported significantly more eye irritation, increased tearing, chest pain on walking, wheezing, loss of appetite, headaches, insomnia, memory trouble than non-exposed.
Fischbein et al /1979 (32)	326 capacitor manufacturer workers	Mid 40s (20s-70s)	Occupational	Reported respiratory, dermatologic, GI, musculoskeletal, neurological symptoms	Plasma PCB levels (mean = 124±229 ppb)	Most prevalent symptoms reported included dermatologic and CNS symptoms (ie. headache, fatigue, nervousness) – however, only dermatologic issues were associated with PCB levels.
Fitzgerald et al/ 1986 (33)	482 individuals potentially exposed from electrical fire	41 years old	Occupational – exposure after electrical transformer fire	Disorders of skin, eyes, liver and neurologic system 6 and 12 months after exposure	Serum PCB levels (mean = 6.9±3.52 ppb at 6months; 6.5±4.12 ppb at 12 months)	Significant correlations only found between serum PCB levels and levels of liver enzymes and lipids.
Kilburn et al/ 1989 (49)	28 fireman (14 exposed; 14 unexposed)	33 years old	Occupational – exposure after a transformer fire	Battery of tests: Profile of Mood State; Digits forward and backward; Block design score; Purdue Pegboard; Trails A and B; Culture Fair; Embedded Figures; Choice reaction time; Balance; and others	Serum PCB levels as Aroclor 1248 (median = 6 ppb; range= 1.9-15 ppb)	PCB levels did not correlate with neurobehavioral test scores. However, exposed individuals had impaired short-term memory, interpretation of designs, spatial relation integration, decision making and coordination when compared to unexposed.
Kilburn et al/ 2000 (34)	98 exposed and 58	40 for exposed	Environmental – exposure	Visual field performance, color	Serum PCB levels from 22 subjects	Exposed individuals scored significantly lower on simple and choice reaction

Table A2 (continued). Studies that examine neurotoxic effects of PCBs in adults

Author/ Year (Reference)	Sample Size and population	Mean Age (Range)	Source of exposure	Main outcomes Measured	Main dose measures	Main conclusions
	unexposed living near a pumping station	and 34 for unexposed	was from a pumping station	confusion index, balance, blink reflex latency R-1, hearing, grip strength, simple and choice reaction times, problem solving for Culture Fair and digit symbol, recall memory, peg placement, Trails A and B, POMS	ranged from 0.33 to 4.48 ppb. Exposure was determined more by distance from contaminated site.	times, Culture Fair, digit symbol digit, vocabulary and verbal recall, Purdue Pegboard, color discrimination, visual performance, Trails A and Trails B. Scores on POMS were elevated and sway speeds to measure balance were faster.
Schantz et al/ 1996 (165), 1999 (192), 2001 (30)	101 fisheaters and 78 non-fisheaters living in Great Lakes region	Ages ranged from 49-86 years old	Environmental – Fish consumption	Grooved Pegboard Test (GPT) and Static Motor Steadiness test (SMST). Weschler Memory Scale (WMS), WAIS-R vocabulary subtest, Grooved pegboard test, California Verbal Learning Test (CVLT), Wisconsin Card Sorting Test, Stroop test, Trails A and B, Digit Symbol test, Hooper Visual Organization Test, Short Category Test	Total serum PCB levels (sum of 25 congeners); values ranged from below the limit of detection (3 ng/mL) to 75 ng/mL	PCB exposure was not associated with GPT results. Scores on the SMST improved slightly as PCB exposure increased. PCB exposure was associated with impairments in memory and learning; PCBs associated with lower scores on delayed verbal recall of the WMS and on the CVLT.

APPENDIX B: Predictors of PCBs in non-occupational human epidemiologic studies

Table B1. Summary of PCB associations found in non-occupational human epidemiologic studies among adults

Author/year	Population (N)	PCB Biomarker	Variables of Interest	Study Findings
Bloom et al/ 2003 (174)	White New York State sportsmen (66)	Nine PCB congeners (19, 28, 47, 118, 153, 169, 180, 183, 187)	Mailed questionnaires - Time of sample collection, serum triglycerides, cholesterol, HDL, LDL, age, BMI, smoking, serum total thyroxine	Increases in serum triglyceride, cholesterol and LDL levels were associated with increases in many of the PCB congeners.
Bloom et al/ 2005 (3)	White New York Male State Anglers (203)	Sera levels of 57 lipid-adjusted PCB congeners	Questionnaire- Age, BMI, cigarette smoking, water/wildfowl consumption index variable, years of sampling. Collected dietary information for sportsfish (brown trout, catfish, salmon, lake trout, rainbow trout)	Long-term dietary consumption of sports fish from Lake Ontario contributes to PCB levels; More specifically, PCB 105, 132, 153, 118, 163, 183, 187, 188 were associated with fish consumption.
Devoto et al/ 1998 (11)	Elderly males and females in Germany (297)	Total PCBs not lipid-adjusted	Dietary journal and interviews- Fish consumption, BMI, plasma cholesterol, beef, pork and poultry consumption, age, sex, parity	Saltwater fish consumption was correlated with higher levels of PCBs (r=.12) and beef/pork consumption (r=.13)
Falk et al/ 1999 (4)	Sports anglers around Lake Michigan (100)	4 coplanar PCBs were measured. (lipid-adjusted total coplanar PCB TEq used in the analysis)	Telephone survey – Demographic variables (age, height, weight, sex), total fish consumption for the past year, and specific questions about Great Lakes sport fish consumption (specific types of fish, location of fish caught, years sports fish consumed)	Higher levels of PCBs were associated with increased consumption of lake trout and salmon, as well as increases in age and BMI; Males had significantly higher PCB levels than females after controlling for all other variables.
Finklea et al/ 1972 (149)	Urban and rural volunteers in South Carolina (723)	Plasma residues of PCBs	Interviews Age, race, sex, place of residence	PCBs were found in 43% of the population and ranged from below the limit of detection *(not specified)* to 29 ppb. Urban blacks were less likely to have detectable levels of plasma PCBs than urban whites; however, when plasma PCBs were detected, they had significantly higher levels than urban whites. Rural blacks had lower levels than rural whites.
Fiore et al/ 1989 (5)	Men and women Wisconsin anglers (192)	Sum of 13 PCB congeners in the serum	Mailed questionnaire - Mean number of total sport-caught meals and meals including only fish listed on fish advisory. Demographic information,	PCB levels ranged from non-detectable (below 0.6μg/l) to 27.1μg/l. 31% was below LOD. Positive correlations between PCB levels and total number of sport-caught fish meals

Table B1. Summary of PCB associations found in non-occupational human epidemiologic studies among adults

Author/year	Population (N)	PCB Biomarker	Variables of Interest	Study Findings
			including age, sex, race, education level, place of residence, was also collected, but not used in PCB analysis.	(Chinook, salmon, lake trout and brown trout) were noted. Fish consumption accounted for 10-15% of the variance in PCB levels.
Fitzgerald et al/ 2004 (6)	Mohawk women potentially exposed to PCBs in a hazardous waste site (111)	Total and sum of 7 serum PCB congeners (74, 99, 118, 138, 153, 180, 187 and 181) Lipid adjustment was done only when data were available.	Interview – Age, education, height, weight, BMI, marital status, cigarette use, alcohol use, coffee consumption, prescription drugs, weeks breastfeeding parity, estimated air and soil exposures and occupational exposure. Fish consumption was estimated by multiplying the total number of local fish meals reported during each time period by contaminant level in each type of fish and adjusting for cooking methods. Consumption of local beef, pork, chicken, duck.	Mean PCB level was 1.8 ppb (SD 1.6 ppb); median 1.2 ppb. 41% were below the LOD (1 ppb). Maximum value was 7.8 ppb. Age was positively associated with all PCB congener levels. Fish consumption was positively associated with PCB 74, 99 and 118 levels.
Furberg et al/ 2002 (199)	Female population in Norway (47)	Seven PCB congeners (105, 118, 138, 153, 180, 183, 187)	Questionnaire- Fish consumption (mostly lean fish from Norwegian waters), consumption of seagull eggs, BMI, lifetime lactation, residence	Congeners 138 and 153 were associated with intake of sea gull eggs. Length of residence was positively associated with most congeners. Lactation was negatively associated with all but two of the congeners. BMI was positively associated with congeners 105 and 153. Fish consumption was not a significant predictor.
Glynn et al/2003 (12)	Swedish women (205) Ages 54-75 years	Seven PCB congeners (105, 188, 138, 153, 156, 167, 180) lipid-adjusted	Interview- Age, BMI, body weight change over three months, place of residence, smoking, diabetes, duration of breastfeeding. Consumption of fruits, vegetables, legumes, high fiber grains, dairy products, fish, chicken, meat. Only fish data were examined. Information was gained regarding consumption of fatty fish (herring, mackerel, salmon) and total fish consumption (never consumed, <1 portion per week, ≥1 portion per week.	Positive associations found between serum PCB concentrations, age, fatty fish intake, body weight change, and place of residence. BMI was negatively associated with certain congeners and positively associated with others. Significant association found between fatty fish consumption and PCB153, even after adjusting for BMI, age, weight change, residence. (PCB levels were 1.2 times higher for those who ate fatty fish.) No significant associations for total fish consumption. Also note: PCBs were negatively associated with weight change for a three-month period.

Table B1. Summary of PCB associations found in non-occupational human epidemiologic studies among adults

Author/year	Population (N)	PCB Biomarker	Variables of Interest	Study Findings
Hanrahan et al/ 1999 (168)	Great Lakes Fish consumers, males and females (538)	Sum of 89 PCB congeners (not lipid-adjusted)	Telephone interviews – Age, sex, height, weight, Great lakes fish consumption (species and amount consumed for past year, lakes fished, methods of preparation, years eating sports fish), reproductive history (number of pregnancies, fish consumption while pregnant)	Fish consumption was the strongest predictor of PCB levels. However, males who consumed Great Lakes fish had significantly higher PCB levels than any other group. PCBs were also correlated with age, BMI and sport fish consumption.
Hovinga et al/ 1993 (143)	Great lakes fisheaters (115) and non-fish eaters (95)	Total Serum PCB using Aroclor standard 1260 (limit of detection 3 ppb)	Interview – Fish consumption (previous fisheater versus nonfisheater status, years of fish consumption, number of Great Lakes fish meals consumed in one year), tobacco use, alcohol consumption, self reported chemical exposure, age, sex	Mean PCB levels in fish eaters was 19 ppb (range= 4.9-173.9 ppb). Mean in nonfisheaters was 6.8 ppb (range= <LOD – 42.1.) Fish consumption was the most important predictor of PCB levels. Age was also significant. Body size was also a positive predictor of PCB levels; however, body size for males may be a more significant predictor than body size in females. Past fish consumption was a more significant factor in predicting PCB levels than current fish consumption.
James et al/ 2002 (7)	Pregnant women from the 1960s living in the San Francisco Bay area (399)	11 PCB congeners and their sum (lipid-adjusted)	Interview data - Age, race, place of birth, date of blood draw, BMI, occupation, past residence on a farm, parity, duration of pregnancy at time of blood draw	Age was positively associated with the sum of PCBs and the highly chlorinated congeners. Nonwhites had significantly higher levels of PCB congeners 180 and 187 than whites. Higher BMI was significantly associated with lower levels of some congeners and total PCBs. PCBs were also significantly associated with date of blood draw.
Kearney et al/ 1999 (8)	Fish license holders (men and women) living in Ontario (232)	Plasma levels of 14 pcb congeners and total PCBs (as Aroclor 1260) lipid-adjusted. Limit of detection = 0.2 µg/l	Interview data – Frequency of Great Lakes fish, waterfowl and ocean fish consumption within past year, age, sex, community	Mean PCB level was 4.0 ug/L and median value was 3.4 ug/L among non-fisheaters. Among fisheaters, the arithmetic mean was 6.1 ug/L. Great Lakes fish consumption was associated with high PCB levels in men only. Waterfowl consumption was associated with higher PCB levels in men and women. Age was also positively associated with PCB levels.

Table B1. Summary of PCB associations found in non-occupational human epidemiologic studies among adults

Author/year	Population (N)	PCB Biomarker	Variables of Interest	Study Findings
Kiviranta et al/ 2002 (200)	420 Fishermen from the Finnish Baltic Sea area	Serum samples of 36 PCB congeners	Questionnaires – Fish consumption (greater than twice a week), age, BMI, place of residence	Reported level of fish consumption was strongly associated with PCB levels. Age and place of residence were also strongly associated with PCBs
Kreiss et al/ 1981 (167)	Community population in Alabama (458)	Total serum PCB	Age, sex, fish consumption, race, obesity, serum cholesterol level, alcohol consumption, weight loss, blood pressure	Geometric mean of serum PCBs was 17.2 µg/l (SD 20.8). Age was significantly associated with higher PCB levels. Males had higher PCB levels than females. Fish consumption was associated with higher PCB levels, independent of age. Alcohol consumption was positively associated with PCB levels after controlling for age, sex, fish consumption and serum cholesterol. An association between PCB level and BP was also noted.
Kreiss et al/ 1982 (151)	Michigan residents (1631)	Total serum PCB levels based on Aroclor 1254 (not lipid-adjusted)	Sex, age (major focus of study was polybromated biphenyls)	Total PCB levels ranged from <1 to 57 µg/l. The geometric mean was 6.4 µg/l and the median was 6 µg/l. Males had significantly higher levels than females. PCB levels increased with age.
Laden et al/ 1999(13)	Women from nurses breast cancer study (240)	Sum of 16 congeners measured from plasma. Limit of detection <1 ppb.	Age, serum cholesterol, region of residence, adiposity, lactation, dietary intake from a semi-quantitative food frequency questionnaire. Food variables of interest included: meat, chicken, fish, eggs, dairy, vegetables, fruits and grains.	Total plasma PCB levels ranged from 1.61 to 16.62 ppb (mean = 5.22; sd 2.35) in individuals without breast cancer and 1.55 to 17.44 ppb (mean = 5.15; sd 2.77) in cases. Women living in the northeast and Midwest had significantly higher PCB levels than those living elsewhere. Fish consumption was positively associated with PCB levels for women living in Northeast and Midwest. Age and serum cholesterol were positively associated with PCB levels. Eggs and fish were statistically significant predictors for PCB levels, controlling for age, cholesterol and residence in the Northeast or Midwest.

Table B1. Summary of PCB associations found in non-occupational human epidemiologic studies among adults

Author/year	Population (N)	PCB Biomarker	Variables of Interest	Study Findings
Millikan et al 2000/ (152)	African American and white women enrolled in a breast cancer study in North Carolina (1407)	35 PCB congeners measured in plasma. Also measured total lipid-adjusted PCBs as sum of congeners 118, 138, 153 and 180.	Interview data- Race, breast cancer, BMI, farming history, income, parity, breast feeding, reported pesticide exposure.	Race, BMI, breastfeeding were all associated with PCB levels.
Moysich et al/ 2002 (148)	Postmenopausal western New York women (192)	Sum of 56 PCB congeners for a total PCB level lipid-adjusted	Interview data - Medical history, reproductive history, BMI, age, smoking, alcohol consumption, diet (assessed by 172-item food frequency questionnaire covering two years prior to interview)	Age was a significant predictor for total PCBs; however, the strength of association was stronger for moderately and higher chlorinated PCBs but non-existent for lower chlorinated PCBs. BMI was inversely associated with higher chlorinated PCBs. Inverse associations were also noted with serum lipids for moderate and higher chlorinated PCBs. Parity was weakly associated with lower PCB levels. Fish consumption (reported as intake of fresh, frozen, or canned fish, shrimp and other shellfish) was weakly associated with PCB levels. Mean total PCB based on 56 congeners = 4.12 ng/g of serum.
Paris-Pombo et al/2003 (201)	Benign breast disease patients in Ontario (190)	14 congeners (28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, 187) lipid-adjusted measured in adipose tissue	Questionnaire Food frequency questionnaire that averaged 67 foods over a two-year period (nonfatty foods, dairy, oil, fish, chicken, other meats were considered in this study), age, time of breast biopsy, BMI, number of pregnancies, occupational exposure, duration of lactation, place of birth, location of hospital, energy intake	Fish intake was noted to be the strongest dietary predictor, explaining 8% of the variation in PCB levels. Age was positively associated with tissue PCB levels and was the strongest predictor overall; It explained 5-24% of the variation in PCB levels. Lactation was inversely associated with PCB levels for most congeners. Dairy and chicken intake were inversely related to PCBs and at a level of significance for some congeners. Geometric mean for total PCBs (excluding 28, 52, 101, 128) = 0.89 μg/g lipid.

Table B1. Summary of PCB associations found in non-occupational human epidemiologic studies among adults

Author/year	Population (N)	PCB Biomarker	Variables of Interest	Study Findings
Sala et al/1999 (9)	Population living near electrochemical factory in Spain (608)	Sum of seven PCB congeners in serum (28, 52, 101, 118, 153, 138, 180) Limit of detection 0.05ng/mL	Questionnaire - Age, sex, alcohol consumption, smoking habits, medication, parity, social class, pesticide use, occupation, years of employment, fish consumption, BMI, weight loss	Mean sum of PCB congeners = 4.3 ng/mL. Age, local fish consumption and reported pesticide use were associated with higher levels of PCBs after controlling for other variables.
Schantz et al/ 1994/ (10)	Mothers (28) and children (38) from Michigan farms	Total serum PCB (using Aroclor 1016 and 1260 as reference standards) Limit of detection was 3 ng/mL	Interview - Age, years of residence on a farm with a PCB contaminated silo, Great Lakes fish consumption	Mean serum concentrations were 9.6 ng/mL (range 3.1 to 23.3). Detectable PCB levels were found in 86% of the mothers. Years of residence on farm and fish consumption were significantly associated with higher PCB levels in the mothers.
Wolff et al/ 2004 (150)	Pregnant women in New York (194)	Sum of four serum PCB congeners (118, 138, 153, 180) Limit of detection = 0.3 µg/l	Questionnaire - Age, race, country of birth, BMI, education, smoking, pre-pregnancy alcohol intake, fish consumption (types of fish purchased and frequency of consumption), other dietary variables collected through FFQ (fruit, vegetable, dairy, fish and meat intake)	Only 6% of the values were below the LOD. The median lipid-adjusted PCB value was 0.79 ug/l (IQR 0.548-1.28). BMI was inversely associated with PCB levels. Age and purchase of fresh fish were significantly associated with higher levels. A decision was made not to lipid adjust due to residuals; however, triglycerides were retained in final model.

APPENDIX C: Examination of the role of lipid-adjustment in regression models

The relationship between lipophilic compounds (such as PCBs) and serum lipids is not completely understood. Schisterman and colleagues proposed eight directed acyclic graphs that illustrate potential causal relationships between PCB levels, serum lipids and a dichotomous outcome (157). They applied four methods of analyses (unadjusted, standardized, adjusted and two-stage), discussed how each model related to the directed acyclic graphs and examined the potential for bias. We opted to use a standardized model for lipid adjustment (described in Methods) that has been frequently used in other studies. However, we examined the influence of each method proposed by Schisterman in our final models that investigated the relationship between PCBs and neurobehavioral function, as described below.

Tables 1 and 2 show results from regression analyses without serum lipid adjustment. Compared to the standardized models (presented in Chapter 5), for several tests, this approach showed a weaker association with PCB levels. However, using this method of adjustment is only appropriate if lipids are not related to PCBs or the outcome of interest (157). In our data, a correlation between PCB serum levels and total lipids exists (correlation coefficient = 0.26), thus indicating that this method may not be appropriate.

In Tables 3 and 4, serum lipids were entered into the regression models as an independent variable. Schisterman and colleagues referred to this method as "adjusted" analyses and predicted that this method would result in the least biased estimates of the relationship between lipophilic compounds and outcome. However, this prediction was based on hypothetical models with breast cancer as an outcome, and did not consider

other potential confounders. Compared to the standardized models presented in Chapter 5 (Tables 5.4 and 5.5), the main differences occurred in the visual memory and verbal memory domains. The strength of association between PCB levels and both visual memory tests (the Rey Complex Figure delayed recall and Symbol Digit) decreased in these models (p-values went from 0.06 to 0.10 and from 0.09 to 0.23, respectively); whereas two verbal memory tests (the Rey Auditory Verbal Learning Test immediate recall and Rey Auditory Verbal Learning Test delayed recall) demonstrated stronger associations (p-values went from 0.12 to 0.06 and from 0.17 to 0.09, respectively).

The two-stage method treated the residuals of serum lipids when regressed on PCB levels as independent variables. Results were identical to those of the "adjusted" model. (Not shown.)

We were unable to determine which method produced the most unbiased estimates for our data. Therefore, the standardized method of adjustment as described by Philips et al. was considered appropriate for this study.

Table C1. Results of multiple linear regression analyses showing standardized neurobehavioral test scores (z-scores) per unit increase in total serum PCB level (not adjusted for lipids)

	Model 1 including race/ethnicity (n=198)*			**Model 2 excluding race/ethnicity (n=198)****		
Cognitive Domain	β Coefficients for "unadjusted" serum PCB (95% CI)	P-value	Adjusted R-squared	β Coefficients for "unadjusted" serum PCB (95% CI)	P-value	Adjusted R-squared
Language						
Category Fluency	-0.06 (-0.13, 0.02)	0.12	0.39	-0.09 (-0.16, -0.01)	0.02	0.32
Letter Fluency[a]	-0.06 (-0.12, -0.01)	0.08	0.37[†]	-0.08 (-0.14, -0.02)	0.01	0.30[†]
Visual Memory						
Rey Complex Figure delayed recall	-0.03 (-0.11, 0.05)	0.46	0.23	-0.05 (-0.13, 0.03)	0.25	0.21
Verbal Memory						
Rey Auditory Verbal Learning Immediate Recall	-0.03 (-0.11, 0.05)	0.45	0.26	-0.06 (-0.14, 0.02)	0.16	0.21
Visuoconstruction and Visuoperception						
Rey Complex Figure copy	-0.02 (-0.10, 0.05)	0.52	0.36	-0.05 (-0.12, 0.03)	0.23	0.33
Motor and Manual Dexterity						
Finger Tapping						
Dominant Hand[b]	0.02 (-0.06, 0.10)	0.64	0.25	0.004(-0.08, 0.08)	0.92	0.24
Nondominant Hand[b]	-0.001 (-0.08, 0.08)	0.98	0.28	-0.01 (-0.09, 0.07)	0.75	0.27
Purdue Pegboard						
Dominant Hand	-0.03 (-0.11, 0.05)	0.44	0.28	-0.05 (-0.12, 0.03)	0.26	0.27
Nondominant Hand[b]	-0.05 (-0.13, 0.03)	0.23	0.22	-0.07 (-0.15, 0.01)	0.11	0.20
Both Hands[b]	-0.05 (-0.13, 0.03)	0.21	0.32	-0.07 (-0.15, -0.007)	0.07	0.29
Simple Reaction Time (negated)	-0.02 (-0.07, 0.11)	0.63	0.09	-0.003 (-0.08, 0.09)	0.94	0.07
Executive Function						
Purdue Pegboard Assembly[b]	-0.02 (-0.09, 0.07)	0.71	0.24	-0.04 (-0.12, 0.04)	0.36	0.21
Stroop Test (negated)	-0.02 (-0.06, 0.10)	0.65	0.20	-0.01 (-0.09, 0.08)	0.89	0.16
Trail Making						
Test A (log transformed/negated)	-0.01 (-0.07, 0.10)	0.81	0.16	-0.01 (-0.10, 0.08)	0.80	0.12
Test B (log transformed/negated)[b]	-0.01 (-0.09, 0.06)	0.72	0.35	-0.04 (-0.12, 0.04)	0.31	0.30

* All coefficients standardized; adjusted for race/ethnicity, age, sex, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication, total serum PCBs; **Includes all variables from Model 1 except race/ethnicity. [a] One influential point removed; [b] One missing data point. [†]Value indicates R-squared unadjusted.

Table C2. Results of Logistic Regression Analyses showing the odds of scoring in the lowest 25% for neurobehavioral tests per unit increase in PCB levels (not adjusted for lipids)

	Model 2 including race/ethnicity* (n=198)			**Model 1 excluding race/ethnicity** (n=198)**		
Cognitive Domain Test	Odds Ratio for "unadjusted" serum PCB (95% CI)	P-value	Pseudo R-squared	Odds Ratio for "unadjusted" serum PCB (95% CI)	P-value	Pseudo R-squared
Language						
Boston Naming	1.09 (0.85, 1.41)	0.48	0.34	1.21 (0.96, 1.51)	0.10	0.26
Verbal Memory						
Rey Auditory Verbal Learning						
Recognition	1.07 (0.86, 1.34)	0.55	0.17	1.14 (0.93, 1.41)	0.21	0.12
Delayed Recall	1.13 (0.91, 1.40)	0.27	0.21	1.18 (0.97, 1.45)	0.11	0.18
Visual Memory						
Symbol Digit	1.04 (0.84, 1.29)	0.70	0.10	1.09 (0.89, 1.34)	0.41	0.08

*All coefficients have been standardized; adjusted for race/ethnicity, age, sex, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication and total serum PCBs

** Adjusted for all variables in Model 1 excluding race/ethnicity.

Table C3. Results of multiple linear regression analyses showing standardized neurobehavioral test scores (z-scores) per unit increase in total serum PCB level with serum lipids entered as an independent variable ("adjusted method" as determined by Schisterman et al)

	Model 1 including race/ethnicity (n=198)*			**Model 2 excluding race/ethnicity (n=198)****		
Cognitive Domain Test	β Coefficients for "adjusted" serum PCBs (95% CI)	P-value	Adjusted R-squared	β Coefficients for "adjusted" serum PCBs (95% CI)	P-value	Adjusted R-squared
Language						
Category Fluency	-0.06 (-0.13, 0.02)	0.14	0.38	-0.10 (-0.17, -0.02)	0.02	0.32
Letter Fluency[a]	-0.08 (-0.14, -0.02)	0.01	0.38[†]	-0.10 (-0.16, -0.04)	0.001	0.36[†]
Visual Memory						
Rey Complex Figure delayed recall	-0.05 (-0.14, 0.03)	0.22	0.24	-0.07 (-0.15, 0.01)	0.10	0.23
Verbal Memory						
Rey Auditory Verbal Learning Immediate Recall	-0.05 (-0.13, 0.03)	0.25	0.27	-0.08 (-0.16, 0.003)	0.06	0.23
Visuoconstruction and Visuoperception						
Rey Complex Figure copy	-0.03 (-0.11, 0.04)	0.40	0.36	-0.06 (-0.14, 0.02)	0.13	0.33
Motor and Manual Dexterity						
Finger Tapping						
Dominant Hand[b]	-0.01 (-0.08, 0.09)	0.88	0.26	-0.01 (-0.09, 0.07)	0.80	0.25
Nondominant Hand[b]	-0.02 (-0.10, 0.06)	0.57	0.29	-0.04 (-0.12, 0.04)	0.36	0.29
Purdue Pegboard						
Dominant Hand	-0.02 (-0.11, 0.06)	0.57	0.28	-0.04 (-0.12, 0.04)	0.31	0.26
Nondominant Hand[b]	-0.05 (-0.14, 0.03)	0.23	0.21	-0.07 (-0.16, 0.01)	0.09	0.20
Both Hands[b]	-0.06 (-0.14, 0.02)	0.14	0.32	-0.09 (-0.16, -0.006)	0.04	0.29
Simple Reaction Time (negated)	-0.01 (-0.08, 0.10)	0.86	0.09	-0.02 (-0.10, 0.08)	0.78	0.07
Executive Function						
Purdue Pegboard Assembly[b]	-0.01 (-0.09, 0.07)	0.84	0.24	-0.04 (-0.12, 0.05)	0.36	0.20
Stroop Test (negated)	-0.03 (-0.08, 0.09)	0.95	0.20	-0.03 (-0.11, 0.06)	0.55	0.17
Trail Making						
Test A (log transformed/negated)	-0.02 (-0.11, 0.06)	0.60	0.19	-0.05 (-0.13, 0.04)	0.30	0.17
Test B (log transformed/negated)[b]	-0.03 (-0.11, 0.05)	0.47	0.35	-0.06 (-0.14, 0.02)	0.14	0.31

* All coefficients standardized; adjusted for race/ethnicity, age, sex, technician, education, household wealth, history of diabetes, homocysteine, antihypertensive use, serum lipids, serum PCBs; **Includes all variables from Model 1 except race/ethnicity. [a] One influential point removed; [b] One missing value. [†]Value indicates R-squared unadjusted.

Table C4. Results of logistic regression analyses showing the odds of scoring in the lowest 25% for neurobehavioral tests per unit increase in PCB level when serum lipids are entered as independent variables

	Model 1 including race/ethnicity* (n=198)			**Model 2 excluding race/ethnicity** (n=198)**		
Cognitive Domain Test	Odds Ratio for "adjusted" serum PCB (95% CI)	P-value	Pseudo R-square	Odds Ratio for "adjusted" serum PCBs (95% CI)	P-value	Pseudo R-square
Language						
Boston Naming	1.17 (0.89, 1.52)	0.26	0.35	1.29 (1.02, 1.63)	0.03	0.28
Verbal Memory						
Rey Auditory Verbal Learning						
Recognition	1.15 (0.92, 1.45)	0.24	0.18	1.23 (0.99, 1.53)	0.07	0.14
Delayed Recall	1.13 (0.91, 1.41)	0.28	0.21	1.20 (0.97, 1.48)	0.09	0.18
Visual Memory						
Symbol Digit	1.09 (0.87, 1.36)	0.47	0.11	1.14 (0.92, 1.41)	0.23	0.09

*All coefficients have been standardized; adjusted for race/ethnicity, age, sex, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication, total lipids and total serum PCBs.

** Adjusted for all variables in Model 1 except race/ethnicity.

APPENDIX D: Raven's Coloured Progressive Matrices as a "hold" measure

Hold measures are meant to be fairly resistant to the effects of neurotoxicants and are expected to remain relatively stable over time (202). They are often considered better measures of intellectual ability than education alone, and, therefore, are used to adjust for differences in intellectual ability between subjects (202). While many studies have used reading or vocabulary tests as hold measures, in one study of lead exposure vocabulary tests did not conform to these assumptions (203). As the Baltimore Memory Study was originally designed to examine the relationship between lead exposure and cognitive function, the Raven's Coloured Progressive Matrices (CPM), was intended to serve as a "hold" measure. However, our results were similar to the lead study described above. For our population, scores on this test were associated with serum PCB concentrations at the $p<0.01$ level, thus suggesting that the CPM also failed as a hold measure. We therefore did not use these test scores to adjust for native intelligence.

APPENDIX E: Additional demographic information

Basic Demographic Information Comparing PCB population and BMS population (153)

Table E1. Racial/Ethnic distribution for PCB and BMS populations

Race/Ethnicity	PCB Population		BMS Population	
	N	%	N	%
White	123	62.1	598	52.5
White/Native American	5	2.5	14	1.2
Black/African American	62	31.3	474	41.7
African American/mixed	3	1.5	30	2.6
Asian or Hawaiian	1	0.5	9	0.8
Native American	4	2.0	11	1.0
Missing or refused	0	0	4	0.4
Total	198	100.0	1,140	100.0

The Census 2000 method, which allows an individual to identify with more than one racial/ethnic group, was used in this study. Overall, the PCB population has a higher percentage of whites and lower percentage of blacks compared to the BMS population. Our sampling design that was a stratified random sample based on eNOS genotype may partially explain this difference. In general, 11% of whites are TT homozygotes compared to 2% of African Americans (refer to Tables 2.2 and 2.3 in Background Chapter). Therefore, purposely oversampling TT homozygotes may have resulted in oversampling of whites within our sample population. In addition, exclusion of individuals without complete neurobehavioral data may have resulted in the differences, as a higher percentage of nonwhites in the BMS population were found to have incomplete neurobehavioral data compared to whites.

Table E2. Distribution of genotypes with the PCB population based on race/ethnicity

Race	Number of GG homozygotes	Number of TG heterozygotes	Number of TT homozygotes
Nonwhites	54	20	1
Whites	45	65	13

Table E3. Levels of education for the PCB and BMS populations.

	PCB Population			BMS Population (153)		
Education	**No. (%)**	**% Nonwhite**	**% White**	**No. (%)**	**% Black**	**% White**
< High school education	15 (7.5)			*154 (13.5)*		
<10th Grade	7 (3.5)	3.0	3.8	48 (4.2)	5.3	3.1
≥ 10th Grade	5 (2.5)	6.1	0.8	76 (6.7)	8.2	5.6
Completed Trade School	3 (1.5)	3.0	0.8	30 (2.6)	4.6	1.0
High school graduate (or equivalency)	60 (30.3)			438 (38.5)		
Without trade school	23 (11.6)	18.2	8.3	194 (17.0)	21.7	12.8
With trade school	37 (18.7)	25.8	15.2	244 (21.4)	31.9	13.4
Some college or associate degree	17 (8.6)	12.1	6.8	66 (5.8)	7.8	4.3
Baccalaureate degree	40 (20.2)	12.1	24.2	136 (11.9)	7.4	15.7
Some post baccalaureate education	21 (10.6)	7.6	12.1	110 (9.7)	4.9	13.3
Post baccalaureate degree	45 (22.7)	12.1	28.0	235 (20.6)	8.2	30.9

Overall, the PCB population tends to be a fairly educated group of individuals with more than half having a baccalaureate degree or higher education. When compared to the overall BMS population, the PCB population tends to be slightly more educated; however, as educational attainment varies dramatically by race in the BMS population, our sampling design may help explain the difference (described on the previous page).

Table E4. Distribution of chronic illnesses among the PCB population

Disease	**Yes** Number (Percentage)	**Suspect/ Probable** Number (Percentage)	**No** Number (Percentage)	**Don't know/ Refused** Number (Percentage)
Heart Attack/ Coronary Thrombosis	7 (3.5%)	3 (1.5%)	188 (94.9%)	0 (0%)
Stroke or Brain Hemorrhage	4 (2.0%)	0 (0%)	194 (98.0%)	0 (0%)
Cancer, Malignancy, Tumor	35 (17.7%)	10 (5.1%)	153 (77.3%)	0 (0%)
Diabetes, sugar in the urine, high blood sugar	29 (14.6%)	3 (1.5%)	166 (83.8%)	0 (0%)
Congestive Heart Failure	3 (1.5%)	0 (0%)	195 (98.5%)	0 (0%)
Major depressive disorder, including bipolar disease	29 (14.6%)	1 (0.5%)	168 (84.8%)	0 (0%)
Anxiety or panic disorder	25 (12.6%)	7 (3.5%)	165 (83.3%)	1 (0.5%)
Lung disorder such as emphysema or bronchitis	23 (11.6%)	4 (2.0%)	171 (86.4%)	0 (0%)
Chronic Kidney Disease	1 (0.5%)	0 (0%)	197 (99.5%)	0 (0%)
High Cholesterol	78 (39.4%)	7 (3.5%)	113 (57.1%)	0 (0%)
High Blood Pressure	80 (40.4%)	8 (4.0%)	110 (55.6%)	0 (0%)
Arthritis	74 (37.4%)	13 (6.6%)	111 (56.1%)	0 (0%)
Parkinsons Disease	0 (0%)	0 (0%)	197 (99.5%)	1 (0.5%)
Venous thrombosis or blood clot in leg	5 (2.5%)	1 (0.5%)	192 (97%)	0 (0%)
Transient ischemic attack or mini strokes	2 (1.0%)	0 (0%)	196 (99.0%)	0 (0%)
Amputation	4 (2.0%)	0 (0%)	193 (97.5%)	1 (0.5%)
Post-menopausal (women only)	54 (41.5%)	73 (56.2%)	0 (0%)	3 (1.5%)

Table E5. Medication use within the PCB population

Type of Medication Used within the past 2 weeks	**Used** Number (Percentage)	**Not Used** Number (Percentage)
Antidepressant	29 (14.7%)	169 (85.3%)
Antidiabetics	20 (10.1%)	178 (89.9%)
Antihypertensive	76 (38.4%)	122 (61.6%)
Antihyperlipidemic	38 (19.2%)	160 (80.8%)
Aspirin	56 (28.3%)	142 (71.7%)
Nonsteroidal Anti-inflammatory	40 (20.2%)	158 (79.8%)
Thyroid Medication	18 (9.1%)	180 (90.9%)

Table E6. Distribution of other measurements within the PCB population

Measure	**Units**	**N**	**Mean**	**Median**	**Range**
Blood lead	µg/dL	198	3.6	3.2	0.1 - 12.2
Homocysteine	umol/L	198	9.4	8.8	4.4 - 24.9
Mercury	µg/l	91	2.31	1.70	0.07 – 9.2
Tibia lead levels	ppm	178	16.6	16.4	-32.2 - 52.5
Triglycerides	mg/dL	198	179.7	147.0	40.0 – 763.0
Total cholesterol	mg/dL	198	210.2	205.5	112 - 348
Total PCBs	ppb	198	2.1	1.8	0.2 - 10.9
Total lipid-adjusted PCBs	µg/g lipid	198	0.30	0.25	0.04 - 1.57

APPENDIX F: Regression models examining quartiles of lipid-adjusted PCBs and neurobehavioral function.

Table F1. Results of multiple linear regression analyses showing standardized neurobehavioral test scores (z-scores) compared to the less than 25th percentile per quartile of lipid-adjusted serum PCB levels

	Lipid adjusted PCBs >25th ≤50th percentile		Lipid adjusted PCBs >50th ≤75th percentile		Lipid adjusted PCBs >75th percentile		Adjusted R^2
Cognitive Domain Test	**β Coefficients for lipid-adjusted PCBs (95% CI)**	**P-value**	**β Coefficients for lipid-adjusted PCBs (95% CI)**	**P-value**	**β Coefficients for lipid-adjusted PCBs (95% CI)**	**P-value**	
Language							
Category Fluency	0.12 (-0.21, 0.42)	0.47	0.05 (-0.28, 0.38)	0.76	-0.10 (-0.43, 0.23)	0.56	0.38
Letter Fluency[a]	0.09 (-0.25, 0.42)	0.61	0.01 (-0.32, 0.35)	0.93	-0.22 (-0.56, 0.12)	0.21	0.32
Visual Memory							
Rey Complex Figure delayed recall	-0.00 (-0.36, 0.36)	0.99	-0.29 (-0.65, 0.08)	0.12	0.01 (-0.36, 0.38)	0.97	0.23
Verbal Memory							
Rey Auditory Verbal Learning							
Immediate Recall	0.29 (-0.07, 0.64)	0.11	-0.12 (-0.48, 0.39)	0.49	0.07 (-0.29, 0.42)	0.72	0.28
Visuoconstruction and **Visuoperception**	-0.28 (-0.60, 0.05)	0.09	-0.38(-0.70,-0.05)	0.02	0.00 (-0.33, 0.33)	0.99	0.38
Rey Complex Figure copy							
Motor and Manual Dexterity							
Finger Tapping							
Dominant Hand[b]	0.05 (-0.31, 0.41)	0.77	0.08 (-0.29, 0.44)	0.68	-0.02 (-0.38, 0.35)	0.93	0.25
Nondominant Hand[b]	0.01 (-0.34, 0.36)	0.95	0.11(-0.24, 0.47)	0.53	-0.12 (-0.48, 0.24)	0.51	0.28
Purdue Pegboard							
Dominant Hand	0.08 (-0.27, 0.43)	0.66	0.05 (-0.30, 0.40)	0.78	-0.03 (-0.39, 0.33)	0.87	0.27
Nondominant Hand[b]	0.11 (-0.26, 0.48)	0.56	-0.08 (-0.45, 0.29)	0.67	-0.14 (-0.52, 0.23)	0.46	0.21
Both Hands[b]	0.02 (-0.32, 0.36)	0.91	-0.07 (-0.42, 0.27)	0.68	-0.23 (-0.58, 0.12)	0.20	0.32
Simple Reaction Time (negated)	0.10 (-0.30, 0.50)	0.62	0.09 (-0.30, 0.49)	0.65	0.12 (-0.28, 0.52)	0.55	0.08
Executive Function							
Purdue Pegboard Assembly[b]	0.06 (-0.30, 0.42)	0.74	0.02 (-0.34, 0.39)	0.90	-0.07 (-0.43, 0.30)	0.72	0.24
Stroop Test (negated)	-0.00 (-0.37, 0.37)	0.98	-0.17 (-0.55, 0.20)	0.35	-0.20 (-0.58, 0.17)	0.29	0.20
Trail Making							
Test A (log transformed/negated)	-0.17 (-0.55. 0.21)	0.38	-0.16 (-0.54, 0.22)	0.41	-0.19 (-0.57, 0.20)	0.34	0.15
Test B (log transformed/negated)[b]	-0.02 (-0.35, 0.32)	0.91	-0.11 (-0.44, 0.23)	0.52	-0.12 (-0.47, 0.22)	0.48	0.34

; [a]One influential point removed from analysis; [b]One missing value. All coefficients have been standardized; adjusted for age, sex, race, technician, education, household wealth, history of diabetes, use of hypertension medication, homocysteine, lipid-adjusted total serum PCBs (quartiles)

Table F2. Results of logistic regression analyses showing the odds of scoring in the lowest 25% for neurobehavioral tests per PCB quartile

	Lipid adjusted PCBs >25th ≤50th percentile		**Lipid adjusted PCBs >50th ≤75th percentile**		**Lipid adjusted PCBs >75th percentile**		**Pseudo R^2**
Cognitive Domain Test	**Odds Ratio for lipid-adjusted PCBs (95% CI)**	**P-value**	**Odds Ratio for lipid-adjusted PCBs (95% CI)**	**P-value**	**Odds Ratio for lipid-adjusted PCBs (95% CI)**	**P-value**	
Language							
Boston Naming	1.04 (0.29, 3.70)	0.95	0.92 (0.27, 3.10)	0.89	2.40 (0.73, 7.86)	0.15	0.36
Verbal Memory							
Rey Auditory Verbal Learning							
Recognition	0.53 (0.16, 1.74)	0.29	2.26 (0.77, 6.58)	0.14	1.28 (0.43, 3.84)	0.66	0.20
Delayed Recall	0.36 (0.12, 1.09)	0.07	0.90 (0.32, 2.51)	0.84	0.63 (0.22, 1.81)	0.39	0.22
Visual Memory							
Symbol Digit	0.85 (0.27, 2.63)	0.77	2.30 (0.83, 6.37)	0.11	1.75 (0.62, 4.92)	0.29	0.12

All coefficients have been standardized; adjusted for age, sex, race/ethnicity, technician, education, household wealth, history of diabetes, use of hypertension medication, homocysteine, lipid-adjusted total serum PCBs

APPENDIX G: Example showing the influence of race in regression models

Table G1: Results from linear regression models showing differences in beta coefficients for independent variables after exclusion of race.

	Model for Category Fluency Score including race/ethnicity	**Model for Category Fluency Score excluding race/ethnicity**
Variable	**Beta coefficient (95% CI)**	**Beta coefficient (95% CI)**
Age	-0.02 (-0.04, -0.00)*	-0.02 (-0.04, -0.00)*
Female	0.24 (-0.01, 0.48)	0.22 (-0.04, 0.48)
Testing Technician	0.05 (-0.18, 0.27)	0.04 (-0.20, 0.28)
Household wealth (log transformed)	0.05 (-0.03, 0.13)	0.10 (0.02, 0.18)*
Homocysteine	-0.04 (-0.07, 0.003)	-0.04 (-0.08, 0.000)*
Less than High School Education	-0.49 (-0.94, -0.03)*	-0.44 (-0.92, 0.03)
Baccalaureate	0.43 (0.11, 0.75)**	0.53 (0.20, 0.86)**
Higher than a baccalaureate	0.59 (0.30, 0.89)***	0.67 (0.36, 0.97)***
Antihypertensive use	-0.01 (-0.26, 0.25)	-0.06 (-0.33, 0.21)
History of Diabetes	-0.06 (-0.40, 0.29)	-0.09 (-0.45, 0.28)
Lipid adjusted PCB levels	-0.46 (-1.01, 0.09)	-0.73 (-1.29, -0.16)**

*$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$

APPENDIX H: Examination of the role of tibia lead in regression models

Table H1. Results of multiple linear regression analyses showing standardized neurobehavioral test scores (z-scores) per unit increase in lipid-adjusted serum PCB levels with and without controlling for tibia lead measures.

	Model 1 excluding tibia lead (n=178)*			**Model 2 including tibia lead(n=178)****		
Cognitive Domain Test	β Coefficients for lipid-adjusted total serum PCB (95% CI)	P-value	Adjusted R-squared	β Coefficients for lipid-adjusted total serum PCB (95% CI)	P-value	Adjusted R-squared
Language						
Category Fluency	-0.54 (-1.11, -0.04)	0.07	0.37	-0.52 (-1.10, 0.05)	0.08	0.37
Letter Fluency[a]	-0.68 (-1.17, -0.18)	0.008	0.38[†]	-0.68 (-1.17, -0.18)	0.008	0.38[†]
Visual Memory						
Rey Complex Figure delayed recall	-0.60 (-1.23, 0.03)	0.06	0.25	-0.58 (-1.21, 0.05)	0.07	0.26
Verbal Memory						
Rey Auditory Verbal Learning						
Immediate Recall	-0.17 (-0.78, 0.44)	0.59	0.28	-0.15 (-0.77, 0.46)	0.62	0.27
Visuoconstruction and Visuoperception						
Rey Complex Figure copy	-0.36 (-0.91, 0.20)	0.20	0.39	-0.33 (-0.89, 0.22)	0.23	0.39
Motor and Manual Dexterity						
Finger Tapping						
Dominant Hand[b]	-0.13 (-0.73, 0.47)	0.68	0.28	-0.16 (-0.76, 0.43)	0.59	0.30
Nondominant Hand[b]	-0.37 (-0.96, 0.22)	0.21	0.32	-0.39 (-0.98, 0.20)	0.20	0.32
Purdue Pegboard						
Dominant Hand	-0.27 (-0.89, 0.36)	0.40	0.20	-0.29 (-0.92, 0.33)	0.36	0.20
Nondominant Hand[b]	-0.41 (-1.05, 0.24)	0.21	0.17	-0.43 (-1.08, 0.21)	0.19	0.17
Both Hands[b]	-0.54 (-1.14, 0.06)	0.08	0.29	-0.56 (-1.16, 0.04)	0.07	0.30
Simple Reaction Time (negated)	-0.27 (-0.91, 0.37)	0.41	0.08	-0.27 (-0.91, 0.37)	0.41	0.08
Executive Function						
Purdue Pegboard Assembly[b]	-0.27 (-0.91, 0.38)	0.41	0.21	-0.28 (-0.93, 0.36)	0.39	0.21
Stroop Test (negated)	-0.11 (-0.74, 0.52)	0.74	0.20	-0.09 (-0.72, 0.54)	0.78	0.20
Trail Making						
Test A (log transformed/negated)	-0.28 (-0.93, 0.37)	0.40	0.17	-0.28 (-0.93, 0.37)	0.40	0.16
Test B (log transformed/negated)[b]	-0.31 (-0.87, 0.26)	0.29	0.35	-0.32 (-0.88, 0.25)	0.28	0.34

* All coefficients have been standardized; adjusted for age, sex, race/ethnicity, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication, total lipid-adjusted serum PCBs; **Includes all variables from Model 1 and tibia lead levels. [a] One influential point removed; [b] One individual did not complete the test. Value treated as missing. [†] Value indicates R-squared unadjusted due to unequal variance.

Table H2. Results of logistic regression analyses showing the odds of scoring in the lowest 25% for neurobehavioral tests per unit increase in lipid-adjusted serum PCBs with and without controlling for tibia lead measures.

	Model 1 excluding tibia lead* (n=178)			**Model 2 including tibia lead** (n=178)**		
Cognitive Domain Test	Odds Ratio for lipid-adjusted total serum PCB (95% CI)	P-value	Pseudo R-square	Odds Ratio for lipid-adjusted total serum PCB (95% CI)	P-value	Pseudo R-square
Language						
Boston Naming	4.46 (0.53, 37.49)	0.17	0.37	5.58 (0.68, 46.05)	0.11	0.38
Verbal Memory						
Rey Auditory Verbal Learning						
Recognition	2.48 (0.46, 13.44)	0.30	0.18	2.39 (0.44, 13.08)	0.32	0.18
Delayed Recall	2.23 (0.41, 12.17)	0.35	0.20	2.04 (0.36, 11.47)	0.42	0.21
Visual Memory						
Symbol Digit	1.68 (0.32, 8.89)	0.54	0.09	1.78 (0.34, 9.48)	0.50	0.10

*All coefficients have been standardized; adjusted for age, sex, race/ethnicity, technician, education, household wealth, history of diabetes, homocysteine, use of hypertension medication and total lipid-adjusted serum PCBs

** Adjusted for all variables in Model 1 and tibia lead.

APPENDIX I: Relationships between genotypes and neurobehavioral tests

Differences in Neurobehavioral Test Scores by Genotype

Based on several animal and *in vitro* studies (discussed in Chapter 2), we hypothesized that TG heterozygotes or TT homozygotes would perform significantly worse on neurobehavioral tests compared to GG homozygotes. Differences in neurobehavioral test score by genotype was not the main focus of this study, but was investigated in the BMS population. We examined this issue within our sample population to better understand possible interactions between PCB concentrations and genotype in predicting neurobehavioral function. Table I.1 shows one-way ANOVA results for differences in standardized neurobehavioral test scores by genotype. The overall trend indicates that GG homozygous individuals scored lower on tests than TG or TT genotypes, which contradicts our original hypothesis. However, the differences noted in Table I.1 are not controlled for other potential founders and may be partially explained by race as nonwhites – who scored significantly lower on neurobehavioral tests – contributed to a significant proportion of GG genotypes (55%), while the TG and TT genotypes contained a much smaller proportion of nonwhites (24% and 7% respectively.) Appendix J discusses the relationship between genotype and neurobehavioral outcome after adjusting for other covariates.

Table I1. One-way ANOVA results for differences in standardized neurobehavioral test scores by genotype (GG, TG, TT)

Neurobehavioral Test	**GG Genotype (n=99) Mean Z-score (SD)**	**TG Genotype (n=85) Mean Z-score (SD)**	**TT Genotype (n=14) Mean Z-score (SD)**	**F-score**	**P-value**
Category Fluency	-0.21 (0.97)	0.13 (0.97)	0.66 (1.01)	6.30	0.002
Letter Fluency	-0.12 (1.05)	0.06 (0.92) [a]	0.29 (0.63)	1.55	0.21
Rey Complex Figure delayed recall	-0.06 (1.07)	-0.02 (0.94)	0.54 (0.74)	2.27	0.11
RAVLT Trials 1-5	-0.10 (1.04)	0.05 (0.97)	0.40 (0.86)	1.80	0.17
Rey Complex Figure copy	-0.14 (1.02)	0.05 (0.98)	0.68 (0.66)	4.51	0.01
Finger tapping dominant hand	-0.29 (1.07)	0.23 (0.82)	0.71 (0.83) [b]	10.63	0.000
Finger tapping nondominant hand	-0.27 (0.97)	0.18 (0.93)	0.88 (0.86) [b]	10.85	0.000
Purdue Pegboard dominant Hand	-0.12 (1.13)	0.13 (0.86)	0.02 (0.75)	1.48	0.23
Purdue Pegboard nondominant hand	-0.04 (1.09)	-0.01 (0.88)	0.35 (1.05) [b]	0.88	0.41
Purdue Pegboard both hands	-0.04 (1.15)	0.03 (0.81)	0.18 (0.94) [b]	0.35	0.70
Simple Reaction Time (negated)	-0.11 (1.10)	0.10 (0.90)	0.18 (0.83)	1.30	0.28
Purdue Pegboard assembly	-0.15 (1.10)	0.13 (0.83)	0.31 (1.09) [b]	2.39	0.09
Stroop Test (negated)	-0.19 (1.07)	0.16 (0.87)	0.36 (1.06)	3.82	0.02
Trails Making Test A (log/negated)	-0.18 (0.98)	0.13 (1.02)	0.53 (0.65)	4.48	0.01
Trails Making Test B (log/negated)	-0.24 (1.05)	0.20 (0.92)	0.55 (0.51)	6.92	0.001
Raven's Coloured Progressive Matrices	-0.21 (0.97)	0.13 (0.97)	0.66 (1.01)	6.30	0.002
Boston Naming	-0.21 (1.14)	0.17 (0.83)	0.44 (0.49)	4.96	0.008
RAVLT recognition	-0.12 (1.13)	0.11 (0.86)	0.17 (0.74)	1.40	0.25
RAVLT delayed recall	-0.11 (1.04)	0.11 (0.97)	0.11 (0.87)	1.26	0.29
Symbol Digit	-0.16 (1.06)	0.07 (0.93)	0.76 (0.55)	5.90	0.003

[a]One outlier excluded from analysis. [b]One individual did not complete the test. Value treated as missing data.

APPENDIX J: Examination of the interaction between PCB levels and genotype in predicting neurobehavioral test scores

Tables J1 and J2 present the data discussed in Chapter 6. The tables show results of multiple linear and logistic regression models that were used to evaluate interaction between genotype and PCB levels in predicting scores on the neurobehavioral tests. For each neurobehavioral outcome, Model 1 was adjusted for age, race/ethnicity, sex, household wealth, education, testing technician, history of diabetes, use of antihypertensive medications, homocysteine levels and lipid-adjusted PCB levels. Model 2 controls for all variables in Model 1, as well as eNOS genotype (TG/TT versus GG as the reference), while Model 3 introduces an interaction term to variables included in Model 2.

Given a difference in slopes between the two genotype groups (GT versus TG/TT) of -0.61 (from the Letter Fluency model that includes race/ethnicity), a root mean square of 0.80, a sample size of 99 in each genotype group, and an alpha level of 0.05, the estimated power we had to detect this difference was 20% (PS: Power and Sample Size Calculation by Dupont and Plummer). In contrast, we would have had 80% power, using the same sample size and other parameters, to detect a difference in slopes between the two genotype groups of at least 1.53.

Table J1 Results from Multiple Linear Regression Models examining the role of the eNOS polymorphism in predicting neurobehavioral function and as an effect modifier in the relationship between PCB exposure and neurobehavioral function.

Neurobehavioral Test	**PCB Beta coefficient** (95% CI)	**eNOS Beta coefficient** (95% CI)	**PCB*eNOS Beta coefficient** (95% CI)	**Adjusted R-Squared**
Category Fluency				
Model 1	-0.46 (-1.01, 0.09)			0.39
Model 2	-0.49 (-1.05, 0.06)	0.09 (-0.15, 0.33)		0.39
Model 3	-0.51 (-1.46, 0.44)	0.08 (-0.33, 0.49)	0.03 (-1.13, 1.19)	0.38
Letter Fluency[a]				
Model 1	-0.63 (-1.11, -0.14)**			0.38[†]
Model 2	-0.61 (-1.11, -0.12)*	-0.05 (-0.30, 0.20)		0.38[†]
Model 3	-0.37 (-1.15, 0.41)	0.06 (-1.38, 0.64)	-0.37 (-1.38, 0.64)	0.38[†]
Rey Complex Figure delayed recall				
Model 1	-0.44 (-1.05, 0.18)			0.23
Model 2	-0.40 (-1.02, 0.22)	-0.11 (-0.38, 0.15)		0.23
Model 3	-0.71 (-1.78, 0.35)	-0.25 (-0.70, 0.20)	0.47 (-0.82, 1.77)	0.23
RAVLT Trials 1-5				
Model 1	-0.24 (-0.84, 0.36)			0.26
Model 2	-0.24 (-0.85, 0.37)	-0.01 (-0.27, 0.26)		0.26
Model 3	-0.15 (-1.20, 0.89)	0.02 (-0.42, 0.47)	-0.12 (-1.40, 1.16)	0.26
Finger tapping dominant hand[b]				
Model 1	-0.01 (-0.62, 0.59)			0.25
Model 2	-0.13 (-0.73, 0.48)	0.33 (0.07, 0.59)*		0.27
Model 3	-0.27 (-1.31, 0.77)	0.27 (-0.17, 0.72)	0.21 (-1.05, 1.48)	0.27
Finger tapping nondominant hand[b]				
Model 1	-0.21 (-0.81, 0.38)			0.28

Table J.1. (Continued).

Neurobehavioral Test	**PCB Beta coefficient** (95% CI)	**eNOS Beta coefficient** (95% CI)	**PCB*eNOS Beta coefficient** (95% CI)	**Adjusted R-Squared**
Finger tapping nondominant hand[b]				
Model 2	-0.31 (-0.91, 0.28)	0.28 (0.02, 0.54)*		0.30
Model 3	-0.43 (-1.45, 0.59)	0.23 (-0.21, 0.67)	0.18 (-1.07, 1.42)	0.29
Rey Complex Figure copy				
Model 1	-0.26 (-0.82, 0.30)			0.36
Model 2	-0.26 (-0.82, 0.31)	-0.01 (-0.26, 0.24)		0.36
Model 3	-0.69 (-1.66, 0.28)	-0.20 (-0.61, 0.22)	0.65 (-0.53, 1.84)	0.26
Purdue Pegboard dominant hand				
Model 1	-0.21 (-0.80, 0.39)			0.28
Model 2	-0.23 (-0.84, 0.37)	0.07 (-0.19, 0.33)		0.28
Model 3	-1.19 (-2.22, -0.17)*	-0.34 (-0.78, 0.10)	1.45 (0.20, 2.69)*	0.29
Purdue Pegboard nondominant hand[b]				
Model 1	-0.41 (-1.03, 0.21)			0.22
Model 2	-0.36 (-0.99, 0.27)	-0.14 (-0.41, 4.28)		0.22
Model 3	-1.03 (-2.10, 0.04)	-0.42 (-0.88, 0.04)	1.01 (-0.30, 2.31)	0.23
Purdue Pegboard both hands[b]				
Model 1	-0.49 (-1.06, 0.09)			0.32
Model 2	-0.43 (-1.02, 0.15)	-0.15 (-0.41, 0.10)		0.32
Model 3	-0.99 (-1.98, 0.00)	-0.38 (-0.82, 0.04)	0.84 (-0.38, 2.05)	0.33
Simple Reaction Time				
Model 1	-0.07 (-0.74, 0.60)			0.09
Model 2	-0.08 (-0.76, 0.60)	0.03 (-0.27, 0.32)		0.08
Model 3	-0.68 (-1.84, 0.48)	-0.22 (-0.72, 0.27)	0.90 (-0.52, 2.31)	0.08

Table J.1 (Continued.)

Neurobehavioral Test	PCB Beta coefficient (95% CI)	eNOS Beta coefficient (95% CI)	PCB*eNOS Beta coefficient (95% CI)	Adjusted R-Squared
Purdue Pegboard assembly[b]				
Model 1	-0.21 (-0.82, 0.39)			0.24
Model 2	-0.23 (-0.85, 0.38)	0.06 (-0.21, 0.33)		0.24
Model 3	-0.82 (-1.87, 0.23)	-0.19 (-0.64, 0.26)	0.88 (-0.40, 2.17)	0.25
Stroop				
Model 1	-0.10 (-0.73, 0.53)			0.20
Model 2	-0.10 (-0.17, 0.38)	0.10 (-0.17, 0.38)		0.20
Model 3	-0.63 (-1.71, 0.46)	-0.11 (-0.57, 0.36)	0.74 (-0.58, 2.07)	0.20
Trails Making Test A				
Model 1	-0.22 (-0.86, 0.42)			0.16
Model 2	-0.27 (-0.92, 0.39)	0.13 (-0.15, 0.42)		0.16
Model 3	-1.35 (-2.45, -0.25)*	-0.33 (-0.80, 0.14)	1.64 (0.30, 2.98)*	0.18
Trails Making Test B[b]				
Model 1	-0.24 (-0.81, 0.32)			0.35
Model 2	-0.30 (-0.87, 0.27)	0.17 (-0.08, 0.42)		0.35
Model 3	-0.17 (-1.15, 0.81)	0.23 (-0.19, 0.65)	-0.20 (-1.39, 1.00)	0.35

*P≤0.05; **P≤0.01

Model 1- Adjusted for age, sex, race/ethnicity, testing technician, household wealth, education, history of diabetes, antihypertensive medication use, homocysteine and lipid-adjusted serum PCB levels.

Model 2- Adjusted for all variables in Model 1 plus eNOS genotype (Reference group is GG homozygotes).

Model 3- Model 2 plus PCB*genotype interaction term.

[a]One influential point removed.

[b]One individual did not complete the test. Value treated as missing.

Table J2. Results from Logistic Regression Models examining the role of the eNOS polymorphism and the odds of scoring in the lowest 25th percentile of neurobehavioral function and as an effect modifier in the relationship between PCB exposure and neurobehavioral function.

Neurobehavioral Test	PCB Odds Ratio (95% CI)	eNOS Odds Ratio (95% CI)	PCB*eNOS Odds Ratio (95%CI)	Pseudo R-Squared
Boston Naming				
Model 1	3.89 (0.56, 26.93)			0.35
Model 2	4.02 (0.56, 28.67)	0.92 (0.39, 2.18)		0.35
Model 3	207.20 (1.99, 22141.33)*	4.37 (0.71, 26.73)	0.23 (0.00, 1.10)*	0.37
RAVLT Recognition				
Model 1	2.56 (0.49, 13.23)			0.17
Model 2	2.54 (0.48, 13.43)	1.02 (0.47, 2.21)		0.17
Model 3	0.62 (0.0.04, 10.30)	0.54 (0.14, 2.03)	7.93 (0.25, 253.16)	0.17
RAVLT Delayed Recall				
Model 1	1.97 (0.38, 10.14)			0.21
Model 2	2.02 (.38, 10.62)	0.93 (0.38, 10.6)		0.21
Model 3	1.81 (0.10, 31.42)	0.88 (0.24, 3.30)	1.17 (0.34, 40.01)	0.21
Symbol Digit				
Model 1	2.69 (0.56, 12.87)			0.11
Model 2	2.90 (0.59, 14.29)	0.80 (0.38, 1.69)		0.11
Model 3	15.25 (0.75, 311.78)	1.71 (0.45, 6.47)	0.08 (0.00, 3.21)	0.12

*P≤0.05; **P≤0.01

Model 1- Adjusted for age, sex, race/ethnicity, testing technician, household wealth, education, history of diabetes, antihypertensive medication use, homocysteine and lipid-adjusted serum PCB levels.

Model 2- Adjusted for all variables in Model 1 plus eNOS genotype. (Reference group is GG genotypes)

Model 3- Adjusted for all variables in Model 2 plus PCB*genotype interaction term.

CURRICULUM VITAE

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Date and Place of Birth
October 17, 1973
West Chester, Pennsylvania
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Education

Johns Hopkins Bloomberg School of Public Health
Department of Environmental Health Sciences, Division of Occupational and Environmental Health, Baltimore, MD
Doctorate of Philosophy, Completed Requirements November 2005

Johns Hopkins Bloomberg School of Public Health and School of Nursing
Department of Environmental Health Sciences, Division of Occupational and Environmental Health, Baltimore, MD
Masters of Public Health and Masters of Community Health Nursing, May 2000

University of New Hampshire, Durham, NH
Bachelors of Science in Nursing, May 1997

Norwich University, Northfield, VT
Associate Degree in Nursing, May 1994

Professional Licensure and Additional Training

Current	Maryland Board of Nursing, Registered Nurse, License Number: R142082
May 2001	Risk Sciences and Public Policy Certificate, Johns Hopkins Bloomberg School of Public Health
May 2000	Certificate in Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health

Experience

01/00 – Present **The National Center for Healthy Housing,** Columbia, MD
Project Coordinator/Research Assistant
Assisted in the development of a national Healthy Homes Training course for environmental, public health, and housing practitioners. Participated in the development of a national program to educate public health practitioners (primarily nurses) on the CDC's case management recommendations for lead poisoned children and helped deliver training to more than 400 individuals at eight sites. Assisted in the development and implementation of a national survey to evaluate case management of lead poisoned children within state and local health departments.

05/99 – 1/04 **Johns Hopkins Bloomberg School of Public Health,** Baltimore, MD
Teaching Assistant
Primary teaching assistant for several courses ranging in class size from 30-200 students. Responsibilities included conducting review sessions and grading papers, presentations, and exams. Courses included: *Introduction to Environmental Health* (involved with 9 deliveries on-site and on-line), *Food and Waterborne Diseases*, and *Global Environment and Public Health*

09/99 – 8/00 **Johns Hopkins Bloomberg School of Public Health**, Baltimore, MD
Research Assistant
Community-based exposure study in South Baltimore. Provided assistance with sampling design, helped develop study protocol, conducted air monitoring (personal and home monitoring of ambient air for VOCs, particulate matter, and contaminants). Interviewed study participants.

Study to examine the effectiveness of an air purifier in reducing particulate matter. Conducted experiments in the laboratory and home environment and performed data analysis, prepared graphs and charts.

01/99 – 01/00 **Future Care of Canton Harbor,** Baltimore, MD
Staff Nurse (part-time)
Provided nursing care to patients in a skilled care unit and supervised Certified Nurses Aides in a 160 bed in nursing home.

08/98 – 01/99 **Mariner Health of Bel Air,** Bel Air, MD
Staff Nurse (part-time)
Provided nursing care to patients in a skilled care unit and supervised Certified Nurses Aides in 155 bed nursing home.

01/98-9/98 **Seacoast Health Center**, Hampton, NH
MDS Coordinator
Conducted assessments and developed individualized care plans for all skilled care patients. Coordinated multi-disciplinary team meetings to develop plan of care. Completed and transmitted all Minimum Data Sheets (MDS) and Resident Assessment Protocols (RAPs) to the state for Medicaid reimbursement in 117 bed nursing home.

05/97 – 08/98 **Seacoast Health Center,** Hampton, NH
Certified Nurses Aide Instructor
Primary instructor for Certified Nurses Aide course. Lectured and provided clinical instruction and oversight to prepare students for the state certification exam.

08/94 – 01/98 **Seacoast Health Center**, Hampton, NH
Charge Nurse
Charge nurse for a 30 bed skilled care unit. Responsible for all aspects of patient care including oversight of nurses aides and LPNs working on the floor in 117 bed nursing home.

Other Experience

05/02 – 10/05	Student Representative to Departmental Academic Affairs Committee, Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health
05/02 – 05/03	Organized and served as President of Environmental Health Sciences Student Organization, Johns Hopkins Bloomberg School of Public Health

Community Involvement/ Affiliations

05/04 – Present	Member of the Board of Directors for the Baltimore Harbor Watershed Association; Chair of Membership Committee
08/04 - Present	Member of the American Public Health Association, Environment section
1997	Volunteer for American Red Cross, Disaster Relief Services

Awards

2000-Present	NIOSH training grant (T42CCT310419)
2003	NIOSH Pilot Project Grant
1997	Sigma Theta Tau, Nursing Honors Society
1991	4-year Air Force R.O.T.C. Scholarship

Presentations

2004 Morley, R., McLaine, P., **Gaitens, J.**, Zerbe, D., Ettinger, A. "Development of a National Healthy Homes Training Center and Network" poster presented at American Public Health Association 132nd Annual Meeting and Exposition, Washington, D.C.

2004 **Gaitens, J.** Ettinger, A., Morley, R., McLaine, P., Zerbe, D. "A Systematic Evaluation of Trainings Related to Healthy Homes" poster presented at American Public Health Association 132nd Annual Meeting and Exposition, Washington, D.C.

2004 Ettinger, A., Morley, R., **Gaitens, J.**, McLaine, P., Tohn, E. "Defining the Technical Competencies for Healthy Homes Training" poster presented at American Public Health Association 132nd Annual Meeting and Exposition, Washington, D.C.

2004 **Gaitens, J.**, McLaine, P. "National Healthy Homes Training Center and Network-Blueprint for Success" presented at 2004 National Lead and Healthy Homes Grantee Conference, Orlando, FL

2002 **Gaitens, J.**, McLaine, P. "Update and Lessons Learned: State Policies and Practices for Case Management and Environmental Investigation for Lead-

Poisoned Children" presented at National Lead Safe Conference, Washington D.C.

2000 **Gaitens, J.**, McLaine, P. "Another Link in the Chain Update: State Policies and Practices for Case Management and Environmental Investigation for Lead-Poisoned Children" presented at 2000 National Lead Grantee Conference, Atlanta, GA.

Publications

McLaine, P. and **Gaitens, J.** *Another Link in the Chain Update: State Policies and Practices for Case Management and Environmental Investigation for Lead-Poisoned Children*, National Center for Healthy Housing and Alliance to End Childhood Lead Poisoning, 2001.