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COMPARING QUESTIONNAIRE BASED MEASURES OF GREAT LAKES SPORT FISH CONSUMPTION FOR PREDICTION OF HUMAN SERUM POLYCHLORINATED BIPHENYL (PCB) LEVELS

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By

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ABSTRACT

COMPARING QUESTIONNAIRE BASED MEASURES OF GREAT LAKES SPORT FISH CONSUMPTION FOR PREDICTION OF HUMAN SERUM POLYCHLORINATED BIPHENYL (PCB) LEVELS

By

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Human consumption of Great Lakes sport fish has been associated with increased PCB exposure and adverse health outcomes. This thesis evaluates the criterion-related validity of four simple questionnaire based measures of sport fish consumption for prediction of human serum total PCB level.

The study population was a cohort of Michigan sport fish consumers (n=129). Measures of consumption included: 1) lifetime years (YEARS); 2) number of meals in past year (MEALS); 3) lifetime meals (MXY1); and 4) modified lifetime meals (MXY2). Multivariable regression was used to construct adjusted regression equations. Predictors were compared using a test for comparing non-nested models.

All adjusted predictors were significantly associated with serum PCBs (YEARS [R-Square delta=4%], MEALS [5%], MXY1 [7%], MXY2 [9%]). Only MXY2 showed a statistically significant increase in predictive power over YEARS and MEALS. However, the observed trend for predictive power was consistent with theory.

The modified index for lifetime sport fish meals (MXY2) is simple to attain and maximizes predictive power among the evaluated predictors. However, no test for model reliability was performed due to insufficient sample size. This study was performed on a population of high sport fish consumers; therefore these findings may not apply to other populations.

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CHAPTER I

INTRODUCTION

Polychlorinated Biphenyls (PCBs) are a group of synthetic compounds with unusual properties that made them desirable in many commercial and industrial applications. Between 1929 and 1977, PCBs were produced in massive quantities. Large quantities of PCBs were released into the environment leading to exposures in wildlife and humans. By the mid-1960's concerns arose over PCB pollution and hazards. PCBs were found to persist in the environment, to be toxic to humans exposed to high doses, and to be toxic to some wildlife even when exposed to very low doses. In 1977, these discoveries led to the outright ban of PCBs resulting in major reductions in wildlife and human exposures. However, the redistribution of PCBs already in the environment continues to pose a diminishing but significant hazard to wildlife and humans.

Since the ban, an extensive body of PCB toxicology and epidemiology research has been produced. A number of excellent reviews are available that explicate the contribution of this research toward PCB risk assessment. The toxicology research, animal models, observational studies of wildlife, and other sources have proven indispensable in contributing to an evidence based approach to PCB risk assessment. However, only epidemiologic investigations can provide direct information about the PCB exposure-response relationship in humans.

Many epidemiologic studies have been conducted that report associations between PCB exposure and health effects such as cancer, and adverse reproductive and hormonal related outcomes. These studies have been influential in the classification of PCBs as carcinogens, neurobehavioral toxicants, and suspect endocrine disruptors.

However, controversy surrounds these designations because of limitations with existing studies. Although the current weight of epidemiologic evidence suggests an association between exposure to PCBs and some adverse health outcomes in humans, strong evidence for causal relationships has not been established.

In the Great Lakes Basin, consumption of PCB contaminated sport fish is the primary route for human exposure to PCBs. The population of Great Lakes sport fish consumers, because of its increased risk for exposure to PCBs, has been of special interest in researching the PCB exposure-response relationship. This exposure route is of similar concern for other regions internationally. For example, PCB contamination in the Baltic Sea fishery poses similar exposures to fish-consuming populations in northern Europe.

This thesis presents the background on PCB risk assessment and limits the scope of the literature review to epidemiologic studies reporting PCB exposure-response relationships in populations of Great Lakes sport fish consumers. The emphasis is on methods of exposure assessment, but the main findings for study outcomes are also considered. The rationale for this thesis is two-fold. First, the comparability of existing research is hindered by the lack of minimum standards for reporting exposures. Second, no evaluation of methods of exposure assessment has been performed that may offer meaningful reductions in exposure measurement error. The main objective is to evaluate a set of predictors by testing hypotheses comparing the predictive power of four simple, but representative, methods for exposure assessment. Finally, recommendations are made and the strengths and limitations of questionnaire and biosample based approaches are discussed.

CHAPTER II

BACKGROUND

Chemistry, Production, Industrial Use and Environmental Fate

Polychlorinated Biphenyls (PCBs) are a class of synthetic chemicals that possess a unique chemistry. Their qualities have made them valued and widely used in industry, but also pose special concern as environmental pollutants.

Chemistry. PCBs are a class of synthetic organic chemicals, composed of a 12-carbon biphenyl ring with 1 to 10 chlorine substitutions. Depending on the number and position of chlorine substitution, up to 209 individual congeners are possible (1). PCBs vary from hydrophobic/lipophilic oily liquids to waxy solids, and are chemically stable, non-flammable, have high boiling points, and electrical insulating properties (2).

Production. An estimated 1.5 billion pounds of PCBs were produced in the United States between 1929 and 1979 (3). Most PCBs were produced and sold commercially as Aroclor® mixtures with signature levels of individual congeners. Aroclor® names reflect the number of carbon atoms and the percent of chlorine in the mixture (e.g., 'Aroclor® 1242' has 12 carbon atoms and is 42% chlorine by weight).

Industrial use. PCBs had hundreds of practical uses in industrial and commercial applications (e.g., in electrical and hydraulic equipment, as heat exchange fluids, as plasticizers in paints, plastics and rubber products, in pigments, dyes and carbonless copy paper, and many other applications) (2, 3).

Environmental fate. PCBs are also currently released to the environment from landfills containing PCB waste materials and products, incineration of municipal refuse and sewage sludge, and improper disposal of PCB materials, such as waste transformer

fluid, to open areas (4). The EPA's Toxic Chemical Release Inventory estimates that in the U.S. between 1987 and 1993, over 74,000 pounds of PCBs were released into land and water (4).

Current evidence suggests that the major source of PCB release to the environment is an environmental cycling process of PCBs previously introduced into the environment; this cycling process involves volatilization from ground surfaces (water, soil) into the atmosphere with subsequent removal from the atmosphere via wet/dry deposition and then revolatilization (5).

Volatization is recognized as a major mode of transport for PCB redistribution (4-7). The International Joint Commission estimates that atmospheric deposition accounts for the majority of PCB inputs to the upper Great Lakes. According to the *Mass Balancing of Toxic Chemicals in the Great Lakes: The Role of Atmospheric Depositions*, a 1988 EPA report, the atmosphere accounts for 90% of PCB deposition into Lake Superior, 58% in Lake Michigan and 63% in Lake Huron (4). The figures for the Lower Great Lakes are much smaller, but may still be significant. The majority of inputs of PCBs to Lakes Erie and Ontario is from other sources, including the upper lakes. Recent information continues to support the relative role of the atmosphere for Lakes Superior and Huron. The relative role of atmospheric deposition of toxics in Lakes Michigan may be somewhat less and is under active investigation (4).

PCBs that find their way into watersheds become sequestered in sediments where they persist indefinitely and slowly release into ecosystems. Because of their lipophilic properties, PCB congeners biologically magnify by moving from external media into biological compartments of living organisms. Similarly, these same PCBs magnify up

food chains, when one organism feeds on another, and can result in a million-fold increase in the PCB levels in apex species (8, 9). In 1966, Jensen discovered PCBs as a contaminant in wildlife (10). Shortly thereafter, elevated PCBs levels were reported among apex species, such as predatory fish, piscivorous birds and humans (11-16).

Risk Assessment

In 1968, Japanese investigators reported on an outbreak called "Yusho" or oil disease (17). The source of this poisoning was linked to accidental contamination of rice oil with heat exchange fluids containing high levels of PCBs, polychlorinated dibenzofurans and quarterphenyls (17). The identification of these chemicals as one of the causative agents for this outbreak spurred investigation of PCBs.

Subsequently, the relationship between PCB exposure and adverse health outcomes have been the focus of considerable research. Toxicological research including investigation of wildlife and animal models has linked exposure to PCB contaminated sport fish to adverse effects including cancers and dysfunctions of immune, reproductive, nervous, and endocrine systems (18).

A weight of evidence based approach indicates that significant adverse health outcomes are also associated with human exposure to PCBs through consumption of large amounts of contaminated sport fish (2, 19-21). These studies report associations between PCB exposure and a variety of adverse health outcomes including: disruption of reproductive function, neurobehavioral deficits, developmental deficits, self-reported liver disease, self-reported diabetes, cancer risk, and effects on the thyroid and immune systems (19).

Hazard Control

In the 1970's, rising concern among scientists and the public over PCB toxicity, water contamination, and risk of human and animal exposure, prompted aggressive hazard control.

Ban on production and use. In 1977, the EPA banned the manufacture of PCBs, and regulated their removal from industrial use, under the Toxic Substances Control Act (22). PCBs are now regulated under many federal and state laws. The EPA has broad authority to regulate virtually all aspects of the manufacture, distribution, use, and disposal of PCBs (3, 22).

Fish consumption advisories. Since the mid 1970s, all Great Lakes States have issued annual sport fish consumption advisories that aim to limit human exposure to PCBs by this route (23-26). While PCBs are a common contaminant that triggers advisories, several other contaminants do so as well. These include mercury, polychlorinated dibenzodioxin, polychlorinated dibenzofurans, and persistent pesticides such as Chlordane (26, 27). To reduce risk of exposure, sport fish consumers are urged to decrease the amount consumed and to switch to less contaminated species (26). A long term goal stated in the Great Lakes Strategy 2002 is that all Great Lakes fish should be safe to eat without restriction (28).

Time trends. Environmental PCB levels have declined greatly since their ban. However the rate of reduction has decreased, and some authors are reporting having reached a near steady state in environmental PCB levels. In any case, current levels continue to pose a sufficiently likely hazard to warrant continuance of sport fish consumption advisories (3, 26).

PCB Exposures in the Great Lakes Basin

Residents of the Great Lakes Basin are exposed to PCBs through multiple routes including background, occupational and dietary exposures. The distribution of PCB levels in the entire population is positively skewed. A positively skewed distribution describes an asymmetrical distribution in which most members of the population exhibit very low levels of exposure near, but not at, zero. The remaining and much smaller portion of the population exhibit higher, more positive, levels of exposure that taper off toward higher positive values and form what is called the positive tail of the distribution. The sources of exposure that contribute toward the formation of this distribution include background exposures, occupational exposures, and dietary exposures (2).

Background exposures. The general Great Lakes population experiences low level background exposures to PCBs from contaminated air, water, soil and food (2). Higher PCB exposure levels have been reported among individuals living in contaminated areas (29, 30).

Occupational exposures. Prior to their ban, occupational exposures to commercial PCB mixtures were more common but since the implementation of hazard control, and near elimination of use in industry, occupational exposures have decreased greatly. However, utility workers, firefighters and a handful of other occupations are still at significant risk for exposure (31, 32). Sweeney et al. (33) and Schantz et al. (34) report on a special exposure source originating from PCB-lined silos. Risk for exposure from this source occurred between 1941 until identification and remediation in the early 1970s.

Dietary exposures. Dietary intake studies of PCBs indicate that >80% of cases of human exposure to chlorinated organic compounds occur through eating contaminated food including meat and dairy products but primarily from eating sport fish (35-37).

Interestingly, a recent investigation comparing contaminant levels in farmed salmon to wild salmon, in a large international sample of farm, market and wild collected fish tissue, report that even the least contaminated farmed salmon had significantly higher contaminant loads than wild salmon (38). The authors findings suggest that farmed salmon may be an important new, but as yet unconfirmed, source for dietary exposures to PCBs and other contaminants (38).

Exposure from eating sport fish. Among the Great Lakes population, fish consumption has been reported as the primary route for exposure to PCBs (35-37). Several studies have shown that individuals who consume large amounts of PCB contaminated Great Lakes sport fish have PCB body burdens that average three-fold higher than the general population (37, 39-46).

Sport fish consumption advisories have been reported to have resulted in reductions in the PCB exposure level for the overall population. He (47) and Tee et al. (48) have evaluated changes in sport fish consumption and serum levels of PCBs among individuals in Michigan during three time periods in 1974-75, 1979-82, and 1989-91. The researchers found that over time, there was a decline both in the number of sport fish meals consumed (median = 66, 54, and 31 per year, respectively), and the total amount of sport fish consumed (median = 40, 38, and 16 pounds per year, respectively) (48).

General population. In the Great Lakes Basin, the general population experiences nearly ubiquitous PCB exposures that originate from background sources and meat and dairy products. Only special subpopulations tend to experience substantially higher exposures from dietary or occupational exposures. For the entire population, most members experience very low levels of exposure to PCBs, a small proportion experience

medium levels of exposure, and only an even smaller fraction experience high exposure levels.

Special populations. Special subpopulations have been identified as either especially vulnerable to exposure or at increased risk for exposure. Factors that identify these populations include: gender, age, ethnicity, lifestyle, and geography.

Vulnerable populations include couples attempting to reproduce, fetuses, the very young and the very old. These populations, and especially reproductive age females, typically receive special consideration in advisories and outreach programs. Even so, a significant proportion of women of reproductive age fail to heed such warnings (23, 49-52).

Populations at risk for elevated exposure include: sports anglers, subsistence anglers, and their family members and acquaintances who eat significant quantities of contaminated sport fish. Men on average annually consume more sport fish than do women (37, 47, 49). Similarly, elevated levels of sport fish consumption are often observed in minority groups such as Native Americans and Asian Americans. Elevated sport fish consumption among minority populations is largely attributable to cultural practices, but may in part be explained by a decreased awareness of fish consumption advisories (53, 54).

Pharmacodynamics and Body Burden

Pharmacodynamics describes the uptake, biotransformation, detoxification, elimination and accumulation of PCB exposures. Ingested PCBs rapidly absorb into the blood stream, with an observable spike in serum PCB level, and are then absorbed by liver, muscle, and other soft tissues before finally compartmentalizing in fatty tissues (55-

57). PCBs sequestered in fatty tissue will approach an equilibrium state with all other tissues in proportion to the respective tissue/blood ratios and the body burden (56).

In humans, PCB half lives for all 209 congeners vary immensely from months to decades (58-60), but for the main congeners associated with sport fish, half lives range from 2 to 6 years (60-65). Therefore, current PCB body burden is expected to be at least moderately associated with past exposure. For example, He (47) concluded that sport fish consumers who decreased their sport fish consumption (in a cohort whose fish consumption habits and serum PCB levels were measured at three time periods in 1974-75, 1979-82, and 1989-91) did not experience a parallel decline in serum PCB levels.

Other important mediators of PCB body burden are metabolic and compartmental factors (29, 30, 66, 67). Metabolic factors include detoxification systems and enzyme induction. Compartmental factors include compartment ratios and weight change. Compartment ratios describe relative compartment volumes of blood and soft tissues to fat tissues. Compartment ratio is directly associated with PCB half lives, since PCBs in blood and soft tissue compartments are more readily excreted than PCBs sequestered in fat tissue (68). Increasing Body Mass Index (BMI) is associated with a decreasing blood and soft tissue to fat tissue compartment ratio that dilutes the level of PCBs in the blood, decreases the rate of excretion, and consequently increases half lives (68). Increased BMI has been associated with increased PCB body burden (29, 62, 69-74). Decreasing BMI is associated with an increasing compartment ratio that concentrates the level of PCBs in the blood, increases the rate of excretion, and consequently decreases half lives (75, 76).

Breast feeding is especially important because it can result in a transfer of a significant proportion of the mothers PCB body burden directly to the infant (77). Lactation mobilizes and excretes fatty tissue stored lipids and PCBs in breast milk (56). Schecter et al. (78) estimated, in a case study, that breast feeding for two and half years reduced the mother's total level lipid-adjusted serum non-coplanar PCB levels by 78% from 285 to 63 ng/g.

Bioaccumlation and body burden. Bioaccumulation describes the net result of pharmacodynamics acting upon an individual's cumulative exposure (9). Body burden is a point-in-time measure of bioaccumulation. Pharmacodynamic factors are powerful mediators of PCB body burden that introduce considerable variation between a cumulative exposure and an observed point in time body burden (9). These factors warrant special consideration when investigating associations between PCB exposures, body burdens and responses of interest.

Exposure Assessment

In environmental epidemiology, direct assessment of a dose-response relationship is typically not feasible, since a direct measure of the biologically effective dose is rarely available (79). Investigators therefore investigate exposure-response relationships by basing inferences upon the best available proxy measures. Measures of the characteristics of exposure can be based upon laboratory measures of biological samples or derived from self-reported information (80).

Characteristics of exposure. Fundamental characteristics of exposure include rate, duration, and timing. Theoretically, the product of rate and duration produce the cumulative exposure, while timing describes when the exposure occurred (80).

Information on timing is also often important when there are critical periods for exposure (81). Timing of exposure is of particular concern for the investigation of reproductive and developmental outcomes, especially since the biological mechanism involves soft tissues that experience post-ingestion spikes (42, 82, 83).

The importance of appropriately characterizing an exposure is crucial to detecting the true relationship between an exposure and response (81). A theoretically optimal measure would ascertain rate, duration and timing to calculate an index of cumulative exposure (80). For many exposure-related outcomes, it may be insufficient to base exposure assessment upon a single characteristic, such as rate or duration. When timing is crucial, even an index of cumulative measures may be insufficient. An exposure measure that fails on theoretical grounds is very likely to introduce serious exposure misclassification (80).

Biosamples. Biosamples are the putative "Gold Standard" for PCB exposure assessment. PCB levels are most commonly measured from venous blood, but cord blood, fatty tissue, human milk, and neonatal meconium are also sampled (84, 85). Meconium analysis is a new and potentially sensitive tool for the detection of fetal exposure to environmental toxins (86-88).

Biosamples do face limitations however; an important issue is whether or not a particular biosample measure actually characterizes exposure in a way that best approximates the hypothetical biologically effective dose. Biosamples provide an index of body burden, but typically do not measure cumulative exposure and offer no direct information about the duration or timing of exposure. A biosample measure, that does

not measure the biologically effective dose inevitably introduces measurement error resulting in exposure misclassification.

However, even serum PCB levels are not always an accurate predictor of past PCB exposure. In most other exposure studies, serum PCB levels have been used to estimate body burden. However, as these other studies have reported, PCB levels in blood can vary daily according to the food consumed and the state of the individual. Pregnant women may have more PCBs in their blood due to the mobilization of fat stores during pregnancy. Consequently, in interpreting relationships between fish consumption and outcomes, the uncertainty of the actual PCB intake must be realized.

Serum PCB levels can be significantly affected by serum lipid content (total cholesterol, free cholesterol, triglycerides, and phospholipids) because of partitioning between plasma and serum lipids. Non-fasting levels also tend to be higher. Therefore, lipid-adjusted serum PCB levels are a better biomarker for body burden. Non-fasting and/or non-lipid adjusted serum PCB levels introduce bias and/or measurement error resulting in an additional source of exposure misclassification.

A further, largely unacknowledged, limitation is encountered in reporting exposure levels for individual congeners. The majority of the epidemiological studies report exposure simply as summed or total PCBs, but a large body of data clearly demonstrates different effects following exposure to different classes of PCB congeners (89). In this light, summed or total PCBs is a conceptual construct and must be recognized as a proxy measure for any biologically plausible disease causing agent. Regardless of precision, the effect of reporting exposure as summed or total PCBs, is a loss of sensitivity that, like measurement error, dilutes strength of association (90).

DeVoto et al. (91) and Gladen et al. (92) report that congeners tend to be well correlated with each other (Pearson > 0.80). However such correlations still introduce a considerable loss of sensitivity that contributes to exposure misclassification.

Still other limitations strain sample selection and resource allocation. Biosample collection is invasive, inconvenient and costly. Fear of needles, travel to clinics, waiting rooms, and procrastination all decrease and potentially bias participation. Laboratory analyses of collected biosamples are technically difficult and costly (~$250 per sample for full PCB profile).

In summary, the most important advantage of biosamples is the ability to provide an index of body burden for individual PCB congeners. Disadvantages of biosamples include a lack of information about the duration and timing of exposure, a loss of sensitivity when reporting individual congeners as summed or total PCBs, and a decrease in participation rates. Moreover, the collection and analysis is costly. Sometimes a researcher may decide that the disadvantages of biosample collection outweigh their advantages, forgo biosample measures, and rely solely on questionnaire-based measures of exposure.

Questionnaires. Even though questionnaire-based measures offer several attractive advantages, they are generally regarded as inferior to biosamples. Advantages of questionnaire-based measures are that they are relatively noninvasive, convenient and cheap. Questionnaires are flexible; they can be used in mass mailings, random digit dialing, or face-to-face interviews. Because they are relatively noninvasive and convenient, their use produces higher participation rates.

Self-reported sport fish consumption can serve as a proxy measure for PCB dose. The rate of sport fish consumption is exposure per unit time (e.g., the number of cigarettes per day). In this thesis, measures of sport fish consumption based upon the number of meals in the past 12 months are considered rates of exposure where year is the unit of time. However, a theoretical limitation of rate based-measures of exposure is that it captures no information about the duration of exposure.

Duration of sport fish consumption is nearly always based on number of years. Information about PCB half lives suggests that historical fish consumption should exert a diminishing effect on current body burden (48). However, such reductions may be attenuated by historically higher sport fish contaminant levels and rates of consumption (61). In fact, some studies have reported that historic fish consumption predicts current PCB levels better than recent consumption (48, 72). Hanrahan et al. (44) reported that total number of years of eating sport fish was the best predictor of PCB body burden when compared to rate-based measures.

Cumulative sport fish consumption is a calculated index based on the product of exposure rate and duration. Several studies have used such measures of exposure (93-96). Schwartz et al. (97), report a moderate correlation (r=0.21-0.29) between cumulative fish consumption with PCB levels in maternal serum and milk. A theoretical limitation of this measure of exposure is that it does not capture information about the timing of exposure.

An index of cumulative PCB exposure (ng) is provided by the product of exposure rate (meals/year), duration (years), meals size (grams), and is weighted for the average fish tissue PCB level (ng/g) by species consumed (50, 98, 99). Dar et al. (40)

used a similar weighting technique, but limited questions about fish consumption to the 12 months prior to pregnancy, and found a much higher correlation between fish consumption and serum PCB levels than did studies that included years of consumption.

An ostensible limitation of questionnaire-based measures is that they all tend to have inadequate criterion-related validity with serum PCB level (100). Problems with obtaining accurate retrospective dietary information may be one factor contributing to the relatively low associations between PCB body burdens and estimated fish consumption based upon questionnaires (fish consumption diaries reduce the error but are limited to use in prospective studies) (42, 101).

An alternative explanation that clearly limits the strength of association is that questionnaire-based measures estimate cumulative exposures, not body burden. A significant proportion of the variance in serum PCB levels not explained by questionnaire-based measures of exposure may be attributable to pharmacodynamic factors. This suggests that serum PCB level may not be an entirely meaningful criterion for judging the accuracy, potential validity, or true value of questionnaire-based measures.

Complementary measures of exposure. Consideration of the strengths and limitations of biosample and questionnaire-based measures of exposure suggests that both methods offer complementary information that help produce more meaningful and accurate characterizations of exposure (102, 103). Although biologically-effective dose (e.g., the level of particular PCB congeners in a body tissue during a specific time window) is the ideal measure, hazard control is based on external exposures (e.g., number

of fish meals eaten by species per unit time), and therefore a researcher may want to measure both exposure and dose.

Sample Selection

Sample selection for study subjects is a fundamental consideration for any study design. Characteristics of the study sample determine statistical power (81, 104). In the case of epidemiological investigations of exposure-response relationships, the researcher strives to maximize statistical power while staying within budget and time constraints.

The skewed distribution of PCBs in the Great Lakes population complicates sample selection for exposure-response studies, since it is difficult to sample a sufficient proportion of exposed subjects. The ideal solution would be to perform stratified sampling based on actual serum total PCB level. Unfortunately, such a process would require a highly inefficient use of resources since biosample based screening of the general population is impractical due to the expense and inconvenience of biosample collection.

Targeting special populations. One successful approach has been to target special populations such as charter boat captains, Native Americans, or Hmong Americans (105) (Table 1). These studies may effectively capture a more highly exposed subpopulation, but even such subpopulations exhibit positively skewed distributions for PCB body burden and they may have limited generalizability.

Oversampling. Another possible strategy is oversampling with screening surveys. Oversampling is a procedure designed to selectively sample a segment of a population in larger proportion than its actual representation in the target population. Many of these studies use sample allocation strategies designed to over select for

increased exposure. For example, screening surveys that ascertain estimators of exposure such as meals in the previous 12 months are used to target individuals more likely to have elevated serum PCB levels.

Selected Great Lakes Cohorts

Table 1 provides a review of selected cohorts for the epidemiologic investigation of PCB exposure-response relationships among consumers of Great Lakes sport fish. All seven cohorts targeted special populations at elevated risk for exposure to PCBs by consumption of Great Lakes sport fish. Four of the studies employed some form of oversampling based on a screening survey to measure sport fish consumption. All of these sampling strategies boost the study sample mean serum PCB levels however the distributions are still highly positively skewed suggesting the potential for improved oversampling.

Table 1. Selected cohorts for epidemiologic investigations of PCB exposure-response relationships among Great Lakes sport fish consumers.

Cohort (Enrollment Period)	Target Population Risk Factors for PCB Exposure	Geographic area sampled	Sample Allocation
Michigan Department of Public Health Cohort (MDPHC), 1982 (16)	Anglers and geography	Ten Lake Michigan shoreline communities in the State of Michigan	Weighted lifetime PCB exposure based on sport fish meals. Pounds of sport-caught Great Lakes fish consumed annually
Lake Michigan Maternal Infant Cohort study (LMMIC), 1983 (106)	Geography	Western side of the State of Michigan in close proximity to Lake Michigan	Pounds of Lake Michigan fish consumption in past 6 years
New York Anglers Cohort, 1991 (107)	Anglers	Sixteen State of New York counties in close proximity to Lake Ontario	n.r.
Oswego Cohort, 1991-1994 (108)	Geography	Oswego county on southeastern shore of Lake Ontario	Weighted lifetime PCB exposure based on Lake Ontario sport fish meals
Great Lakes Charter Boat Captains Cohort, 1993-1995 (109)	Currently active Great Lakes charter boat captains	Statewide in Wisconsin, Michigan, Indiana, Illinois and Ohio	Stratified sample of Great Lakes sport fish consumers
Fisheater Reproductive Health Screening Survey Cohort, 1993-1995 (49)	Anglers and geography	Ten State of Michigan counties containing or adjacent to Areas of Concern	n.r.
Fisheater Family Health Project Cohort-A, 1997-2000	Anglers and geography	Ten Michigan counties containing or adjacent to Areas of Concern	n.r.

n.r., none reported.

Studies Reporting on Exposure-response Relationships

This section reviews the methodologies and key findings of 23 studies that have reported on PCB exposure-response relationships for subjects in the selected cohorts (Tables 1 & 2). Sample selection in these studies employs a variety of strategies to capture populations with elevated exposure to PCBs. The majority of the studies target populations at increased risk for exposure.

Table 2. Selected epidemiology studies reporting on PCB exposure-response relationships among Great Lakes sport fish consumers.

		Self-report Sport Fish Consumption		Biosample PCB Level	
Author	**Year**	**Measure**	**Findings**	**Specimen**	**Findings**
Jacobson et al.	1984	Cumulative	+	Cord serum	–
Fein et al.	1984	Cumulative	+	Serum	+
Jacobson et al.	1985	Cumulative	+	Cord serum	+
Jacobson et al.	1990	n.r.	.	Cord serum	+
Dar et al.	1992	Cumulative	–	Serum	–
Mendola et al.	1995	Multiple	–	n.r.	.
Lonky et al.	1996	Cumulative	+/–	n.r.	.
Jacobson et al.	1996	n.r.	.	Composite	+/–
Buck et al.	1997	Years	–	n.r.	.
Mendola et al.	1997	Multiple	+	n.r.	
Buck et al.	1999	Multiple	–	n.r.	.
Courval et al.	1999	Cumulative	+/–	n.r.	.
Schantz et al.	1999	n.r.	.	Serum	–
Buck et al.	2000	Multiple	+/–	n.r.	.
Darvill et al.	2000	Cumulative	–	Cord serum	+
Stewart et al.	2000	Cumulative	+	Cord serum	+
McGuinness et al.	2001	Multiple	+/–	n.r.	.
Persky et al.	2001	Multiple	+/–	Serum	+/–
Schantz et al.	2001	n.r.	.	Serum	+
Karmaus et al.	2002	n.r.	.	Serum	+
Stewart et al.	2003	n.r.	.	Cord serum	+
Weisskopt et al.	2003	n.r.	.	Serum	+
Karmaus et al.	2004	n.r.	.	Serum	+

n.r., none reported. + Positive findings. – Negative findings. +/– Both positive and negative findings on multiple responses.

Findings from the 23 studies in Table 2 have been very influential. Eighteen of the studies report at least one positive finding of either a questionnaire or biosample based exposure-response relationship.

Limitations. Several authors have suggested concerns over the validity of findings drawn from this body of research (101, 110-116). A summary of these limitations include: 1) weak associations; 2) potential bias; 3) lack of specificity; and 4) inconsistent findings. Together these limitations bring into question the validity of arguments that make causal inferences about PCB exposures and adverse health outcomes.

These studies are vulnerable to non-differential selection bias, differential selection bias, and recall bias. Non-differential selection bias occurs when the study sample is not randomly sampled from the target population. Stein et al. (117), in a cross sectional survey called the Fisheater Reproductive Health Screening Study, report that among men, nonresponders had fished fewer days in the past year (12% reported no fishing, compared to 4. 3% of responders). Almost one half of nonresponders reported no fish consumption in the past year, compared to one quarter of responders. Non-differential selection may affect the external validity of study findings, but not the internal validity.

Differential selection bias is potentially much more problematic because it can result in spurious associations between the exposure measure and response, or the study's internal validity. This form of bias occurs when there is interaction between selection bias for exposure and a selection bias for the response. It is likely to be directly associated with the inconvenience and invasiveness of a study design since motivation

becomes an important factor influencing participation. Stein et al. (117) found no evidence for differential selection bias in survey data from the Fisheater Reproductive Health Screening Study. Biosamples are more likely to be vulnerable to differential selection bias, because of the increased difficulty and inconvenience of participation, but as yet there are no publications on its actual impact. Also, Larsen et al. (118) report findings that subfertile men were more likely to participate in their reproductive health study. Considered together, these two reports should serve as a warning that differential selection bias may occur.

Recall bias is another serious limitation that can result in spurious associations between an exposure and a response. Recall bias occurs when error in ascertaining exposure status is not random but varies by response status. Biosamples plainly avoid this limitation, but self-report based measures are vulnerable to recall bias and may result in spurious associations.

Confounding is a study design limitation that can introduce spurious associations between the putative exposure and response. Both questionnaire and biosample-based measures of exposure are vulnerable to confounding. Confounding occurs when the effects of the exposure factor and the confounder factor are not separated. An exposure that has no causal relationship with a response, but is associated with an agent that is causally related to the response, will be associated with the response, distorting the apparent effect of the exposure on risk for exhibiting the response (119). Sport fish is an exposure source not only for PCBs but also a complex mixture of other bioaccumulative pollutants (e.g., methylmercury, dioxins, furans, Lindane isomers, Chlordane isomers, DDE, Polybrominated Diphenyl Ethers, and toxic metabolites such as hydroxlated and

methylsulfone PCBs, and other identified and unidentified contaminants) (13, 15, 104, 120). Given the complex mixture of contaminants in sport fish, PCBs exposures from consuming sport fish are almost certainly associated with other contaminants.

Another potential confounder is disease-induced weight change (e.g., weight loss associated with cancer). Recent weight loss is associated with increased serum PCB level, probably caused by mobilization of lipid-soluble PCB from body fat (121-125). Breast feeding also mobilizes organochlorines from body stores, and the body burden of organchlorines usually declines during breast-feeding (71). If the response under investigation is associated with weight change, than an increased serum PCB levels may be associated with the outcome, without being causally related to it.

Sport fish consumption may also be confounded with known risk factors for a response such as demographic and lifestyle factors. For example, a few studies have indicated that smoking may be positively related to organochlorines level in plasma (126-129).

Another limitation includes the inconsistent use of methods for measuring exposure (Table 2). Of the 23 studies, 7 report both questionnaire and biosample-based measures of exposure, 8 report biosample based measures of exposure only, and the remaining 8 report questionnaire-based measures of exposure only. Of the studies reporting questionnaire-based measure of exposure, no consistent measure were used. These inconsistencies in methods of exposure assessment suggest that suboptimal characterizations of exposure may have been used in at some of the studies. Studies using suboptimal measure of exposure may be vulnerable to substantial exposure

misclassification that dilutes the true strength of association between an ideal, or true, characterization of exposure and the response (80).

Methodological inconsistencies for biosamples include: questions of comparability of biosamples between laboratories (including PCB quantification and what set of congeners are reported), different protocols for imputation of values below the levels of detection, and methods by which total PCBs are calculated (130). Together these methodological limitations almost certainly impact observed exposure-response relationships and hinder between-study comparability and metaanalysis for this body of research.

Assessing the impact, and properly addressing, this set of limitations poses a daunting challenge. Some of the limitations are inherent to this area of research and are by in large intractable. But others are not. For example, it may be possible to decrease measurement error by developing improved measures of exposure and response, the effect of bias can be investigated, and comparability between studies can be improved with standard measures (81). Presently, the extent to which the weight of evidence in this body of research is affected by these limitations is unknown. However, it would be a mistake to ignore these concerns.

Rationale and Aims

Investigation of PCB exposure-response relationships faces serious methodological limitations that introduce exposure misclassification and reduce statistical power to discern exposure-response relationships. Evaluation of measures of exposure may improve screening surveys, reduce measurement error and promote standardization of measures of exposure.

Improve screening surveys. The effectiveness of sample selection methods that utilize a screening survey to over-sample for subjects with increased serum PCB levels is directly proportional to predictor criterion-related validity. Criterion-related validity, also referred to as instrumental validity, is used to demonstrate the accuracy of a measure by comparing it with another measure which has been demonstrated to be valid. Identification of measures with improved criterion-related validity can increase the predictive power and effectiveness of screening surveys.

Reduce measurement error. Poor criterion-related validity reduces precision, increases exposure misclassification, and dilutes the observed strength of association between exposure and response. Exposure measurement error decreases the power of a study or increases the sample size required to detect a significant health risk. If error is random and does not vary by response status (i.e., there is no recall bias), the sample size needed to detect a statistically significant effect is inversely related to the squared correlation (R-Square; i.e., a criterion for criterion-related validity) between the imperfect exposure measure and the perfect exposure measure ($n_{error}= n_{true}/R\text{-}Square$) (80). Therefore, even small improvements in criterion-related validity, if cost effective, are meaningful. For example, if the study exposure measure has a criterion-related validity of an R-square of 0.1, then the sample size (n_{error}) necessary to detect a significant health effect would be approximately 10 times higher than the sample size (n_{true}) necessary if the perfectly measured dose was used ($n_{error}= n_{true}/0.1 = 10*n_{true}$).

Promote standard measures. Inconsistent measures of exposure limit comparability and metaanalysis. Studies that adopt a minimum standard for measuring

and reporting exposure promote comparability and thereby increase the likelihood of discerning real exposure-response relationships in metaanalysis.

In conclusion, existing epidemiologic investigations of PCB exposure-response relationships utilize suboptimal methods for sample selection and inconsistent measures of exposure. Evaluation of self-report based measures of sport fish consumption for their criterion-related validity to serum PCB level may distinguish strengths and limitations of individual predictors, promote adoption of a minimum set of standards for future research in this area, and contribute toward less controversial research permitting strong causal inference.

Objectives

This thesis investigates the predictive power of questionnaire-based measures of sport fish consumption as predictors of serum total PCB level. The thesis has three main objectives: 1) Evaluate individual questionnaire-based measures of sport fish consumption as predictors of human serum PCB levels (H_0: $\beta_1 = \beta_2 = \beta_3 = \beta_4 = 0$), 2) Compare individual measures for statistically significant improvement in the predictive power (H_0: $MS_1 = MS_2$ versus H_1: $MS_1 \neq MS_2$), and 3) Predict the performance of individual predictors to determine if differences are meaningful.

CHAPTER III

MATERIALS & METHODS

Study Population

This thesis uses data from the Fisheaters Family Health Project (FFHP) Cohort-A. This cohort was created to investigate the relationship between exposure to polychlorinated biphenyls (PCBs) through consumption of Great Lakes sport fish and the reproductive health of males and females. This cohort is a sample of couples, one or both of whom is a licensed Michigan angler, and resident in one of ten Michigan counties abutting Lake Michigan, Lake Huron, or Lake Erie and within International Joint Commission designated Areas Of Concern (AOC), because of their proximity to water bodies contaminated with high levels of PCBs and other persistent and toxic critical pollutants. The full cohort enrolled 305 subjects between July, 1997, to November, 2000. However, the thesis data is limited to subjects with data available for serum PCB level (n=141) and complete data for important demographic and exposure related information (n=129).

Questionnaire-based Measures of Sport Fish Consumption

Cohort members performed a telephone questionnaire with separate male and female sections for reproductive history, but shared common sections for exposure assessment including the base questions of the predictors (APPENDICES A & B). The four measures of sport fish consumption evaluated in this thesis include: 1) lifetime years eating sport fish (YEARS); 2) sport fish meals in the previous 12 months (MEALS); 3) lifetime sport fish meals (MXY1); and 4) modified lifetime sport fish meals (MXY2).

Lifetime Years Eating Sport Fish (YEARS). Lifetime years eating sport fish (YEARS) characterizes the duration of exposure. This predictor was assessed as a single question with the response broken into five year age groups (APPENDIX A).

Sport Fish Meals in Previous 12 Months (MEALS). Sport fish meals in previous 12 months (MEALS) characterizes the rate of exposure. This predictor assessed a single question with the response broken into four intervals, of three months each, that distinguish between winter, spring, summer, and fall in order to distinguish seasonal variation (APPENDIX B).

Lifetime Sport Fish Meals (MXY1). Lifetime sport fish meals (MXY1) is an index that measures cumulative exposure. MXY1 combines rate and duration of exposure to provide an index of the total number of lifetime sport fish meals. MXY1 is calculated as the product of YEARS times MEALS. This index assumes that fish consumption patterns are relatively consistent across years that any fish is consumed. A potential limitation of this index is that subjects who report having eaten sport fish during their lifetime (YEARS > 0), but report eating zero sport fish meals during the previous 12 months (MEALS = 0), will be misclassified as never having eaten sport fish during their lifetime, since the product of zero meals times any number of total years is always zero. Consequently, lifetime sport fish consumers who did not eat sport fish in the previous 12 months, are misclassified as non-consumers, regardless of how long or what amount of sport fish they may have eaten in previous years.

Modified Lifetime Sport Fish Meals (MXY2). Modified lifetime sport fish meals (MXY2) is also an index that measures cumulative exposure. This index improves on MXY1 by imputing rate of sport fish consumption when zero meals were consumed in

the previous 12 months. The modified index is calculated as the product of multiplying non-zero responses for number of sport fish meals consumed in the previous 12 months by the total number of lifetime years in which any sport fish was consumed. For subjects reporting zero sport fish meals in the previous 12 months, zero is replace by an imputed value calculated as the overall sample median value of sport fish meals in the previous 12 months (8 meals for thesis data) and multiplied by the total number of lifetime years in which any sport fish was consumed. The status of true non-fish eaters is preserved since the product of multiplying the median value for sport fish meals in the previous 12 months by zero total years still results in an index value of zero. This method treats a response of zero to sport fish meals for the previous 12 months as missing information since no information has been provided about a subject's exposure frequency when he or she may have eaten sport fish in prior years. This salvages exposure information captured by lifetime years. MXY2 also assumes that fish consumption patterns are relatively consistent across years that any fish is consumed. A theoretical limitation of this measure of exposure is that it captures no information about the rate of exposure.

Biosample Collection and Analysis

Upon recruitment into the study, subjects were asked to report for a blood drawn at one of several Quest Clinical Laboratories located in the study counties. Cohort members who failed to have their blood drawn at a clinic were given the option of having a field phlebotomist visit their home to draw the blood sample. Subject inclusion in either group occurred by self-selection with 103 subjects reporting to a clinic and the remaining 26 requesting field collection.

Protocols for both sample groups specified collection of blood samples into 10 ml glass vacutainers without an anticoagulant, clotting at room temperature and decanting into storage containers. Processing of samples between the two groups was identical. However, sera processed at Quest Clinical Laboratories were decanted into polystyrene storage containers, while those collected and processed by the field phlebotomist were decanted into glass storage containers. Serum-containing storage containers from both groups were then stored at $-20^{\circ}C$ until analysis. Storage container type is the only known difference between the two sample groups. The median storage time was 8 months with a range of 1 to 32 months. All samples were analyzed within 3 months of each other.

Storage of serum in polystyrene containers was an unplanned violation of the study protocol that raises at least two concerns. First, the polystyrene storage containers used to store serum may contain PCBs, or other substances, that may contaminate the serum samples and introduce experimental error. Second, container type is perfectly confounded with clinic and field sample groups. This limits the comparability of data between the two groups. It also complicates evaluation of the potential effects of this protocol violation, since the two groups are likely to vary on other factors besides type of storage container. However, for the purposes of this thesis, the preceding concerns are considered a limitation of the data. The risk of experimental error from this source is plausible, but highly unlikely, and is not of sufficient concern to prevent its use for this thesis.

The analysis was performed, between October and November of 1999, at the Michigan Department of Community Health (MDCH), Bureau of Laboratories Analytical Chemistry Section. The MDCH laboratory adheres to strict internal and external quality

assurance and quality control practices and has over twenty years of experience in developing and conducting chemical analyses on human specimens.

Sample preparation involves extraction of the serum sample using a liquid/liquid method. The extract is then cleansed of interfering lipids using a micro florisil column, and the PCBs are separated from the pesticides with a micro-silica gel 60 column technique. The two PCB fractions (coplanar PCBs and other PCBs) are separated by an automated carbon column/casium silicate-acid silica/alumina separation and cleanup device. Capillary column gas chromatography (Varian model 3800) was used to separate and identify polychlorinated biphenyl (PCB) congeners. Individual PCB congeners were quantified using custom calibration mixtures. Separate calibration standard mixtures were obtained commercially for the PCB congeners (composed of 83 individual PCB congeners).

Derived measures for PCB exposure

The laboratory did not report values for individual congeners below the level of detection (LOD) that varied by congener from 0.2 to 1.3 parts per billion (ppb). For values below the LOD, the square root of the LOD was recorded when less than 40% of the subjects sampled were reported below the level of detection on any individual congener. When greater than 40% of the subjects sampled were reported below the level of detection on an individual congener, the zero value was retained. No adjustment was made for serum lipid levels. Total PCBs were determined by summing individual congeners.

Descriptive Statistics

All statistical analyses were performed using SAS software versions 8.02. Descriptive statistics are presented for selected demographic factors, questionnaire-based measures of sport fish consumption, and serum PCB levels. Frequencies are presented for categorical variables. Means, the minimum (Min), the 10^{th} percentile (P10), the median (Med), the 90^{th} percentile (P90), and the maximum (Max) are presented for continuous variables.

Correlation Matrix

A Pearson nonparametric correlation matrix is presented to show unadjusted associations between modeled variables. These associations aid in the interpretation of relationship between variables in the multivariable regression models. For example, age is likely to be associated with total years eating sport fish and act as an intervening variable that limits the maximum number of total years that a subject may have eaten fish.

Multiple Regression Modeling

SAS regression procedures were used to model serum PCB level (y) on control variables (x_i) and predictors (x_j). Criteria for selection include partial sum of squares and R-Square delta. Model diagnostics are assessed for violation of model assumptions. Transformations of model variables are implemented where appropriate to remedy violations of model assumptions. Standardization procedures are used to facilitate comparisons between modeled predictors.

To interpret control and predictor variables, partial regression coefficients are provided. Partial regression coefficients represent the expected increase in y per unit

increase in x_j, with all other variables held constant and are estimated by the parameter estimate b_j (131). If there is potential confounding between predictor and control variables, then the predictor partial regression coefficient may differ considerably from the simple linear-regression coefficient obtained by modeling the predictor without considering control variables (131).

The strategy for fitting models is based upon forward selection. The procedure uses four steps: 1) Specify the maximum model to be considered including candidate control variables and the four predictors; 2) Model control variables; 3) Add predictor variables to the control variables as separate models (Models A, B, C & D), and individually examine predictor partial f-value with an α of .05 for significance, 4) for each model evaluate for goodness of fit and transform variables as indicated.

Assumptions. Assumptions for multiple regression are: 1) Existence: For each specific combination of values of the independent variables $X_1, X_2, \ldots, X_k$, Y is a random variable with a certain probability distribution having finite mean and variance. 2) Independence: the Y observations are statistically independent of one another. 3) Linearity: the mean value of Y for each specific combination of $X_1, X_2, \ldots, X_k$ is a linear function of $X_1, X_2, \ldots, X_k$. 4) Homoscedasticity: The variance of Y is the same for any fixed combination of $X_1, X_2, \ldots, X_k$. 5) Normality: for any fixed combination of X_1, $X_2, \ldots, X_k$, the variable Y is normally distributed (132).

Goodness of fit. Residuals are important in model critique for detecting outliers, evaluating goodness of fit, and diagnosing departures from assumptions. A SAS macro was used to produce an "influence plot" for regression model residuals, leverage and their combined effect as influence. Residuals are calculated as the observed value minus the

predicted value for each observation ($e_{ij} = Y_{ij} - \hat{Y}_i$). This plot shows Studentized residuals versus hat values for leverage, with Cook's Distance as the size of a bubble symbol for influence. The value of Cook's distance for each observation represents a measure of the degree to which the predicted values change if the observation is left out of the regression. Observations exhibiting unusually large values for the Cook's distance are suspect outliers and considered candidates for deletion. Horizontal reference lines added to the plots delimit observations whose Studentized residuals are individually significant at p=0.05. A vertical reference line in the plot shows observations which are of "high leverage" (133).

Transformations. Models with non-normally distributed residuals can usually be corrected by transforming variables. Many of the full model variables are highly skewed. Skewed data is transformed to approximate normality using rescaling and fractional exponentiation (134). The exponent for transformation is selected based upon an iterative procedure for convergence of the distribution mean and median (135).

Standardizations. Two standardizations procedures are used to ease comparisons of predictors with different units. First, standardized regression coefficients are used to compare the predictive value of each measure of sport fish consumption (x_j). This method is useful for comparing estimators with different units of measurement (131). It describes the predicted increase in standard deviation units of serum PCB level (y) that would be expected per standard deviation increase in each questionnaire-based measure (x_j). Second, for comparison of overlay plot and predicted means, exposure units are standardized to a 0 to 1 scale by dividing each exposure measure value by its maximum value.

Partial residual plots with fitted splines. Partial residual plots are used to visualize the relationship between serum PCB level (y) and the predictors (x_j), while adjusting for control variables. Partial-residual plots are constructed as follows: 1) A multiple regression is performed for y on all control variables (i.e., gender, age, BMI) and the residuals are saved. 2) A multiple regression is performed for x_j on all other control variables. 3) The partial-residual plot is then constructed as a scatter plot of the residuals in step 1 on the y axis versus the residuals in step 2 on the x_j axis (131).

To assess the plausibility of a linear relationship between y and x_j, smoothed regression lines are fitted to plotted data using spline routines with 90% bootstrap confidence intervals (136). This method allows great flexibility in fitted-line curvelinearity. A linear model is considered plausible when an approximately straight line is fit, or a straight line can be fit within the confidence intervals.

Comparing Non-nested Multiple Regression Models

When comparing the performance of two non-nested regression functions for predicting y the partial F-test does not apply. Graybill and Iyer (137), describe a procedure for comparing predictors between non-nested models.

First the full model is described; superscripts A and B are used to represent the predictor variables in the two comparison subsets. The full model includes all the covariates and predictor variables that are in model A and model B, $X_1^A,....,X_r^A$ and $X_1^B,....,X_m^B$, and is denoted by the collection $X_1,....,X_k$.

Then the criterion for selection is declared; this thesis uses the model partial regression root mean square, $RMS(X_4 \mid X_1, X_2, X_3)$, of the predictor. The partial regression root mean square is a measure of the magnitude of variance explained by a

predictor after adjusting for the control variables already in the model. Therefore, to compare predictors, it is of interest to know how much bigger or smaller the partial RMS_B is than the partial RMS_A. The approach used in this thesis is to examine the ratio $RMS(X_4^B \mid X_1^B, X_2^B, X_3^B) / RMS(X_4^A \mid X_1^A, X_2^A, X_3^A)$, or for brevity RMS_B/RMS_A, and make a practical decision based on these results.

The test for significance computes Bonferroni two-sided confidence intervals for RMS_B/RMS_A with confidence coefficient greater than or equal to 1-α. The procedure for its construction is given below:

1) Let r and m denote the number of independent variables in models A and B, respectively, and let n be the number of sample observations.

2) Regress y on the independent variables in model A and obtain the partial regression sum of squares SS_A for model A predictor x_j.

3) Regress y on the independent variables in model B and obtain the partial regression sum of squares for SS_B for model B predictor x_j.

4) Compute a 1 – α/2 two-sided confidence interval for RMS_A, which is given by $C[L_A \leq MS_A \leq U_A] = 1 - \alpha/2$.

Where

$$L_A = \sqrt{\frac{SS(X_4^A \mid X_1^A, X_2^A, X_3^A)}{\chi^2_{1-\alpha/4:n-r-1}}} \quad \text{and} \quad U_A = \sqrt{\frac{SS(X_4^A \mid X_1^A, X_2^A, X_3^A)}{\chi^2_{\alpha/4:n-r-1}}}$$

5) Compute a 1 – α/2 two-sided confidence interval for RSS_B, which is given by $C[L_B \leq MS_B \leq U_B] = 1 - \alpha/2$.

Where

$$L_B = \sqrt{\frac{SS(X_4^B \mid X_1^B, X_2^B, X_3^B)}{\chi^2_{1-\alpha/4:n-m-1}}} \quad \text{and} \quad U_B = \sqrt{\frac{SS(X_4^B \mid X_1^B, X_2^B, X_3^B)}{\chi^2_{\alpha/4:n-m-1}}}$$

6) Compute the following confidence statement for MS_B/MS_A:

$$C[L_B/U_A \leq RMS_B/RMS_A \leq U_B/L_A] = 1 - \alpha/2.$$

Predicted Mean Serum PCB Levels at Selected Exposure Levels

For each modeled predictor, the serum PCB level predicted means, with 95% confidence limits, are presented at three sample selection related exposure levels: 1) full sample (midpoint=0.50); 2) oversampling top 50% (midpoint=0.75); and 3) oversampling top 20% (midpoint=0.90).

CHAPTER IV

RESULTS

Characteristics of the Study Data

55.0% of study subjects were male and 45.0% were female (Table 1). The mean age was 36.7 years, with a range of 22 to 53 years. The average body mass index (BMI) was 26.9 kilograms per meters squared, with a range of 16 to 68. One subject (ID, 0009-1) reported an extremely high BMI of 68. This subject was a 36 years old male with a recorded weight of 462 pounds and height of 5 feet 9 inches. Therefore, while this is an extremely high BMI it is nevertheless correctly calculated from plausible base parameters. During modeling, this subject was deleted from models as a influential outlier. For highest level of education attained, 3.1% received less than high school, 20.9% received GED/high school, 46.5% reported some college, 24.0% reported being college graduates, and 5.4% reported Graduate school.

Table 1. Demographic and sport fish related characteristics of 129 subjects in the Fisheaters Cohort-A, 1997-2000.

	N	%	Mean	Min	P10	Med	P90	Max
Gender (GENDER)								
Female	58	45.0						
Male	71	55.0						
Age in years (AGE)			36.7	22	31	35	45	53
Body Mass Index (BMI)			26.9	16	21	26	34	68
Education (EDUCAT)								
Less than high school	4	3.1						
GED/high school	27	20.9						
Some college	60	46.5						
College graduate	31	24.0						
Graduate school	7	5.4						
Grams of sport fish eaten per day in past 12 months			9.0	0	0	5	27	58
Sport fish meals in past 12 months (MEALS)			11.8	0	0	8	32	56
Lifetime years eating sport fish (YEARS)			23.8	0	10	25	35	35
Lifetime years eating sport fish among subjects reporting zero meals in the past 12 months (n=23)			16.8	0	5	15	25	35
Index of lifetime sport-caught fish meals (MXY1)			306.7	0	0	160	910	1,960
Modified index of lifetime sport-caught fish meals (MXY2)			330.7	8	50	200	910	1,960

The average number of grams of sport fish eaten per day in the past 12 months was 9.0, the median value was 5, and the range was 0 to 58 (Table 1). The average number of meals eaten in the previous 12 months was 11.8, the median value was 8, and the range was 0 to 56. The average number of lifetime years eating sport fish was 23.8 and the median value of 25, and the range 0 was 35. The average of the index for lifetime meals was 306.7, the median value was 160, and the range was 0 to 1,960. The

average of the modified index for lifetime meals was 330.7, the median was 200, and the range was 8 to 1,960.

Table 2. Serum PCB level (ppb) for the Fisheaters Cohort-A, 1997-2000.

	N	%	Mean	Min	P10	Med	P90	Max
Serum PCB level (ppb)			1.9	0.1	0.2	0.8	4.8	13.5
Serum PCB group (ppb)								
<2	95	73.6						
2 to <4	17	13.2						
4 to <6	6	4.7						
6 to <8	4	3.1						
8 to <10	4	3.1						
10 to <12	2	1.6						
≥12	1	0.9						

The mean serum PCB level was 1.9 ppb, with a median value of 0.8 ppb, and a range of 0.1 to 13.5 (Table 2). Nearly three quarters of the sample (73.6%) exhibited serum PCB levels in the lowest exposure level grouping, or less than 2 ppb. The relationship between the mean, median and range relative to zero suggests a highly positively skewed, or lognormal, distribution. To illustrate the distribution, a histogram was constructed and fitted with a lognormal curve (Figure 1). The resulting histogram verified the highly positive skew of the distribution and indicated that a transformation was required to meet the assumptions for multiple regression.

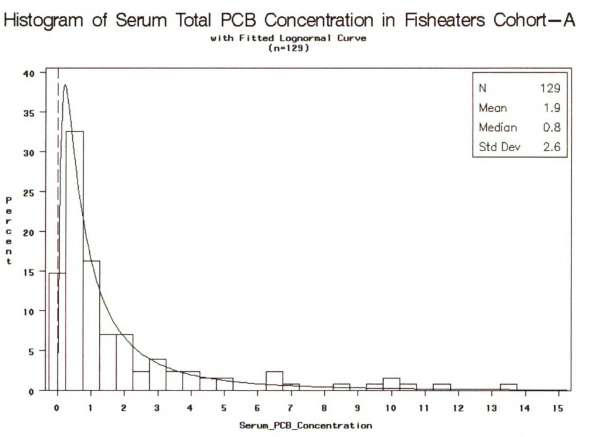


Figure 1. Distribution of serum PCB level in the Fisheaters Cohort-A (n=129).

Correlation Matrix

Inspection of the Pearson correlation matrix reveals that AGE is strongly correlated with SERUMPCB, and GENDER and BMI are less strongly associated with SERUMPCB (Table 3). All of the measures of sport fish consumption, YEARS (r=0.36), MEALS (0.35), MXY1 (0.37) and MXY2 (0.38) are all strongly correlated with SERUMPCB.

MXY1 and MXY2 are extremely highly correlated (0.99) suggesting that they may be indistinguishable in the regression. MEALS, MXY1 and MXY2 are all highly co-linear (greater than 0.6). Therefore, these three variables should not be considered in

the same model, since they are likely to measure the same phenomenon; including them may cause the model to appear to explain the observed data better than is really does.

Table 3. Pearson correlations (prob > |r| under H_0: r=0) between serum PCB level, control variables, and measures of sport fish consumption in study data (n=129).

	Serum Total PCB	GENDER†	AGE	BMI	YEARS	MEALS	MXY1
GENDER	-.20*	—					
AGE	.49***	-.27**	—				
BMI	.19*	-.22*	.17	—			
YEARS	.36***	-.40***	.39***	.23**	—		
MEALS	.35***	-.01	.19*	.01	.21*	—	
MXY1	.37***	-.12	.27**	.06	.42***	.93***	—
MXY2	.37***	-.13	.26**	.05	.42***	.91***	.99***

†Male, 0; Female, 1; *p<.05; **p<.01; ***p<.001

Multiple Regression Models

Candidate multiple regression models were developed to predict serum PCB level, identify control variables, and compare predictors. The four models are presented sequentially and each section is organized similarly and presents the model ANOVA and a partial residual plot for serum PCB level versus each predictor.

The maximum model (X_1 ,....., X_k) to be considered was SERUMPCB on candidate control variables GENDER, AGE, BMI, EDUCAT, and the four predictors YEARS, MEALS, MXY1, and MXY2. Candidate control variables were identified by a review of literature and theoretical considerations. All four models were modeled for GENDER, AGE, BMI and EDUCAT as control variables. EDUCAT was included in the initial full model but finally restricted on the grounds that it did not meaningfully impact

estimates for predictors, did not improve precision, and was not statistically significant in any of the models.

Gender and age are significantly associated with SERUMPCB. BMI is not found to be significantly associated with SERUMPCB. Of the control variables age is the most strongly associated with SERUMPCB (Table 4).

Table 4. ANOVA and parameter estimates for regression of log10 serum total PCB level on control variables: gender, age, and BMI.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	3	12.81618	4.27206	23.68	<.0001
Error	124	22.36991	0.18040		
Corrected Total	127	35.18608			

Root MSE	0.42474	R-Square	0.3642
Dependent Mean	-0.05429	Adj R-Sq	0.3489
Coeff Var	-782.39850		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Standardized Estimate
Intercept	1	-1.82193	0.32251	-5.65	<.0001	0.37722	0
gender1	1	-0.18904	0.07956	-2.38	0.0190	3.75943	-0.17948
age	1	0.04913	0.00708	6.94	<.0001	9.04616	0.52405
BMI	1	0.00195	0.00804	0.24	0.8090	0.01059	0.01805

Model A: Model PCBs on Lifetime Years Eating Sport Fish (YEARS)

A standard multiple regression was performed modeling SERUMPCB on YEARS while controlling for GENDER, AGE and BMI. Results of evaluation of assumptions led to transformation of the variables to reduce skewness, reduce the number of outliers, and improve the normality, linearity, and homoscedasticity of the residuals. A logarithmic transformation was used on SERUMPCB (t_SERUMPCB=Log10[SERUMPCB]). An exponential transformation was used on YEARS (t_YEARS=[YEARS + 1]**2.8). With the use of Cook's D, visualized on the influence plot, one extreme outlier was identified.

The outlying observation was identified as the subject exhibiting the extreme BMI (ID, 0009-1). This subject was deleted from the analysis data as an extreme outlier (n=128). The model was refitted and the influence plot redrawn (APPENDIX C: MODEL A GOODNESS OF FIT STATISTICS).

A partial residual plot was constructed relating t_SERUMPCB to t_YEARS (Figure 2). A spline with 90% confidence intervals was fitted to this plot and is consistent with a linear relationship between t_SERUMPCB and t_YEARS.

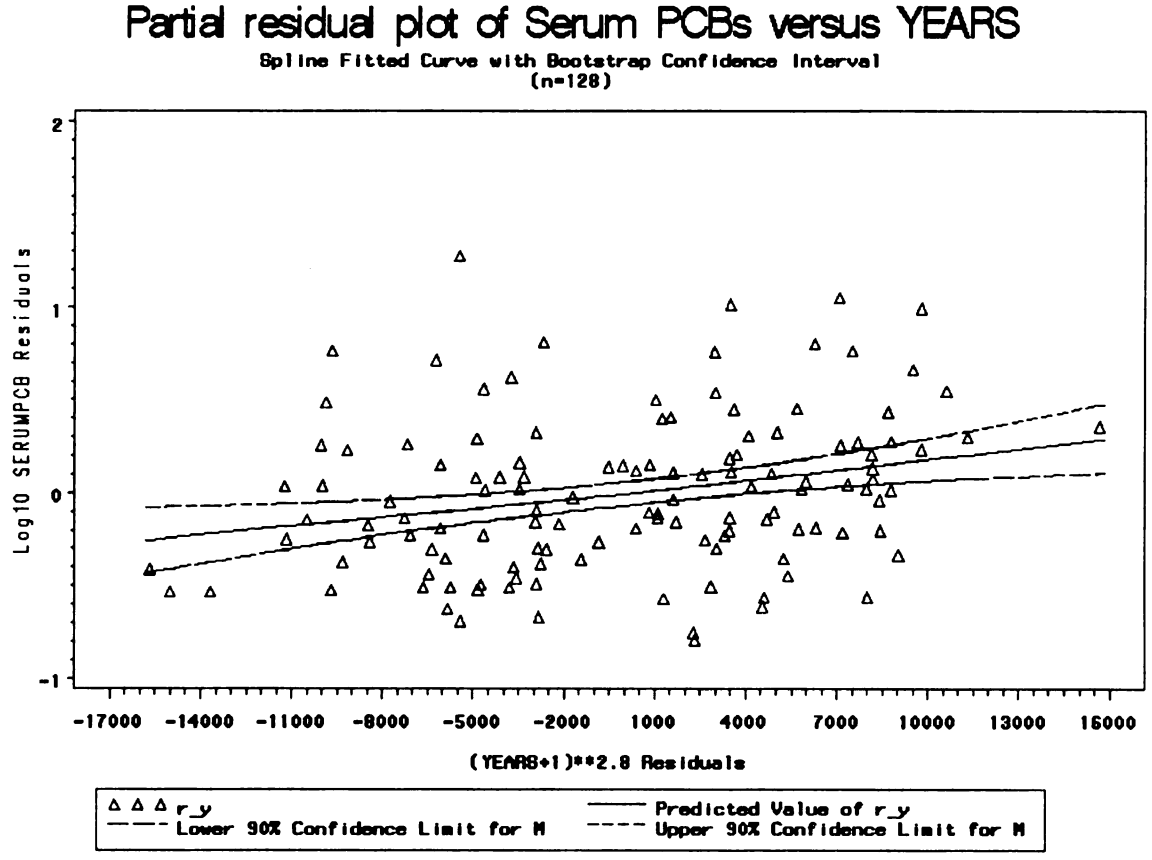


Figure 2. Partial residual plot of SERUMPCB on YEARS, with spline regression line, and 90% confidence intervals, after correcting for GENDER, AGE and BMI.

The t test for t_YEARS added to the model last is statistically significant (t-value=3.01, Pr<0.0032) (Table 5). The R-Square delta indicates that t_YEARS explained an additional 4% of the overall variation in t_SERUMPCB after controlling for gender, age and BMI.

According to the parameter estimation column, the regression equation is given by SERUMPCB = (-1.627 -0.105*GENDER +0.040*AGE -0.001*BMI +0.0000171 *[YEARS+1]**2.8)**10.

The partial-regression coefficient for t_YEARS=b_4=0.0000171*[YEARS+1] **2.8 represents the expected increase in log10 serum total PCB level per unit increase in total years eating sport fish for subjects of the same gender, age and BMI (Table 5).

The standardized estimate for age decreases from 0.524 to 0.427 suggesting that AGE and YEARS are confounded. The standardized estimate for GENDER decreases from -0.179 to -0.100 suggesting that GENDER and YEARS are confounded.

Table 5. ANOVA and parameter estimates for regression of log10 serum total PCB level on gender, age, BMI and YEARS.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.34842	3.58711	21.17	<.0001
Error	123	20.83766	0.16941		
Corrected Total	127	35.18608			

Root MSE	0.41160	R-Square	0.4078
Dependent Mean	-0.05429	Adj R-Sq	0.3885
Coeff Var	-758.19118		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Standardized Estimate	R-Square Delta
Intercept	1	-1.62657	0.31921	-5.10	<.0001	0.37722	0	
gender1	1	-0.10533	0.08197	-1.28	0.2012	3.75943	-0.10001	
age	1	0.04006	0.00750	5.34	<.0001	9.04616	0.42723	
BMI	1	-0.00110	0.00786	-0.14	0.8885	0.01059	-0.01023	
t_YEARS	1	0.00001705	0.00000567	3.01	0.0032	1.53224	0.25776	0.0436

Model B: Model PCBs on Sport Fish Meals in Previous 12 Months (MEALS)

A standard multiple regression was performed modeling SERUMPCB on MEALS while controlling for GENDER, AGE and BMI. A logarithmic transformation was used on SERUMPCB (Log10[SERUMPCB]). An exponential transformation was used on MEALS (t_MEALS=[MEALS + 1]**0.002). With the use of Cook's D, visualized on the influence plot, ID 0009-1 was again identified as an extremely influential outlier and was deleted from the analysis data (n=128). The model was refitted and the influence plot redrawn (APPENDIX D: MODEL B GOODNESS OF FIT STATISTICS).

A partial residual plot was constructed relating t_SERUMPCB to t_MEALS (Figure 3). A spline with 90% confidence intervals was fitted to this plot and is consistent with a linear relationship between t_SERUMPCB and t_MEALS.

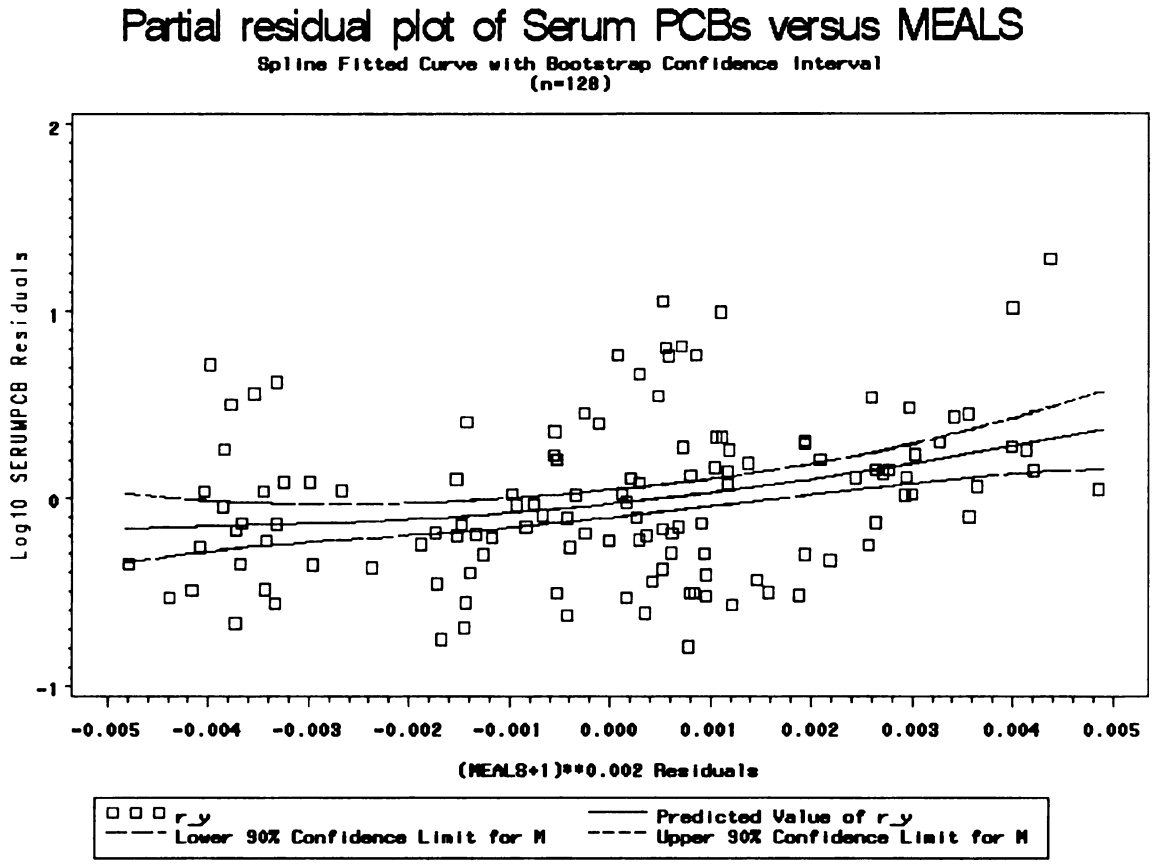


Figure 3. Partial residual plot of SERUMPCB on MEALS, with spline regression line, and 90% confidence intervals, after correcting for GENDER, AGE and BMI.

The t test for t_MEALS added to the model last is statistically significant (t-value=3.27, Pr<0.0014) (Table 6). The R-Square delta indicates that t_MEALS explained an additional 5% of the overall variation in t_SERUMPCB after controlling for gender, age and BMI.

According to the parameter estimation column, the regression equation is given by SERUMPCB = (-53 -0.18*GENDER +0.045*AGE +0.001*BMI+ 51.2*[MEALS+1] **0.002)**10.

The partial-regression coefficient for t_MEALS=b_4=51.20703*[MEALS+1] **0.002 represents the expected increase in log10 serum total PCB level per unit increase in MEALS for subjects of the same gender, age and BMI (Table 6).

The standardized estimate for age decreases from 0.524 to 0.475 suggesting that AGE and MEALS are confounded. The standardized estimate for GENDER decreases from -0.179 to -0.175 suggesting that GENDER and YEARS are not meaningfully confounded.

Table 6. ANOVA and parameter estimates for regression of log10 serum total PCB level on gender, age, BMI and MEALS.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	14.60432	3.65108	21.82	<.0001
Error	123	20.58176	0.16733		
Corrected Total	127	35.18608			

Root MSE	0.40906	R-Square	0.4151
Dependent Mean	-0.05429	Adj R-Sq	0.3960
Coeff Var	-753.52124		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Standardized Estimate	R-Square Delta
Intercept	1	-53.04125	15.67136	-3.38	0.0010	0.37722	0	
gender1	1	-0.18431	0.07664	-2.40	0.0177	3.75943	-0.17500	
age	1	0.04451	0.00697	6.39	<.0001	9.04616	0.47477	
BMI	1	0.00115	0.00775	0.15	0.8820	0.01059	0.01067	
t_MEALS	1	51.20703	15.66452	3.27	0.0014	1.78814	0.23156	0.0509

Model C: Model PCBs on Lifetime Sport Fish Meals (MXY1)

A standard multiple regression was performed modeling SERUMPCB on MXY1 while controlling for GENDER, AGE and BMI. A logarithmic transformation was used on SERUMPCB (Log10[SERUMPCB]). An exponential transformation was used on MXY1 (t_MXY1=[MXY1 + 10]**0.05). With the use of Cook's D, visualized on the influence plot, one extreme outlier was identified (ID, 0009-1). This subject was deleted from the sample on the basis of its extreme BMI reducing the modeled data to 128 subjects. The model was refitted and the influence plot redrawn (APPENDIX E: MODEL C GOODNESS OF FIT STATISTICS).

A partial residual plot was constructed relating t_SERUMPCB to t_MXY1 (Figure 4). A spline with 90% confidence intervals was fitted to this plot. The fitted spline was only approximately linear for the relationship between SERUMPCB and MXY1. However, a more effective transformation was not found.

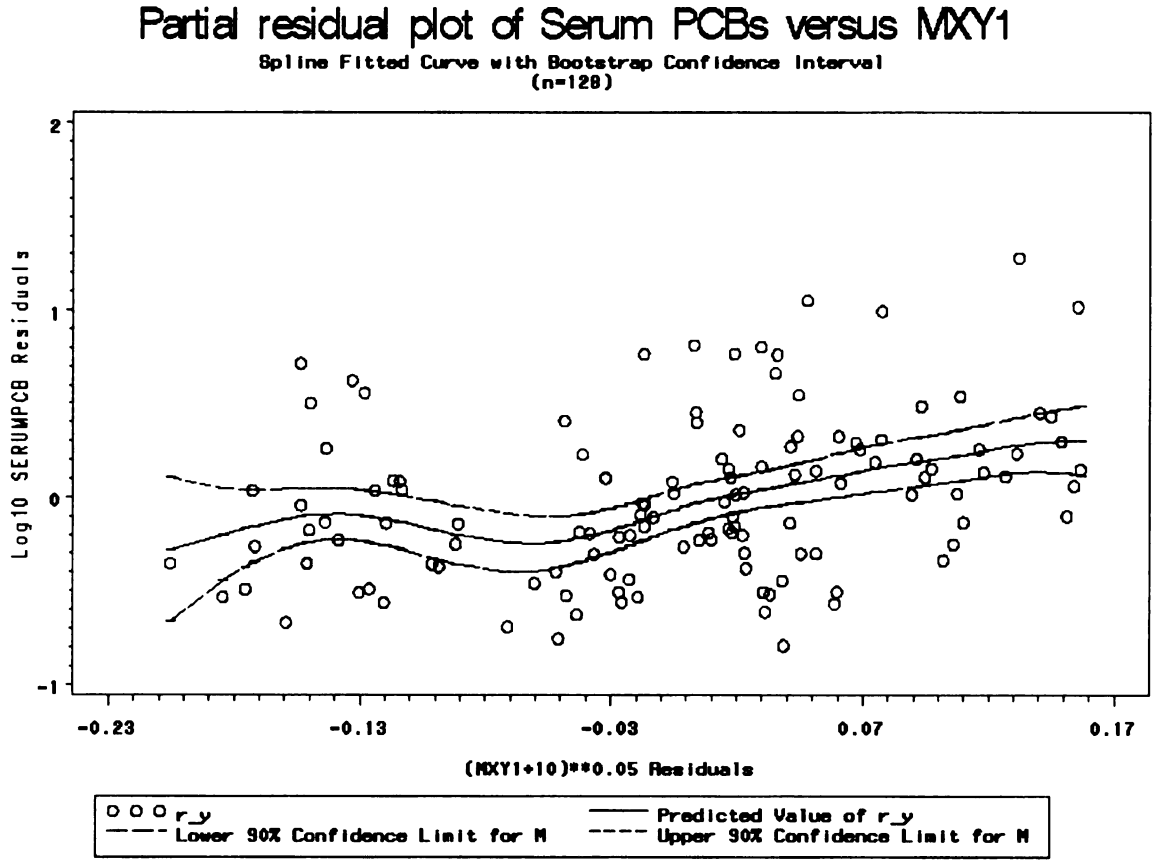


Figure 4. Partial residual plot of SERUMPCB on MXY1, with spline regression line, and 90% confidence intervals, after correcting for GENDER, AGE and BMI.

The t test for t_MXY1 added to the model last is statistically significant (t-value=3.27, Pr<0.0014) (Table 7). The R-Square delta indicates that transformed MXY1 explained an additional 7% of the overall variation in t_SERUMPCB after controlling for gender, age and BMI.

According to the parameter estimation column, the regression equation is given by SERUMPCB = (-3.46 -0.161*GENDER +0.0425*AGE -0.000239*BMI + 1.50202 *[MXY1+10]**0.05)**10.

The partial-regression coefficient for t_MXY1=b_4=1.50202*[MXY1+10]**0.05 represents the expected increase in log10 serum total PCB level per unit increase in MXY1 for subjects of the same gender, age and BMI (Table 7).

The standardized estimate for age decreases from 0.524 to 0.454 suggesting that AGE and MEALS are confounded. The standardized estimate for GENDER decreases from -0.179 to -0.153 suggesting that GENDER and YEARS are confounded.

Table 7. ANOVA and parameter estimates for regression of log10 serum total PCB level on gender, age, BMI and MXY1.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	15.24092	3.81023	23.50	<.0001
Error	123	19.94517	0.16216		
Corrected Total	127	35.18608			

Root MSE	0.40269	R-Square	0.4332
Dependent Mean	-0.05429	Adj R-Sq	0.4147
Coeff Var	-741.77651		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Standardized Estimate	R-Square Delta
Intercept	1	-3.45682	0.52177	-6.63	<.0001	0.37722	0	
gender1	1	-0.16140	0.07577	-2.13	0.0352	3.75943	-0.15324	
age	1	0.04253	0.00693	6.14	<.0001	9.04616	0.45359	
BMI	1	-0.00023899	0.00765	-0.03	0.9751	0.01059	-0.00221	
t_MXY1	1	1.50202	0.38843	3.87	0.0002	2.42474	0.27722	0.0690

Model D: Model PCBs on Modified Lifetime Sport Fish Meals (MXY2)

A standard multiple regression was performed modeling SERUMPCB on MXY2 while controlling for GENDER, AGE and BMI. A logarithmic transformation was used on SERUMPCB (Log10[SERUMPCB]). An exponential transformation was used on MXY2 (t_MXY2=[MXY2+ 10]**0.125). With the use of Cook's D, visualized on the influence plot, ID 0009-1 was again identified as an extremely influential outlier and was

deleted from the analysis data (n=128). The model was refitted and the influence plot redrawn (APPENDIX F: GOODNESS OF FIT STATISTICS).

A partial residual plot was constructed relating transformed SERUMPCB to MXY2 (Figure 5). A spline with 90% confidence intervals was fitted to this plot and is consistent with a linear relationship between SERUMPCB and MXY2.

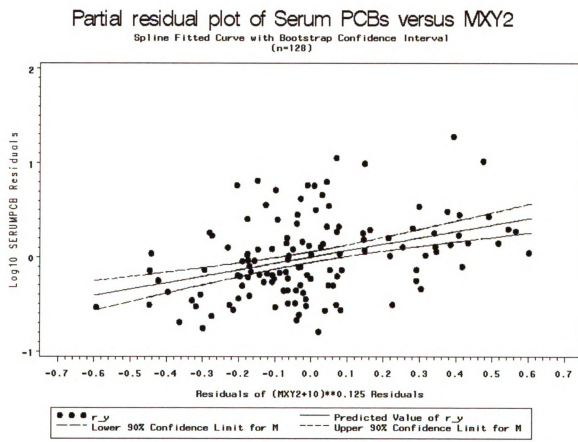


Figure 5. Partial residual plot of SERUMPCB on MXY2, with spline regression line and 90% confidence intervals, after correcting for GENDER, AGE and BMI.

The t test for t_MXY2 added to the model last is statistically significant (t-value=4.64, Pr<.0001) (Table 8). The R-Square delta indicates that transformed MXY2

explained an additional 9% of the overall variation in t_SERUMPCB after controlling for gender, age and BMI.

According to the parameter estimation column, the regression equation is given by SERUMPCB = (-2.90 -.140*GENDER +0.043*AGE -0.000726*BMI +0.684 *[MXY2+10]**0.125)**10.

The partial-regression coefficient for t_MXY2=b_4=1.50202*[MXY1+10]**0.05 represents the expected increase in log10 serum total PCB level per unit increase in MXY1 for subjects of the same gender, age and BMI (Table 8).

The standardized estimate for age decreases from 0.524 to 0.454 suggesting that AGE and MEALS are confounded. The standardized estimate for GENDER decreases from -0.179 to -0.133 suggesting that GENDER and YEARS are confounded (Tables 4 & 8).

Table 8. ANOVA and parameter estimates for regression of log10 serum total PCB level on gender, age, BMI and MXY2.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	4	16.15437	4.03859	26.10	<.0001
Error	123	19.03171	0.15473		
Corrected Total	127	35.18608			

Root MSE	0.39336	R-Square	0.4591
Dependent Mean	-0.05429	Adj R-Sq	0.4415
Coeff Var	-724.59133		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|	Type I SS	Standardized Estimate	R-Square Delta
Intercept	1	-2.89992	0.37825	-7.67	<.0001	0.37722	0	
gender1	1	-0.13964	0.07445	-1.88	0.0631	3.75943	-0.13258	
age	1	0.04323	0.00668	6.47	<.0001	9.04616	0.46104	
BMI	1	-0.00072628	0.00747	-0.10	0.9227	0.01059	-0.00672	
t_MXY2	1	0.68379	0.14721	4.64	<.0001	3.33819	0.32322	0.0949

Comparing Predictors

An overlay plot of the four partial residual plots (Figures 2-5) was constructed relating log10 serum PCB level residuals to standardized predictor residuals with regression lines fitted for all four predictors (Figure 6). Comparing standardized regression coefficients for t_SERUMPCB we observe the following estimates: t_YEARS, 0.25776; t_MEALS, 0.23156; t_MXY1, 0.27722; t_MXY2, 0.32322. These coefficients tell us that the predicted increase in standard deviation units of y that would be expected per standard deviation increase in x, holding GENDER, AGE and BMI constant. Thus the rank order for least to most increase in predictive power is: MEALS, YEARS, MXY1, MXY2.

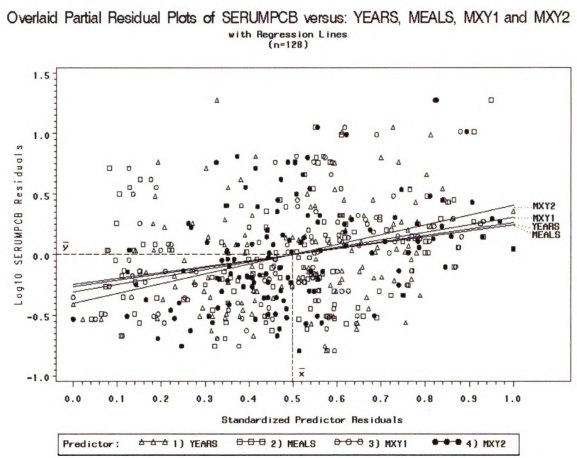


Figure 6. Overlay of partial residual plots, and regression lines, of SERUMPCB on standardized: YEARS, MEALS, MXY1 and MXY2 after controlling for GENDER, AGE and BMI.

The portion of the variance in serum total PCB level explained by individually modeled predictors was YEARS (R-Square delta=4%), MEALS (5%), MXY1 (7%), and MXY2 (9%).

Table 9. Partial regression mean square ratios with 95% confidence intervals for comparing non-nested models (n=128, alpha=0.05).

MS_B/MS_A (CI 95%)	YEARS (MS_A)	MEALS (MS_A)	MXY1 (MS_A)
MEALS (MS_B)	1.08 (0.81, 1.43)		
MXY1 (MS_B)	1.26 (0.95, 1.67)	1.16 (0.88, 1.55)	
MXY2 (MS_B)	1.48 (1.11, 1.96)	1.37 (1.03, 1.81)	1.17 (0.88, 1.56)

Only two pairs of predictors, MXY2 versus YEARS and MXY2 versus MEALS, have root mean square ratios significantly greater than 1 (Table 9). MXY2 explains 1.48 times more variance than YEARS, and 1.37 times more variance than MEALS. MXY2 explained 1.17 time more variance than MXY1 but is not significantly better (Table 9). A general trend was observed for increasing overall explanation of variance from MEALS, YEARS, MXY1 to MXY2.

Predicted Mean Serum PCB Levels with Oversampling

Predicted mean SERUMPCB levels are presented at standardized exposure levels (0.50, 0.75, 0.90) for the four predictors (Table 10). At 0.50, all four predictors share the overall same predicted mean and supports the appropriateness of selected transformations used in the models. At 0.75, average levels are: 1.18 ppb for YEARS, 1.16 ppb for MEALS, 1.23 ppb for MXY1 and 1.37 ppb for MXY2. At 0.90, average levels are: 1.41 ppb for YEARS, 1.37 ppb for MEALS, 1.52 ppb for MXY1 and 1.80 ppb for MXY2.

The ratio of the 0.50 standardized exposure level mean to the higher exposure level means gives the predicted percent increase. At 0.75, the percent increases are: 32% for YEARS, 30% for MEALS, 38% for MXY1 and 53% for MXY2. At 0.90, the percent

increases are: 58% for YEARS, 53% for MEALS, 70% for MXY1 and 102% for MXY2 (Table 10).

Table 10. Predicted adjusted mean serum PCB levels (ppb) with 95% confidence intervals and percent above full sample mean with no oversampling, oversampling top 50%, and oversampling top 20% for four questionnaire-based measures of sport fish consumption (n=128).

	Predicted adjusted mean serum PCB level (ppb) with 95% C.I. and percent increase over full sample		
Predictor	Full sample (midpoint=.50)	Oversampling top 50% (midpoint=.75)	Oversampling top 20% (midpoint=.90)
YEARS	0.89 (0.72, 1.10), 0%	1.18 (0.92, 1.51), 32%	1.41 (0.99, 2.00), 58%
MEALS	0.89 (0.72, 1.10), 0%	1.16 (0.92, 1.46), 30%	1.37 (1.00, 1.87), 53%
MXY1	0.89 (0.72, 1.10), 0%	1.23 (0.97, 1.56), 38%	1.52 (1.09, 2.10), 70%
MXY2	0.89 (0.72, 1.10), 0%	1.37 (1.06, 1.77), 53%	1.80 (1.26, 2.58), 102%

CHAPTER V

DISCUSSION

Applied Epidemiology

Paneth (138) points out the importance of relating measures of response to meaningful public health outcomes. Of similar value is the ability to link characterization of exposure to investigations of dose-response relationships. This is not simply a matter of converting units, or even necessarily quantifying available or ingested dose, since many factors mediate the relationship between a meal of PCB-contaminated sport fish and a hypothetical biologically effective dose. Study designs able to simultaneously relate sport fish consumption to laboratory based measures of the dose and the response are likely to contribute most meaningfully to risk assessment and hazard control. Of the 23 studies reviewed in this thesis (Table 2), only six report both measures of sport fish consumption and biosample based measures. This limits the comparability of existing studies and may partially explain inconsistent findings.

Findings

Discussion of this study's findings will include consideration of theoretical validity, criterion-related validity, sample selection, exposure measurement, questionable criterion, theoretical model, strengths, and limitations.

Theoretical validity. The four measures of sport fish consumption compared in this thesis provide a logical basis for relating reported exposure to actual absorbed dose and a hypothetical biologically effective dose. The thesis findings show that the relative increases in criterion-related validity, with R-Square delta as the criterion, correspond to

theory and suggest that this approach is theoretically valid. Overall, the findings support the general assertion that indexes of cumulative sport fish consumption that account for both rate and duration of exposure are more strongly associated with observed serum PCB level than are measures that account for either rate or duration, but not both.

Thesis findings might be misinterpreted so as to suggest that increasing the criterion-related validity of a measure is simply a matter of constructing more and more complex measures of sport fish consumption. This is a false interpretation, since there is certain to be a threshold at which the theoretical advantage of a more complex measure is overcome by disadvantages of costs, lower participation rates, and the compounding of measurement errors associated with the individual components of more complex measures of exposure.

Criterion-related validity. Comparisons between predictors indicated statistically significant variation in criterion-related validity. Predicted mean serum PCB levels for oversampling (Table 10) would increase the proportion of more highly exposed subjects and result in a 30 to 100% increase in predicted mean serum PCB levels, and provide a meaningful increase in statistical power for detecting exposure-response relationships.

The study findings indicate that the modified index for lifetime meals (MXY2) offers a meaningful improvement over other predictors tested, based upon the proportion of variance explained and predicted values. MXY2 explained a similar amount of variance (R-Square, 9%) as the measure reported by Jacobson et al. (1983) (R-Square, 8%). There are only negligible differences between the relative costs of collecting and calculating each of the four predictors.

Sample selection. The findings support use of questionnaire-based measures of fish consumption as instruments in sampling strategies that screen target populations for more highly exposed individuals. Sampling strategies that target special populations such as charter boat captains, or minority groups, appear to result in more highly exposed study samples. However, such sample strategies may not be appropriate for all study questions. The high positive skew of the distribution of serum PCB level data in the study sample mirrors the general population and illustrates the inefficiency of existing sampling strategies. These predictors may be of use to screen target populations and over sample for individuals more likely to exhibit elevated serum PCB body burdens, or may be used when a researcher lacks sufficient resources, or cannot justify analyzing a full set of biosamples.

Exposure measurement. The relatively small proportion of variation explained (R-Square, 4 to 9%) by the four questionnaire-based predictors of serum PCB level can be attributed to at least four sources, including: 1) other unmeasured sources of PCB exposure; 2) measurement error for serum PCB level; 3) important but unmeasured pharmacodynamic factors; and 3) measurement error for the questionnaire-based predictors.

In data from this thesis, twenty three (18%) subjects reported zero sport fish meals in the previous 12 months. Of those, the average number of years eating sport fish was 16.8, and only one reported never eating sport fish (Table 1). The mean age of these subjects was 34.2 years, so they were actually younger than the mean age for the remainder of the cohort. This highlights a vulnerability of self-reported measures that do not account for lifetime duration of fish consumption. Questionnaire-based measures of

sport fish consumption limited to consumption during the previous 12 months misclassify sport fish consumers as unexposed no matter how much sport fish they consumed in previous years. This is a particular problem considering the participation bias for studies that collect biosamples. Therefore evaluation of criterion-related validity in such samples is questionable.

The findings of this study suggest that strength of association may be subject to excessive exposure misclassification. For example, Courval et al. (93) report findings of an association between male sport fish consumption and reduced time to pregnancy. They based exposure upon the index of lifetime sport fish meals (MXY1). However, my thesis shows this measure misclassifies sport fish consumers who have not eaten sport fish in the previous 12 months.

The findings also suggest that, questionnaire-based measures of fish consumption are, by themselves, poor proxies measures of PCB body burden. With the R-Square deltas explaining no better than 9% of the sample variation in serum total PCB level, this suggests that excessively high measurement error that probably overrides any advantage gained by ease of acquisition.

Questionable criterion. It can be argued that poor criterion-related validity for questionnaire-based measures of sport fish consumption in predicting serum PCB levels is insufficient evidence against their use as exposure measures. A meaningful proportion of variance in serum PCB level is attributable to pharmacodynamic factors that mediate the true cumulative dose. Even though regression models include control variables that approximate pharmacodynamic factors (e.g., Gender and Body Mass Index), it is unlikely that they are entirely effective and a meaningful portion of model error is almost certainly

attributable to this limitation. Given mediation by these factors, it is logical to infer that no measure of cumulative exposure would be highly correlated with an unadjusted endpoint such as point-in-time body burden, and other methods for evaluating the value of questionnaire-based measure of sport fish consumption may be more appropriate.

Theoretical model. Figure 7 illustrates a theoretically plausible scenario consistent with existing research in which neither self-report (*E1*) nor biosample-based (*E2*) measures of exposure are strongly associated with a hypothetical biologically effective dose (*D*). In this scenario, the true strength of association (*DR*) is strongly diluted by measurement error in both measures of exposure. Plausible examples of this scenario include: 1) both *E1* and *E2* are confounded with an unidentified toxic agent, *D*, that contaminates sport fish; 2) the true biologically effective dose is sensitive to timing, resulting in serious measurement error in *E1* and *E2*; or 3) one or both *E1* and *E2* are spuriously associated with the response due to bias.

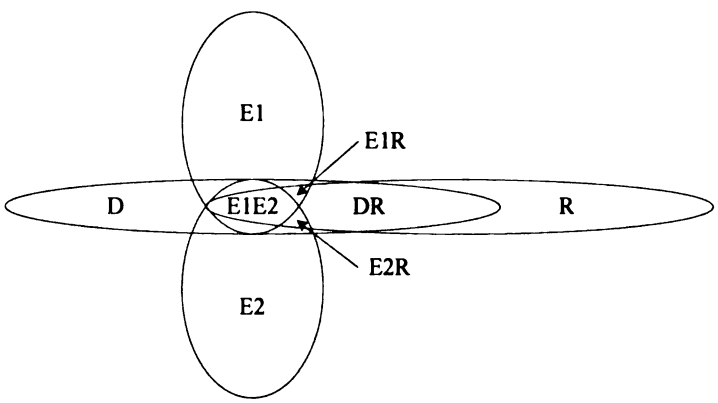


Figure 7. Diagram of a theoretically plausible relationship between self-reported sport fish consumption (E1), biosample based measures (E2), relevant dose (D), and response (R).

Strengths

This study has a number of strengths. This study contributes to the literature on exposure assessment methods for the investigation of sport fish consumption. Four representative questionnaire-based measures of sport caught fish consumption were modeled providing: 1) evaluations of predictive power; 2) between-predictor comparisons; and 3) predictions of performance. In the statistical analysis, special consideration was given to compliance with model assumptions and where appropriate utilized transformations; 4) standardizations to facilitate comparisons between predictors with different units, and 5) an appropriate statistical test for comparing non-nested models; 6) results show cohesive statistical analyses between both nonparametric (Pearson correlation matrix) and parametric (multivariable regression models) procedures; 7) findings are also consistent with theory, in that criterion-related validity was positively associated with improved characterization of rate, duration, and cumulative exposure.

Limitations

There are several limitations with this study: 1) we were unable to test predictive values of indexes of PCB intake that adjust for variation in PCB exposure by species consumed; 2) the use of serum PCB levels unadjusted for lipids probably contributes meaningful measurement error; 3) among the available modeled predictors, no test for model reliability was performed due to insufficient sample size; 4) it is difficult to interpret how confounding of predictors with control variables may affect the validity of the model. However, it is important to remember that the objectives of this thesis relate

to predictive models, and therefore they are not necessarily subject to the same strict requirements as are models aimed at performing causal inference.

The application of these findings to other populations warrants special consideration, since the predictive power of these measures very likely varies with the characteristics of target populations. For example, among the very young, total years probably carries less weight than meals consumed in the previous 12 months, and vice versa, in an older population, total years consuming sport fish probably carries greater weight than meals consumed in the previous 12 months. However, an interaction term for age and meals in the previous 12 months was not statistically significant (Model B adding AGE*t_MEALS, p=0.781).

The study sample does not represent a random sample of the general population. Study sample subjects are self selected from a defined target population. They are know to differ from the general population on demographic and exposure related characteristics and may respond differently to self-reported information. Considering these limitations, findings from this study can not be directly extrapolated to other populations but may be considered suggestive.

Implications for Future Research

This study identifies sources of substantial measurement error in many existing studies that could account for the observed weak associations between exposure and response. Similarly, if observed exposure-response relationships are indeed causal, the true biologically effective dose-response relationship could be much stronger than observed due to measurement error related dilution of the strength of association. If

future research is able to more effectively control these sources of error then it is plausible that causal inferences may be made with much more confidence.

Comparability. Between study differences in methodologies limit comparability and interpretation of the consistency of findings. It would be advantageous if future research reported comparable measures of exposure. Given the limitations and misinterpretation of existing literature, it would be of benefit for studies to adopt a minimum standard for exposure measures based upon self-reported sport fish consumption. Future research should consider adopting an approach that allows evaluation of the predictive power and reliability of their sampling strategies.

Large sample size random digit dial telephone surveys are often used at the state level to assess the exposure level of the general population. These surveys are also performed to investigate the effectiveness of fish consumption advisories and to assess the economic impact of sports fishing.

Nested study design. Given all the special considerations (e.g., oversampling, assessing differential participation bias, and desire for external validity), a possible study design that takes best advantage of this approach is a design that nests screening of the general population within a large scale fish consumption survey. The Charter Boat Captains Cohort is the only existing cohort to utilize a nested study design (109). In a nested design, interviewees who score highest on the measure of sport fish consumption could then be recruited into the exposure-response study. This study design may act to limit recall bias since subjects would response to the fish consumption survey would be unaware that they may be contacted later for information about outcomes. Such a study design offers several potential strengths: 1) Nesting sample selection with a survey

contacts a large population for oversampling; 2) allows assessment of non-participants that permit evaluation of non-differential participation bias; 3) combining resources is more cost effective.

Conclusions

The confidence with which causal inferences may be made is limited in this body of research. Limitations include problems of weak associations, vulnerability to bias, loss of sensitivity, measurement error resulting in excessive exposure misclassification, and dilution of strength of association. One strategy to address these limitations, in future research, is to identify measures of exposure that reduce measurement error. This study investigated four common predictors. In a population of Great Lakes Fisheaters, the index of modified lifetime sport fish meals (MXY2) is a simple and effective predictor of human serum total PCB levels that offers a modest but meaningful improvement over other predictors considered including lifetime years eating sport fish (YEARS), sport fish meals in the prior 12 months (MEALS), or the index of lifetime sport fish meals (MXY1). These findings are consistent with theory, but no test for reliability was performed due to insufficient data, and no validation has yet been performed on other sources of data.

CHAPTER VI

APPENDICES

APPENDIX A: SOURCE QUESTION FOR YEARS

L.3. I will read a list of age groups. Please tell me at which ages you ate sport-caught fish from any of Michigan's Great Lakes and tributaries. **Answer yes only if you ate at least one Great Lakes sport-caught fish meal each year while you were in that age group. Did you eat any sport-caught fish when you were...**

(CIRCLE ANY THAT APPLY. DO NOT ASK ABOUT PERIODS BEYOND RESPONDENT'S AGE)

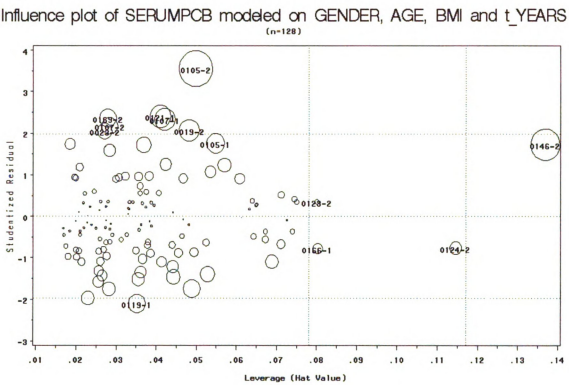
	No	***Yes***
a. 5 – 9 yrs old	0	1
b. 10 - 14 yrs old	0	1
c. 15 - 19 yrs old	0	1
d. 20 - 24 yrs old	0	1
e. 25 - 29 yrs old	0	1
f. 30 - 34 yrs old	0	1
g. 35 + yrs old	0	1

APPENDIX B: SOURCE QUESTION FOR MEALS

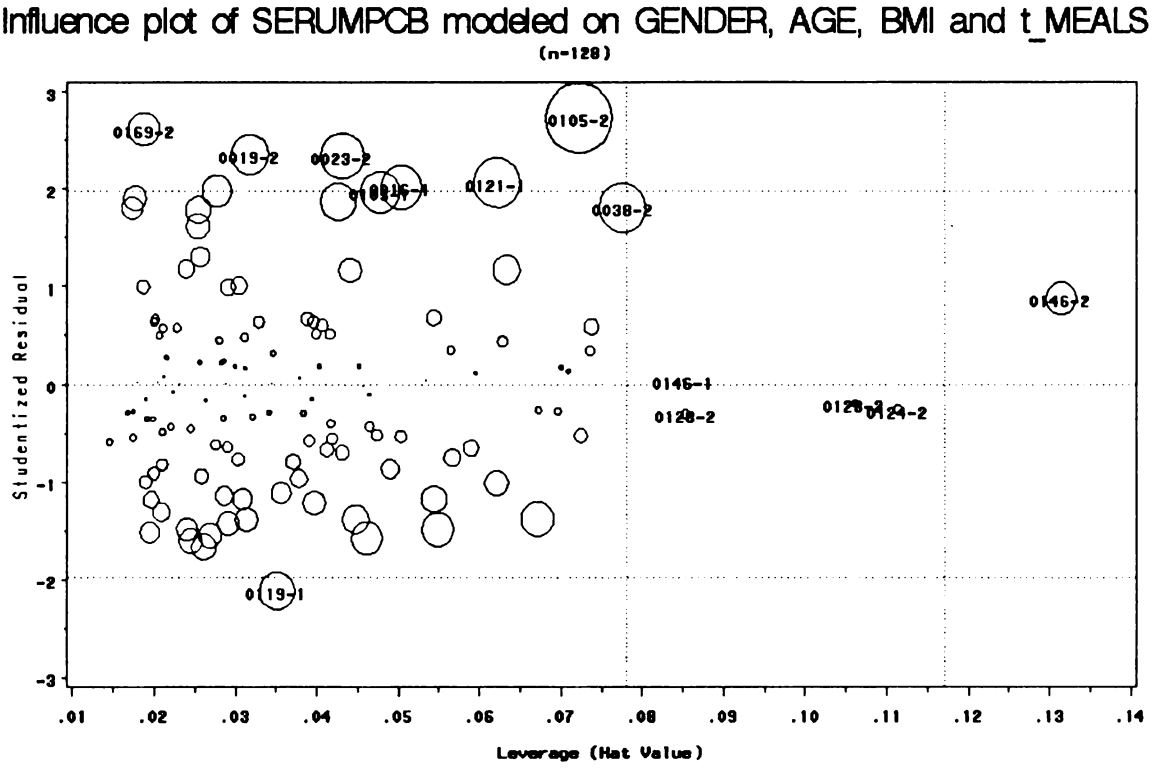
L.4. Please describe your usual **sport-caught fish** consumption during each of the past 12 months. Considering only fish that you, a family member, or an acquaintance caught in Michigan waters; how many meals did you eat in the three months of...

	None	*1 to 3 meals*	*4 to 6 meals*	*7 to 9 meals*	*10 to 12 meals*	*13 or more*
April, May, and June						
July, August, & September						
October, November & December						
January, February, & March						

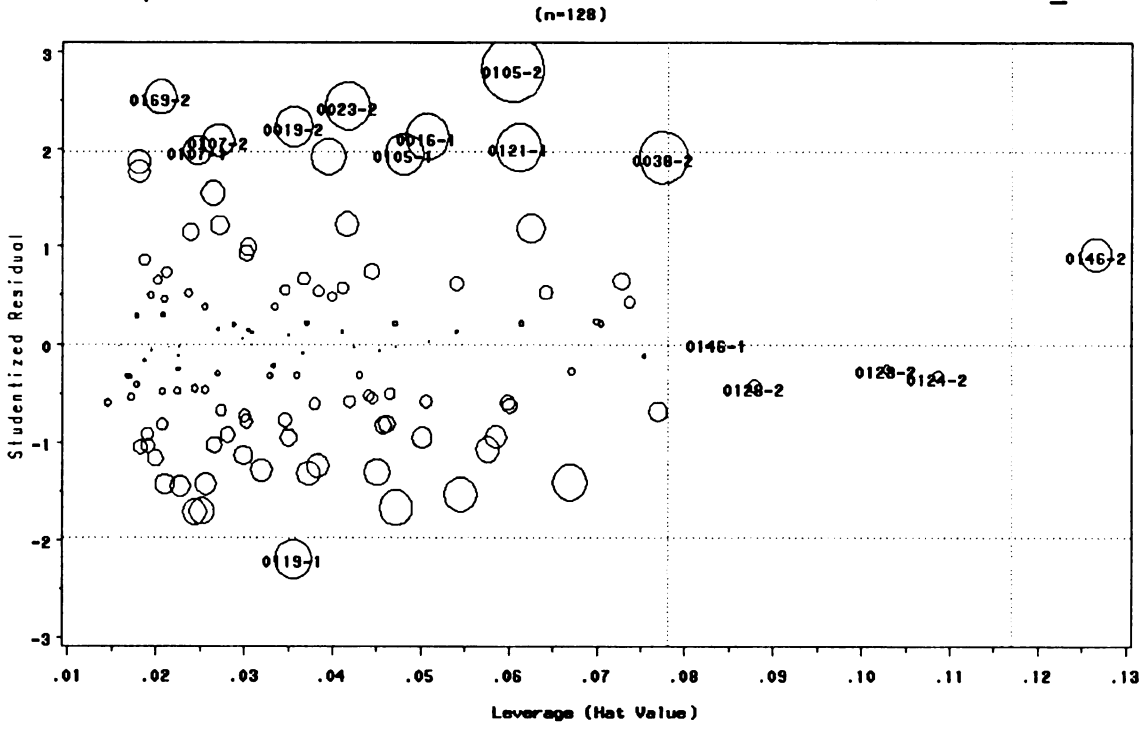
APPENDIX C: INFLUENCE PLOT FOR MODEL A



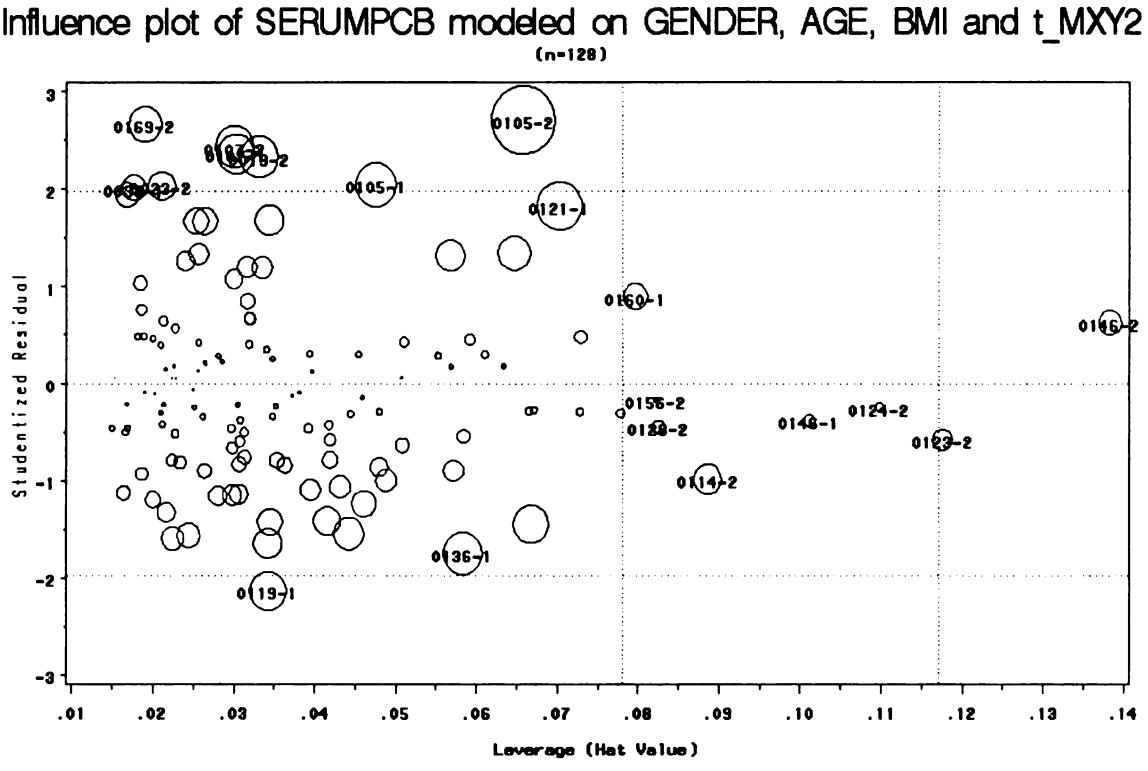
APPENDIX D: INFLUENCE PLOT FOR MODEL B



APPENDIX E: INFLUENCE PLOT FOR MODEL C



APPENDIX F: INFLUENCE PLOT FOR MODEL D



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