Review

Journal of INTERNAL MEDICINE

doi: 10.1111/j.1365-2796.2012.02536.x

Can persistent organic pollutants and plastic-associated chemicals cause cardiovascular disease?

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Abstract. Lind L, Lind PM (Uppsala University, Uppsala, Sweden). Can persistent organic pollutants and plastic-associated chemicals cause cardiovascular disease? (Review). *J Intern Med* 2012; **271**: 537–553.

During the last decade, associations between persistent organic pollutants (POPs), such as polychlorinated biphenyls, dioxins and pesticides, and cardiovascular (CV) risk factors and overt CV disease (CVD) have been reported in humans. Recently, associations between plastic-associated chemicals (PACs), such as bisphenol A and phthalates, and CVD have also begun to emerge. Several approaches to evaluating such associations have been used: accidents with a high level of exposure, occupational exposure studies, geographical studies of subjects living near a contaminated area and traditional case–control or cohort studies with measurements of circulating levels of different environmental contaminants in the general population. Exposure to POPs has consistently been associated with diabetes using all the approaches described above, including prospective studies. The evidence regarding associations between exposure to POPs and other CV risk factors, such as hypertension, obesity and lipids, is less strong and is mainly based on cross-sectional data. Associations between overt CVD and POPs have been reported using all the above approaches, but prospective data from population-based studies are still lacking to provide firm evidence of an important and independent role of POP exposure in the pathogenesis of CVD. Nevertheless, taken together, current evidence suggests that further longitudinal and experimental studies should be conducted to investigate the effect of exposure to both POPs and PACs, such as bisphenol A and phthalates.

Keywords: cardiovascular disease, environmental contaminants, persistent organic pollutants (POPs), plastic-associated chemicals (PACs).

Introduction

Since the Second World War, humans have been exposed to a growing number of man-made chemicals. To date, about 150 000 chemical substances have been registered in the database of the European Chemicals Agency (http://www.echa.europa.eu/). Whilst chemical substances to be used as pharmaceuticals are subjected to rigorous regulatory control, chemicals intended for most other purposes require little or no regulation before entering the market. Very few of these chemicals have been tested for toxicity, and only around 20% of the many chemicals on the market are tested to a sufficient degree to enable proper assessment of their risks to health and their effects on the environment.

The term persistent organic pollutants (POPs) refers to a number of highly divergent chemicals with the common characteristics of toxicity and resistance to degradation. Many POPs are highly lipophilic and thereby accumulate in adipose tissue with a half-life from 1 month up to several years. The best-known POPs are polychlorinated biphenyls (PCBs), dioxins, brominated flame retardants and organochlorine (OC) pesticides, such as dichlorodiphenyltrichloroethane (DDT).

Another group are high-volume produced chemicals used in the production of plastics and include bisphenol A and phthalates. Most of these compounds are not lipophilic, but because of their widespread use in daily life, they are measureable in the circulation in almost all individuals in the industrialized world. Table 1 provides a summary of some of the POPs and plastic-associated chemicals (PACs) discussed in this review.

A common feature of several POPs and PACs is an effect on hormonal systems, and therefore, they have been collectively termed ‘endocrine disruptors’. Because these chemicals can act both as agonists and antagonists on sex hormone receptors in different tissues, studies have been carried out to investigate

Table 1 *Overview of some of the toxicants discussed in this review*

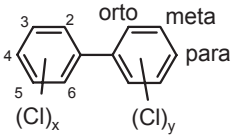
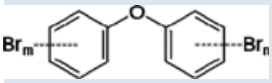
Chemicals	Uses and sources of exposure
Persistent organic pollutants (POPs)	
Polychlorinated biphenyls (PCBs) 	PCBs are mixtures of up to 209 individual chlorinated compounds (known as congeners) with between two and 10 chlorine atoms attached to a biphenyl, which is a molecule composed of two benzene rings. Twelve of the PCBs have 'dioxin-like' properties. PCBs have been used commercially since 1930. The use of PCBs was banned in Sweden in 1972 and PCB production was banned by the US Congress in 1979 because of evidence that they build up in the environment and can have harmful effects on health. PCBs are either oily liquids or solids that are colourless or pale yellow; some can exist as a vapour in air. PCBs are very persistent and accumulate in food webs. They have been used as coolants and lubricants in transformers, capacitors and other electrical equipment because they do not burn easily and are good insulators. Products that may contain PCBs include old fluorescent lighting fixtures, electrical devices containing PCB capacitors and micro scope and hydraulic oils. PCBs are fat-soluble compounds that accumulate in individuals and magnify in the food chain, so although there are manufacturing and use restrictions for PCBs in many industrialized countries, they are still present in food (fish and dairy produce are most contaminated) and in the body.
Polybrominated diphenyl ethers (PBDEs) 	PBDEs are added to plastics and foam products to make them difficult to burn; they are the predominant constituents of flame-retardant agents. PBDEs exist as mixtures of similar chemicals known as congeners. There are different kinds of PBDEs; some have only a few bromine atoms, whereas others have as many as 10 bromine atoms attached to the central molecule. Because they are mixed into plastics and foams rather than bound to them, PBDEs can leak from products and enter the environment. They are distributed in terrestrial and aquatic environments worldwide. The penta- and octabromodiphenyl ether mixtures were banned in Europe in August 2004.

Table 1 *(Continued)*

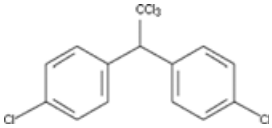
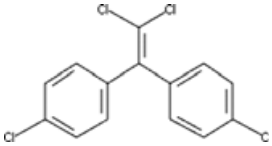
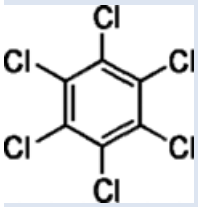
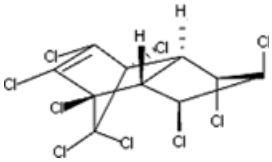
Chemicals	Uses and sources of exposure
DDT and DDE: 1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane (DDT)  1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene (p,p′-DDE) 	DDT is a lipid-soluble organochlorine pesticide extensively used as an agricultural insecticide since the 1940s. DDT and its major and very persistent metabolite p,p′-DDE are biomagnified at different trophic levels in the environment. p,p′-DDE is considered an anti-androgen as it inhibits androgen binding to its receptor. In 1972, the US Environmental Protection Agency banned all use of DDT except when essential to public health. Similar bans were instituted by Sweden in 1975 and later in most developed countries. DDT is still being used in developing countries to control disease, in particular for control of malaria-carrying mosquitoes. Because DDT was banned in most Western countries, the residual levels in the environment have de creased locally. However, residual low levels of DDT remain worldwide because of its high chemical stability and transport over long distances
Hexachlorobenzene (HCB) 	HCB is a very persistent organochlorine chemical that was introduced as early as the 1930s and was widely used as a fungicide until 1965. It was also used to make fireworks, ammunition and synthetic rubber and may be created as either a byproduct of or an impurity in the manufacturing process for other chemicals and pesticides. HCB is now banned worldwide for use as a fungicide, but it may still contaminate the environment as it is formed as a by-product of other chemicals, in the waste streams of chloralkali and wood-preserving plants, and when burning municipal waste.
Trans-nonachlordane (*trans*-nonachlor, TNC, t-NCHL) 	Technical chlordane is a mixture of >140 related compounds; 60–85% of technical chlordane consists of the stereoisomers cis- and trans-chlordane and trans-nonachlordane (*trans*-nonachlor, TNC, t-NCHL). Chlordane is a broad-spectrum insecticide known for its toxic effects and its capacity to persist and accumulate in the environment. It is stable in soil and breaks down very slowly, so can remain for decades. Chlordane is lipid soluble and very resistent to degradation, and the main exposure route is ingestion of high-fat foods such as meat, fish and dairy produce. Chlordane was introduced in 1945 and was commonly used from 1948 to 1988, but was banned for agricultural use in Sweden 1971 and in the EU in 1981 because of concern about damage to the environment and harm to human health.

Table 1 *(Continued)*

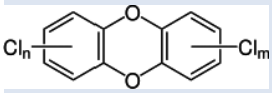
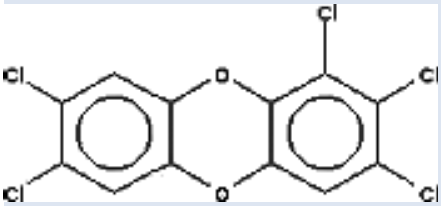
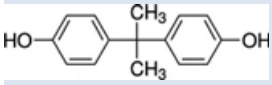
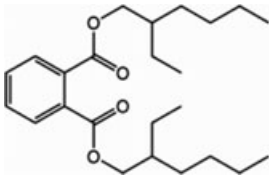
Chemicals	Uses and sources of exposure
Dioxins – polychlorinated dibenzo-*p*-dioxins and dibenzofurans:  Polychlorinated dibenzo-*p*-dioxin (PCDD)  2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)	The term dioxins usually refers to polychlorinated dibenzo-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). PCDDs are a family of 75 chemically related compounds commonly known as chlorinated dioxins. In PCDDs, chlorine atoms are attached at any of eight different places on the molecule. There are 75 different PCDD congeners. One of these compounds, 2,3,7,8- tetrachloro-dibenzo-*p*-dioxin (TCDD), is amongst the most toxic of the PCDDs and also the most studied. As TCDD has the highest toxic potential, the toxicity of other PCDDs, PCDFs and dioxin-like PCBs is defined in comparison to TCDD. Toxic Equivalents (TEQs) are used to report the toxicity-weighted masses of mixtures of dioxins. Dioxins are formed as byproducts of numerous types of industrial activity and all combustion processes. Dioxins build up primarily in fatty tissues over time (bioaccumulate), so even small exposures may eventually reach dangerous levels. Apart from occupational or accidental exposures, most human exposure to PCDD/PCDF occurs as a result of dietary intake, mainly of meat, milk, eggs, fish and related products.
Perfluorinated chemicals (PFCs):  Perfluorooctane sulphonate (PFOS)  Perfluorooctanoic acid (PFOA)	PFCs are a large group of chemicals that, in recent years, have been used increasingly as surfactants in various industrial and consumer products because of their unique properties as repellents of dirt, water and oils. Fluoropolymer coatings are used in such varied products as clothing, furniture, adhesives, food packaging, heat-resistant nonstick cooking surfaces and the insulation of electrical wire. The best-known PFCs are PFOS and PFOA, and their derivatives. Industrial production of PFOS and its derivatives ceased in 2000, and the EU has banned most uses. However, hundreds of related chemicals, homologues with shorter or longer alkyl chains, PFOA and telomeres, which may degrade, are not regulated. PFCs have been found in rivers and lakes and in many types of animals on land and in water. Bioaccumulation also occurs in humans, and individuals in Western societies have traces of these PFCs in their blood and internal organs such as the liver, kidneys, spleen, gall bladder and testes. In the blood, PFOS and PFOA are bound to serum proteins.

Table 1 *(Continued)*

Chemicals	Uses and sources of exposure
Plastic-associated chemicals (PACs)	
 Bisphenol A (BPA)	BPA has been used for over 50 years in the manufacture of polycarbonate plastics and epoxy resins, in thermal paper production, and as a polymerization inhibitor in the formation of some polyvinyl chloride plastics. Polycarbonates are used to make products such as compact discs, car parts, baby feeding bottles, plastic dinnerware, eyeglass lenses, toys and impact-resistant safety equipment. Epoxy resins containing bisphenol A are used in protective linings of some canned food containers, wine barrel linings, epoxy resin-based paints, floorings and dental composites. General exposure to BPA at low levels comes from eating food or drinking water stored in containers that at least in part are composed of the compound. Young children may be exposed by hand-to-mouth and direct oral contact with materials containing BPA. Dental treatment with BPA-containing sealants also results in short-term exposure. In addition, workers can be exposed during manufacture of BPA-containing products.
Phthalates  Di-2-ethyl hexyl phthalate (DEHP)	Phthalates or phthalate esters are a large group of chemicals used mainly to make polyvinylchloride (PVC) more flexible and less breakable. The most widely used phthalates are di-2-ethyl hexyl phthalate (DEHP), diisodecyl phthalate and diisononyl phthalate. DEHP is the dominant plasticizer used in PVC. These kinds of phthalates (plasticizers) are used in hundreds of PVC products, such as vinyl flooring, adhesives, detergents, automotive plastics, raincoats, garden hoses, inflatable toys, blood-storage containers, medical tubing and children's toys. Some phthalates are used as solvents in perfumes, soaps, shampoos, hair sprays and nail polishes. Individuals are exposed to phthalates by eating and drinking foods that have been in contact with containers and products containing phthalates. To a lesser extent, exposure can occur from breathing in air that contains phthalate vapours or dust contaminated with phthalate particles. Young children may have a greater risk of exposure to phthalate particles in dust than adults because of their hand-to-mouth behaviour. Once phthalates enter the body, they are converted into metabolites, for example, DEHP is metabolised to mono-(2-ethylhexyl) phthalate, which is rapidly excreted in the urine.

their effects on reproduction and hormone-sensitive cancers. In recent years, associations with cardiovascular (CV) factors and overt CV disease (CVD) have also been reported.

In the present review, we focus on evidence from human studies regarding a link between POPs/PACs and the development of atherosclerosis and CVD. Five different research tools have been used: data from accidents in which some subjects were exposed to very high concentrations of a pollutant, occupational exposure at moderate to high levels, geographical exposure by living close to a contaminated area, case–control studies, cross-sectional studies and traditional longitudinal population studies with measurements of circulating levels of different chemical compounds (see Table 2 for summary of study types). Studies with CVD as outcomes are summarized in Table 3.

Table 2 *Studies to evaluate the link between environmental contaminants and human disease*

Type of study	Comments
Accidental exposure (e.g. of a geographical area or workplace) to very high contaminant levels	Resembles classical toxicity studies in experimental animals. Catches effects on the right part of a dose–response curve
Occupational exposure studies	Usually used to evaluate a moderately high degree of expo sure. Could evaluate the effects of a single compound, but more likely to determine the effects of a mixture of compounds
Geographical area exposure studies (e.g. proximity to a waste site)	Usually used to evaluate a low degree of exposure, and the effects of a mixture of compounds
Case–control studies	Used to assess exposure either by history or through blood samples
Cross-sectional population-based studies	Usually used to evaluate a low degree of exposure. Able to assess exposure either by history or through blood samples
Prospective population-based studies	Usually used to evaluate a low degree of exposure. Able to assess exposure either by history or through blood samples. Reverse causality has less effect than in case–control and cross-sectional studies

Risk factors for CVD

Diabetes

Accidents. In Seveso in northern Italy, a manufacturing plant exploded in 1976, and the surrounding area was heavily contaminated with the highly toxic dioxin 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD). After 25 years of follow-up, the diabetes mortality rate in Seveso was elevated [relative risk (RR) 1.7, 95% confidence interval (CI) 1.1–2.7] compared with the rate in a nonexposed surrounding reference area [1].

In the late 1970s, the consumption of rice-bran oil laced with PCBs and polychlorinated dibenzofurans (PCDFs) poisoned thousands of Taiwanese people in Yucheng. At follow-up 24 years later, 378 subjects from Yucheng were compared with 370 matched nonexposed controls. The risk of diabetes was significantly increased for women [odds ratio (OR) 2.1, 95% CI 1.1–4.5], but not for men (OR 1.0), amongst the Yucheng population. Furthermore, women from Yucheng diagnosed with chloracne, a sign of severe PCB/dioxin exposure, had an adjusted OR for diabetes development of 5.5 [2].

Occupational studies. During the Vietnam War, the US Air Force used the herbicide Agent Orange, a chemical contaminated with TCDD. A large number of studies have been conducted with various follow-up periods, most often using Air Force soldiers who served in Vietnam but were not exposed to Agent Orange as controls. In general, these studies showed an increased risk of diabetes development in the exposed Vietnam veterans (RR values between 2.0 and 4.0) as significant in some, but not all, studies [3–8].

Geographical studies. A study team led by Carpenter investigated >900 waste sites in New York, NY, USA, and quantified the extent of POP contamination. Merging data of individual's zip codes and health records, the investigators concluded that subjects living close to a contaminated waste site showed a 23% (95% CI 15–32%) increased risk of diabetes [9].

Population-based studies. Although a large number of cross-sectional studies investigating POP levels in different populations have been reported, we were only able to identify five prospective studies. In the

Table 3 *Summary of studies relating POP or PAC levels to CVD*

Type of study	Study [references]	Study design	Number of subjects/cases	Endpoint	Results
Accident	Seveso [1]	Prospective/25 years' follow-up	278 108/2402	CVD mortality	RR 1.8 (95% CI 1.0–3.1) for 5- to 9-year follow-up, NS thereafter
Accident	Yucheng [2]	Prospective/24 years' follow-up	748/90	Incident CVD	RR 1.4 (95% CI 0.7–2.4)
Occupational exposure	Vietnam veterans [3]	Prospective/>25 years' follow-up	1499/243	Incident CHD	RR 1.4 (95% CI 1.0–1.8) for sprayers of TCDD
Occupational exposure	IARC [64]	Prospective	21 863/158–1183	CHD/stroke mortality	RR 1.6 (95% CI 1.2–2.2) for CHD, RR 1.5 (95% CI 0.8–2.8) for stroke
Geographical study	New York waste sites [29]	Cross-sectional	>5 M	Prevalent MI	RR 1.2 (95% CI 1.0–1.3) for POP contamination
Geographical study	New York waste sites [60]	Cross-sectional	>5 M/28 216	Prevalent stroke	RR 1.1 (95% CI 1.0–1.2) for POP contamination
Population-based study	NHANES 2003–2004 [26]	Cross-sectional	1455/42–79	Prevalent CVD/MI	For 1 SD of bisphenol A: RR 1.3 (95% CI 1.1–1.6) for CVD, RR 1.4 (95% CI 1.1–1.7) for MI
Population-based study	NHANES 2005–2006 [27]	Cross-sectional	1331/38–76	Prevalent CVD/MI	For 1 SD of bisphenol A: RR 1.2 (95% CI 0.9–1.5) for CVD, RR 1.3 (95% CI 1.0–1.6) for MI
Population-based study	NHANES 1999–2002 [66]	Cross-sectional	2032/143	Prevalent PAOD	RR 1.2 (95% CI 1.0–1.5) in obese subjects for sum of all OC pesticides. NS in nonobese subjects
Population-based study	NHANES 1999–2002 [63]	Cross-sectional	889/108	Prevalent CVD	RR for Q4 versus Q1: dioxin-like PCBs 5.0 (95% CI 1.2–20), nondioxinline PCBs 3.8 (1.1–12), OC pesticides 4.0 (1.0–17); NS for women alone, PCDD and PCDFs
Population-based study	PIVUS [56]	Cross-sectional	1016/616	Carotid artery plaque	RR 1.8 (95% CI 1.3–2.6) for a summary measure of PCBs for Q4 versus Q1 RR 1.49 (95% CI 1.0–2.0) for sum of all pesticides
Population-based study	PIVUS [62]	Cross-sectional	1016/616	Carotid artery plaque	RR 1.4 (95% CI 1.0–1.9) for Q4 versus Q1 for mono-methyl-phthalate levels

MI, myocardial infarction; CVD, cardiovascular disease; PAC, plastic-associated chemicals; PAOD, peripheral artery occlusive disease; POP, persistent organic pollutants; CHD, coronary heart disease; NS, not significant; M, million; Q, quartile.

Michigan polybrominated biphenyl (PBB) cohort of >1300 subjects living in an area of known POP contamination, 180 subjects received a diagnosis of diabetes during a 25-year follow-up. Comparing the highest versus the lowest quintile of a summary measurement of PBBs revealed an OR of 2.0–3.0 in women and 1.7 in men; only the OR value in women was statistically significant [10]. As there are many chemical forms of PBBs and PCBs, it is common to present a summary measure instead of analysing individual compounds. The potential drawback of this procedure is that different compounds might have different actions or effects because of variations in half-lives.

In a cohort of fish consumers from sport fishing in the Great Lakes followed for more than 10 years, 36 new diabetes cases were observed. Circulating levels of 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene, DDE (a metabolite of DDT; OR 7.1, 95% CI 1.6–31 for the highest tertile), but not a summary measure of PCBs (OR 1.8, 95% CI 0.6–5.0 for the highest tertile), predicted development of diabetes [11].

In the population-based CARDIA study, a nested case–control study was performed after 17 years of follow-up and 90 new cases of diabetes were identified. Circulating levels of the pesticide trans-nonachlordane (TNC) and several PCB congeners predicted diabetes development. This effect was evident even for very low doses of TNC (OR 5.3 for second versus first sextile) and was most pronounced in obese subjects [12].

In a nested case–control study conducted in women from Lund, Sweden, DDE (OR 5.5, 95% CI 1.2–25 for the highest quartile), but not PCB153 (OR 1.6, 95% CI 0.6–4.0), predicted development of diabetes that was diagnosed more than 7 years after the baseline examination [13].

In the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study, a cohort study including >1000 subjects all aged 70 years, we investigated the associations between POPs and diabetes using both a cross-sectional design (113 cases) and prospective data during 5 years of follow-up (36 cases). Using both approaches, a number of PCB congeners, as well as a summary measure of pesticides including DDE and TNC, were found to be associated with diabetes [14]. Of note, the summary measure of PCBs was a stronger risk factor for incident diabetes (OR 7.5, 95% CI 1.4–38) than body mass index (BMI) (OR 4.5, 95% CI 1.2–22), even following adjustment for BMI.

The results of most, but not all, of the cross-sectional studies confirmed the findings of the prospective studies, suggesting a role of POPs in diabetes [15–25]. It is noteworthy that all cross-sectional studies analysing TNC found a significant association with diabetes [12, 14, 16, 19, 24, 25].

In the US National Health and Nutrition Examination Survey (NHANES) of 2003–2004, urinary bisphenol A levels were analysed in >1400 subjects. Using self-reported diabetes as outcome in a cross-sectional analysis, there was an RR of 1.3 (95% CI 1.2–1.6) for a one standard deviation (SD) change in urinary bisphenol A levels [26]. However, an investigation of the NHANES 2005–2006 survey cycle did not confirm these findings (OR 1.0, 95% CI 0.8–1.3) [27].

Brominated flame retardants, such as polybrominated diphenyl ethers (PBDEs) or PBBs, were also evaluated in a cross-sectional analysis of the NHANES data. Prevalent diabetes was significantly associated with serum concentrations of PBB-153 and PBDE-153 (ORs 1.8–3.1 for highest versus lowest quartiles) [28].

Hypertension

Compared with studies of diabetes, investigations of the association between hypertension and POPs are rare.

In the Yucheng PCB accident, women diagnosed with chloracne showed an adjusted OR of 3.5 for hypertension compared with those without chloracne during 24 years of follow-up [2].

In one of the above-mentioned studies of US Vietnam veterans, an increased incidence rate of hypertension was found amongst those who had sprayed Agent Orange compared with controls (increased risk of 32%, 95% CI 8–61%) [3].

In the above-mentioned study of contaminated waste sites in New York, a 19% increased risk (95% CI 9–31%) in hypertension was observed amongst those living close to a contaminated site [29].

Population-based studies. In a cross-sectional analysis of the NHANES 1999–2002 data, it was found that the RR for hypertension was 1.8 (95% CI 1.2–2.7) for the highest quartile of a number of PCBs [30], whilst another evaluation of the same survey including 524 subjects showed that dioxin and PCDF concentrations in serum were related to newly diagnosed

hypertension in women only (RR 5–6 for highest versus lowest quartiles) [31].

In a cross-sectional analysis of data from 758 participants living in a polluted environment in Anniston, AL, USA, RR for hypertension for the highest versus the lowest tertile of PCBs was 4.1 (95% CI 1.3–14).

In some of these studies, the diagnosis of hypertension was based on the combination of blood pressure measurements and/or history of antihypertensive medication, which is appropriate for determining the prevalence of hypertension, as more than half of all hypertensive cases are unknown in population surveys in adults [32].

Metabolic syndrome

Population-based studies. With regard to the metabolic syndrome (MetS), there are only cross-sectional data on the effect of these pollutants. Most investigations have used the National Cholesterol Education Program/Adult Treatment Panel III, NCEP/ATP III criteria for the diagnosis of the MetS [33].

In a nationwide survey of >1300 Japanese subjects, circulating levels of dioxins and PCBs were related to the occurrence of the MetS (OR 3.2–4.8 for the highest versus lowest quartiles). Levels of these POPs were associated with all components of the syndrome [34].

Using data from >700 nondiabetic subjects in the NHANES 1999–2002 survey, it was demonstrated that levels of pesticides were strongly related to the MetS (OR 5.3, 95% CI 2.5–11.0, for the highest versus lowest quartile). Pesticide levels were significantly related to all criteria of the syndrome except blood pressure [ORs (highest versus lowest quartile): 2.4 for waist circumference, 7.1 for triglycerides, 2.3 for HDL, 5.6 for glucose and 1.8 for blood pressure] [35].

In a case–control study of 50 nondiabetic Korean subjects with the MetS and 50 controls, two POP pesticides, beta-hexachlorocyclohexane and heptachlor epoxide, were associated with the MetS (OR 4.4 and 6.0, respectively, for the highest quartiles) [36].

Because a common feature of the MetS is insulin resistance, the relationship between POPs and insulin resistance has also been analysed using NHANES data in 749 nondiabetic participants. The homoeostasis model assessment-estimated insulin resistance (HOMA-IR) index was used to define insulin resistance. Nineteen POPs were investigated. Amongst the POPs, pesticides were most strongly associated with HOMA-IR (OR 3.8 for highest quartile). The association between pesticides and HOMA-IR tended to strengthen as waist circumference increased [37].

Obesity

As with the MetS, most of the data regarding associations between obesity and environmental contaminants are from cross-sectional studies.

Population-based studies. In a cross-sectional study of the PIVUS cohort, we investigated the associations between POP levels and fat mass using dual-emission X-ray absorptiometry. PCBs with a low degree of chlorination were found to be positively related to fat mass, whereas PCBs with a high degree of chlorination were inversely related to fat mass [38]. Levels of pesticides were therefore positively related to fat mass. This unexpected finding of divergent effects of different PCBs might be explained by different pharmacokinetic properties, and the fact that PCBs with a low degree of chlorination have a shorter half-life than highly chlorinated PCBs, as well that peak exposure to PCBs was in the 1970s. As noted by Wolff et al. [39], the relationship between POP concentrations and fat mass (BMI) is always negative immediately after the main exposure because of the fact that POPs are mainly stored in fat tissue. However, following 2–3 half-lives of the compound, this relationship becomes positive if there is no further exposure. The timing of the change from a negative to a positive relationship is governed mainly by the half-life of the compound, but also by changes in body fat and by any recurrent exposure. An inverse relationship between highly chlorinated PCBs and obesity was also reported by Dirinck et al. [40] in a smaller case–control study of obese and lean subjects.

In a cross-sectional analysis of NHANES data, gender interactions were seen in the relations between pesticide levels and BMI, whilst a positive relationship between levels of a dioxin and BMI was seen in both men and women [41].

In another cross-sectional Japanese study of >13 000 subjects in the community, a positive relationship was found between the level of all PCBs and BMI [34]. Similar findings were seen in a smaller substudy using the CARDIA cohort, where subjects with high levels of PCBs had a high BMI and waist circumference after 25 years of follow-up [12].

Using NHANES 2003–2004 data, positive associations were found between different phthalate metabolites and BMI or waist circumference. Most often, these associations were found only in either men or women [42].

One problem with the study of associations between POPs and obesity/MetS is that POPs are stored in adipose tissue and thus the amount of fat determines the circulating levels for a given exposure, as discussed above. Thus, it is very hard in cross-sectional studies to determine whether high POP exposure could induce obesity/the MetS, as has been suggested based on animal studies [43–46]. Prospective studies would in part overcome this difficulty, but very few such studies have been reported.

In a prospective analysis of the PIVUS cohort, 100 new cases of abdominal obesity were discovered over a 5-year follow-up period. Similar to our previously reported findings of the cross-sectional analysis [38], PCBs with a low degree of chlorination were positively related to the risk of future abdominal obesity, whereas PCBs with a high degree of chlorination were inversely related to the risk of future abdominal obesity [47]. Levels of pesticides were positively related to the risk of future abdominal obesity.

Inspired by the Barker hypothesis that intra-foetal programming is a major determinant of obesity, hypertension and CVD in adult life, POP levels have been measured in mothers during pregnancy or in cord blood at delivery, and the children have been followed (mother–child cohorts). As recently reviewed in detail [48], no consistent association has been found between foetal exposure to PCBs and obesity during childhood or early adult life [49–53], whilst a majority of the studies in which foetal exposure to DDE was measured reported positive relationships with later BMI or body weight [50–54]. It should be noted, however, that only one of these studies [50] evaluated obesity during a prolonged follow-up (20–50 years), so the full potential of these mother–child studies remains unclear at present.

Lipids

Most POPs are highly lipid-soluble compounds that are transported by lipoproteins, and therefore, any associations between levels of POPs and lipids could be hard to distinguish in a meaningful way. Furthermore, traditionally POP levels have been measured as concentration normalized with respect to serum lipids.

Occupational and geographical studies. Although traditional population-based studies might not be suitable to determine the relationship between POP levels and lipids, some occupational exposure and geographical studies have addressed this issue.

In a case study of workers so heavily exposed to TCDD, it was shown that even 40 years after exposure, they still showed levels >100% higher than normally found in humans, 91% of the subjects were on anti-lipid medication and 100% showed atherosclerotic plaques in the carotid arteries at ultrasound [55]. Although no control group was included in this case study of 11 Caucasian subjects with a mean age of 66 years, both the use of anti-lipid medication and the prevalence of carotid atherosclerosis were higher than usually found in this age group (13% and 66%, respectively, in the PIVUS study in 70-year-old subjects) [56].

In a cross-sectional study amongst 133 male workers at municipal waste incineration sites, serum cholesterol levels were higher in those with a high Toxic Equivalent (TEQ; a marker for dioxin exposure) compared with those with a low value [57].

A total of 281 workers from two chemical plants in the USA and 260 control referents participated in a case–control study; those with high levels of dioxins showed increased levels of serum triglycerides [58].

In a cross-sectional study of >1000 subjects in a POP-polluted area of East Slovakia, serum triglyceride levels were increased compared with a control population from a nonpolluted nearby area [59].

Population-based studies. In contrast to other POPs, perfluorinated compounds are not highly lipid soluble (they are oil and water repellants) and lipid normalization is not needed. An association between perfluorooctane sulphonate/perfluorooctanoic acid (PFOA) levels and serum cholesterol was found in a large US cross-sectional study [60]. In a cross-sectional study of 1025 workers with potential exposure to PFOA, the circulating levels of this compound were related to LDL cholesterol concentration [61].

Atherosclerosis

Population-based studies

Very few studies on the relationship between human atherosclerosis and levels of POPs or PACs have been reported. In the PIVUS study, the carotid arteries

were examined by ultrasound. Following adjustment for lipids and a further 10 known CVD risk factors, a number of PCBs were found to be related to the occurrence of plaques in a cross-sectional analysis. In addition, levels of highly chlorinated PCBs were related to an echolucent (dark) vascular wall indicative of lipid infiltration in the intima space. Furthermore, there was a relationship between the phthalate metabolite mono-methyl phthalate and plaque occurrence, even at levels only slightly higher than the lowest levels in the sample (low-dose effect) [62]. Levels of bisphenol A and several phthalate metabolites were related to lipid infiltration of the vascular wall.

CVD

Nonspecific CVD

Accidents and occupational studies. Mortality rates were investigated in a 25-year follow-up of the Seveso accident. It was found that CVD mortality was increased during the first 10 years following the accident, but thereafter no further increase was observed. The highest CVD mortality rates were found in the 5- to 9-year follow-up interval (RR 1.8, 95% CI 1.0–3.1) [1].

In the 24-year follow-up of the Yucheng accident, a nonsignificant trend for self-reported CVD was reported in the PCB- and PCDF-exposed cases, compared with controls (OR 1.5, 95% CI 0.8–2.7) [2].

In one of the above-mentioned US Vietnam veteran studies, an increased incidence rate of CVD was found after 30 years of follow-up in those who had sprayed Agent Orange compared with veterans who did not handle the herbicide (OR 1.5, 95% CI 1.1–1.9) [3].

Population-based studies. In one cross-sectional analysis of 889 adults from the NHANES 1999–2002, concentrations of PCBs and pesticides in serum were positively related to self-reported CVD, defined as a history of myocardial infarction, angina pectoris or stroke. PCBs and OC pesticides were associated with the prevalence of CVD in women only (OR 3.8–5.0 for the highest versus the lowest quartiles), whereas the total level of all three evaluated dioxins was related to CVD in both sexes (OR 1.7 for the highest versus the lowest quartile) [63].

In a cross-sectional analysis of 1455 adults from the NHANES 2003–2004, urinary levels of bisphenol A were positively associated with self-reported CVD, with an RR of 1.6 (95% CI 1.1–2.2) for a change of 1 SD in urinary bisphenol A levels [26]. An analysis of the NHANES 2005–2006 confirmed these findings [27]. In these studies, no distinction was made between different CV disorders.

Coronary heart disease

Because the results of several small occupational studies pointed towards an association between dioxin exposure and coronary heart disease, the IARC international cohort was formed, consisting of 36 cohorts from 13 countries, including 21 863 workers followed for >20 years. A significant association was found between dioxin exposure and coronary heart disease in this powerful analysis (RR 1.6, 95% CI 1.2–2.2) [64].

In the above-mentioned study of contaminated waste sites in New York, an increased risk of 20% (95% CI 3–39%) for a diagnosis of myocardial infarction was found for those living close to a contaminated site [29].

In the cross-sectional analysis of the combined NHANES 2003–2004 and 2005–2006 data, there was an RR of 1.2 (95% CI 1.1–1.4) for a change of 1 SD in urinary bisphenol A levels for prevalent myocardial infarction [27].

Stroke

In the IARC international cohort including 21 863 workers exposed to dioxin followed for >20 years, a nonsignificant tendency for an association with incidence of stroke was noted (RR 1.5, 95% CI 0.8–2.8) [64].

Similar to the findings for myocardial infarction, living close to a POP-contaminated waste site in New York was associated with an increased risk of being hospitalized for stroke (RR 1.1, 95% CI, 1.0–1.2) [65].

Peripheral artery disease

In a recent evaluation of 2032 subjects from the NHANES 1999–2004, it was shown that OC pesticides were associated with peripheral artery disease (PAD), defined as an ankle–brachial index <0.9 (a commonly used definition of PAD). This association was most pronounced in obese subjects (OR 1.1, 95% CI, 1.0–1.3) [66].

Mechanisms of action

As discussed above, many of the POPs have been shown to interact with hormonal systems. Indeed, different effects on sex hormone receptors have been demonstrated with both agonist and antagonist properties of different POPs in different tissues and at different levels of exposure. Bisphenol A was termed an oestrogen as early as the 1930s, when it was shown to stimulate the reproductive system in female rats. There were plans for its introduction as an oestrogenic pharmaceutical agent, although it never reached the market because more potent agonists were found [67]. A number of other steroid nuclear receptors are also known to be affected by POPs. With regard to dioxin and dioxin-like PCBs, a main target is the aryl hydrocarbon receptor (AHR). Following activation by dioxin, this receptor, with no clearly identified endogenous ligand or known action, will increase the activity of the cytochrome P450 enzyme CYP1A1, resulting in formation of reactive oxygen species (ROS) and inflammation [68, 69]. In addition, alterations in the apoptosis rate and cell cycling have been described following dioxin activation of the AHR [70–73]. Thus, several mechanisms that are currently discussed in relation to the pathogenesis of atherosclerosis are known to be triggered by POPs, including induction of ROS during oxidation of LDL cholesterol in the vascular wall, ROS-induced low-grade chronic inflammation within the atherosclerotic plaque, and apoptosis of vascular smooth muscle cells making the plaque vulnerable to rupture and thrombosis formation.

Other effects of environmental contaminants might be due to the activation of other nuclear receptors. One such example is the well-established action of phthalates on Peroxisome proliferator-activated receptors, PPAR receptors, which will affect adipocyte differentiation, lipid metabolism and insulin resistance, and thereby the risk of diabetes development [74].

Validation issues

As shown above, there is evidence to suggest that exposure to POPs, and to some extent PACs, is associated with major CV risk factors, as well as with the risk of atherosclerosis and overt CVD. However, compared with other traditional risk factors, such as high LDL cholesterol, smoking and hypertension, it is not possible to perform clinical trials, as for example, for lipid-lowering or smoking cessation medication, to establish whether these environmental contaminants are true risk factors or merely risk markers. It would be unethical to administer these contaminants in clinical trials, and there are no known ways to induce their rapid elimination. Furthermore, the Mendelian randomization approach, as recently used to determine whether C-reactive protein is a risk factor or a risk marker [75], is not applicable to nonendogenous potential risk factors, as in this case. Therefore, at present, the only other way to validate the human findings reported in the present review is to challenge appropriate experimental *in vivo* and *in vitro* models with dosages relevant for human exposure to seek biological evidence of causal relationships.

In summary, there is experimental evidence to support the human findings, showing that POPs and/or PACs (i) induce diabetes because of impairments in insulin secretion and/or resistance [76–78], (ii) induce obesity by means of altered adipocyte differentiation [79], (iii) raise blood pressure by increasing the sensitivity to angiotensin II [80] and impairing endothelium-dependent vasodilatation [81, 82], (iv) induce dyslipidaemia both by interfering with lipid storage in adipocytes and by altering lipid-regulating enzymes in the liver, leading to hepatic steatosis [83, 84], (v) accelerate atherosclerosis formation in ApoE knockout mice (a well-established atherosclerosis model) [85], (vi) induce cardiac hypertrophy [86] and (vii) alter the composition of contractile myocardial elements and induce cardiac fibrosis [87]. However, it is noteworthy that in many of these studies, no attempts have been made to transfer relevant human exposure data to the experimental setting, and the exposure levels are often much higher than are seen in humans.

Problems associated with in human studies

The two major problems with conducting proper population-based large prospective studies in this field are the high cost and the analytical volume needed. Therefore, it is necessary to improve the analytical procedures to increase the speed of the analysis and to reduce the sample volume without loss of precision. In the PIVUS study, van Bavel and co-workers have been able to analyse >20 POPs with high precision in 0.5 mL plasma [88, 89]. However, even more improvements in analytical capacity at lower volumes must be achieved before it is realistic to use frozen samples from the major long-term prospective studies of CVD that have been performed in the USA and Europe. If these improvements can be achieved, knowledge of the relationships between environmental contaminants and CVD will increase significantly.

Another problem concerns the pharmacokinetics of POPs. It is not possible to determine true exposure to POPs from one sample alone because of their very long half-lives; maximal exposure in the Western world occurred about four decades ago, but widespread use of these compounds continues in Africa, Asia and Latin America with global spread by sea and air. Thus, repeated samples over time together with pharmacokinetic modelling may overcome this problem and thus obtain a proper estimate of exposure for POPs. Some PACs, including bisphenol A, are less persistent in the body than POPs. Nevertheless, repeated sampling might also result in a more precise estimate of exposure to PACs.

Humans are not exposed to one chemical at a time, but are chronically exposed to a simultaneous cocktail of chemicals. The potential for synergistic or additive effects from such multiple exposures must be taken into account to evaluate the combined effects of contaminant exposure on health. This issue has recently been highlighted by the World Health Organization [90], the Swedish Chemical Agency [91], the US Centers for Disease Control and Prevention [92], the European Commission [93] and in a report by Silins and Hogberg [94]. Thus, it is essential to develop new statistical strategies to identify highly potent mixtures with additive or synergistic effects of relevance for CVD development. Traditional statistical models of interactions would demand much larger samples than are available today to search for interactions between multiple contaminants.

It has been shown both experimentally and in human studies that a nonmonotonic relationship between POP exposure and different outcomes, such as conditions that predispose towards CVD (CV risk factors) or overt CVD, might exist [12, 47, 62]. In these studies, effects were also seen at very low levels of exposure, and no further effect size was noted if the level of exposure was increased. In some cases, the effect even disappeared during high exposure. This feature of some POPs will make the interpretation of dose–response relationships very challenging, especially when the effects of mixtures are evaluated. Figure 1 shows the odds ratios for a PCB (congener 138) divided into quintiles versus three outcomes in the PIVUS study. With regard to prevalent carotid artery atherosclerosis, the main effect was seen in the highest quintile; however, the highest OR values were not seen in the highest quintile for the other two outcomes, incident abdominal obesity and incident diabetes. Indeed, a considerable decrease in risk was seen in the highest quintile for these two outcomes,

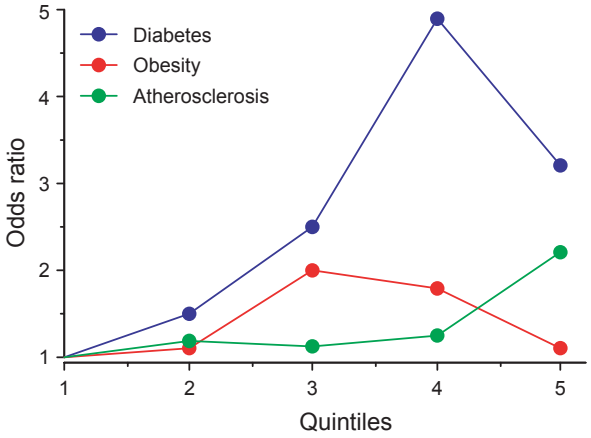


Fig. 1 *Summary showing published data of the relationships between a PCB (congener 138) divided into quintiles and three different outcomes in the PIVUS study: incident diabetes [14], incident abdominal obesity [47] and prevalent carotid artery atherosclerosis [56]. Odds ratio values are given for quintiles 2–5 versus quintile 1 (given as 1.0) following adjustment for age, gender, lipids, exercise habits, education level, smoking, body mass index (for atherosclerosis and diabetes), hypertension and glucose (for atherosclerosis only). Please see text for discussion of the nonmonotonic relationships for obesity and diabetes.*

exemplifying the nonmonotonic relationships often observed in studies of POPs.

Another problem in this field is that large prospective studies with long follow-up periods are ideal to generate confidence in the results. However, if the length of the follow-up period is increased, the contaminants measured at baseline may have been banned by the authorities by the time of the analysis and therefore evaluation would no longer be relevant. These chemicals might have been replaced by others during the follow-up period. Thus, there is always a risk that when waiting for the results of 'ideal' studies to emerge, the range of contaminants will have changed. An example is that PCBs were banned in the 1970s and were in part replaced by brominated compounds (i.e. bromine rather than chlorine in PCBs). The chlorinated and brominated chemicals have now to some extent been replaced by fluorinated compounds, but neither the brominated nor the fluorinated compounds were measured at baseline in the prospective studies that are currently being conducted with prolonged follow-up periods. Repeated sampling in longitudinal studies might be one way to deal with this problem.

A major source of the environmental contaminants discussed in this review is food. Fatty fish, such as salmon and herring, are heavily contaminated with POPs in some parts of the world. Fish are also known to be contaminated with mercury, for example, in the Baltic Sea. Thus, the associations between POPs and some of the phenotypes discussed here might be confounded by the health effects of fish oils, as well as the effects of mercury. These other additional effects of diet and contaminants that are closely associated with POP exposure are unfortunately very hard to control for accurately, because detailed dietary assessments together with measurements of multiple contaminants are rare in environmental research.

Strengths and limitations

The major strength of the reviewed data is the level of congruence of the different approaches (accidents, occupational exposure, geographical exposure and population-based studies). In the case of the existing data regarding diabetes, prospective population-based studies with measured circulating levels contribute the highest degree of evidence. Five such prospective studies have been reported, and all but one show significant associations between PCB exposure and future diabetes. In addition, there is agreement amongst the five studies regarding DDT exposure and diabetes. These data are largely supported by cross-sectional studies, accidents and occupational and geographical exposure studies.

Few prospective studies of the effects of POP exposure on conditions that increase the risk of CVD, atherosclerosis and overt CVD have been conducted, and therefore, any reported associations are based on a lower level of evidence. However, cross-sectional data suggest that further longitudinal studies of the effects of POPS would be highly worthwhile. Similarly, despite the availability of fewer reported studies, further longitudinal studies of PACs, including bisphenol A and phthalates, should be considered.

Conflict of interest statement

No conflict of interest to declare.

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