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# **Persistent Organic Pollutants and Inflammatory Markers in a Cross-Sectional Study of Elderly Swedish People: The PIVUS Cohort**

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**Running title:** POPs and inflammatory markers

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## **Abstract**

**Background and Objective:** Persistent organic pollutants (POPs) are compounds that are generated through various industrial activities and released in the surrounding environment. Different animal studies have shown effects of different POPs on various inflammatory markers. Since there are very few studies conducted in humans, we assessed the associations between different POPs and inflammatory markers in a large population-based sample of elderly men and women (all aged 70 years) from Sweden.

**Methods:** This cross-sectional study investigated the concentrations of several polychlorinated biphenyls (PCBs), organochlorine pesticides, polychlorinated dibenzo-*p*-dioxin, and brominated diphenyl ether congener and their association with a number of inflammatory markers (VCAM-1, ICAM-1, E-selectin, C-reactive protein or CRP, total leucocyte count, TNF- $\alpha$ , MCP-1 and IL-6) in 992 individuals. These individuals were recruited from the Prospective Investigation of the Vasculature in Uppsala Seniors cohort. The concept of total toxic equivalency (TEQ) value that measures toxicological effects utilizing the relative potencies of various POPs was used.

**Results:** Following adjustment for potential confounders, the TEQ value (mainly driven by PCB-126) was found to be significantly associated with levels of ICAM-1 ( $p < 10^{-5}$ ). A similar trend was also observed between sum of PCBs and VCAM-1 ( $p < 0.001$ ). No significant associations were observed between levels of POPs and other inflammatory markers.

**Conclusions:** TEQ values were found to be associated with levels of ICAM-1, to a lesser degree also to VCAM-1, but not to CRP and several other inflammatory markers. These findings suggest an activation of vascular adhesion molecules by POPs, and in particular by PCB-126.

## Introduction

Persistent organic pollutants (POPs) are the organic compounds that are present in surrounding environment and not easily degradable through various environmental processes. Due to industrial revolution in the past few centuries, a plethora of hazardous POPs generated directly or as byproducts has been released in the environment. Once released, these chemicals persist for a long time and may reach concentrations that induce adverse health effects (<http://www.chem.unep.ch/Pops/default.htm>). Several of the chemical entities, including polychlorinated biphenyls (PCBs) congeners, organochlorine (OC) pesticides, polychlorinated dibenzo-*p*-dioxins, and brominated diphenyl ether (BDE) congeners fall into this category. An overwhelming number of studies have shown that large majority of individuals born after mid twentieth century have been and still are exposed to a number of POPs in everyday life (Hertsgaard 1996; Schechter et al. 2010). Bioaccumulation of POPs in the general population occurs primarily through ingestion of contaminated food (like fish, meat or dairy products) as well as via air and dust in indoor environment (Johnson et al. 2010; Letcher et al. 2010). A number of cross-sectional and prospective studies have reported positive associations between levels of POPs with different diseases like obesity, atherosclerosis, diabetes, allergies, and cancers (Dirinck et al. 2011; Elobeid et al. 2010; Hardell et al. 2006a; Hardell et al. 2006b; Lee et al. 2007; Lee et al. 2011; Lee et al. 2012a; Lee et al. 2012b; Lind and Lind 2012; Lind et al. 2012; Noakes et al. 2006; Persky et al. 2011; Persky et al. 2012; Ronn et al. 2011; Verner et al. 2008).

Some of the POPs, like dioxins and PCBs, exhibit their biochemical and toxic effects through aryl hydrocarbon receptors (Ahr) mediated response (Beischlag et al. 2008; Hao and Whitelaw 2013). Ahr is a member of the family of basic helix-loop-helix transcription factors that are very

important for developmental processes and involved in regulation of biological responses to aromatic hydrocarbons by inducing the expression of target genes (Beischlag et al. 2008; Hao and Whitelaw 2013). A concept of total toxic equivalency (TEQ) value utilizing relative potencies of various POPs to bind to Ahr has been developed to measure toxicological effects and risk characterization in different organisms (Van den Berg et al. 1998). The TEQ values can be calculated utilizing toxic equivalency factors (TEF) that expresses the toxicity of various POPs in relation to the most toxic dioxin, called 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TEF=1) (Van den Berg et al. 2006). TEF is used for human health risk assessment for different POPs (Van den Berg et al. 1998). The applicability of TEF approach for determining toxicity for different PCB mixtures has been substantiated utilizing both *in vivo* and *in vitro* studies (Bradlaw et al. 1980; Harris et al. 1993).

There are clear indications through experimental studies that POPs can induce inflammation (Cheon et al. 2007; Hennig et al. 2002; Nishiumi et al. 2010; Peltier et al. 2013). Inflammation is a cluster of different responses to a trauma (for example exposure to toxic compounds) and may be initiated in different ways involving various pathways. Although inflammation plays an important role in the defense mechanism in biological systems, it may also lead to apparent damage in cases of severe response (Medzhitov 2008). Various toxic compounds may trigger abnormal inflammatory response directly or indirectly through interfering with normal physiological functioning of cells or tissues (Medzhitov 2008). In a cross-sectional study including large number of non-diabetic individuals, Kim KS et al. have analyzed the influence of POP concentrations on inflammation and insulin resistance, showing the association of pesticides with increases levels of CRP (KS Kim et al. 2012). Another study by Kim MJ et al., studying the impact of POPs on human adipose cells have shown that both precursor cells and adipocytes are

targets of POPs and these pollutants trigger mainly the inflammation pathway (MJ Kim et al. 2012). In a study from Japan involving 40 Yusho patients and 40 controls, Kawatsuka et al., have also shown that serum levels of IL-17, IL-1b, IL-23, and TNF- $\alpha$  are higher in these patients who are exposed to POPs including PCBs through consumption of contaminated rice (Kuwatsuka et al. 2013). Circulating inflammatory biomarkers like C-reactive protein (CRP), interleukin-6 (IL-6), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), monocyte chemotactic protein-1 (MCP-1), Intercellular Adhesion Molecule 1 (ICAM-1), Vascular cell adhesion protein 1 (VCAM-1) and E-selectin have been associated with a number of metabolic disorders and associated outcomes (Goldberg 2009).

Although several animal studies have been performed to show that POPs are related to inflammation, very little data exists in humans, and in the existing studies only limited numbers of individuals have been included (Fang et al. 2012; Glynn et al. 2008; Hennig et al. 2002; Imbeault et al. 2012; KS Kim et al. 2012; Noakes et al. 2006; Sipka et al. 2008; Sipos et al. 2012; Weisglas-Kuperus et al. 1995). Therefore, we conducted this study utilizing measurements of various circulating POPs in a large population-based sample of men and women aged 70 years from the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) cohort (<http://www.medsci.uu.se/pivus/>). In this cross-sectional study, our primary aim was to investigate the association of TEQ values derived from seven dioxin-like PCBs and octachlorodibenzo-*p*-dioxin (OCDD), sum of OC pesticides and sum of PCBs with a variety of inflammatory markers (VCAM-1, ICAM-1, E-selectin, TNF- $\alpha$ , and IL-6, CRP, total leucocyte count or TLC). As an exploratory effort, our secondary aim was to analyze the associations between different individual POPs measured with these inflammatory markers.

## **Materials and Methods**

### *Study participants*

All the participants in this study were 70 years old individuals living in the community of Uppsala, Sweden and were chosen from population register. A total of 1,016 individuals agreed to participate (participation rate 50.1%) and provided their written informed consent. The Ethics Committee of Uppsala University, Uppsala, approved the study. All the study participants were asked to observe overnight fast and not to use any medication or smoke after midnight before being evaluated the next morning. Venous blood samples were drawn in the morning between 8 and 10 am by one of three experienced nurses after an overnight fast. Blood samples were centrifuged within 30 min and plasma/serum were frozen within one hour and stored at  $-70^{\circ}\text{C}$  until analysis. The temperature in the sampling room was kept constant at  $22^{\circ}\text{C}$ . A detailed questionnaire concerning smoking, medications and medical history was filled in (can be obtained from corresponding author at request). Standard laboratory techniques were employed to measure the fasting blood glucose as well as different lipid variables (see [http://www.medsci.uu.se/digitalAssets/109/109917\\_56-article-56.pdf](http://www.medsci.uu.se/digitalAssets/109/109917_56-article-56.pdf)). A calibrated mercury sphygmomanometer (SD-43 (Omron Healthcare, Myata, Japan)) was used to measure blood pressure, and an average of three recordings was used. Kidney function was measured by calculating glomerular filtration rate following Modification of Diet in Renal Disease formula (Levey et al. 2007). Details about recruitment of study participants and clinical characteristics could be found elsewhere (Lind et al. 2005). Out of 1,016 individuals, POPs could be measured in 992 participants only. In these 992 individuals, 11.6% had diabetes and 1.1% were on lipid-lowering medication, while 10.6% of the individuals were current smokers.

### ***Measurement of POPs***

POPs were measured in 992 participants from stored serum samples employing a slightly modified method by Sandau et al. (Sandau et al. 2003). The samples were kept in -70°C freezer for approximately 5 years before analysis. The sampling, collection, and storage processes were performed in rooms kept free from POPs as far as possible. Details regarding measurements of different POPs can be found from a previous study by our group (Salihovic et al. 2012). Briefly, 1 mL formic acid was added to 0.5 mL plasma sample to denature proteins followed by sonication for 15 min. After 60 minutes, 1 ml of 3% isopropanol in water along with labeled <sup>13</sup>C internal standards were added and sonicated again. Samples were loaded on conditioned Oasis® HLB SPE (Waters, Milford, MA, USA) cartridge (6 cm<sup>3</sup>/150 mg) to perform Solid-phase extraction. The cartridge was preconditioned with 3 mL of methanol followed by 3 mL of dichloromethane, 6 mL of methanol/dichloromethane (1:1), 4.5 mL of methanol and ultimately with 4.5 mL of water. 3% isopropanol in water (6 mL) and 40% methanol in water (6 mL) were used to rinse the cartridge so as to remove all the proteins and interferences from sorbent phase. After drying under vacuum and nitrogen for 40 min, the target compounds were eluted with 6 mL dichloromethane/hexane (1:1) into 8 mL amber glass vials pre-spiked with 25 µL of N-tetradecane. Samples were then dried up under nitrogen and reconstituted on hexane (500 µL). A small, activated multilayer silica gel column (2 mL, 1.5 g) was utilized for further cleanup. 7.5 mL of hexane was passed through the column to achieve elution of analytes. After evaporation and addition of the <sup>13</sup>C-labeled recovery standard, the final volume was adjusted to 25 µL N-tetradecane. A Micromass Autospec Ultima (Waters, Mildford, MA, USA) high-resolution gas chromatograph/high-resolution mass spectrometer was employed to perform the final measurements. The two most abundant ions of the chlorine or bromine cluster in addition to one

ion for  $^{13}\text{C}$ -labeled internal and recovery standards were monitored by injecting 2  $\mu\text{L}$  on a 6890N gas chromatograph (Agilent Technologies, Atlanta, GA, USA) containing a 30 m  $\times$  0.25 i.d.  $\times$  0.25  $\mu\text{m}$  DB-5 capillary column (SGE Analytical Science, Victoria, AUS). The levels of POPs were normalized for the lipid content in plasma. To calculate the total amount of lipid present in each plasma sample, a summation formula based on the concentrations of serum cholesterol and triglyceride was utilized (Rylander et al. 2006).

### ***Quality control***

In each batch of 10 samples, quality control plasma samples and procedural blank samples were incorporated to ensure the quality. The blank samples were devoid of any target compounds at levels  $>5\%$  of the levels in samples except for *cis*-chlordanes and *trans*-chlordanes. Both of the chlordanes were present below detection limit in 95% of the samples analyzed. The recovery of internal standards ranged from 60–110% and was satisfactory. The relative standard deviation of 100 quality assurance/quality control (QA/QC) samples was less than 25% for all the compounds measured except for one present at low levels and just above the limit of detection (LOD) in the QA/QC sample. Further details of quality controls may be found elsewhere (Salihovic et al. 2012). Samples with POPs having concentration below LOD were imputed and given  $\text{LOD}/2^{-0.5}$  values.

### ***TEQ value calculation***

TEQ value was calculated using seven mono- and non-ortho-substituted dioxin-like PCBs (PCB-126, 169, 105, 118, 156, 157, 189) and OCDD as described by Van den Berg et al. (Van den Berg et al. 2006). The concentrations of PCBs and OCDD were multiplied by their respective

TEF and then added. We also calculated sum of concentrations of all PCBs (sum of PCBs) measured and a separate sum of three OC pesticides (sum of OC pesticides).

### ***Measurement of inflammatory markers***

A total of eight inflammatory markers were utilized in this study. Serum high sensitive CRP was measured by ultrasensitive particle enhanced immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland) on a Konelab 20 autoanalyser (Thermo Clinical Labsystems, Espoo, Finland). The inter-assay coefficient of variation was 3.2%. Cytokines, chemokines and adhesion molecules were analyzed on Evidence® array biochip analyzer (Randox Laboratories Ltd., Crumlin, UK). The method is detailed by Fitzgerald et al. in a previous article (Fitzgerald et al. 2005). The functional sensitivities for different inflammatory markers measured were as follows: CRP: 0.1 mg/l, TLC: 0.2, IL-6: 0.3 pg/ml, TNF- $\alpha$ : 1.8 pg/ml, MCP-1: 19.4 pg/ml, ICAM-1: 18.6 pg/ml, VCAM-1: 3.1 pg/ml and E-selectin: 3.1 pg/ml.

### ***Statistical analysis***

Variables were evaluated for non-normality, and variables showing skewed distribution were log transformed in order to achieve normal distribution. Linear regression was used to analyze the association of TEQ values, sum of PCBs or sum of OC pesticides (independent variables) with different inflammatory markers (dependent variables). A variety of covariates including sex, education (three discrete levels), physical activity (four different groups), waist circumference (cm), smoking (current smokers, previous smokers and non-smokers), kidney function (glomerular filtration rate), fasting blood glucose, systolic blood pressure, body mass index (BMI), lipid profile (levels of high- and low-density lipoproteins cholesterol, triglycerides) were considered and adjusted for. Two different statistical models were employed in regression

analysis: model A, considering sex and kidney function as covariates; and model B, considering all covariates. Additionally, a squared term of POPs was included in the models to evaluate potential nonlinear relationships. Interactions between levels of POPs and sex for POPs and all outcomes were evaluated by introducing an interaction term between POP and sex together with the POP and sex terms. In the primary analysis, TEQ values, sum of PCBs and sum of OC pesticides were analyzed for their association with different inflammatory markers studied. The alpha threshold was set by dividing 0.05 by the number of inflammatory markers analyzed (i.e. a Bonferroni correction;  $0.05/8=0.0063$ ). Further analysis was performed in a sub-sample of presumably healthier individuals (non-smokers, non-diabetic and non-hyperlipidemic individuals) in order to confirm the findings from primary analysis. Additionally, participants were divided into two groups based on median BMI and the significant findings were confirmed. Smoking was also analyzed in the similar manner. Since the studied population is older, we also investigated whether there is an impact of certain medications (aspirin, cortisone and non-steroidal anti-inflammatory drugs) on significant outcomes. In the secondary exploratory analysis, levels of individual POPs were examined for their association with inflammatory markers. No Bonferroni adjustment was made in secondary analysis, but p-value  $<0.0063$  was considered significant. The statistical software package STATA (version 12; StataCorp, College Station, TX, USA) was employed to perform all statistical analysis.

## Results

The general/clinical characteristics of the individuals recruited in this study are shown in Table 1. There were an almost equal percentage of males and females in the study group. Sixty-seven percent individuals were overweight ( $BMI \geq 25.0 \text{ kg/m}^2$ ) while 22% individuals were obese ( $BMI \geq 30.0 \text{ kg/m}^2$ ). Two of the OC pesticides (*trans*-chlordane and *cis*-chlordane) were not

detectable in >30% of the study population and hence were not analyzed further. A total of 21 POPs involving 16 PCBs, 3 OC pesticides, 1 BDE and 1 OCDD were detected in >70% of individuals and were taken up further for analysis (Table 2). Distribution of all 21 POPs in the studied population is shown in Table 2. Median (interquartile range) of TEQ values was calculated using eight POPs (7 different PCBs and one OCDD) and found to be 9.8 (6.7 – 13.9). Logarithmic transformation was performed on these values to achieve normal distribution (Table 2).

***Association of TEQ value, sum of PCBs and sum of OC pesticide concentrations with cell adhesion molecules***

The levels of different cell adhesion molecules ICAM-1, VCAM-1 and E-Selectin were analyzed for their association with the TEQ values utilizing two different statistical models in linear regression. In model A, TEQ value was found to be significantly associated with levels of ICAM-1 in a positive fashion (p-value= $2.7 \times 10^{-5}$ ). In model B, the significance level did not change much and remained highly significant (p-value= $3.6 \times 10^{-5}$ ) (Table 3). When the concentrations of individual PCBs were analyzed for their association with levels of ICAM-1, mainly PCB-126 showed significant association in both models A and B (p< $10^{-8}$ , see Supplemental Material, Tables S1 and S2).

When the TEQ values were analyzed for their association with levels of VCAM-1, both models A and B showed results similar to ICAM-1 but less significant (p-value=0.006 and 0.005 respectively, Table 3). In this case, the association was driven by a number of PCBs (p-value=0.001 for sum of PCBs), and not only PCB-126. (See Supplemental Material, Table S2). Sum of OC pesticides showed significant positive association with VCAM-1 in model A (p-value=0.004, Table 3) but not in model B (p-value=0.17, Table 4).

No significant association between TEQ value or sum of PCBs and levels of E-selectin was observed in our study (Table 3). Further, no significant association for sum of OC pesticides was observed in model B with levels of cell adhesion molecules studied (Table 3). When individual POPs were analyzed, PCB-209 showed significant association with levels of E-selectin in model A, but this significance was attenuated when adjusted for additional covariates in model B (see Supplemental Material, Tables S1 and S2).

#### ***Association with downstream inflammation indicators***

When the TEQ value, sum of OC pesticides and sum of PCBs concentrations were analyzed for their association with downstream inflammatory indicators (CRP and TLC) in both statistical models A and B, no significance was observed (Table 3). Although levels of some of individual PCBs (156, 157, 170, 180, 206, 209) were found to be significantly associated with CRP in model A ( $p < 0.001$ ), the significance disappeared when model B including a large number of covariates was employed (see Supplemental Material, Tables S1 and S2). Further, PCB-126 and *p,p'*-DDE showed significance association with TLC ( $p$ -value=0.006 and 0.003 respectively) but only in model A (see Supplemental Material, Tables S1 and S2).

#### ***Association with cytokines***

In linear regression analysis, no statistical significance was observed when TEQ values, sum of PCBs or sum of OC pesticides were analyzed for their association with levels of different cytokines (IL-6, TNF- $\alpha$  and MCP-1) in both models A and B (Table 3). Further, similar non-significant results were observed with individual POPs (See Supplemental Material, Tables S1 and S2).

### ***Additional analysis***

Since a large proportion of individuals studied were either overweight or obese in the studied cohort, we divided these individuals into two groups based on median BMI (26.6 kg/m<sup>2</sup>) and compared the association of TEQ and PCB-126 on ICAM-1 between these groups. The association observed for TEQ values was stronger in the group having higher median BMI as compared to others (see Supplemental Material, Table S3). Further, TEQ value and PCB-126 showed significant association with levels of ICAM-1 only in non-smokers (see Supplemental Material, Table S3).

Additionally, in order to investigate the influence of medications on significant findings, we excluded individuals on medications in a stepwise manner (individuals taking aspirin, cortisone or NSAID at each step). Association of TEQ values, sum of PCBs and sum of OC pesticides with ICAM-1 or VCAM-1 was similar in all three groups (see Supplemental Material, Table S4).

In order to understand association of confounders with inflammatory markers as well as POP exposures, univariate associations were performed and presented in supplementary data (see Supplemental Material, Table S5-S8).

No consistent non-linear effects or sex/POP interactions were disclosed (data not shown).

### ***Association in sub-sample***

In order to exclude the impact of smoking, diabetes and hyperlipidemia, a sub-sample of presumably healthy individuals including non-smokers, non-diabetic and normal lipid profile was extracted (n=776). When the association analysis was performed between POPs and inflammatory markers in this sub-sample, significant associations observed did not differ from total samples (data not shown).

## Discussion

In the present study, we primarily report the association of TEQ values, sum of PCBs and sum of OC pesticides with a number of inflammatory markers. While analyzing the association of TEQ values (representing concentration of seven dioxin-like PCBs and OCDD) with cell adhesion molecules that are considered to be classical inflammatory mediators, we found significant association mainly with two adhesion molecules, ICAM-1 and VCAM-1. Both of these molecules belong to immunoglobulin supergene family mediating attachment of leukocytes to vascular endothelium and their trans-endothelial migration, thereby having an important influence on inflammatory reactions (Muller 2009).

### *Cell adhesion molecules*

Several experimental studies have shown potent vascular effects of different PCBs and their interaction with endothelial cells through inflammatory response (Hennig et al. 2002). Studies performed in porcine endothelial cells to analyze impact of coplanar PCBs (like PCB-77, 126 or 169) have shown that they have a concentration-dependent oxidative stress response and subsequent proinflammatory events (Hennig et al. 1999; Hennig et al. 2002). When cultured endothelial cells are exposed to individual PCBs (PCB-77, 126 and 169) with different concentration (0.5, 1.0 and 2.5  $\mu$ M), concentration-dependent increase in cellular oxidative stress have been observed as compared to the control media (Hennig et al. 2002). When incubated with PCBs, these cells have shown increased production of IL-6. Further, the levels of VCAM-1 mRNA in endothelial cells treated with PCB-77 have been found to be significantly higher compared to control culture (Hennig et al. 2002). The most common route of PCBs exposure to humans is through food chain (<http://www.atsdr.cdc.gov/ToxProfiles/tp17.pdf>). Sipka et al. have utilized *in vivo* mice model to understand orally administered PCBs mediated inflammatory

mediators (Sipka et al. 2008). Oral administrations of individual PCBs (150  $\mu\text{mol/kg}$  body weight by oral gavage) have been found to activate a variety of specific inflammatory mediators. The mRNA levels of both ICAM-1 and VCAM-1 have been found to be significantly increased in various organs like liver, lungs, and brain in response to the exposure to different PCBs (Sipka et al. 2008). This increase in mRNA expression is both time and dose dependent. Another study in mice analyzing the impact of PCB-118 (oral gavage with 150  $\mu\text{mol/kg}$  body weight) on formation of brain metastases has shown increased levels of VCAM-1 mRNA levels when exposed to PCB-118 (Sipos et al. 2012).

Both ICAM-1 and VCAM-1 mediate adhesion dependent cell-to-cell interactions in vascular system and have an important influence on inflammatory reactions (Muller 2009). They play an important role in the initial phase of pathogenesis of atherosclerosis through migration of leukocytes and their adherence to the endothelium, one of the initial steps in pathogenesis of atherosclerosis (Cybulsky and Gimbrone 1991). The present findings are therefore in accordance with recently published data from the PIVUS study, where we found TEQ and PCBs to be related to carotid artery atherosclerosis measured by ultrasound (Lind et al. 2012). The toxicity due to coplanar PCB exposure, which binds to AhR with high affinity, has been well documented. However, recent studies have also suggested that non-coplanar PCBs may also produce adverse effects. Several non-coplanar PCBs (PCB-170, 180, 206, 209) have shown positive association with inflammatory response via VCAM-1. Although, how non-coplanar PCBs exactly exert their effects remains unclear, it may be possible that these non-coplanar PCBs may bind to not yet identified PCB receptor(s).

### *Downstream inflammation indicators*

In a cross-sectional study including the non-diabetic individuals, Kim et al. have analyzed the association of levels of various serum POPs with CRP (KS Kim et al. 2012). In that study, two different statistical models (including a number of covariates) were considered for analysis. Kim et al. observed that OC pesticides follow a significant positive trend with levels of CRP but the association turns insignificant when additional adjustment for waist circumference and BMI are made. In the present study, similar observations were made with regard to some of the PCBs. Although a few of individual PCBs showed significant associations with levels of CRP in model A, the association was attenuated to non-significance in model B. We did not observe any significant association between TEQ values, sum of PCBs or sum of OC pesticides with CRP in our cohort.

When total leucocyte counts were studied for their association with different TEQ values, sum of PCBs or sum of OC pesticides, we did not see any significant association. There are a few studies, mainly in infants, that have looked at POPs exposure and their association with total leucocyte counts (Glynn et al. 2008; Weisglas-Kuperus et al. 1995). Glynn et al. performed a study in infants from a location similar to ours showing that infants with greater exposure to different POPs have significantly higher mean numbers of total WBCs than infants in the reference category with the lowest exposure. Although two of the individual POPs (PCB-126 and PCB-209) were found to be significantly associated with TLC in our study, these POPs became insignificant when adjusted for all the covariates in model B. The difference in age between the present study and the infants from study by Glynn et al, along with the respiratory infections experienced by these infants may account for the differences in the observations. Another study

in Dutch infants did not find any significant differences in total leucocyte counts due to the exposure to different POPs (Weisglas-Kuperus et al. 1995).

### ***Cytokines***

Only a very few studies are available today that have looked at the effects of POPs on the levels of cytokines in humans (Imbeault et al. 2012; MJ Kim et al. 2012). In a study including 109 individuals from Canada, where the association of elevated levels of POPs with activation of immune response was studied, a weak but significant association was observed (Imbeault et al. 2012). We did not observe any association between levels of POPs and pro-inflammatory cytokines (IL-6, MCP-1 and TNF- $\alpha$ ). Differences in results between two studies may be due to various factors. The presence of other diseases like arthritis, cardiovascular and chronic respiratory diseases experienced by these Canadian people may enhance the levels of various cytokines. Further, number of individuals recruited in the Canadian study was small.

Studies have shown positive correlations between concentrations of different POPs with different measures of adiposity like BMI, fat mass or waist circumference (Kim et al. 2011; Pelletier et al. 2003; Porta et al. 2010; Roos et al. 2013). The effect size of POPs on significant inflammatory markers observed in our study was more than double in individuals with BMI above median than with those below the median. Since increased adiposity leads to higher burden of POPs that may augment the inflammatory response (La Merrill et al. 2013), higher effect size of POPs in individuals with BMI above median was in the expected direction. In a recent study by Baker et al., it has been shown that mice treated with PCBs (dose of 2.5-248 mg/kg for PCB-77 and 0.3-3.3 mg/kg for PCB-126 by oral gavage) have increased glucose and insulin tolerance due to increased accumulation of PCBs in adipose tissue thereby resulting into higher expression of inflammatory marker like TNF- $\alpha$  (Baker et al. 2013).

It has been hypothesized that consuming fishes or fish oil may have beneficial health effects. On one hand, we may see favorable impact on health due to nutrients like omega-3 present in fish food that may control inflammation, these fishes may be high in POPs concentration that may revert the positive outcomes (Turunen et al. 2013). Although we don't have such data in our cohort, the results observed in our study may be influenced by diet and nutrients consumed (especially fish and fish oil) by the participants.

### ***Strengths and limitations***

The main strength of this study is inclusion of a large number of POPs and a variety of inflammatory markers. The study was conducted in a large community-based sample from the general population. A large number of POPs as well as inflammatory markers were analyzed.

Since the study was conducted in a cohort of elderly Caucasian individuals only, caution should be taken while generalization of results to individuals of other age groups or ethnicities. Individuals were recruited from a limited geographical location, thereby limiting the extrapolation of results to other locations. The associations for individual POPs shown in the supplementary materials were not corrected for multiple test corrections and therefore should be treated with caution. The TEF/TEQ approach may overestimate the inflammatory response of various POPs studied due to other agents causing inflammation. There are natural dietary components present as well that are formed during cooking and mimic the AhR agonists, thereby augmenting inflammatory response, but these are not accounted for in the estimation of TEQ exposure (Denison and Nagy 2003).

## **Conclusion**

The TEQ values were found to be associated with levels of ICAM-1, to a lesser degree also to VCAM-1, but not to CRP and several other inflammatory markers. These findings suggest an activation of vascular adhesion molecules by POPs and in particular by PCB-126. These findings might in part explain why high levels of POPs are related to atherosclerosis and other disorders in which vascular inflammation plays an important part.

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**Table 1.** General/clinical characteristics and inflammatory markers of the studied individuals.

<b>Variable</b>	<b>Number</b>	<b>Median (IQR)</b>	<b>Min-Max</b>
BMI (kg/m <sup>2</sup> )	991	26.6 (24.0–29.6)	16.6–49.8
WC (cm)	980	90 (84–98)	60–134
Glucose (mmol/L) <sup>a</sup>	988	5.0 (4.6–5.4)	2.8–19.9
SBP (mmHg)	987	148 (134–164)	84–230
LDL (mmol/L)	986	3.3 (2.8–3.9)	0.8–6.9
HDL (mmol/L)	988	1.4 (1.2–1.8)	0.6–3.8
TG (mmol/L) <sup>a</sup>	988	1.15 (0.87–1.51)	0.35–4.8
GFR (mL/min/1.73m <sup>2</sup> ) <sup>a</sup>	987	78.9 (65.8–94.9)	23.2–210.8
ICAM-1 (mg/L)	991	226 (193–266)	88–886
VCAM-1 (mg/L)	991	520 (457–601)	217–1661
E-Selectin (mg/L) <sup>a</sup>	991	14.8 (11.1–18.9)	3.5–98.8
IL-6 (pg/mL) <sup>a</sup>	974	4.2 (2.2–15.0)	0.3–800
TNF- $\alpha$ (pg/mL) <sup>a</sup>	987	3.7 (2.9–4.9)	1.2–183.2
MCP-1 (pg/mL)	982	382 (308–467)	16–973
CRP (mg/L) <sup>a</sup>	991	1.20 (0.62–2.32)	0.18–93.46
TLC (x10 <sup>9</sup> cells/L)	986	5.5 (4.7–6.5)	1.7–15.2

Abbreviations: IQR: Interquartile Range; Min: Minimum; Max: Maximum; BMI: Body Mass Index; WC: Waist Circumference; SBP: Systolic Blood Pressure; LDL: Low Density Lipoprotein; HDL: High Density Lipoprotein; TG: Triglyceride; GFR: Glomerular Filtration Rate; ICAM-1: Intercellular Adhesion Molecule 1; VCAM-1: Vascular cell adhesion protein 1; IL-6: Interleukin 6; TNF- $\alpha$ : Tumor Necrosis Factor  $\alpha$ ; MCP-1: monocyte chemotactic protein-1; CRP: C-reactive protein; TLC: Total Leucocyte Count.

<sup>a</sup>Log transformed values are reported.

**Table 2.** Distribution of POPs studied along with their summary measures.

POPs	n	Mean <sup>a</sup>	Median (IQR) <sup>a</sup>	Median (IQR) <sup>b</sup>	Min-Max <sup>b</sup>	%<LOD
PCB-74	991	88.4	91.4 (63.8–128.2)	2.7 (2.4–3.0)	0.4–4.1	0
PCB-99	991	87.8	90.7 (62.4–132.0)	2.7 (2.3–3.0)	0.2–4.9	0.5
PCB-105	991	31.6	32.0 (21.0–46.8)	1.6 (1.3–2.0)	-0.3–4.0	0
PCB-118	990	194.5	200.6 (136.4–281.0)	3.5 (3.1–3.8)	1.5–5.5	0
PCB-126	985	38.4	40.4 (21.6–71.8)	1.9 (1.3–2.4)	-2.3–4.1	4.5
PCB-138	991	807	819 (619–1115)	4.9 (4.6–5.2)	2.6–6.0	0.4
PCB-153	991	1394	1428 (1114–1846)	5.5 (5.2–5.7)	2.9–6.5	0
PCB-156	991	151.5	154.2 (118.6–197.6)	3.2 (3.0–3.5)	0.8–4.3	0
PCB-157	991	28.0	28.0 (21.4–37.0)	1.5 (1.2–1.8)	-0.2–3.6	0
PCB-169	985	166.4	171.4 (130.6–219.8)	3.1 (2.8–3.3)	-0.3–4.5	0.3
PCB-170	991	489.0	497.2 (385.4–632.8)	4.4 (4.2–4.6)	2.3–5.8	0
PCB-180	991	1147	1165 (917–1487)	5.2 (5.0–5.5)	3.2–6.4	0
PCB-189	991	20.2	19.2 (14.6–25.8)	1.1 (0.9–1.4)	-1.1–5.0	0
PCB-194	991	101.9	119.4 (87.6–158.8)	3.0 (2.7–3.2)	-0.8–4.4	1.4
PCB-206	991	26.2	26.8 (20.8–35.2)	1.5 (1.2–1.8)	-1.1–3.3	0
PCB-209	991	25.0	26.2 (19.6–34.6)	1.5 (1.2–1.7)	-1.3–2.9	0
OCDD	986	2.7	2.6 (1.4–4.1)	-0.9 (-1.4 – -0.4)	-2.1–1.1	19.4
HCB	991	255.5	254.0 (189.2–336.6)	3.7 (3.4–4.0)	2.6–6.4	1.4
<i>p,p'</i> -DDE	991	1847	1858 (1024–3451)	5.7 (5.1–6.3)	0.7–8.4	0
BDE-47	991	15.1	12.6 (9.0–19.4)	0.7 (0.5–1.1)	-0.2–6.2	27.8
TNC	991	137.3	139.2 (91.6–211.2)	3.1 (2.7–3.5)	0.8–4.9	0
TEQ	979	9.6	9.8 (6.7–13.9)	0.5 (0.1–0.8)	-2.1–1.9	–
Sum PCBs	984	4908	4988 (3942-6300)	47.3 (43.3-50.9)	16.6-62.5	–
Sum OCP	991	2402	2298 (1475-3945)	12.6 (11.6–13.5)	4.8–17.4	–

Abbreviations: n: number of samples; IQR: interquartile range; Min: minimum; Max: maximum; LOD: limit of detection; TEQ: total equivalency value; PCB: polychlorinated biphenyls; OCDD: octachlorodibenzo-*p*-dioxin; HCB: hexachlorobenzene; TNC: *trans*-nonachlordane; *p,p'*-DDE: 2,2-bis (4-chlorophenyl)-1,1-dichloroethene; BDE: bromodiphenyl ether; OCP: organochlorine pesticide. Geometric mean is reported a: actual concentrations of pollutants (pg/ml) in the plasma; b: lipid normalized and log transformed concentrations of POPs (ng/g of lipid).

**Table 3.** Associations [ $\beta$  (95% CI)] of TEQ values, sum of PCBs and sum of OC pesticide concentrations with inflammatory markers studied.

Marker	TEQ: Model A	p-value	TEQ: Model B	p-value	Sum of PCBs: Model A	p-value	Sum of PCBs: Model B	p-value	Sum of OC Pest: Model A	p-value	Sum of OC Pest: Model B	p-value
ICAM-1	16.35 (8.76, 23.94)	2.7*10 <sup>-5</sup>	16.36 (8.64, 24.08)	3.6*10 <sup>-5</sup>	0.05 (-0.59, 0.68)	0.89	0.11 (-0.55, 0.76)	0.75	0.67 (-1.98, 3.32)	0.62	-0.17 (-2.93, 2.59)	0.91
VCAM-1	25.26 (7.40, 43.11)	0.006	26.06 (7.85, 44.27)	0.005	2.25 (0.76, 3.73)	0.003	2.55 (1.02, 4.07)	0.001	9.20 (3.017, 15.37)	0.004	4.50 (-1.98, 10.99)	0.17
E-Selectin	-0.02 (-0.07, 0.03)	0.44	-0.01 (-0.06, 0.04)	0.69	-0.002 (-0.01, 0.002)	0.36	-0.001 (-0.01, 0.003)	0.55	0.02 (-0.002, 0.03)	0.07	-0.003 (-0.02, 0.02)	0.76
IL-6	0.02 (-0.18, 0.22)	0.82	0.05 (-0.16, 0.26)	0.62	0.01 (-0.01, 0.02)	0.43	0.01 (-0.01, 0.03)	0.41	0.01 (-0.06, 0.08)	0.76	0.004 (-0.07, 0.08)	0.92
TNF- $\alpha$	0.01 (-0.06, 0.09)	0.77	0.01 (-0.07, 0.09)	0.78	-0.003 (-0.01, 0.004)	0.41	-0.003 (-0.01, 0.004)	0.37	-0.01 (-0.03, 0.02)	0.62	-0.01 (-0.04, 0.02)	0.35
MCP-1	3.74 (-13.47, 20.95)	0.67	2.96 (-14.78, 20.70)	0.74	-0.32 (-1.76, 1.11)	0.66	-0.29 (-1.78, 1.20)	0.7	1.11 (-4.87, 7.10)	0.72	-1.42 (-7.73, 4.89)	0.66
CRP	-0.04 (-0.16, 0.09)	0.57	0.06 (-0.07, 0.18)	0.36	-0.01 (-0.02, -0.001)	0.03	-0.002 (-0.01, 0.01)	0.7	-0.004 (-0.05, 0.04)	0.86	-0.05 (-0.09, -0.01)	0.03
TLC	0.11 (-0.08, 0.30)	0.24	0.09 (-0.09, 0.27)	0.33	-0.01 (-0.03, 0.003)	0.11	-0.01 (-0.02, 0.01)	0.26	0.06 (-0.004, 0.13)	0.06	0.004 (-0.06, 0.07)	0.91

Abbreviations:  $\beta$ : beta coefficient; CI: confidence interval; PCB: polychlorinated biphenyls; OC: organochlorine; Pest: pesticide; ICAM-1: intercellular adhesion molecule 1; VCAM-1: vascular cell adhesion protein 1; IL-6: interleukin 6; TNF- $\alpha$ : tumor necrosis factor alpha; CRP: C-reactive protein; MCP-1: monocyte chemotactic protein-1; TLC: total leucocyte count. Model A: Linear regression model adjusted for sex and kidney function; Model B: Linear regression model adjusted for sex, kidney function, smoking, BMI, waist circumference, blood glucose, systolic blood pressure, HDL, LDL, triglycerides, exercise habits and education.

**Table 4.** Association [ $\beta$  (95% CI)] of individual OC pesticides with inflammatory markers<sup>a</sup> studied.

Marker	HCB	p-value	TNC	p-value	<i>p,p'</i> -DDE	p-value
ICAM-1	-11.83 (-21.32, -2.34)	0.02	-7.251 (-14.19, -0.31)	0.04	3.69 (-0.65, 8.03)	0.10
VCAM-1	2.19 (-19.93, 24.32)	0.85	5.10 (-11.12, 21.33)	0.54	7.12 (-3.01, 17.24)	0.17
E-Selectin	-0.022 (-0.08, 0.04)	0.50	-0.008 (-0.05, 0.04)	0.75	0.001 (-0.03, 0.03)	0.95
IL-6	0.21 (-0.05, 0.46)	0.11	-0.04 (-0.23, 0.15)	0.67	-0.01 (-0.13, 0.10)	0.81
TNF- $\alpha$	-0.03 (-0.12, 0.07)	0.55	-0.005 (-0.07, 0.07)	0.89	-0.02 (-0.07, 0.02)	0.28
CRP	-0.02 (-0.17, 0.13)	0.79	-0.09 (-0.20, 0.02)	0.11	-0.083 (-0.15, -0.02)	0.016
MCP-1	0.78 (-20.92, 22.48)	0.94	-11.35 (-27.29, 4.6)	0.16	0.69 (-9.15, 10.53)	0.89
TLC	-0.014 (-0.23, 0.2)	0.9	-0.14 (-0.30, 0.02)	0.09	0.05 (-0.05, 0.15)	0.29

Abbreviations:  $\beta$ : beta coefficient; CI: confidence interval; HCB: hexachlorobenzene; TNC- *trans*-nonachlordane; *p,p'*-DDE: 1,1-bis-(4-chlorophenyl)-2,2-dichloroethene; ICAM-1: Intercellular Adhesion Molecule 1; VCAM-1: Vascular cell adhesion protein 1; IL-6: Interleukin 6; TNF- $\alpha$ : Tumor Necrosis Factor alpha; CRP: C-reactive protein; MCP-1: monocyte chemotactic protein-1; TLC: Total Leucocyte Count.

<sup>a</sup>Linear regression model adjusted for sex, kidney function, smoking, BMI, waist circumference, blood glucose, systolic blood pressure, HDL, LDL, triglycerides, exercise habits and education.