Environmental Research 142 (2015) 148–154

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

# Levels of perfluoroalkyl substances and risk of coronary heart disease: Findings from a population-based longitudinal study

Kristina Mattsson [a,*], Anna Rignell-Hydbom [a], Sara Holmberg [b], Anders Thelin [c], Bo AG Jönsson [a], Christian H Lindh [a], Andréa Sehlstedt [a], Lars Rylander [a]

[a] Division of Occupational and Environmental Medicine, Institute of Laboratory Medicine, Lund University, SE-221 85 Lund, Sweden
[b] Department of Research and Development, Region Kronoberg, Box 1223, SE-351 12 Växjö, Sweden
[c] Department of Public Health and Caring Sciences, Family Medicine and Preventive Medicine Sections, Uppsala University, Uppsala, Sweden

ARTICLE INFO

*Article history:*
Received 31 March 2015
Received in revised form
26 June 2015
Accepted 28 June 2015
Available online 3 July 2015

*Keywords:*
Coronary heart disease
Perfluorinated compounds
Epidemiology
Longitudinal study

ABSTRACT

*Background:* Cross-sectional studies have shown an association between exposure to perfluoroalkyl substances (PFASs) and coronary heart disease (CHD). These findings need to be evaluated in longitudinal settings.
*Objectives:* To investigate the risk of CHD in relation to PFAS levels in a longitudinal setting among Swedish rural residents.
*Methods:* In a population-based prospective cohort of male farmers and rural residents recruited in 1990–1991, all men who received a CHD diagnosis between 1992 and 2009 were identified from national registers ($n=253$). For each CHD case, one control, matched for age, was chosen randomly from the cohort. For all cases and controls, levels of eight PFASs at baseline were measured in stored blood samples. In addition, for a subsample, PFAS levels were also measured in serum samples collected at a follow-up in 2002–2003.
*Results:* There were no statistically significant associations between levels of seven of the eight PFASs at baseline and risk for developing CHD. There was a significant association between perfluoroheptanoic acid (PFHpA) and CHD (OR=2.72; 95% CI: 1.52, 4.84) for the 3rd quartile and (OR=2.45; 95% CI: 1.40, 4.29) for the 4th quartile compared to the lowest quartile. Changes in levels of PFCs between baseline and follow-up did not differ systematically between cases and controls.
*Conclusions:* This longitudinal study does not lend support to the previously reported cross-sectional relationship between PFAS levels and CHD risk. We found a significant association with PFHpA, but this could be a chance finding, considering its chemical resemblance to other PFASs.


## 1. Introduction

Cardiovascular disease (CVD), in particular coronary heart disease (CHD) and stroke, is one of the main causes of morbidity and mortality worldwide, and in spite of significant treatment improvements, it remains the most common cause of death globally (World Health Organization, 2011) and in Sweden (National Board of Health and Welfare, 2013).

CVD is a preventable disease; the largest part of its etiology is explained by factors other than genetic influence (Stampfer et al., 2000; Willett, 2002). Although well-known life-style risk factors such as diet and smoking explain most of the non-genetic fraction, there is growing evidence that exposure to environmental toxins could play a role in disease development (Bhatnagar, 2006; O'Toole et al., 2008).

Perfluoroalkyl substances (PFASs), such as perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS), are man-made, highly stable compounds that accumulate in the environment as well as in humans. Properties like grease repellency and flame retardation have lead to a wide-spread use of PFASs in industrial and consumer products since the introduction of the compounds, and they can be found in a variety of common materials such as Teflon and Gore-Tex.

There are detectable levels of PFASs in populations all over the

*Abbreviations:* BMI, body mass index; CHD, coronary heart disease; CVD, cardiovascular disease; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; LOD, limit of detection; PFAS, perfluoroalkyl substances; PFDA, perfluorodecanoic acid; PFDoDA, perfluorododecanoic acid; PFHpA, perfluoroheptanoic acid; PFHxS, perfluorohexanesulfonic acid; PFNA, perfluorononanoic acid; PFOA, perfluorooctanoic acid; PFOS, perfluorooctane sulfonate; PFUnDA, perfluoroundecanoic acid

* Corresponding author.

http://dx.doi.org/10.1016/j.envres.2015.06.033
0013-9351/

world (Lau et al., 2007). Human exposure routes primarily include the oral pathway (contaminated drinking water and food). Studies in rats have shown that the compounds are not metabolized, poorly eliminated and can be found in blood, kidney and liver (Fromme et al., 2009; Lau et al., 2007).

Concerns have been expressed regarding potential cardiovascular health effects following PFAS exposure, but epidemiological studies on the subject are scarce and findings inconsistent (Steenland et al., 2010). Reports have demonstrated a relationship between PFOA and PFOS exposure and higher serum levels of total cholesterol, low-density lipoprotein cholesterol (LDL) and triglycerides (Eriksen et al., 2013; Fu et al., 2014; Geiger et al., 2014; Nelson et al., 2010; Starling et al., 2014; Steenland et al., 2010), however not consistently (Chateau-Degat et al., 2010; Fisher et al., 2013). Recently, an association between levels of PFOA and CVD was reported in a nationally representative cohort in the U.S. (Shankar et al., 2012), and another study has found a higher prevalence rate of self-reported angina and myocardial infarction among individuals exposed to PFOA in contaminated drinking water (Anderson-Mahoney et al., 2008). Other studies, on the contrary, did not find higher mortality rates due to heart disease in occupationally exposed individuals (Leonard et al., 2008; Lundin et al., 2009; Sakr et al., 2009; Steenland and Woskie, 2012).

However, limited conclusions regarding potential causality can be drawn from these earlier studies, as many have used a cross-sectional study design or have focused on occupationally exposed cohorts with higher levels of PFASs, rather than on the general population. Also, the focus of these studies has often been on mortality rather than on morbidity. We could find only one longitudinal study investigating CHD morbidity in relation to PFOA exposure (Winquist and Steenland, 2014). Additionally, most previous studies are restricted to having studied only PFOA and PFOS. Less is known about potential risks of exposure to other perfluorinated compounds.

The aim of this study was to investigate the risk of CHD in relation to levels of eight different perfluorinated compounds using a population-based cohort and a longitudinal study design.

## 2. Methods

### 2.1. Study population

This study used data from a population-based prospective cohort of farmers and other rural residents, originally aiming at investigating health determining factors in relation to farming. The cohort was established in 1989 and has been described in more detail elsewhere (Holmberg et al., 2002). In brief, male farmers born during 1930–1949 from nine different rural districts of Sweden were identified from the Swedish National Farm Register. The districts were chosen in order to mirror the previously shown geographical gradient of CVD mortality in Sweden (Nerbrand et al., 1991). A population of men living in rural areas was identified from the National Population Register and matched to the farmers on age, sex and residential area. In total, 1220 farmers and 1130 rural residents were invited to take part in an extensive health survey including questionnaires, laboratory tests and physical measurements. Of the total 2350 men, 1782 (75.8%) agreed to participate at baseline. Reasons for non-participation and a more exhaustive description of the sampling procedure have been presented previously (Holmberg et al., 2002). There were no notable differences between participants and non-participants. The baseline examination was done in 1990–1991 and there was one follow-up examination from 2002 to 2003.

From the cohort we identified all individuals who were deceased or had been hospitalized due to CHD between 1992 and

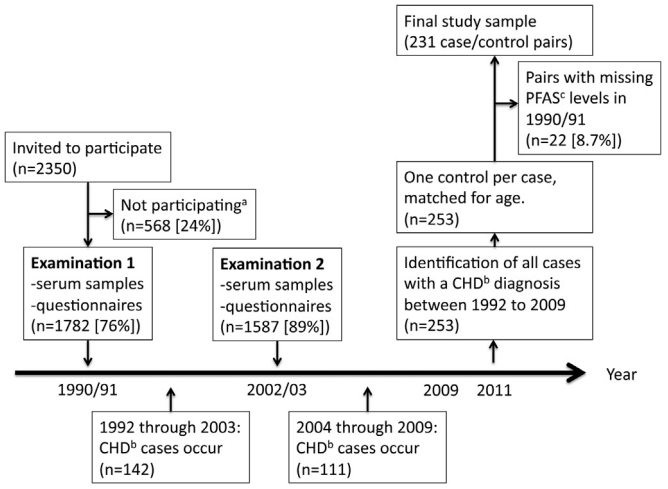


**Fig. 1.** Sampling procedure and selection of final study cohort. Figure notes: (a) Reasons for non-participation: declined ($n=157$), illness ($n=36$), moved from area ($n=7$), could not be retrieved ($n=11$), not at home during examination period ($n=48$), deceased after sampling but before examination ($n=6$) and unknown reason ($n=303$). (b) Coronary heart disease. ICD-9 codes 410-414 and ICD-10 codes I20-I25. (c) Perfluoroalkyl substance. Valid levels for all of PFOS, PFOA, PFDA, PFNA, PFHpA, PFHxS, PFDoDA and PFUnDA.

2009. Information on the number of deceased individuals as well as the cause of death was obtained from the National Cause of Death Register. From the National Patient Register, diagnoses for hospital admissions were retrieved for the years 1992–2009. The register contains nation-wide information on all admissions to hospitals in Sweden since 1987, with one main and up to seven auxiliary diagnoses. Diagnoses used were from the Swedish version of the International Classification of Diseases (ICD) version 9 (for the years 1992–1996) and version 10 (for the years 1997–2009). CHD was defined as ICD-9 codes 410-414 and ICD-10 codes I20-I25. In total, 253 men developed CHD during this period. For each case, one control without a diagnosis of any cardiovascular disease or diabetes and born the same year was chosen randomly from the cohort. Fig. 1 shows the sampling procedures and selection of participants. Additionally, for 104 case-control pairs, serum samples stored from the second examination in 2002/03 were also analyzed. These were chosen randomly from the included study sample analyzed at baseline, with the condition that time of CHD diagnosis among the cases should be between the years of 1993–2000 for around half of them (55 pairs) and between 2004–2009 (49 pairs) for the other half.

The study was approved by the Ethics Committee at Lund University, Lund, Sweden. All men included in the cohort gave their informed consent.

### 2.2. Exposure assessment and laboratory analyses

Blood samples from cohort members were taken at both sampling times (in 1990–1991 and 2002–2003) and were stored at −20 °C or below. Blood samples were non-fasting.

The analysis of PFASs was performed by LC/MS/MS using the method previously described by Lindh et al. (2012). In brief, aliquots of 100 μl serum were added with glucoronidase, ammonium acetate buffer and $^{13}C$- or $^{18}O$-labeled internal standards and digested at 37 °C for 90 min. The proteins were precipitated with 200 μl acetonitrile and vigorously shaken for 30 min. The samples were thereafter centrifuged and 3 μl of the supernatant injected on a LC (UFLC$^{XR}$, Shimadzu Corporation, Kyoto, Japan; LC/MS/MS) using hybrid triple quadrupole linear ion trap mass spectrometry equipped with a TurboIonSpray source (QTRAP 5500, Applied Biosystems, Foster City, CA, USA).