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High serum PCBs are associated with elevation of serum lipids and cardiovascular disease in a Native American population ☆

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Abstract

We have investigated the relationships among the concentration of total serum polychlorinated biphenyls (PCBs), various PCB congener groupings, and three pesticides to total serum lipids in humans with and without self-reported cardiovascular disease. Blood samples were obtained from 335 adult Akwesasne Mohawks, and were analyzed for 101 PCB congeners, mirex, dichloro-diphenyl-dichloro-ethylene (DDE), and hexachlorobenzene (HCB), as well as serum triglycerides and cholesterol. Structural equation modeling, following the definition of latent variables by means of confirmatory factor analysis, was used to analyze the relationships between serum lipids with PCBs and heart disease. There were significant associations among PCBs, lipids, age, and body mass index (BMI), a fact which justified the application of the structural equation model. Gender of the participant was unrelated to any of the remaining study variables. The results of this study are consistent with a model in which age is considered as *both* an exogenous explanatory variable *and* a biological driving mechanism for the acquisition of PCBs. Moreover, the results of this study are consistent with the conclusion that PCBs, acting through P450 enzymes, are directly responsible for increased synthesis of cholesterol and triglycerides, substances known to be major risk factors for cardiovascular disease.

Keywords: Cholesterol; Triglycerides; Hypertension; Age; BMI; Superfund sites

1. Introduction

Cardiovascular disease is the most common cause of adult mortality in developed countries. The major risk factor for cardiovascular disease is elevated serum lipids, secondary to a variety of factors including diet, lack of exercise, genetic factors, gender, smoking, hormone levels and stress (Basu et al., 2005; Cohn and Colucci, 2006; Kratz, 2005; Poirier et al., 2006). However, exposure to certain environmental agents can also increase the risk of cardiovascular disease (Dalton et al., 2001; Konig et al., 2005; Navas-Acien et al., 2005).

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There are a number of reports indicating that exposure to persistent, fat-soluble chlorinated organics, such as dioxins, furans, polychlorinated biphenyls (PCBs) and chlorinated pesticides, including dichloro-diphenyl-trichloroethane (DDT) and its metabolite, dichloro-diphenyl-dichloro-ethylene (DDE), is associated with an elevation of serum lipids. For instance, Bell et al. (1994) exposed non-human primates to PCBs and found an elevation in triglycerides and a decrease in the "good" HDL-cholesterol. Likewise, an elevation in serum lipid levels has been reported in several studies of workers exposed to PCBs and/or dioxin (Baker et al., 1980; Chase et al., 1982; Martin, 1984). Moreover, an elevation in rates of cardiovascular disease and stroke in populations of individuals who have been exposed to high levels of dioxin and PCBs has also been reported (Dalton et al., 2001; Gustavsson and Hogstedt, 1997; Tokunaga et al., 1999).

Because PCBs, dioxins, and chlorinated pesticides are fat-soluble, there is the possibility of significant confounding with serum lipids. A question remains as to how this apparent PCB–lipid relationship can be analyzed: specifically, are these variables confounded, or are they part of a discernible causal structure? Does exposure to dioxin or PCBs cause an elevation in level of serum lipids, or does an elevation in serum lipids, due to other causes, lead to only an apparent elevation in the serum levels of dioxins and PCBs? In addition, the nature of the relationships between PCBs and cardiovascular events, and between serum lipids and cardiovascular events, evaluated on the same set of data, merits investigation. This is particularly true since there is some evidence that exposure to PCBs is associated with excess mortality due to cardiovascular events (Gustavsson and Hogstedt, 1997).

We obtained measurements of serum levels of PCBs and three pesticides, and serum levels of cholesterol and triglycerides, from 335 Native Americans of the Mohawk Nation who reside at Akwesasne. We analyzed these complex relationships in what we believe to be a theoretically defensible, mediated structural equation model, to demonstrate that elevated PCBs increase the synthesis of serum lipids, the latter in turn being significantly associated with elevated risk in cardiovascular disease.

Akwesasne is a Mohawk community of approximately 12,000 persons at the juncture of New York State, and the Provinces of Ontario and Quebec. The Mohawks were a traditional fish-eating community before local fish became highly contaminated with PCBs as a result of discharges from three local aluminum foundries, operated by General Motors, ALCOA, and Reynolds Metals, and located immediately upstream from the Reservation. PCBs, used as hydraulic fluids at these foundries, leaked and were washed into the St. Lawrence River and tributaries, which comprised the local fishing grounds. Prior to advisories against consumption of local fish, there was a significant relationship between levels of PCBs in breast milk and rates of fish consumption (Fitzgerald et al., 2004; Hwang et al., 1996). Consumption of local fish has declined, but it has not stopped completely. Furthermore, PCBs are highly persistent compounds. Average PCBs levels in the Mohawks (4 ppb) (DeCaprio et al., 2005) remain somewhat higher than those levels found in the overall US population lacking unusual PCB exposure (reported to be 0.9–1.5 ppb; ATSDR, 2000). The persistence of each PCB congener and their relative contribution to the body burden varies with chemical structure (Shirai and Kissel, 1996). Highly chlorinated congeners and those with *para-* (i.e., 4,4′-) chlorine-ring substitution generally persist longer, whereas less highly chlorinated congeners are more rapidly metabolized.

The objectives of this research were: (1) to determine the relationship between contaminant concentrations and serum lipids; (2) to determine which PCB congeners or pesticides are responsible for this relationship; (3) to determine the relationships between PCBs and self-reported heart disease in this population; and (4) to test a theoretical, mediated structural model of the complex relationships among PCBs, serum lipids, and heart disease. We tested alternative theoretical models in order to assess the possible causal ordering among these variables. The manner in which we analyzed the data accommodates the roles of both PCBs and lipids on a selected disease endpoint, thereby removing the necessity to decide between the use of either wet weight or lipid-adjusted contaminant levels in studies of this type.

2. Materials and methods

2.1. Study population and blood chemistry analytic procedures

Randomized cluster sampling techniques were utilized to collect samples and are described elsewhere (Santiago-Rivera et al., 2007; Schell et al., 2003). After informed consent was obtained, fasting blood samples (5 mL for serum cholesterol and triglycerides and 10 mL for organochlorines) were obtained from 335 adult Mohawks who lived on or near the Akwesasne Reservation in 1995–2000. The tubes were left at room temperature for 1 h to clot, and were then centrifuged; the serum removed and stored on site at −80 °C until shipment to the laboratories.

Organochlorines were extracted and analyzed using a dual column gas chromatograph with electron capture detection as described by DeCaprio et al. (2000), with analysis of 101 congeners (83 individual congeners and 18 congeners as pairs and triplets) and three pesticides [hexachlorobenzene (HCB), DDE, and mirex]. The method detection limit (MDL) for individual congeners ranged from 0.01 to 0.15 ppb (median, 0.02 ppb, where 1 ppb = 1 ng/g). All results were calculated with values below the MDL set to $\frac{1}{2}$ MDL unless otherwise indicated.

In addition to total PCBs and three pesticides, we distinguished eight groups of PCB congeners based on the knowledge of their differing structural properties and biological activities. These are: (1) dioxin-like congeners, which includes 11 non-*ortho* and mono-*ortho* congeners (77, 105, 114, 118, 156, 170, 180, 147 + 109, 123 + 149) that bind to the aryl-hydrocarbon receptor (AhR) and activate CYP 1A1/2 (referred to in the figures as "PCB 1"); (2) total toxic equivalents determined as the sum of the products of the concentration and toxic equivalent factor for each of the major dioxin-like congeners, namely: 77(0.0005), 105(0.0001), 114(0.0005), 118(0.0001), 123(0.0001), 156(0.0005), 170(1), and 180(0.00001), referred to as "PCB 2"; (3) phenobarbitol-type congeners, consisting of 5 di-*ortho* congeners (99, 153, 180, 183, 203) which induce CYP 2B1/2 ("PCB 3"); (4) estrogenic congeners, a mixture of six mono

and di-*ortho* congeners (44, 52, 70, 177, 187, 201) which directly or through metabolites mimic estrogen and share affinity to the E_2 receptor ("PCB 4"); (5) the sum of two very persistent non-coplanar, di-*ortho* congeners (138,153) with essentially no activity to AhR but with ability to induce CYP2B ("PCB 5"); and the sum of (6) mono-*ortho* congeners (1, 6, 7, 8, 9, 22, 25, 26, 28, 29, 31, 33, 56, 63, 66, 67, 70, 74, 105, 114, 118, 156) ("PCB 6"), (7) di-*ortho* congeners (4 + 2, 10, 17, 18, 19, 24 + 27, 32 + 16, 40, 42, 44, 47 + 59, 49, 52, 64, 71, 83, 87, 90 + 101, 92, 97, 99, 110, 128, 129, 130, 137, 163 + 164 + 138, 141, 146, 153, 158, 170, 172, 180, 190, 194) ("PCB 7") and (8) tri- and tetra-*ortho* congeners (45, 46, 51, 53, 84, 91, 95, 132, 134, 136, 144, 151, 171, 174, 176, 177, 179, 183, 185, 187, 195, 196, 199, 200, 201, 203, 206) ("PCB 8").

Cholesterol and triglyceride values were measured enzymatically by the Clinical Chemistry Laboratory of the New York State Department of Health using a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis, IN, USA). Total cholesterol was determined as described by Allain et al. (1974). Triglycerides were determined by a glycerol kinase-based procedure that corrects for free glycerol in the specimen (Kohlmeier, 1986), as recommended by the National Cholesterol Education Program Working Group on Lipoprotein Measurement (Stein and Myers, 1995). The laboratory is approved by the Clinical Laboratory Improvement Amendments and is a member of the CDC reference laboratory network for lipid measurements (Myers et al., 2000). Total serum lipids were calculated using the "short" formula proposed by Phillips et al. (1989) and recently validated by the same group (Bernert et al., 2007).

2.2. Statistical analysis

Statistical analyses proceeded in several descriptive and inferential steps. For the 277 participants with complete data, we calculated descriptive statistics (1) on the characteristics of the study participants, (2) on the 17 PCB congeners present in at least 75% of individuals at levels >MDL and the three pesticides, and (3) on all of the independent, dependent, and control variables measured. For inferential tests of hypotheses we used structural equation modeling and path analysis on latent variables (Bollen, 1989). In addition to the PCB congeners, the analyses included age, gender, body mass index (BMI), and serum lipids, based on cholesterol and triglyceride concentrations. Log transformations were implemented for PCBs and pesticides prior to analysis. SAS version 9.1 (SAS Institute Inc., Cary, NC, USA) and SPSS version 15 (SPSS, Inc., 2006) were used for basic statistical analysis. An alpha level of 0.05 was considered to be significant in all statistical tests. Unadjusted, bivariate correlation coefficients with *p*-values were computed and examined for all of the variables of interest. We included the variables of age, BMI, and gender as potential confounders, since they were associated with both individually measured indicator outcome variables and exposure variables at $p < 0.20$.

2.2.1. Statistical analysis of the mediated structural model

The statistical analyses performed on these data (1) began with a confirmatory factor analysis to establish the existence the latent variables of age, gender, BMI, PCBs (LV_{PCBs}), lipids (LV_{LIPIDS}), and cardiovascular disease (LV_{CVD}), (2) were followed by a multivariate multiple regression analysis on latent variables, and (3) concluded with a mediated structural equation model assessing the mediating role of lipids in the relationship between PCBs and cardiovascular disease (Bollen, 1989; Joreskog and Sorbom, 1993; Kline, 2004; Hayduck, 1987; Muthen, 1992). Prior to the analysis of the mediated model, we assessed the correlations among the latent variables of age, gender, BMI, PCBs (LV_{PCBs}), lipids (LV_{LIPIDS}), and cardiovascular disease (LV_{CVD}), in order to establish the preconditions on which the assessment of mediation rests (Baron and Kenny, 1986). All structural equation model analyses were performed with LISREL 8.8 (Joreskog and Sorbom, 1993) and AMOS 4.0 (Arbuckle and Wothke, 1999). The procedures for establishing the scales of the latent variables and allowing for correlated errors among the indicators of LV_{PCBs} are described by Hayduck (1987). The goodness of fit of the models to the data was evaluated by reference to the χ^2-test and several goodness-of-fit indices (CFI, NNFI, RMSEA, and SRMSR) as recommended by Bollen (1989), Hu and Bentler (1999), and McDonald and Ho (2002). We fitted the confirmatory factor model, the multiple regression model on latent variables, and the mediated structural equation model on latent variables by the method of maximum likelihood with standard errors of the parameters estimated by both maximum likelihood (Bollen, 1989) and bootstrap methods (Efron and Tibshirani, 1993). Both standardized and unstandardized parameter estimates were computed, and critical ratio tests of the statistical significance of the parameter estimates were evaluated by the maximum likelihood asymptotically normally distributed *t*-test (Joreskog and Sorbom, 1993) as well as by the bootstrap critical ratio (Efron and Tibshirani, 1993; Arbuckle and Wothke, 1999; Yung and Bentler, 1996). The bootstrap estimates were evaluated so as to accommodate the non-normal distributional properties of parameters estimates associated with the binary measured indicators of heart disease and high blood pressure.[3]

Table 1
Descriptive statistics of the study variables and characteristics of participants ($n = 277$)

Variable	Units	Mean ± SE	Median	Range
Age	Years	38.4 ± 0.82	36.0	18–79
Gender	Female	66 ± 3%	1.00	0–1
BMI	kg/m^2	29.9 ± 0.4	29.2	14.6–54.9
Cholesterol	mg/dL	191 ± 2.3	191	105–410
Triglycerides	mg/dL	144 ± 5.4	121	30–581
Heart disease	Yes	7 ± 2%	0	0–1
High blood pressure	Yes	23 ± 2.5%	0	0–1

3. Results

Descriptive statistics on the study variables and characteristics of the participants are presented in Table 1. The 277 participants for whom complete data existed ranged from 18 to 83 years in age (mean = 39) and comprised 183 females (66%) and 94 males (34%). The average value for the sum of PCB congeners in this cohort was 4.2 ppb (range = 1.2–25.8). While this range of values is relatively low for an exposed group, they are approximately three times the average background level range for persons without unusual exposure in the US (reported to be 0.9–1.5 ppb; ATSDR, 2000). The average values of cholesterol and triglycerides are not extreme for a population of this age range, but the population as a whole is relatively overweight. Table 2 shows total PCB levels and levels of each individual congener present in at least 75% of individuals. We also show total PCB

[3]In addition to the analyses reported here, we performed a series of univariate regression analyses aimed at establishing the fact the ordinary least-squares regression model applied to dichotomous outcome variables would provide conclusions similar to those from a logistic regression analysis of the same variables. Results of these analyses, as well as the means, standard deviations, and full correlation matrix of the 18 measured indicators used in this research can be found at ⟨www.albany.edu/ihe/lipidpaper⟩. In addition more extensive discussion of the underlying rationale for considering age to have separable chronological and biological features can be found at that website, as can more expository discussions of the mechanics of fitting the structural equation models discussed in this paper.

Table 2
Serum PCB congener concentrations (ppb) for all congeners and pesticides found above the MDL in at least 75% of the cohort[a]

	Median	Mean ± SE	Range	Percent > MDL
Total PCBs (sum of 101 congeners)				
Total PCBs (MDL = 0)	2.35	3.54 ± 0.19	0.29–25.18	100
Total PCBs (MDL = $\frac{1}{2}$)	2.94	4.23 ± 0.19	1.21–25.79	100
Total PCBs (MDL = 1)	3.55	4.88 ± 0.18	2.06–26.36	100
Congeners				
PCB–74	0.09	0.25 ± 0.02	< MDL–2.73	97.0
PCB–87	0.04	0.05 ± 0.001	< MDL–0.17	93.7
PCB–90 + 101	0.05	0.07 ± 0.002	< MDL–0.29	98.5
PCB–99	0.12	0.20 ± 0.01	< MDL–1.96	95.8
PCB–105	0.03	0.06 ± 0.005	< MDL–0.96	83.3
PCB–110	0.05	0.07 ± 0.003	< MDL–0.35	97.0
PCB–118	0.14	0.25 ± 0.02	0.02–2.85	100
PCB–146	0.04	0.07 ± 0.005	< MDL–0.64	87.2
PCB–163 + 164 + 138	0.26	0.44 ± 0.02	0.04–3.61	100
PCB–153	0.32	0.51 ± 0.03	< MDL–3.79	99.4
PCB–156	0.04	0.08 ± 0.006	< MDL–0.86	75.5
PCB–170	0.05	0.09 ± 0.005	< MDL–0.79	88.3
PCB–177	0.02	0.03 ± 0.002	< MDL–0.31	77.0
PCB–180	0.21	0.36 ± 0.02	< MDL–2.60	99.7
PCB–183	0.03	0.04 ± 0.002	< MDL–0.35	84.2
PCB–187	0.07	0.14 ± 0.009	< MDL–1.36	94.9
PCB–199	0.04	0.09 ± 0.006	< MDL–0.68	90.1
Pesticides				
HCB	0.06	0.07 ± 0.002	< MDL–0.26	99.1
DDE + 85	1.25	2.60 ± 0.18	0.08–22.51	100
Mirex	0.06	0.09 ± 0.006	< MDL–0.68	78.5

[a]The mean and median were calculated with values below the MDL set to 1/2 MDL, except as indicated.

levels calculated when values below the MDL were considered to be equal to 0, 1/2 the MDL, and the MDL. All other analyses were done with values below the MDL set to 1/2 MDL.

Fig. 1 shows the number of individuals from this sample with total serum lipid levels above or below the mean, in relation to quintiles of total wet weight PCBs. The distribution indicates that individuals with higher levels of PCBs tend to have higher levels of total serum lipids. This analysis, however, does not allow us determination of whether the elevated serum lipids are a consequence of the elevated levels of PCBs or the elevated wet weight PCB values are a consequence of elevated serum lipids, since all of the PCBs are presumed to be in the lipid fraction. Therefore, we tested the hypothesis that elevated PCBs induce elevated serum lipids and cardiovascular disease by application of confirmatory factor analysis and mediated structural equation models.

3.1. Confirmatory factor analysis (CFA) to establish the latent variables

The CFA model was evaluated on the variance–covariance matrix of the 18 measured indicator variables of age, gender, BMI, cholesterol, triglycerides, eight PCB subscores, three pesticides and the endpoint variables of heart disease and high blood pressure. This model defines the latent variables of PCBs, lipids, and cardiovascular disease with multiple indicators for each latent variable, while the latent variables of age, gender and BMI are defined by single indicator variables. Fig. 2 shows the theoretical and empirical relationship between the indicators and their respective latent variables, as well as the correlations among the latent variables. The model parameters and standard errors were estimated by maximum likelihood. Standard errors were also estimated by bootstrap methods to accommodate the potential non-normality of the underlying distributions (Efron and Tibshirani, 1993). By contemporary standards (Hu and Bentler, 1999; McDonald and Ho, 2002) the CFA solution fits the data well ($\chi^2_{(67)} = 232.63$, $p < 0.01$, $\chi^2/\mathrm{df} = 3.5$, CFI = 0.978, NNFI = 0.955, RMSEA = 0.095, SRMSR = 0.07) and we can conclude that the latent variables are well defined. All of the factor loadings of the measured indicators are strongly related to their respective latent variables, and all are statistically significant by both the normal theory *t*-test and the bootstrap critical ratio. It must be noted that none of the PCB subgroups or any of the three pesticides show values that are very different.

The correlations among the latent variables are presented in Table 3. With the exception of its correlation with BMI ($r = -0.169$) gender was statistically *un*related to any of the other variables in the model. Thus it was dropped from any further consideration and does not appear in any

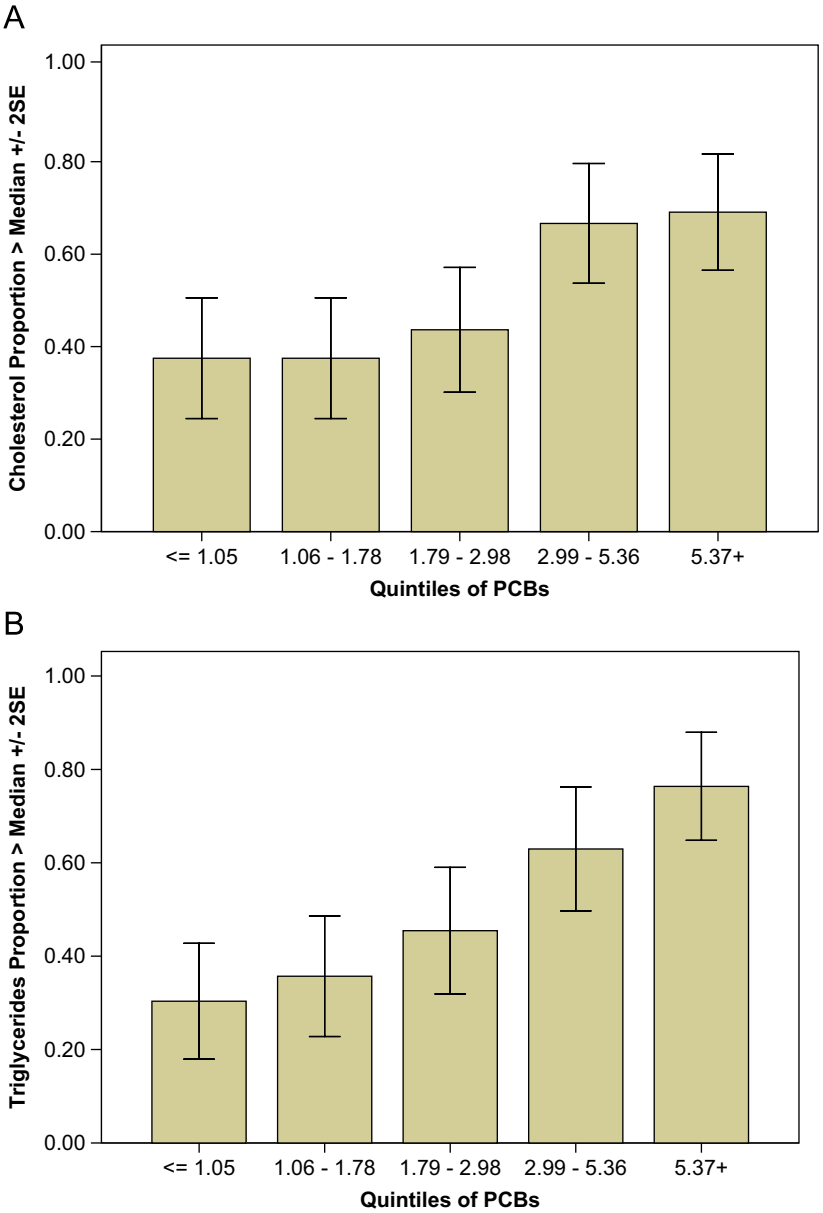

Fig. 1. Cholesterol (A) and triglycerides (B) plotted by quintiles of serum PCB levels in relation to lipid levels above the median. These data are from 299 measures of cholesterol and 304 measures of triglycerides. The mean cholesterol level was 191, while the median was 189. The mean triglyceride level was 144, and the median was 120.

subsequent analysis. The Baron and Kenny (1986) rules for establishing the validity of a mediated model requires that the zero-order correlations among the exogenous, mediator, and endogenous endpoint variables must all be independently substantial and significantly different from zero. Given our hypothesis that the eff ect of PCBs on cardiovascular disease is mediated by increases in serum lipids, it is imperative that the correlations between PCBs and lipids, between PCBs and cardiovascular disease, and between lipids and cardiovascular disease be statistically different from zero. From the CFA analysis the three correlations among these latent variables are substantial and statistically significant: $r_{\text{PCBs.lipids}} = 0.47$, $r_{\text{PCBs.cardiovascular disease}} = 0.57$, $r_{\text{lipids.cardiovascular disease}} = 0.49$ (all p-values <0.001) and the Baron and Kenny (1986) rules are satisfied with respect to the hypotheses that involve mediation.

3.2. Multiple regression model on latent variables

Even though a significant correlation exists between latent variables of PCBs (LV_{PCBs}) and cardiovascular disease (LV_{CVD}) in these data, the variables of age, BMI, and serum lipids (LV_{LIPIDS}) must be considered as potential confounds. Fig. 3 shows a standard multiple regression model on latent variables in which LV_{CVD} was regressed on LV_{PCBs}, LV_{LIPIDS}, age, and BMI. While the overall fit of the regression model is good (the goodness-of-fit statistics

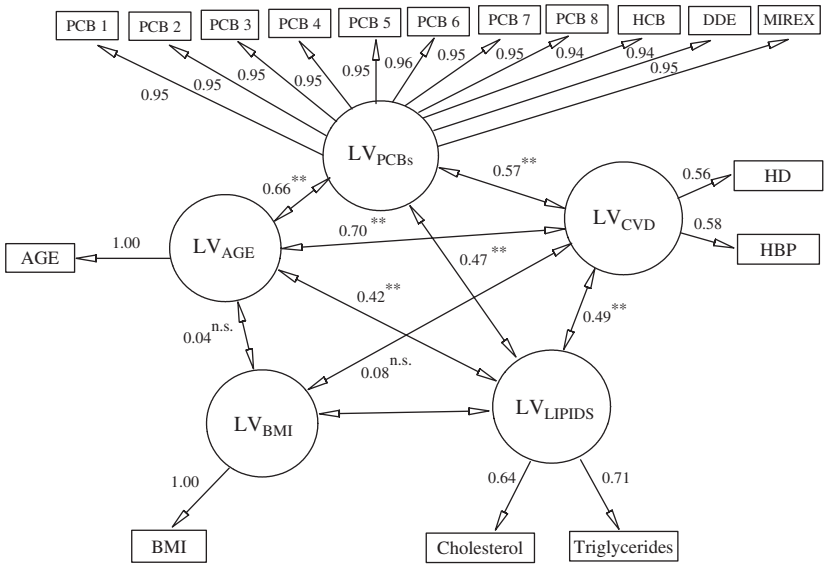

Fig. 2. The measurement model correlations among latent variables. The factor loadings of the measured indicators to the latent variables LV_{PCBs}, LV_{LIPIDS}, and LV_{CVD} are all substantial and significantly different from zero ($p<0.001$). Gender is not significantly related to LV_{PCBs}, LV_{LIPIDS}, and LV_{CVD} and therefore cannot be a confound; it is not included in this or succeeding models. Note that there are only minor differences among the various PCB congener groups and pesticides, whose composition is defined in the Methods section (HD = heart disease; HBP = high blood pressure).

Table 3
Correlations among the latent variables

	Age	Sex	BMI	LV_{PCBs}	LV_{LIPIDS}	LV_{CVD}
Age	1.00					
Sex	0.005	1.00				
BMI	0.038	−0.169	1.00			
LV_{PCBs}	0.661	−0.058	0.058	1.00		
LV_{LIPIDS}	0.423	−0.093	0.256	0.474	1.00	
LV_{CVD}	0.698	−0.012	0.077	0.565	0.494	1.00

A Pearson correlation value of $r \geqslant 0.195$ is significant at $p<0.05$. The prefix "LV" denotes a latent variable constructed from the measured indicators as described in the text. Age is significantly related to LV_{PCBs}, LV_{LIPIDS}, and LV_{CVD}. Gender has no significant relationship to any of the remaining variables and therefore cannot be a confound. BMI is not related to the latent variables of PCBs or CVD and therefore cannot be a confound of a relationship between PCBs and CVD ($N = 277$).

are identical to those for the CFA presented in Fig. 2) the more conventional regression path analytic output associated with Fig. 3 is of greater importance. Cardiovascular disease status (LV_{CVD}) is significantly predicted by the combination of four explanatory variables (PCBs, lipids, age, and BMI) in the model such that 54% of the variance in LV_{CVD} is accounted for ($R^2 = 0.54$, Bootstrap CR = 3.69, $p = 0.004$, $CI_{95} = 0.34, 0.86$). However, because of the extreme multicollinearity among the predictors in the model (see Table 3), neither PCBs ($\beta = 0.11$, Bootstrap CR = 1.04, $p>0.30$), nor serum lipids ($\beta = 0.22$, Bootstrap CR = 1.58, $p>0.13$), nor BMI ($\beta = -0.006$, Bootstrap CR = −0.08, $p>0.95$) make a statistically significant unique contribution to the understanding of cardiovascular disease (LV_{CVD}). Age, however, does make a statistically significant contribution to prediction of LV_{CVD} after adjustment for the remaining predictors ($\beta = 0.53$, Bootstrap CR = 4.81, $p<0.001$). The previously observed unadjusted correlation between the latent variables of PCBs and cardiovascular disease ($r = 0.57$) is made to virtually vanish after adjustment for age, BMI, and lipids (e.g., treatment of them as confounds) in the regression analysis. The predictor variables are so highly correlated that to adjust for one means the eradication of the overlapping effect of the other.

3.3. The role of age

In the multiple regression model, there is no independent effect of PCBs on cardiovascular disease that cannot already be accounted for by age, BMI, and lipids. Most importantly, this regression analysis does not allow us to distinguish statistically between the unique roles of biological and chronological age, which require two very different logical arguments about the potential causal relationships among the variables. We view age as playing two independent roles. In its chronological sense, age is an exogenous variable, given that cardiovascular disease likelihood clearly increases with age. Age is, therefore, a factor related to the acquisition of PCBs, in that exposure continues throughout life, and that rates of intake under most circumstances exceed rates of metabolism and excretion, resulting in slowly rising levels of PCBs with age. In short, the *chronological* aspect of age is a proxy for the passage of time that is relatively independent of an individual's functional mental and physical status. The passage of time, as measured by this aspect of the age variable, is seen to be causally connected to many consequences, including the cumulative acquisition of

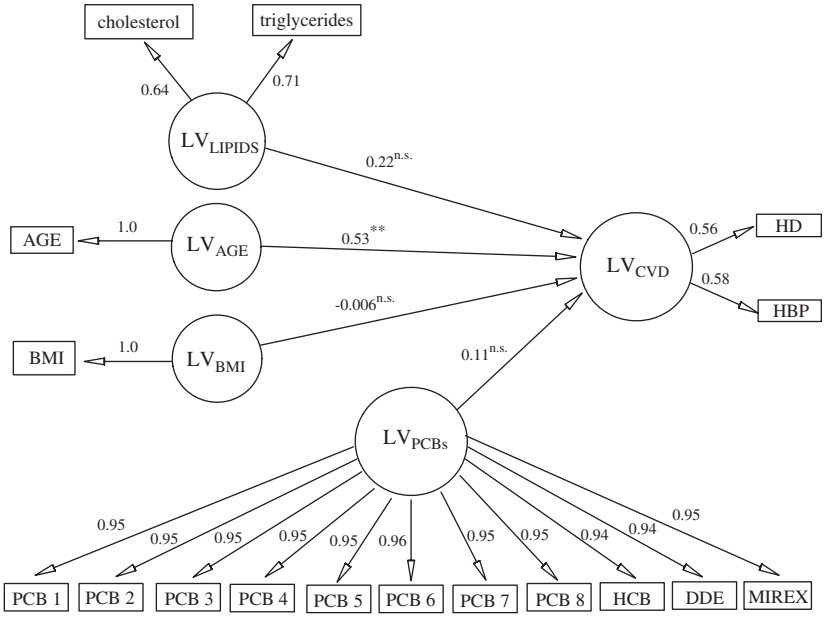

Fig. 3. Standard multiple regression model on latent variables. This model is not adequate for the reasons given in the text.

PCBs. In a very different sense age has a *biological* aspect that is functional, independent of the passage of time, and related to both physical and mental deterioration. This distinction between the disparate roles of chronological and biological age has been found to be theoretically and empirically useful in the study of aging and physical and mental well-being (Antsey and Smith, 1999; Baltes and Lindenberger, 1997). Statistically, it is impossible to distinguish between a confound and a mediator; this distinction must be made logically and by design (Bollen, 1989; MacKinnon et al., 2000; Susser, 1973). While the statistics associated with the regression results displayed in Fig. 3 clearly support the view that age can confound the observed relationship between PCBs and cardiovascular disease, we would suggest that age is not solely a confound, but is also innate to the causal structure of the complex set of relationships observed among PCBs, age, BMI, lipids, and cardiovascular disease. The standard regression model of Fig. 3 does not adequately adjust for these two distinct effects of age.

3.4. The mediated structural equation model

The model of the relationship between PCBs and cardiovascular disease that is mediated by serum lipids is graphically displayed in Fig. 4. The logic of the mediated model suggests that one pathway by which PCBs exert their effects on cardiovascular disease is indirect via P450 action in the liver that we hypothesize to be responsible for raising lipid levels. This elevation of lipids is, in turn, responsible for the increased risk of cardiovascular disease.

The structural path analytical model of Fig. 4 was fitted by the method of maximum likelihood implemented in LISREL 8.8. Bootstrap standard errors based on 500 bootstrap samples were estimated by AMOS 4.0. The scaling of the latent variables and the allowance for correlated errors among the PCBs and pesticides were the same as those conditions imposed on the confirmatory factor model. The statistical tests obtained from the fitted structural model reveal a very good fit to the data (Hu and Bentler, 1999; McDonald and Ho, 2002). The χ^2/df ratio is 3.38 ($\chi^2_{(69)} = 233.52$, $p < 0.01$), Bentler's comparative fit index (CFI = 0.978), the Tucker–Lewis (1973) goodness-of-fit index (NNFI = 0.957), and the root mean square error of approximation (RMSEA = 0.08) meet conventional criteria for an acceptable fit. The standardized residuals of the model fit are also within acceptable bounds (SRMSR = 0.067). As in the CFA, the factor loadings of the individual measured indicators for the latent variables are all substantial and differ significantly from zero (Table 4).

Within the structural model, the path coefficients and their test statistics related to the hypothesized relationships of this mediated model are of great importance. It can be observed in Fig. 4 that age has a significant direct effect on PCBs (Bootstrap CR = 23.61, $p < 0.001$), a marginally significant direct effect on lipids (Bootstrap CR = 1.88, $p < 0.06$), and significant direct effect on cardiovascular disease (Bootstrap CR = 5.03, $p < 0.001$). BMI has a significant relationship to lipids (Bootstrap CR = 2.88, $p < 0.02$). All of the direct paths from age to PCBs, lipids, and cardiovascular disease were retained in this model so as to control for, and adjust for, the influence of *chronological* age that is direct, and is therefore independent of the mediated effect of age on cardiovascular disease through PCBs and lipids. It is in this respect that age is treated as a causally responsible agent (with respect to PCBs and lipids) while simultaneously being treated as a *biological* predictor of cardiovascular disease that is

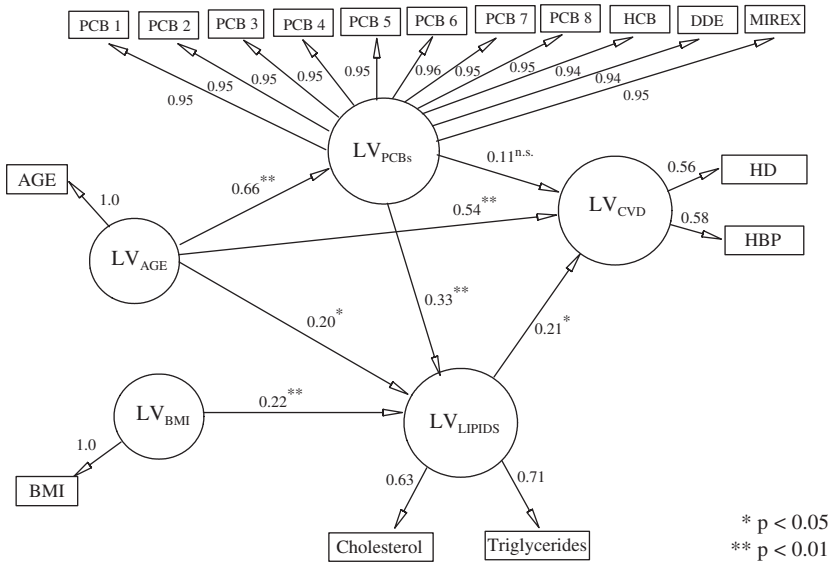

Fig. 4. Mediated model of the relationships among latent variables. This model incorporates that two distinct roles of age, and better explains the data.

Table 4
Maximum likelihood and Bootstrap parameter estimates, standard errors and test statistics, and 95% confidence intervals for the structural model of PCBs → Lipids → CVD

Maximum likelihood					Bootstrap			
Path	ML estimate	Standard error	Critical ratio	p	Standard error	$CI_{0.95}$ LL	$CI_{0.95}$ UL	p
Age → PCBs	0.017	0.001	15.57	0.000	0.001	0.015	0.019	0.004
Age → Lipids	0.909	0.386	2.35	0.019	0.467	−0.158	1.806	0.083
Age → CVD	0.006	0.001	5.10	0.000	0.002	0.002	0.009	0.004
BMI → Lipids	2.329	0.723	3.22	0.001	0.866	0.582	4.047	0.014
PCBs → Lipids	60.345	13.396	4.51	0.000	17.56	28.219	98.396	0.004
PCBs → CVD	0.045	0.033	1.37	0.171	0.039	−0.035	0.116	0.248
Lipids → CVD	0.00123	0.00067	1.85	0.067	0.0006	0.000009	0.001	0.043
Lipids → Chol.	0.387	0.062	6.24	0.000	0.086	0.238	0.554	0.004
Lipids → Trig	1.000	–	–	–	–	1.000	1.000	–
CVD → HD	1.000	–	–	–	–	1.000	1.000	–
CVD → HBP	1.736	0.291	5.96	0.000	0.446	1.179	2.892	0.004
PCBs → PCB1	1.000	–	–	–	–	1.000	1.000	–
PCBs → PCB2	0.974	0.011	89.30	0.000	0.011	0.952	0.996	0.004
PCBs → PCB3	1.063	0.016	67.76	0.000	0.016	1.034	1.097	0.004
PCBs → PCB4	0.886	0.029	30.23	0.000	0.027	0.832	0.946	0.004
PCBs → PCB5	1.045	0.018	58.14	0.000	0.018	1.013	1.082	0.004
PCBs → PCB6	0.782	0.021	36.82	0.000	0.020	0.740	0.821	0.004
PCBs → PCB7	0.795	0.013	62.25	0.000	0.012	0.773	0.819	0.004
PCBs → PCB8	0.692	0.016	42.16	0.000	0.016	0.662	0.723	0.004
PCBs → HCB	0.595	0.027	22.45	0.000	0.030	0.537	0.654	0.004
PCBs → DDE	1.167	0.042	27.67	0.000	0.042	1.089	1.253	0.004
PCBs → MIREX	1.413	0.068	20.65	0.000	0.061	1.309	1.546	0.004

Critical ratio = estimate/standard error. The test statistics and p-values on the standardized estimates of Fig. 4 are identical to those in this table. The measured indicators of PCB1, Triglycerides, and HD were set by design to 1.00 so as to fix the scales of the corresponding respective latent variables. Consequently, these fixed parameters have no test statistics associated with them.

independent of its relationship to PCBs or lipids. The remaining path coefficients suggest that the mediated model is supported by the data: PCBs have a significant direct effect on serum lipid levels (Bootstrap CR = 3.92, $p<0.01$), which in turn are significantly related at $p<0.05$ to cardiovascular disease (Bootstrap CR = 1.83, Bootstrap $CI_{95} = 0.000, 0.001$, Bootstrap $p = 0.043$). The direct effect of PCBs on cardiovascular disease is not significant in this model (Bootstrap CR = 1.15, Bootstrap $CI_{95} = -0.035$, 0.116, Bootstrap $p = 0.043$); rather the effect of PCBs on

cardiovascular disease is largely indirect, through the pathway PCBs→lipids→cardiovascular disease (Fig 4).

As an additional test of the plausibility of the mediation hypothesis, we re-estimated the model of Fig. 4 by constraining the path from PCBs to cardiovascular disease to zero. Constraint of this direct path to zero is consistent with the hypothesis of mediation. Testing of this model against the non-mediated model that includes the direct effect of PCBs on cardiovascular disease yielded a non-significant χ^2 difference test ($\chi^2_{(1)} = 1.84$, $p < 0.275$). Hence, addition of the direct path from PCBs to cardiovascular disease does not significantly improve the fit of the model beyond that of the fully mediated model. When the direct path from PCBs to cardiovascular disease is constrained to zero, the path from lipids to cardiovascular disease also becomes substantially stronger, due to the reduction in multicollinearity that is inherent in the partially mediated model that contains a direct PCB→cardiovascular disease path (Bootstrap CR = 2.44, $p < 0.02$). It is instructive to decompose the effects of age, PCBs, and lipids into direct and indirect components by means of their standardized path coefficients (Cohen et al., 2003).

There are two pathways by which age can exert its effect on cardiovascular disease: the direct effect of age on cardiovascular disease (0.54) and the effect of age on cardiovascular disease mediated by PCBs and lipids [(0.66)(0.33)(0.21) = 0.05]. For this set of paths, a total effect was calculated to be 0.54 + 0.05 = 0.59. Hence, approximately 92% of the effect of age on cardiovascular disease is direct and due to the biological aging process that is exogenous (determined by unknown causes) in the current model. Conversely, about 8% of the total effect of age on cardiovascular disease in this population is mediated by the action of PCBs, an effect which is secondary to the synthesis of lipids. Lipids, in turn, have a deleterious effect on cardiovascular disease. It should be noted that this 8% effect is adjusted for the independent biological effects of age that are not part of the chronological aspect of age that defines this mediated pathway.

Within this model, about 54% of the variation in cardiovascular disease is accounted for by PCBs, lipids, and age ($R^2 = 0.541$), about 29% of the variation in lipids is due to PCBs, age and BMI ($R^2 = 0.292$), and about 44% of the variation in PCBs is due to age ($R^2 = 0.437$). In the entire multivariate model approximately 43% of the aggregate multivariate variance in the set of endogenous variables (PCBs, lipids, and cardiovascular disease) is accounted for by the model.

3.5. Two alternative causal models

3.5.1. Increases in lipids are responsible for increases in PCBs

It is well documented in the structural equation modeling literature that many different theoretical models can in fact produce near-identical statistical fits of the model to the data (Kline, 2004; MacCallum et al., 1993). The primary competing alternative in this instance is a model in which increasing lipids are presumed to be causally responsible for increasing PCBs. The statistical fit of this alternative model is approximately as good as that the fit of original model that makes the opposite prediction (Fig. 4). While the statistical fit is acceptable, we consider the logic of this alternative model to be implausible. It is difficult to imagine how an increase in PCBs, synthetic substances, could be caused by a change in liver activity due to some other disease condition. It is conceivable that an increased synthesis of cholesterol and triglycerides caused by a malfunctioning liver could lead to an increasing failure to metabolize PCBs (compared to some baseline condition), but a causal pathway from lipids→PCBs appears unlikely. The more reasonable explanation is that PCB exposure induces P450s and stimulates liver function, leading to increased synthesis of lipids. Consequently, we consider the causal pathway that is most plausible to be that of PCBs→lipids→cardiovascular disease.

3.5.2. A non-recursive model with reciprocal feedback loops

As further evidence of the plausibility of the PCB→lipid hypothesis, as opposed to the lipid→PCB hypothesis, we have also tested for the possibility of a non-recursive feedback loop between PCBs and lipids. In this test each of the two variables is hypothesized to be reciprocally affecting the other in an infinitely regressive looping structure (Hayduck, 1987; Kenny, 1970). Although we think it implausible that lipids are exerting a causal effect on PCBs (e.g., affecting the liver's ability to metabolize PCBs), it is possible to test, with our current set of cross-sectional data, whether a model that posits a non-recursive, reciprocal feedback loop between lipids and PCBs is plausible (Bollen, 1989; Hayduck, 1987; Kline, 2004). Such models are not uncommon in homeostatic systems (Martens and Haase, 2006; Schaubroeck, 1990). A non-recursive reciprocal model with a feedback loop introduced between the PCBS and lipids was fitted to the data. BMI was used in this model to satisfy the computational and mathematical requirements for an instrumental variable in non-recursive models (Hayduck, 1987; Namboodiri et al., 1975). The paths that are shared with the recursive model of Fig. 3 are similarly substantial and statistically significant in the non-recursive version, which is presented in Fig. 5. Of major interest here are the parameter estimates and their bootstrap test statistics associated with the reciprocal paths of PCBs→lipids ($\beta = 0.55$, Bootstrap CR = 3.93, $p < 0.01$) and the path from lipids→PCBs ($\beta = -0.14$, Bootstrap CR = −0.83, $p > 0.30$). The data support the recursive, unidirectional model. While the path from PCBs→lipids remains strong and significant, the reciprocal path from lipids→PCBs is weak, statistically non-significant, and of the wrong polarity (−). Thus our cross-sectional data do not support a bi-directional,

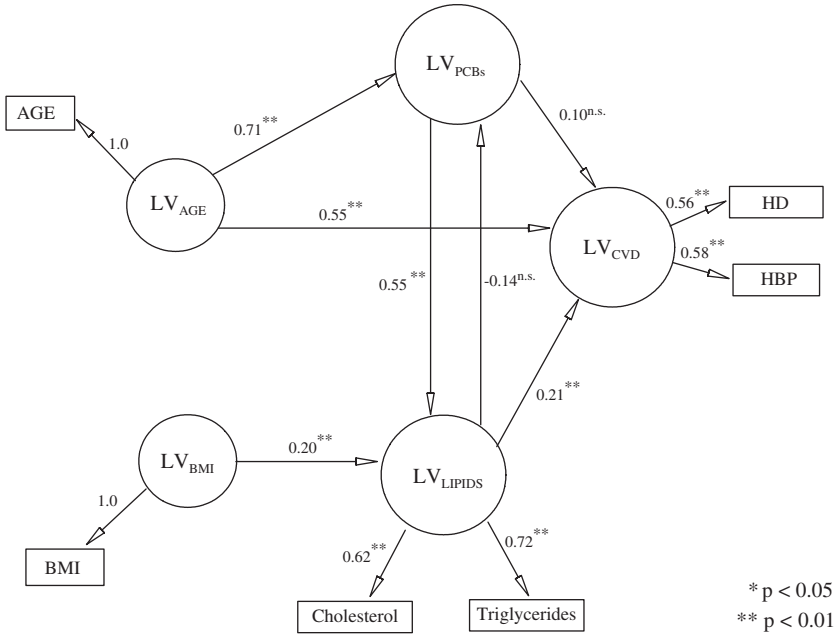

Fig. 5. Non-recursive model with feedback loop between LV_{PCBs} and LV_{LIPIDS}. Measured indicators and their loadings on the PCB latent variable are virtually identical to those presented in Fig. 4.

reciprocal, non-recursive explanation of the effects of lipids and PCBs on cardiovascular disease.

4. Discussion

These results support the conclusion that there is a relationship among serum PCB and pesticide levels, serum lipids, and self-reported cardiovascular disease. The relationship between PCBs and heart disease was found to be mediated by the serum lipids and remains statistically significant even after control for BMI and the direct exogenous effect of biological age. Gender was found to be unrelated to any of the latent variables of the study.

Our study did not find significant differences in the effects of several subgroups of PCBs, identified by commonality of structure and proposed action. This lack of difference may reflect common action of all of these group in regulating serum lipids, or it may merely reflect the fact that the various congeners have common pathways of exposure and persistence, and therefore identification of relative potencies is difficult to elucidate. The biologic activity of individual PCB congeners is a function of the extent and pattern of chlorine substitution. Those congeners lacking multiple *ortho*-chlorine substitution bind to the aryl-hydrocarbon (AhR) receptor, activate certain cytochrome P450 enzymes, and cause responses similar to those of dioxin, through gene transcription. Puga et al. (2000) found a total of 310 known genes to be altered by a factor of 2 or more through exposure to 10 nM tetrachlorodibenzo-*p*-dioxin for 8 h in a hepatoma cell line; some were up-regulated and others down-regulated. Di- and higher *ortho*-substituted PCBs do not bind to the AhR, but instead activate different P450s and have different effects on gene transcription. Metabolites of PCBs may also have biologic effects. Because so many genes are altered, the biologic effects can be many and varied.

Animal studies dating from as far back as the 1970s have demonstrated that exposure to dioxin and PCBs can result in a variety of health effects. From controlled studies of rats and non-human primates there is significant evidence that exposure to both mixtures of PCBs (e.g., Arochlor 1254) as well as single PCB congeners (e.g., PCB 126) results in hepatomegaly, enzyme induction, and elevation in the levels of serum lipids (cholesterol and triglycerides). PCBs have been shown to induce the liver to make more of the enzymes that synthesize lipids, and to cause an increase in plasma triglycerides and a decrease in high density lipoproteins (ATSDR, 2000; Bell et al., 1994, Boll et al., 1998; Mochizuki et al., 2000; Oda et al., 1991). These changes would be expected to increase risk of cardiovascular disease. More recently, Lind et al. (2004) exposed rats to PCB 126, and demonstrated increased cholesterol levels, heart rate and blood pressure. Because these animal studies are not as vulnerable to random variation in levels of serum lipids as are human investigations, they provide strong evidence that, in addition to enzyme induction, exposure to organochlorines induces the liver to increase synthesis of lipids.

Numerous investigators have reported a relationship between PCB levels and serum lipids in humans. Baker et al. (1980) first reported that workers exposed to PCBs showed a significant direct correlation between serum PCB levels and plasma triglyceride levels. They found that blood levels of PCBs in workers in a sewage sludge plant in

Indiana and their families ranged between 17.4 and 75.5 ppb, and that there was a highly significant relationship between plasma triglyceride levels and serum PCB concentrations. Kreiss et al. (1981) determined serum lipids as a function of serum PCB levels in 458 people over 12 years of age in Triana, Alabama, where there was significant contamination. They found that the higher the serum PCB levels, the higher the serum cholesterol level, and the higher the blood pressure. The mean PCB level was 17.2 ppb, with a range from 3.2 to 157.9 ppb. Sixty of these people had levels greater than 30 ppb. Chase et al. (1982) found significantly elevated serum triglyceride levels in exposed workers. Martin (1984) reported that workers accidentally exposed to dioxin still had statistically significant elevations of both serum cholesterol and triglyceride concentrations 10 years later. Moysich et al. (2002) reported a significant correlation between total lipids and serum PCB levels (after age adjustment) in a study of anglers in New York. Tokunaga and Kataoka (2003) reported a relationship between PCB exposure and serum lipids in a Japanese population exposed through contaminated rice oil in the 1960s. These authors found a 10-fold elevation of PCB levels was associated with an elevation of serum total cholesterol by 18.8 mg/dL ($p<0.001$) in men and 17.5 mg/dL in women, and an elevation of serum triglycerides by 43.3% in men and 42.8% in women. In the context of these earlier investigations, what makes our findings important is that the PCB levels in our population are significantly less than those reported in most of these earlier studies, yet the recognized relationship between PCBs and lipids remains robust.

Gustavsson and Hogstedt (1997) studied 242 male capacitor plant workers exposed to PCBs, and found a significantly increased incidence of death from cardiovascular disease for at least a 5-year exposure with a 20-year latency. A similar elevation in mortality from cardiovascular disease has been reported in occupational studies of dioxin-exposed workers (Flesch-Janys et al., 1995; Vena et al., 1998) and Seveso residents exposed to dioxin releases (Bertazzi et al., 1998; Pesatori et al., 1998). Sergeev and Carpenter (2005), examining rates of hospitalization for cardiovascular disease and acute myocardial infarction in residents living in zip codes containing or abutting hazardous waste sites contaminated with persistent organic pollutants, found statistically significant elevations of cardiovascular disease compared to populations not living near such waste sites. Hennig et al. (2002) have demonstrated that dioxin-like PCBs induce oxidative damage of endothelial cells through the generation of reactive oxygen species (Slim et al., 1999). Non-dioxin-like PCBs also induce oxidative stress in endothelial cells, with the induction being mediated via stimulation of inflammatory processes (Choi et al., 2003). Direct damage to endothelial cells by dioxin-like PCB5 is also a mechanism that could increase risk of cardiovascular disease, particularly if such endothelial cell damage is accompanied by an elevation in serum lipids.

Two important conclusions emerge from the present study. Even within this population, which does not have exceptionally elevated PCB levels, there exists a measurable relationship between levels of wet-weight PCBs and serum lipids. PCBs are fat-soluble, and the potential for confounding between PCBs and serum cholesterol and triglycerides is substantial. Typically, controlling for lipids in an analysis, either by formula adjustment (Phillips et al., 1989) or by more direct methods of statistical adjustment for covariation (Rosner, 2006; Schisterman et al., 2005), causes the observed PCB-to-outcome variable relationship to "vanish". In such fully adjusted analyses, the PCB–outcome relationship disappeared after adjustment for lipids, age, and BMI. There is no doubt that PCB levels and levels of serum lipids, as measured by cholesterol and triglycerides, are strongly related. The second important advance of the present study is the reformulation of the theoretical model that can explain the relationships observed among PCBs, age and lipids, as well as the relationship between these variables and the important endpoint, cardiovascular disease. We documented the variability in cardiovascular disease by constructing a latent variable from two single indicators of self-reported heart disease and self-reported high blood pressure. The optimal combination of these two markers, which we termed cardiovascular disease, was found to be significantly related to age, to levels of serum PCBs and pesticides, and to serum lipids.

We chose to focus our attention on a theoretical model that can explain these relationships in a putatively causal manner, and in a manner whereby age is recognized as having multiple layers of meaning. In essence, the role of age in the model of PCBs/lipids/cardiovascular disease is far more complicated than as a mere alternative explanation for variability in heart disease. If age is simply treated as a confound in the analysis of PCB–endpoint relationships, statistical adjustment for age in a traditional linear model equates to removing a variable that lies on the causal pathway of the exposure → disease continuum. This adjustment unintentionally removes much of the variance that is desirable for study as part of the exposure-endpoint process (Greenland, 2000; Rosenbaum, 1995; Rothman and Greenland, 1998, 2005; Rubin, 1974; Sobel, 1994; Susser, 1973). In Fig. 4 we posited and tested a theoretical model that defines age not only as a variable that has some unique direct effect on cardiovascular disease (e.g., its role as a control variable) but also as a chronological marker of the passage of time, since the presence or absence of PCBs is time dependent. Hence, in addition to its exogenous role in the model, age, as we define it, is a part of the mechanism that explains the acquisition of PCBs by environmental exposure over time. We have demonstrated that the chronological and biological aspects of age as a variable are orthogonal components and probably operate independently on study variables. Moreover, we propose that the relative high or low levels of serum lipids in our participants is a causally related function of the magnitude of individual PCB body burden. We make this argument

on the logical grounds that the induction of P450 enzymes for the purpose of riding the body of the PCBs, causes the overactive liver to synthesize excessive amounts of cholesterol and triglycerides, obviously an undesirable side effect.

There are limitations in our study. The cross-sectional design does not allow us to examine the temporal order of the relationship between serum organochlorine levels and serum lipids. The varying persistence of, and the high intercorrelation among, these substances limit our ability to clearly identify which congeners or pesticides are primarily responsible for the observed relationships. The self-reported nature of the measures of heart disease and high blood pressure used in this study is a limitation. While we believe that the participants were able to accurately self-identify their health status, we lack the physical examination data to confirm their self-reports. Lastly, although participants were instructed to fast prior to blood draw, we cannot be certain that this was always the case.

In summary, this study provides evidence that positive relationships exist among serum PCB and pesticide levels, serum lipids, and self-reports of cardiovascular disease. Our data are consistent with a mediated model in which PCBs exert an effect upon the synthesis of cholesterol and triglycerides, elevation of these in turn is significantly associated with an increase in the likelihood of reported heart disease and high blood pressure as key aspects of cardiovascular disease. We propose that this relationship reflects an underlying mechanism by which PCB congeners and chlorinated pesticides increase lipid synthesis in the liver. The resultant elevated serum lipids, perhaps in combination with endothelial cell damage resulting from oxidative stress, may be the basis of the elevation in cardiovascular diseases that has been reported in PCB-exposed populations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.envres.2007.10.006 and at www.albany.edu/ihe/news.htm.

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