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OCCUPATIONAL EXPOSURE TO POLYCYCLIC AROMATIC HYDROCARBONS AND EARLY BIOMARKERS RELATED TO CARDIOVASCULAR DISEASE AND CANCER

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Occupational exposure to polycyclic aromatic hydrocarbons and early biomarkers related to cardiovascular disease and cancer

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I dedicate this thesis to my parents, who have done everything possible and the impossible for their priority no 1 in life – us – (me and my siblings)

أهدي أطروحة الدكتوراه هذه إلى والدي الغاليين الذين لم يدخرا جهدا من أجلي ومن أجل إخوتي

ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are omnipresent environmental pollutants composed of fused benzene rings and mainly produced by incomplete combustion of organic material. PAH exposure has been associated with increased risk of cancer and probably cardiovascular disease (CVD). In one way or another, everyone is exposed to PAH, but the dose and the period of exposure vary between individuals. Workers who remove soot from chimneys (chimney sweeps) are likely exposed to higher levels of PAH compared with the general population. However, whether the current PAH exposure among chimney sweeps leads to disease is not known.

The overall aim of this thesis was to evaluate PAH exposure among currently working chimney sweeps as well as explore early biomarkers related to cardiovascular disease (CVD) and cancer. For this purpose, we recruited 151 chimney sweeps and 152 unexposed control individuals, all males from southern Sweden, from whom we collected questionnaires and biological samples. In one of the studies, we additionally used data and biological samples from 19 creosote-exposed workers, i.e. workers who impregnate wood panels with black oily material rich in PAH known as creosote.

We found that PAH exposure (measured as PAH metabolites in urine) was up to 7 times higher among chimney sweeps compared with unexposed control workers, and the levels of PAH metabolites were positively associated with diastolic blood pressure. Moreover, we found higher serum concentrations of the classical risk markers for CVD (homocysteine and cholesterol) in chimney sweeps, compared with controls. Further, we found 25 putative CVD-related serum proteins differentially expressed between nonsmoking chimney sweeps and controls, among which follistatin (FS), heat shock protein beta-1 (HSP 27), and pro-interleukin-16 (IL-16) showed positive dose-response relationships with PAH metabolites. Pathway analysis demonstrated that these 25 proteins were mainly involved in inflammatory response and immune function.

We also demonstrated hypomethylation (lower methylation) of the genes *F2RL3* and *AHRR*, risk markers for lung cancer, among chimney sweeps and creosote-exposed workers, compared with controls. Notably, creosote-exposed workers had the highest PAH exposure and the lowest DNA methylation, compared with both chimney sweeps and controls, which suggests a dose-response relationship. In addition, we found 17 putative cancer-related serum proteins differentially expressed between nonsmoking chimney sweeps and controls, among which kallikrein-13 (KLK13) showed positive dose-response relationships with the metabolites of carcinogenic PAH (BaP and BaA). Pathway analysis showed that most of the differentially expressed proteins were involved in cell movement, cell migration, and cell invasion.

Overall, findings from this thesis indicate that (i) currently working chimney sweeps are markedly exposed to PAH, (ii) chimney sweeps showed molecular changes related to CVD and cancer, and (iii) some of these molecular changes seem to be, at least partly, induced by PAH exposure. These results stress that protective measures are warranted to reduce PAH exposure among chimney sweeps as well as other occupational groups at risk of PAH exposure. In addition, further research exploring mechanisms of PAH-induced CVD and cancer is encouraged in order to develop strategies of early detection of disease among individuals known to be exposed to PAH.

LIST OF SCIENTIFIC PAPERS

I. **Alhamdow, A**., Lindh, C., Albin, M., Gustavsson, P., Tinnerberg, H., Broberg, K., 2017.
Early markers of cardiovascular disease are associated with occupational exposure to polycyclic aromatic hydrocarbons.
Scientific Reports, 7(1):9426

II. **Alhamdow, A**., Lindh, C., Albin, M., Gustavsson, P., Tinnerberg, H., Broberg, K.
Cardiovascular disease-related serum proteins in workers occupationally exposed to polycyclic aromatic hydrocarbons.
(Submitted manuscript)

III. **Alhamdow, A**., Lindh, C., Hagberg, J., Graff, P., Westberg, H., Krais, A.M., Albin, M., Gustavsson, P., Tinnerberg, H., Broberg, K., 2018.
DNA-methylation of the cancer-related genes *F2RL3* and *AHRR* is associated with occupational exposure to polycyclic aromatic hydrocarbons.
C*arcinogenesis*, 39(7):869-878

IV. **Alhamdow, A**., Tinnerberg, H., Lindh, C., Albin, M., Broberg, K., 2019.
Cancer-related proteins in serum are altered in workers occupationally exposed to polycyclic aromatic hydrocarbons: a cross-sectional study.
Carcinogenesis, [Epub 2019 Feb 8]

LIST OF OTHER PAPERS NOT INCLUDED IN THE THESIS

1. **Alhamdow A**, Gustavsson P, Rylander L, Jakobsson K, Tinnerberg H, Broberg K. 2017. Chimney sweeps in Sweden: a questionnaire-based assessment of long-term changes in work conditions, and current eye and airway symptoms. *International Archives of Occupational and Environmental Health*, *90*(2), pp.207-216.

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5. Li H, Åkerman G, Lidén C, **Alhamdow A**, Wojdacz TK, Broberg K, Albin M. 2016. Alterations of telomere length and DNA methylation in hairdressers: A cross-sectional study. *Environmental and Molecular Mutagenesis*, *57*(2), pp.159-167.

6. Wahlberg K, Kippler M, **Alhamdow A**, Rahman SM, Smith DR, Vahter M, Lucchini RG, Broberg K. 2015. Common polymorphisms in the solute carrier SLC30A10 are associated with blood manganese and neurological function. *Toxicological Sciences*, *149*(2), pp.473-483.

7. Kuehnelt D, Engström K, Skröder H, Kokarnig S, Schlebusch C, Kippler M, **Alhamdow A**, Nermell B, Francesconi K, Broberg K, Vahter M. 2015. Selenium metabolism to the trimethylselenonium ion (TMSe) varies markedly because of polymorphisms in the indolethylamine N-methyltransferase gene. *The American Journal of Clinical Nutrition*, *102*(6), pp.1406-1415.

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CONTENTS

LIST OF ABBREVIATIONS

1-OH-PYR	1-hydroxypyrene
2-OH-PH	2-hydroxyphenanthrene
3-OH-BaA	3-hydroxybenz[a]anthracene
3-OH-BaP	3-hydroxybenzo[a]pyrene
AHRR	Aryl Hydrocarbon Receptor Repressor
ATSDR	Agency for Toxic Substances and Disease Registry
BaA	Benz[a]anthracene
BaP	Benzo[a]pyrene
BEI	Biological Exposure Index
BMI	Body Mass Index
CI	Confidence Interval
CpG	Cytosine-phosphate-Guanine
CRP	C-Reactive Protein
CV	Coefficient of Variation
CVD	Cardiovascular Disease(s)
CYP P450	Cytochromes P450
DEP	Differentially Expressed Protein(s)
DNA	Deoxyribonucleic Acid
F2RL3	Coagulation factor ii (thrombin) Receptor-Like 3
FS	Follistatin
GGT	Gamma-Glutamyl-Transferase
GO	Gene Ontology
GST	Glutathione S-Transferase
HBB	Hemoglobin Beta gene
HDL	High-Density Lipoproteins
HSP 27	Heat Shock Protein beta-1
IARC	International Agency for Research on Cancer
IL-16	Pro-interleukin-16
IPA	Ingenuity Pathway Analysis

KLK13	Kallikrein-13
LC-MS/MS	Liquid Chromatography-tandem Mass Spectrometry
LDL	Low-Density Lipoproteins
LOD	Limit Of Detection
mRNA	Messenger Ribonucleic Acid
mtDNA	Mitochondrial DNA
mtDNAcn	Mitochondrial DNA Copy Number
PAH	Polycyclic Aromatic Hydrocarbons
PCA	Principal Component Analysis
PCR	Polymerase Chain Reaction
PEA	Proximity Extension Assay
qPCR	Quantitative Polymerase Chain Reaction
ROS	Reactive Oxygen Species
SD	Standard Deviation
TG	Triglycerides
TL	Telomere Length
WHO	World Health Organization

1 INTRODUCTION

Work environment can play a substantial role for human health. The World Health Organization (WHO) has estimated that there are around 300 000 work-related mortalities per year in the European region (WHO 2019a). Recently, the occupational burden of disease in Europe constituted 1.6% of total burden of disease, and exposure to carcinogens accounted for 18% of this occupational burden, after injuries (40%) and noise (22%) (WHO 2019b). Exposure to toxic agents is common in workplaces of several professions. It was estimated that about 32 million workers in the European Union are occupationally exposed to toxic agents, of whom 900 000 workers, including 18 000 in Sweden, are exposed to polycyclic aromatic hydrocarbons (PAH) (Kauppinen et al. 2000). Occupations such as chimney sweeping, creosote impregnation, aluminium production, coal tar distillation, coke production, and asphalt paving involve high exposure to PAH (ATSDR 2009; IARC 2010).

Light has been shed on adverse health effects attributable to chimney sweeping as early as 1775. Back then, Sir Percivall Pott, an English surgeon, found overrepresentation of scrotal cancer cases among chimney sweeps who were highly exposed to soot, suggesting causality between exposure to soot and cancer (Pott 1775). Scrotal cancer as well as other types of skin malignancies were frequently reported among chimney sweeps later on (IARC 2012; Melicow 1975). Despite early efforts trying to promote safe work conditions, chimney sweeps still had work-related adverse health outcomes (Kipling and Waldron 1975). Recent epidemiological studies among chimney sweeps have demonstrated increased mortality and incidence of cancer of the lung, esophagus, liver, bladder, prostate, haematolymphatic organs, bowel, colon, and pleura, as well as increased mortality and incidence of cardiovascular disease (CVD) such as coronary heart disease, ischaemic heart disease, and myocardial infarction, (Evanoff et al. 1993; Gustavsson et al. 1987; Gustavsson et al. 1988; Gustavsson et al. 2013; Hogstedt et al. 1982; Hogstedt et al. 2013; Jansson et al. 2012). The main suspect driver of these health problems has been soot, which contains high amounts of PAH. Recently, the International Agency for Research on Cancer (IARC) has classified "soot, as found in occupational exposure of chimney sweeps" as *carcinogenic to humans* (Group 1) (IARC 2012).

However, it is not known what PAH and what levels chimney sweeps are exposed to today, and whether this exposure leads to disease.

2 BACKGROUND

2.1 CHIMNEY SWEEPING PROFESSION

Chimney sweeping traditionally involved removing soot (black sweeping or soot sweeping) from chimneys (Figure 1) and boilers using brushes, scrapers, and other equipment; however, additional work tasks were introduced in the recent decades. Nowadays, chimney sweeps perform soot sweeping tasks (in private homes and industrial facilities) and non-soot sweeping tasks such as inspection of fire safety systems, boilers, and furnaces, as well as cleaning ventilation channels in villas, houses, residential and industrial buildings. Further, cleaning exhaust ducts in restaurants and carrying out mandatory ventilation inspection and administrative work have increasingly been becoming a part of chimney sweeps' work routine.

The use of personal protective equipment has improved over time. In the 18th century when Sir Pott reported his landmark investigation, young chimney sweeps were sent naked inside chimneys and therefore, the soot was lodged in the scrotal skin explaining the high incidence of cancer of the scrotum (Herr 2011; Kipling and Waldron 1975; Pott 1775). Such practice does not exist anymore in Europe. Today, chimney sweeps use gloves, masks, protective clothing, vacuum machines, and other auxiliary equipment during work. Yet, there is a gap between the intended and the actual practice of applying protective measures. There are no mandatory guidelines regarding the use of protective equipment during work. Chimney sweeps usually wear long-sleeved shirts and long trousers especially in winter, but they may also wear T-shirts and shorts in summer, which makes them more exposed to hazardous substances while sweeping (Alhamdow et al. 2017a).

Figure 1. A chimney sweep working in Sweden (photographer; Ayman Alhamdow, 2018).

2.2 WORK ENVIRONMENT OF CHIMNEY SWEEPS

Working as a chimney sweep demands a level of physical capability in order to perform different tasks e.g. climbing up the buildings' roofs to clean chimneys. Such work clearly harbors high risk of falling accidents and other physical injuries. Even though physical injuries are of paramount importance, our focus in this thesis has been chimney sweeps' exposure to PAH from soot. Besides soot, chimney sweeps may be exposed to asbestos used for insulation in different types of ducts and furnaces, dust particles, degreasing chemicals, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDDs/PCDFs), sulfur dioxide, arsenic, cadmium, and lead (Bagchi and Zimmerman 1980; Kapfhammer et al. 1990; Wrbitzky et al. 2001). Therefore, the exposure profile of chimney sweeps is complex; however, soot dominates as soot sweeping is, by far, the main task for most of the chimney sweeps (Alhamdow et al. 2017a).

2.3 SOOT: SOURCE AND COMPOSITION

Soot is a black material resulting as a by-product from incomplete combustion of organic matter (e.g. wood, petroleum oil, gasoline, and coal). The composition and physical characteristics of soot may vary depending on the fuel used and conditions of combustion.

In addition to PAH that are attached to carbon particles, soot may contain metals and metalloids (e.g. nickel, lead, cadmium, chromium, and arsenic), oxides (sulfur dioxide), combustion gases (carbon monoxide), and traces of other compounds (Figure 2). In general, soot produced from burning wood contains very low concentrations of metals, whereas soot produced from heavy petroleum oil contains higher metal concentrations (Fehrmann 1982; cited in IARC 1985). In the same study, Fehrmann found that more PAH were produced at lower combustion temperatures and from burning woods (compared with burning petroleum oil).

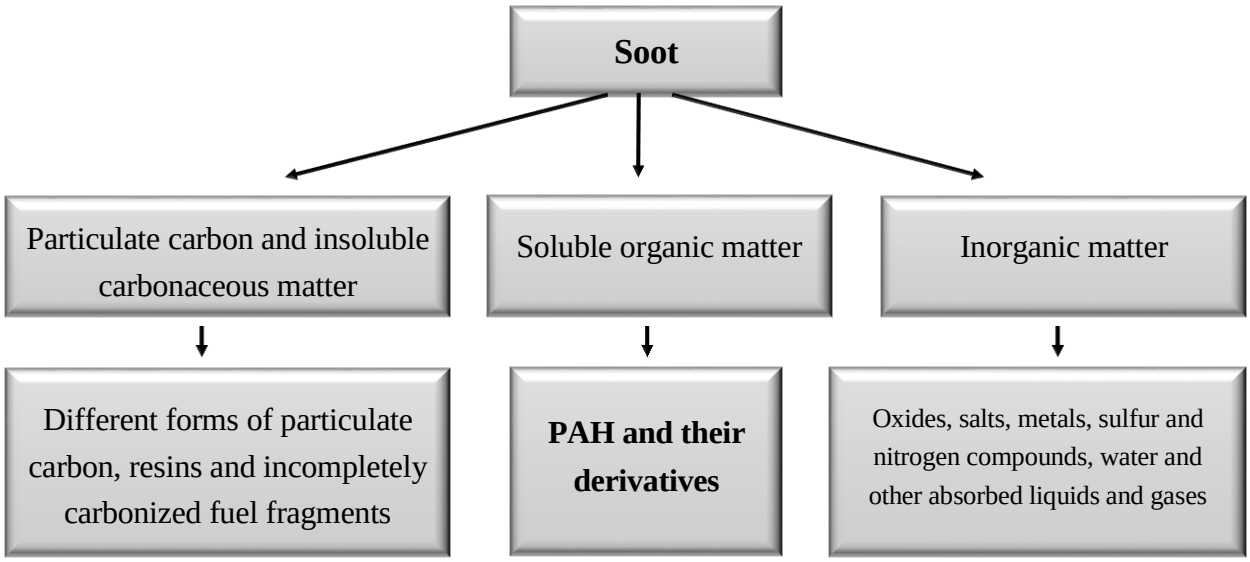

Figure 2. Composition of soot (IARC 1985).

2.4 POLYCYCLIC AROMATIC HYDROCARBONS (PAH)

2.4.1 Structure, characteristics and source of exposure

PAH are a large group of compounds consisting of two or more fused benzene rings (Fetzer 2007) (Figure 3). They may occur naturally (e.g. crude oil and coal) or from incomplete combustion of organic matter such as fossil fuel, coal, and wood (IARC 1985). PAH are usually found as a mixture accompanying each other, unless manufactured at the industrial level as individual pure chemicals (ATSDR 1995). Pure PAH are colorless, white or yellowish powders with faint pleasant odor. Some of PAH with low molecular weight (up to four benzene rings) can be volatile and spread throughout the atmosphere, however, PAH with higher molecular weight are solid and often adsorbed to particles in the ambient air (IARC 2010). Both forms can be precipitated by rainfall contaminating water sources and soil and thereafter, plants and animals (IARC 1985; IARC 2010). PAH can be broken down to simpler compounds by sunlight or microorganisms over a period of days to months (ATSDR 1995). PAH are relatively inert procarcinogens, but they manifest their carcinogenic potentials upon metabolic activation (Gelboin 1980).

Burning fuels for residential heating purposes, automobile exhaust, asphalt and coke production, municipal trash incineration facilities, volcanoes eruptions, wildfires and industrial activities are major contributors to increased ambient air burden of PAH. Other sources of PAH include tobacco smoking, pharmaceutical preparations that contain coal tar, and consumption of grilled, fried and charbroiled food (ATSDR 1995).

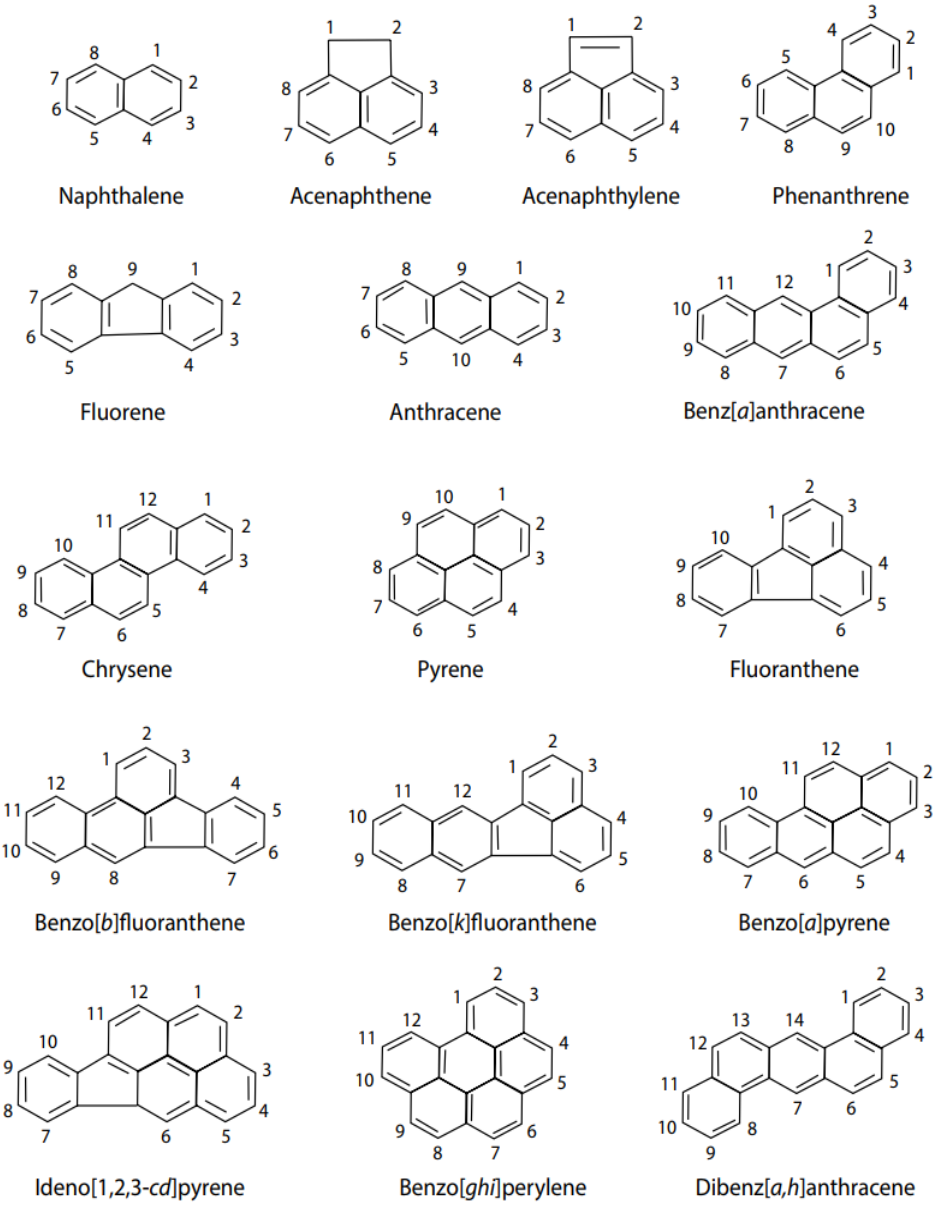

Figure 3. Chemical structure of different PAH (Forsgren 2015).

2.4.2 PAH exposure in the general population

The general population may be exposed to PAH from different sources and through multiple routes. Exposure from ambient air can be dominant, not only in highly polluted residential areas that are in close proximity to industrial activities, but also in areas with regular automobile traffic (Zmirou et al. 2000). In fact, IARC has classified several sources of PAH exposure including "tobacco smoking" (both active and passive), "outdoor air pollution", "indoor emissions from

household combustion of coal", and "diesel engine exhaust" as *carcinogenic to humans* (Group 1) (Table 1) (IARC 2012). Several studies have evaluated non-occupational exposure to PAH in the general population by measuring PAH metabolites in urine. Median concentration of urinary 1-hydroxypyrene (1-OH-PYR; a urinary metabolite of pyrene; see section 2.4.5 for further details) was (0.027 µg/L) for participants from Italy (Tombolini et al. 2018), (0.06 µg/L) Australia, (0.38 µg/L) Vietnam (Thai et al. 2015), (0.38 µg/L) China, (0.075 µg/L) Japan, (0.42 µg/L) India, (0.065 µg/L) Malaysia, (0.10 µg/L) Korea, (0.22 µg/L) Kuwait (Guo et al. 2013), and (0.11 µg/L; 2009–2010) from the U.S. (CDC 2019). The digestive system can also receive considerable doses of PAH from consumption of smoked, broiled, fried, and grilled meat as well as from contaminated water, cow's milk, human breast milk, cereals, bread, vegetables, fruits, and processed and pickled food items (ATSDR 1995). The U.S Environmental Protection Agency (EPA) has determined a "maximum contaminant level" in drinking water for different PAH between 0.1-0.4 ppb (0.2 ppb for Benzo[a]Pyrene (BaP)) (ATSDR 2009). However, the Swedish National Food Agency (Livsmedelsverket) has considered ≥0.1 ppb of BaP in water as "unsuitable" for drinking (Livsmedelsverket 2014). Skin is another route of exposure especially when using topical pharmaceutical preparations that contain coal tar for treatment of some dermal diseases e.g. psoriasis (ATSDR 1995; ATSDR 2002).

2.4.3 PAH exposure in chimney sweeps

A few studies have examined chimney sweeps' exposure to PAH. Knecht et al. (1989) measured benzo[b]fluoranthene, BaP, chrysene, dibenz[a,h]anthracene, and indeno[1,2,3-cd]pyrene in the breathing zone of chimney sweeps during work and in soot samples from oil fuel, solid fuel, and a mixture of both. The PAH air concentrations were fuel-dependent as the highest concentrations (total PAH) were found in oil/solid fuel mixture and pure solid fuel compared with oil fuel (5.06, 5.08, and 2.27 µg/m^3, respectively). The air concentrations of BaP followed the same pattern i.e., 0.83, 0.82, and 0.36 µg/m^3 for the mixture, solid fuel, and oil fuel, respectively). When evaluating the soot content of PAH, the fuel mixture produced the highest amount of PAH compared with oil and solid fuels i.e., 691, 243, and 214 mg/kg, respectively (Knecht et al. 1989). The exposure to PAH was assessed in another group of chimney sweeps from Germany *n*=93 and Poland *n*=7 (Letzel et al. 1999). The median concentration of 1-OH-PYR in the total group was 0.7 µg/L and ranged (<0.1–12.8 µg/L). The authors indicated that the concentrations of 1-OH-PYR were approximately 5 times higher among the Polish chimney sweeps; likely due to higher use of wood and coal in Poland compared with Germany. Further, the concentration of 1-OH-PYR was 1.03 µg/L (median) in another German study of 27 sweeps (Göen et al. 1995) and 1.6 µg/L (mean) in an Italian study including 27 chimney sweeps (Pavanello et al. 2000).

2.4.4 PAH exposure in other occupational groups

Working in coke industry (production of coke from coal by heat in absence of oxygen) is one of the most studied occupations in relation to PAH exposure. Particular attention has been paid to occupational exposure among coke oven workers who were shown to be highly exposed to PAH in multiple studies. A wide spectrum of urinary concentrations of 1-OH-PYR has been reported in the literature e.g. mean concentrations of 1-OH-PYR ranged between 10.1–55.9 µg/L (Strunk et al. 2002) and 3.2–15.7 µg/L (Ovrebø et al. 1995). Rubber industry is another example of work environment with exposure to PAH. A study on nonsmoking rubber workers found that the mean concentrations of 2-naphthol in urine (a urinary metabolite of naphthalene) was increased in the

post-shift samples (13.9 µg/L) compared to pre-shift ones (8.0 µg/L) (Talaska et al. 2012). In the tar distillation industry, Price et al. (2000) reported median urinary 1-OH-PYR of 1.0 µg/L for the low-temperature carbonization of coal and 7.4 µg/L for the high-temperature process (Price et al. 2000; cited in IARC 2012). Further, workers at creosote impregnation plants (creosote-exposed workers) are exposed to PAH from creosote oil used to preserve wooden railway ties (sleepers) (Elovaara et al. 1995; IARC 2010) (Figure 4). Creosote-exposed workers are particularly exposed through skin to PAH with low molecular weight such as naphthalene and phenanthrene (IARC 2010). Table 2 summarizes PAH exposure among several occupational groups.

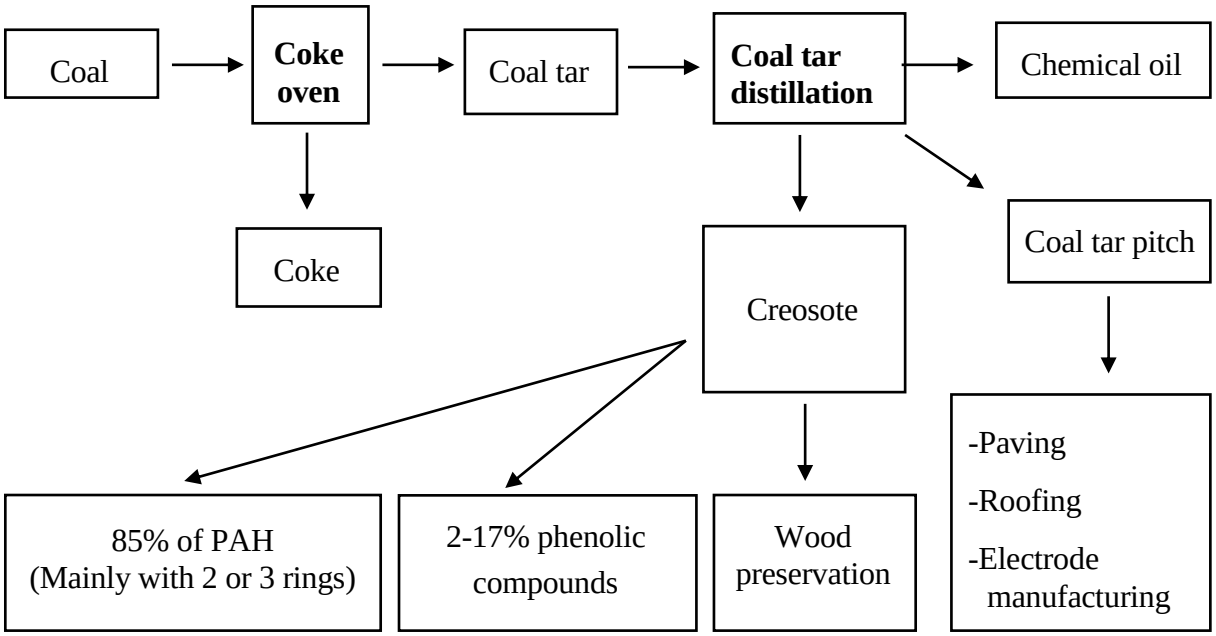

Figure 4. Source and composition of creosote (IARC 2010).

2.4.5 Assessment of PAH exposure

1-hydroxypyrene (1-OH-PYR), a monohydroxylated metabolite of pyrene, has widely been used as a biomarker (see section 2.5 for more details on biomarkers) of occupational exposure to total PAH due primarily to high abundance of pyrene in most PAH mixtures (Hansen et al. 2008; Jongeneelen 2001; Jongeneelen et al. 1988; Jongeneelen et al. 1985). The first international workshop on hydroxypyrene considered 1-OH-PYR as a suitable biomarker for occupational exposure to PAH (Levin 1995). 1-OH-PYR was also suggested as a reliable biomarker for monitoring environmental exposure to PAH owing to its sensitivity, specificity, and analytical feasibility (Bouchard and Viau 1999; Dor et al. 1999). Even though it is a metabolite of non-carcinogenic PAH, 1-OH-PYR was suggested as a comprehensive biomarker for assessment of exposure to carcinogenic PAH in coke oven emissions (Yamano et al. 2014). In workplaces, such as creosote impregnation facilities, where high exposure to low-molecular-weight volatile PAH (two benzene rings) is predominant, 1-OH-PYR might not be the metabolite of choice for exposure assessment since it better correlates with PAH that have 3-6 benzene rings (Elovaara et al. 1995). Thus, assessment of both 1-OH-PYR and additional metabolite(s) is recommended (Elovaara et al. 1995; Heikkilä et al. 1997). 3-hydroxybenzo[a]pyrene (3-OH-BaP), a metabolite of the carcinogen BaP, has been suggested as a biomarker of exposure to carcinogenic PAH in

various workplaces (Förster et al. 2008). However, the levels of 3-OH-BaP in urine, particularly when analysing low occupational exposure levels, are 3 orders of magnitude lower compared with 1-OH-PYR and thus, the analytical method is more challenging (Barbeau et al. 2017; Leroyer et al. 2010).

Recent studies have focused on analysing a wide spectrum of monohydroxylated PAH metabolites in order to obtain a better exposure assessment. Such metabolites include, among others, 1- and 2-OH-naphthalene, 2-, 3-, and 9-OH-fluorene, 1-, 2-, 3-, 4-, and 9-OH-phenanthrene, 3-hydroxybenz[a]anthracene (3-OH-BaA), and alkylated metabolites such as 3-methyl-1-OH-naphthalene (Fan et al. 2012; Li et al. 2012b; Li et al. 2014). Further, dihydroxylated PAH metabolites in urine such as 1,2-dihydroxynaphthalene were considered for exposure assessment (Klotz et al. 2011), however, the method is burdensome due to instability of the free forms of these metabolites (Zobel et al. 2017). Stability of urinary hydroxylated PAH metabolites varies considerably depending on storage temperature and whether the metabolite is in free form or conjugated (e.g. glucuronide-conjugated) (Gaudreau et al. 2016). Although free metabolite forms are less stable than the conjugated ones, studies showed that both types of metabolites can be stable for at least one year of storage at –20Cº (Gaudreau et al. 2016). In general, hydroxylated PAH metabolites can be used for estimation of recent PAH exposure due to their short half-lives (Buckley and Lioy 1992; Castano-Vinyals et al. 2004; Lutier et al. 2016). For assessment of long-term PAH exposure, measurement of PAH-DNA adducts in leukocytes or PAH-protein adducts would be the biomarker of choice since their half-lives are in order of months and weeks, respectively (Castano-Vinyals et al. 2004). However, we did not measure these long-term biomarkers in our studies owing to the fact that the levels of PAH-DNA/protein adducts are much lower than the OH-PAH metabolites and the analysis is more laborious and requires a large amount of sample.

2.4.6 Occupational PAH exposure limits

Several agencies and researchers have proposed exposure limits for workers exposed to PAH at workplace based on either biological monitoring of 1-OH-PYR and 3-OH-BaP in urine, or measurement of BaP in air. Here, two terms should be defined i.e. tolerable risk and accepatable risk. As defined by the UK Health and Safety Executive (HSE), tolerable risk "refers to a willingness by society as a whole to live with a risk so as to secure certain benefits in the confidence that the risk is one that is worth taking and that it is being properly controlled. However, it does not imply that the risk will be acceptable to everyone, ie that everyone would agree without reservation to take the risk or have it imposed on them" (HSE 2001). The acceptable risk is the risk that is generally accepted by the society and the regulators and widely considered insignificant and controlled (HSE 2001). The Health Council of the Netherlands (De Gezondheidsraad) has evaluated the risk of exposure to PAH using BaP as a proxy and set an occupational exposure limit of 550 ng/m^3 for tolerable risk and 5.7 ng/m^3 for acceptable risk (DeGezondheidsraad 2006). The German Federal Institute for Occupational Safety and Health (BAuA) has proposed BaP concentration of 700 ng/m^3 as a tolerable risk limit (4 per 1000 excess mortalities from cancer), and 7 ng/m^3 as an acceptable risk limit (4 per 100 000 excess mortalities) (BAuA 2011). Moreover, the Swedish Work Environment Agency (Arbetsmiljöverket) has set an occupational exposure limit for BaP at 2000 ng/m^3 during an 8-hour working day, and at 20 000 ng/m^3 for a 15-min exposure period. These levels are binding and should not be exceeded at workplaces (Arbetsmiljöverket 2018). The UK Health and Safety Laboratory (HSL) has

suggested urinary 1-OH-PYR concentration of 4.0 µmol/mol creatinine (7.7 µg/g creatinine) in post-shift samples as a Biological Monitoring Guidance Value (BMGV) (HSL 2017). BMGV is not derived from health-based assessment meaning that exceeding this limit does not necessarily pose health risk, but rather a signal for further corrective action in occupational measures and conditions at workplaces. The American Conference of Governmental Industrial Hygienists (ACGIH) has used the limit of 200 000 ng/m^3 of "benzene-soluble coal tar pitch fraction" in air at the workplace as a threshold limit value (TLV) for an 8-hour workday (40 hours a week). However, ACGIH could not set a numerical scientific value (known as Biological Exposure Index; BEI) of exposure to PAH due to lack of sufficient data. Nevertheless, the BEI committee has concluded that the occupational exposure to PAH is evident when 1-OH-PYR concentrations in post-shift urine samples are above 0.5 µmol/mol creatinine (1.0 µg/g creatinine) (ACGIH, 2010; cited in Jongeneelen 2014). Again, the committee of BEI did not indicate health risk related to this level of 1-OH-PYR or higher. Similar to ACGIH, the American Occupational Safety and Health Administration (OSHA) has suggested 200 000 ng/m^3 of "benzene-soluble coal tar pitch fraction" as permissible exposure limit (PEL), and the American National Institute for Occupational Safety and Health (NIOSH) has recommended 100 000 ng/m^3 of airborne coal tar pitch as an exposure limit over a 10-hour workday (ATSDR 2009).

To date, no risk-based occupational limit of 1-OH-PYR has been set due to lack of longitudinal epidemiological data on mortality of cancer in relation to 1-OH-PYR concentrations in urine. Yet, researchers have tried to set a guideline value for urinary 1-OH-PYR of unexposed controls and exposed workers at workplace. Frans J. Jongeneelen has proposed a standard guideline for concentrations of 1-OH-PYR in urine composed of three main levels. First, the 95th percentile of urinary 1-OH-PYR for non-occupationally exposed controls was chosen as a reference value (baseline excretions) and set at 0.46 and 1.47 µg/g creatinine for nonsmokers and smokers, respectively. Second, the level of no-biological-effect (the lowest reported level with no genotoxic effect) among occupationally exposed individuals was suggested at 2.7 µg/g creatinine. Third, the occupational exposure limits for workers in coke and primary aluminium industries were proposed at 4.4 and 9.5 µg/g creatinine, respectively (Jongeneelen 2001). Recently, Jongeneelen has reviewed nine studies on occupational PAH exposure and genotoxicity and suggested 1.0 µmol/mol creatinine (1.9 µg/g creatinine) of 1-OH-PYR in urine as a threshold or guideline value (Jongeneelen 2014). This guideline value corresponded to 5% probability of increased sister chromatid exchange in a study on Polish coke oven workers (Siwinska et al. 2004). Overall, a health-based assessment for occupational exposure limits of 1-OH-PYR and other relevant PAH metabolites is warranted in future studies.

2.4.7 Toxicokinetics of PAH

2.4.7.1 Absorption

PAH are hydrophobic and can be easily absorbed by diffusion through plasma membranes in the airways tracts, gastrointestinal tracts (GIT) and skin. Upon absorption, PAH are distributed to different organs and tissues, particularly the adipose tissue. Regardless of route of exposure, absorption of PAH with two or three rings is generally faster and easier than of those with higher number of aromatic rings (ATSDR 1995). In the airway tracts, exposure may occur to both gaseous PAH (such as naphthalene) and solid PAH (high molecular weight) adsorbed to particles, which results in different absorption profiles. While gaseous PAH are rapidly absorbed, particle-

attached PAH vary in their extent and rate of absorption depending on particle size, molecular weight of individual PAH, reactivity, susceptibility to metabolism and desorbability rate (ATSDR 1995). An essential fraction of particle-attached PAH is deposited in the upper airways and tracheobronchial tracts and thereafter cleared by the mucociliary escalators into the GIT, altering the route of exposure (IARC 2010; Sun et al. 1982; Withey et al. 1994). Other important determinants of absorption include the lipophilicity of PAH, thickness of the epithelium at which the absorption occurs, and local metabolism. High molecular weight PAH can easily pass the first membrane in the respiratory epithelium, but then are slowly transported into the next membrane because of the aqueous environment of the gaps between membranes. This explains the long retention time (several hours) of PAH when passing through the bronchial epithelium (50 μm) compared with few minutes through the alveolar epithelium (0.5 μm) (Gerde et al. 1991; Scott 2001). Local metabolism (phase I and II) gives rise to more water-soluble metabolites accelerating their absorption through lipid membranes and preventing accumulation of unmetabolized PAH in the epithelial cells (Gerde et al. 1997).

PAH may enter the GIT from oral intake, mucociliary clearance in the upper airway tracts, and from biliary excretion. PAH are absorbed from the GIT by either diffusion or normal absorption similar to nutritional lipids (IARC 2010; O'Neill et al. 1991). Absorption of PAH from the alimentary tracts is evident but generally slow in humans (ATSDR 1995; Buckley and Lioy 1992; Kang et al. 1995) and is subjected to interindividual variations (up to eight-fold difference) (Kang et al. 1995). It was estimated that 14-43% of the pyrene administered to nonsmoking individuals in food was detected in urine as 1-OH-PYR (van Maanen et al. 1994). In rats and mice, the absorption is rapid and varies depending on the lipophilicity of individual PAH and can be influenced by concurrent ingestion of oil (Modica et al. 1983; O'Neill et al. 1991). A study in rats showed that at least 30% of BaP dose was absorbed from the GIT (Foth et al. 1988). Another rat study showed that the intestinal absorption of 4- or 5-ringed PAH was facilitated by presence of bile (Rahman et al. 1986).

Dermal absorption of PAH is generally rapid in humans and animals, and the rate of absorption varies depending of the lipophilicity of PAH and the carrying particles or vehicles (ATSDR 1995). Stratum corneum is the outmost layer of the skin and contains several layers of dead cells called keratinocytes. These cells are surrounded by extracellular lipids (sterols, ceramides, and fatty acids) that can retain and release PAH slowly into deeper epidermic strata and then circulation, while less lipophilic PAH and their metabolites can travel faster across the epidermis (Elias and Friend 1975; Long et al. 1985; Melikian et al. 1987; Yardley and Summerly 1981). Animal studies showed that PAH can be locally metabolized in the epidermis forming reactive metabolites (Melikian et al. 1987). Occupational exposure to PAH (e.g. pyrene and BaP) through skin has been reported among workers in different workplaces and industries such as asphalt paving, chimney sweeping, creosote impregnation, petrochemical industry, and coke oven industry (Boogaard and Vansittert 1995; Elovaara et al. 1995; Fustinoni et al. 2010; Kammer et al. 2011; Sobus et al. 2009a; Van Rooij et al. 1993a; Van Rooij et al. 1993b). A study among coke oven workers showed that dermal exposure to pyrene accounted for around 75% total pyrene exposure (Van Rooij et al. 1993a). Another study on creosote-exposed workers found that the use of overall protectors reduced dermal exposure to pyrene by 35% (Van Rooij et al. 1993b). Animal studies have also indicated rapid and extensive dermal absorption of pyrene and BaP (ATSDR 1995; Ng et al. 1992; Sanders et al. 1986; Withey et al. 1993a).

2.4.7.2 Distribution

Animal studies showed that PAH are widely distributed into most of the tissues, particularly fatty tissues, within few hours after administration (ATSDR 1995). Radiolabeled BaP was found in the lungs, blood, liver, kidneys, adipose tissue, and fetus of pregnant rats after a 95-min inhalation exposure (Withey et al. 1993b). Likewise, inhalation exposure to radiolabeled pyrene resulted in distribution to fatty tissue, kidneys, liver, spleen, testes, and brain (Withey et al. 1994). Other inhalation studies reported rapid clearance of pyrene and BaP from airway tracts and distribution to GIT, liver, and kidneys (Mitchell and Tu 1979; Sun et al. 1982).

Oral exposure to BaP in pregnant rats revealed that BaP could accumulate in the placenta and slowly crosses into the fetal tissues (ATSDR 1995; Neubert and Tapken 1988). Orally administered radiolabeled BaP in rats showed high affinity to proteins of the liver, lungs, and kidneys accounting for 50%, 40%, and 65% of the total radioactivity in these organs, respectively after 48 h of the exposure (Yamazaki et al. 1987). Another rat study showed that oral administration of pyrene resulted in distribution to fatty tissue followed by kidneys, liver, and lungs (Withey et al. 1991).

The distribution of PAH following dermal exposure is poorly investigated. A study in rats showed that 1.3% of the total dermal dose of radiolabeled anthracene was found, after 6 days, in the liver and kidneys (Yang et al. 1986). Another study investigated the dermal uptake of pyrene showed that liver, kidneys, lungs, and adipose tissue received the highest levels of pyrene. This study also showed that about 50% the total dose was eliminated over the experiment period (6 days) (Withey et al. 1993a).

2.4.7.3 Metabolism

PAH are procarcinogens, which means they cannot induce carcinogenicity without being transformed into reactive metabolites (Alexandrov et al. 2010; Ramos and Moorthy 2005). Most of the metabolism-related mechanistic studies have used BaP as a model for PAH metabolism because of its well-documented carcinogenicity (ATSDR 1995; IARC 2010). Phase I metabolism of PAH generally involves three pathways; (i) cytochrome P450 monooxygenases (CYP P450; two-electron metabolism; mainly CYP1A1, CYP1A2, and CYP1B1) and epoxide hydrolase, (ii) CYP peroxidase (one-electron metabolism), and (iii) aldo-keto reductase, while phase II metabolism involves glutathione S-transferase, UDP glucuronosyltransferase, and sulfotransferase (IARC 2010). The products of phase I metabolism are radical cations, phenols, epoxides, diols, diol-epoxide, catechols, and quinones. Three members of these metabolites i.e. radical cations, diol-epoxides, and quinones (*ortho*-quinone) are capable of forming adducts with macromolecules (DNA, RNA, proteins, and lipids) (Arlt et al. 2012; Kafferlein et al. 2010; Kwack and Lee 2000; Moorthy et al. 2015). Upon phase I metabolism, enzymes of phase II metabolism are involved to form conjugates (sulfate, glucuronide, and glutathione) of different PAH metabolites (ATSDR 1995; IARC 2010; Saengtienchai et al. 2014). To note, other CYP P450 such as CYP2B, CYP2C, CYP2E, and CYP3A may have a limited role in PAH metabolism (IARC 2010; Shimada et al. 2001; Xue and Warshawsky 2005). Different pathways of PAH metabolism are illustrated in Figure 5.

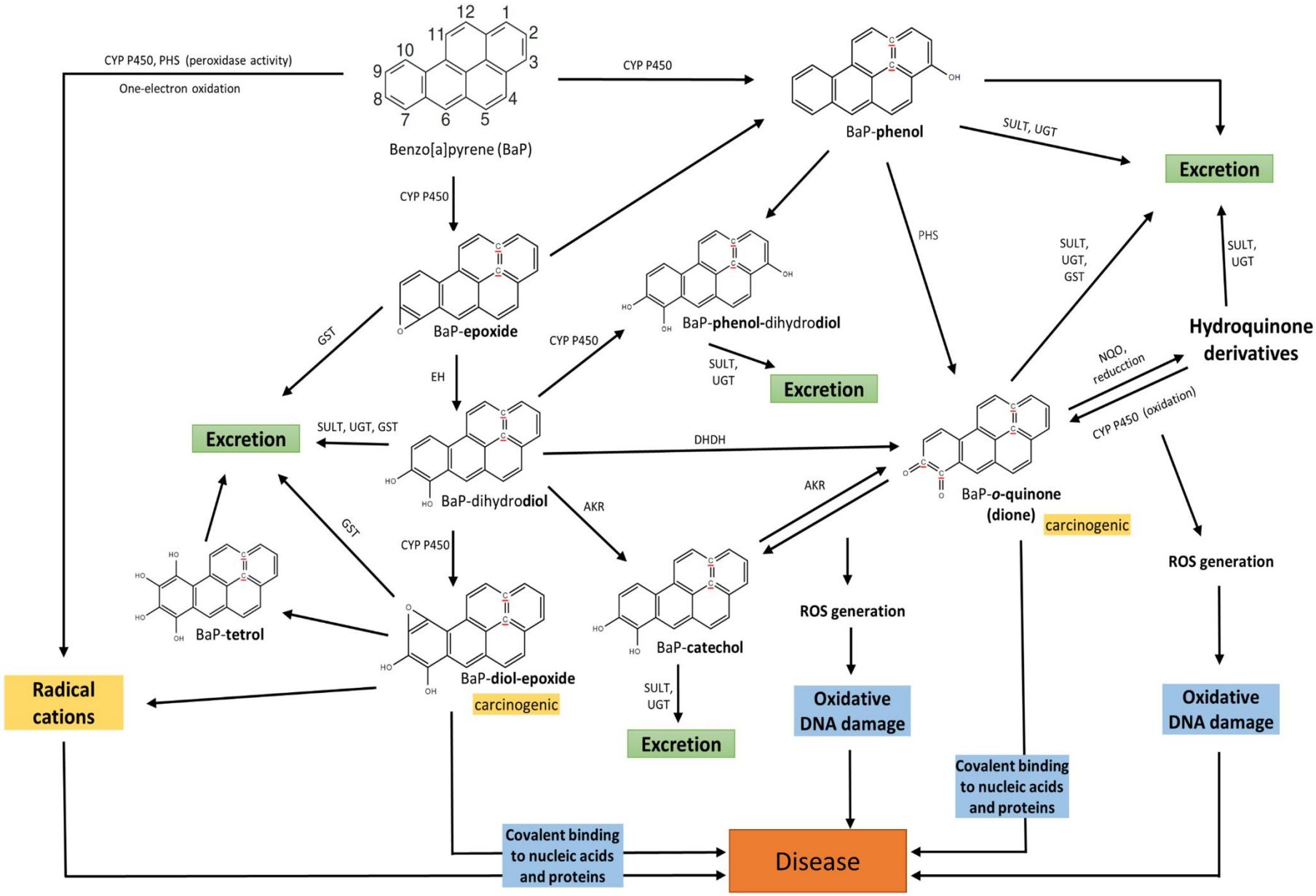
CYP P450, PHS (peroxidase activity)
One-electron oxidation
Benzo[a]pyrene (BaP)
CYP P450
BaP-phenol
SULT, UGT
Excretion
CYP P450
BaP-epoxide
BaP-phenol-dihydrodiol
PHS
SULT, UGT, GST
SULT, UGT
Hydroquinone derivatives
GST
CYP P450
EH
SULT, UGT
Excretion
NQO, reducction
CYP P450 (oxidation)
Excretion
SULT, UGT, GST
DHDH
BaP-dihydrodiol
AKR
BaP-o-quinone (dione)
carcinogenic
GST
CYP P450
AKR
BaP-tetrol
BaP-catechol
ROS generation
ROS generation
BaP-diol-epoxide
carcinogenic
SULT, UGT
Radical cations
Oxidative DNA damage
Oxidative DNA damage
Excretion
Covalent binding to nucleic acids and proteins
Covalent binding to nucleic acids and proteins
Disease

Figure 5. Metabolism of Benzo[a]pyrene; a model for PAH metabolism, depicting different metabolic pathways and reactive metabolites based on the literature (ATSDR 1995; ATSDR 2002; ATSDR 2009; Burczynski et al. 1999; Cavalieri and Rogan 1985; Cavalieri and Rogan 1992; Cavalieri et al. 1988; Devanesan et al. 1992; Hall and Grover 1988; Hrycay and Bandiera 2012; IARC 1985; IARC 2010; Kondraganti et al. 2003; Moorthy et al. 2015; Moorthy et al. 2002; Penning et al. 1999; Smithgall et al. 1988). Chemical structures have been created using an online tool (http://www.chemspider.com). PHS; prostaglandin H synthase. DHDH; dihydrodiol dehydrogenase. AKR; Aldo-keto reductase. GST; Glutathione S-transferase. SULT; Sulfotransferase. UGT; UDP glucuronosyltransferase. EH; Epoxide hydrolase. NQO; NADPH quinone oxidoreductase.

2.4.7.4 Excretion

Upon metabolism, water-soluble PAH metabolites are excreted as free forms or conjugates (sulfate, glucuronide, and glutathione) in the urine and faeces (IARC 2010; van Schooten et al. 1997; Viau et al. 1999); however, unmetabolized PAH can also be excreted; e.g. medians of urinary phenanthrene and pyrene were 0.7 and 0.02 µg/L in coke oven workers (Campo et al. 2010; van Schooten et al. 1997). The half-life of 1-OH-PYR when excreted in urine varied between studies. It was estimated to be 18 h as assessed in workers of coke ovens and graphite electrode industry (Buchet et al. 1992). A study on a creosote worker concluded that urinary 1-OH-PYR is excreted in a biphasic pattern with half-life of 1–2 days for the rapid phase and up to 16 days for the late phase (Jongeneelen et al. 1988). The same authors conducted a study on coke oven workers and found that the half-life of urinary 1-OH-PYR ranged from 6 to 35 h (Jongeneelen et al. 1990). Another study reported a half-life of 4–27 h for 1-OH-PYR among workers exposed to PAH (Boogaard and van Sittert 1994). A study among electrometallurgy workers showed that the half-life for 1-OH-PYR and 3-OH-BaP ranged between 12–18 h and 5–49 h, respectively. The shorter half-life of 1-OH-PYR in comparison with 3-OH-BaP suggests post-shift urine sampling as a preferred method for analysing 1-OH-PYR, but not 3-OH-BaP (Lutier et al. 2016). It was suggested that, for assessment of BaP exposure, pre-shift end-of-the-workweek sampling is advantageous for work environments where the exposure is highly variable, while post-shift end-of-the-workweek sampling is preferred for work environments with invariable exposure (Barbeau et al. 2015; Barbeau et al. 2014). Controlled feeding studies in human volunteers showed that urinary concentrations of 1-OH-PYR were still higher than baseline levels after 24–72 h after exposure and inter-individual variations in 1-OH-PYR concentrations were up to 8 times (Kang et al. 1995). Other studies reported different but comparable figures for the half-life of 1-OH-PYR ranging between 4–29 h (range of medians or means reported in multiple studies) (Figure 6) (Boogaard and Vansittert 1994; Brzeznicki et al. 1997; Buchet et al. 1992; Buckley and Lioy 1992; Chien and Yeh 2010; Huang et al. 2007; Jongeneelen et al. 1990; Lafontaine et al. 2000; Li et al. 2012b; Li et al. 2016; Lutier et al. 2016; Sobus et al. 2009b; St Helen et al. 2012; Viau et al. 1995; Viau and Vyskocil 1995). Overall, the hydroxylated urinary PAH metabolites are short-lived and can reflect recent PAH exposure.

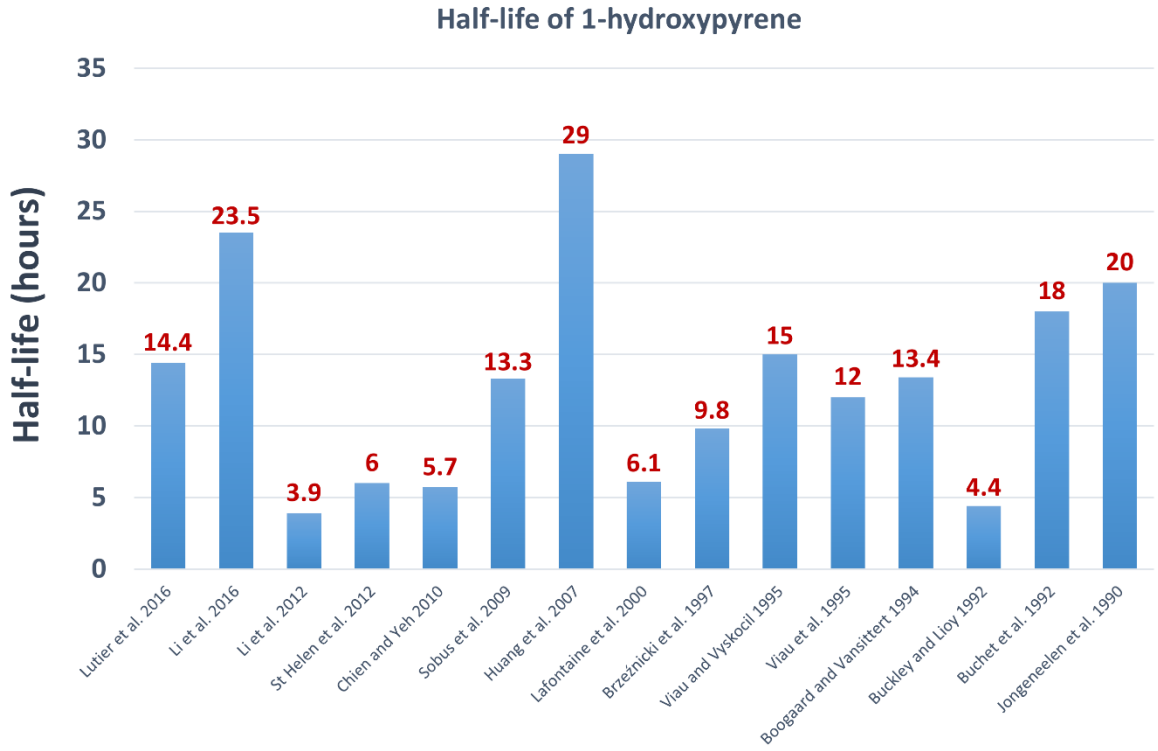

Figure 6. Half-life of 1-hydroxypyrene (1-OH-PYR) reported in human studies.

2.4.8 PAH exposure and adverse health outcomes

Adverse health end-points, particularly cancer and CVD, were investigated in relation to exposure to PAH among workers in several occupations including chimney sweeping.

2.4.8.1 Cancer

In Sweden, the various population registries had enabled researchers to evaluate health risks in relationship with occupations. A large cohort of Swedish chimney sweeps (*n*=6320) was followed up from 1958 to 2006 linking nationwide registry data on cancer and cause of death with occupation as a chimney sweep. The study found increased incidence of cancer from the lung, liver, esophagus, colon, bladder, hematopoietic system, pleura, and from unspecified tissues. In addition, the authors indicated a dose-response relationship between years of employment and incidence of total cancer and bladder cancer (Hogstedt et al. 2013). A further study of the Swedish chimney sweeps (*n*=6374) with a follow-up period 1952-2006 showed that mortality among chimney sweeps was increased for cancers of the bowel, esophagus, liver, and lung, as well as for liver cirrhosis, non-malignant airway diseases, alcoholism, suicide and other causes (Jansson et al. 2012). Moreover, increased risks among chimney sweeps were found, in various studies, for cancers of the prostate, haematolymphatic system, lung, and esophagus, as well as for pulmonary disease, and death from accidents (Evanoff et al. 1993; Gustavsson et al. 1987; Gustavsson et al. 1988; Hogstedt et al. 1982).

Not only chimney sweeps, but also other PAH-exposed occupational groups experience work-related adverse health effects. Several lines of evidence have linked increased risk of cancer with working in coal-gasification (Berger and Manz 1992; Doll et al. 1972; Martin et al. 2000), coal-

tar distillation (Henry 1947; Letzel and Drexler 1998; Maclaren and Hurley 1987; Swaen and Slangen 1997), coal-tar pitch (Hammond et al. 1976; Kennaway and Kennaway 1947; Partanen and Boffetta 1994; Swaen and Slangen 1997), coke production (Costantino et al. 1995; Swaen et al. 1991), aluminium production (Gibbs et al. 2007; Romundstad et al. 2000; Spinelli et al. 2006), and rubber manufacturing (Alder et al. 2006; Kogevinas et al. 1998; Stewart et al. 1999). These work environments, including chimney sweeping, were classified as *carcinogenic to humans* (Group 1) by IARC (Table 1) (IARC 2012). Researchers in collaboration with IARC have established 10 characteristics for human carcinogens, of which all chemicals in Group 1 (*carcinogenic to humans;* IARC) manifest at least one (Smith et al. 2016). These characteristics of carcinogens include (i) electrophilicity, (ii) genotoxicity, (iii) epigenetic toxicity, and the ability to cause (iv) impairment in DNA repair system, (v) oxidative stress, (vi) inflammation, (vii) immune dysfunction, (viii) immortalization, (ix) cell proliferation dysfunction, and (x) modulation of endogenous ligands/receptors (Smith et al. 2016). While PAH are inert, their electrophilic metabolites (such as epoxides and quinones) can manifest some of the above-mentioned characteristics such as forming adducts with DNA (genotoxicity) and giving rise to oxidative stress (Moorthy et al. 2015; Palackal et al. 2002; Smith et al. 2016). Given that, exploring early markers of PAH-induced carcinogenicity would provide important opportunities for promoting occupational health.

2.4.8.2 Cardiovascular disease (CVD)

Cardiovascular health outcomes have been less studied in relation to PAH exposure compared to cancer. Epidemiological studies among chimney sweeps have reported increased incidence and mortality of CVD such as coronary heart disease, ischaemic heart disease, and myocardial infarction (Burstyn et al. 2005; Evanoff et al. 1993; Gustavsson et al. 2013; Hansen 1983; Jansson et al. 2012; Letzel et al. 1992). Further, a number of studies have investigated markers of CVD in association with PAH exposure in the general population. In a study using the National Health and Nutrition Examination Survey (NHANES 2003-2004), the PAH metabolites 2-hydroxyphenanthrene and 9-hydroxyfluorene were positively associated with the marker of acute-phase response to inflammation i.e. C-reactive protein (CRP), suggesting that PAH can induce inflammation and thus contribute to development of atherosclerosis (Everett et al. 2010). Other studies (NHANES 2001-2004) showed that 2-hydroxyphenanthrene was associated with CVD (self-reported), but no associations were found between hydroxylated metabolites of the PAH (naphthalene, fluorene, phenanthrene, and pyrene) and the CVD markers homocysteine (a key player in one-carbon metabolism), fibrinogen (a main component of the coagulation cascade), and white blood cell count (Clark et al. 2012; Xu et al. 2010). Recent results from studies on NHANES 2001-2008 and NHANES 2003-2008 showed positive associations between PAH metabolites (2-naphthalene and 2-phenanthrene) and hypertension (Ranjbar et al. 2015), as well as between PAH metabolites (2-hydroxyphenanthrene and 4-hydroxyphenanthrene) and the CVD markers CRP and gamma glutamyltransferase (participates in glutathione regulation) (Farzan et al. 2016). Similarly, PAH metabolites were associated with CRP levels and white blood cell count in participants from NHANES 2001-2002, 2003-2004, and 2005-2006 (Alshaarawy et al. 2013). Yang et al. *(*2016) measured 10 hydroxylated PAH metabolites (OH-PAH) in urine, plasma proteins, and heart rate variability in a group of coke oven workers (n=489). The authors found that OH-PAH were positively associated with macrophage stimulating protein, activated leukocyte cell adhesion molecule, and CRP, but inversely associated with heart rate variability

(Yang et al. 2016). Correspondingly, other studies in coke oven workers, boilermakers and the general population showed inverse associations between PAH exposure and heart rate variability (Feng et al. 2014; Lee et al. 2011; Li et al. 2012a). Taken together, further studies elucidating the potential mechanisms of PAH-induced CVD as well as exploring new CVD markers in relation to PAH exposure are needed.

2.4.8.3 Other diseases

Lung cancer has been the most studied pulmonary disease among PAH-exposed workers, while non-carcinogenic airway disease and symptoms have received less attention. Epidemiological studies among chimney sweeps have found increased mortality from nonmalignant airway diseases (Jansson et al. 2012) as well as higher risk for asthma, cough with phlegm, dyspnea, and eye symptoms (Alhamdow et al. 2017a; Hansen 1990; Li et al. 2008). A study among workers in rubber industry demonstrated increased risk for dry throat, hoarseness, and dry cough (Jönsson et al. 2007). A further study showed low measurement of ventilatory capacity and increased prevalence of acute and chronic respiratory diseases (cough, dry throat, tightness of the chest, nasal dryness and nasal bleeding) in rubber workers (Zuskin et al. 1996). Findings from a follow-up study demonstrated that coke oven workers had lung function impairment associated with PAH metabolite concentrations (Wang et al. 2016). In line with these studies, the Agency for Toxic Substances and Disease Registry (ATSDR) has linked cough and chronic bronchitis to chronic occupational exposure to PAH (ATSDR 2009). PAH may also affect fertility and embryogenesis, however, these effects are out of the scope of this thesis (Choi et al. 2010).

2.4.9 Mechanisms of toxicity

BaP is a human carcinogen and has extensively been investigated in mechanistic studies in animals. Two main pathways have been shown to be involved in PAH-induced carcinogenicity; PAH-DNA adduct formation, particularly adducts of diolepoxides, radical cations, and *o*-quinones, and production of reactive oxygen species (ROS) (Cavalieri and Rogan 1995; Henderson et al. 1989; IARC 2012; Kwack and Lee 2000).

The BaP metabolites diolepoxides and *o*-quinones are potent reactive metabolites that can form adducts with DNA and other macromolecules (RNA, protein, and lipids) (Balu et al. 2006; Cavalieri et al. 2005; Chakravarti et al. 2008; Henderson et al. 1989; Meehan et al. 1977; Moorthy et al. 2015; Smithgall et al. 1988; Xue and Warshawsky 2005) (Figure 5). In response, the cell can repair depurinating PAH-DNA adducts by base excision repair and stable adducts by nucleotide excision repair (Braithwaite et al. 1998; Wei et al. 1995). However, once these repair systems fail, tumorigenic/non-tumorigenic mutations can be introduced during cell division (Chakravarti et al. 1995; Nelson et al. 1992; Tang et al. 2000; Zhao et al. 2006). Further, one-electron oxidation can be catalyzed by CYP P450 or prostaglandin H synthase giving rise to short-lived, highly reactive radical cations which can form unstable DNA adducts and cause mutations (Banasiewicz et al. 2004; Cavalieri and Rogan 1985; Cavalieri and Rogan 1992; Hrycay and Bandiera 2015a). On the other hand, ROS are produced from redox cycling of quinones/catechols, and quinones/hydroquinones during PAH metabolism (IARC 2010; IARC 2012; Penning et al. 1999). ROS are very reactive and can induce carcinogenesis by causing oxidative DNA damage and cellular lesions in proteins, carbohydrates and lipids (Henderson et al. 1989; Hrycay and Bandiera 2015b; Kwack and Lee 2000).

BaP has been shown to induce atherosclerosis in pigeons, cockerels, and mice (Majesky et al. 1983; Ramos and Moorthy 2005). Moreover, PAH-DNA adducts have been present in human vessels with atherosclerosis, which suggests a role of genotoxic effect in formation of atherosclerotic lesions (Binkova et al. 2001; Ramos and Moorthy 2005). However, intra-nasally administered BaP in rats exhibited neither increased oxidative stress nor adverse effects on the cardiovascular tissue, but showed altered circadian rhythm of blood pressure caused by local inflammation in the lungs (Gentner and Weber 2011). Summary of potential biological adverse effects caused by PAH exposure is provided in Figure 7.

2.5 BIOMARKERS OF DISEASE

The term biomarker (biological marker) encompasses a wide variety of measurable biological characteristics and can be defined as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention" (Naylor 2003). Accordingly, biomarkers can be biological molecules (e.g. proteins and lipids), cellular features (white blood cell count), or genetic/epigenetic characteristics (copy number of a specific DNA sequence and DNA methylation) (Dietze and Patzak 2016; Hussein et al. 2018; Liu et al. 2017). Further, biomarkers may also be classified as biomarkers of exposure (such as urinary 1-OH-PYR; a biomarker for PAH exposure) or biomarkers of disease, which may reflect disease presence (e.g. high levels of glucose in blood is a biomarker of diabetes mellitus) or serve as early signals for future disease development (Dietze and Patzak 2016; Mayeux 2004).

In addition to the classical biomarkers related to CVD (e.g. homocysteine and lipids) (Dhingra and Vasan 2017; Eikelboom et al. 1999) and cancer (e.g. prostate specific antigen; PSA) (Henry and Hayes 2012), newly emerging biomarkers, in association with environmental and occupational exposures, have been evaluated in a vast array of epidemiological studies. Telomere length (TL; copy number of the telomeric sequence AGGGTT) and mitochondrial DNA copy number (mtDNAcn; copy number of the circular double-stranded DNA in the mitochondria) have been investigated in relation to environmental exposures such as methylmercury (Xu et al. 2019; Yeates et al. 2017), arsenic (Ameer et al. 2016), and air pollution (Hou et al. 2012), as well as occupational exposures such as PAH (Pavanello et al. 2013; Pavanello et al. 2010; Xu et al. 2018), and benzene (Bassig et al. 2014; Carugno et al. 2012). Moreover, TL and mtDNAcn have been widely evaluated in relation to cancer and CVD (Ashar et al. 2017; Haycock et al. 2017; Hu et al. 2016; Mi et al. 2015; Wentzensen et al. 2011; Yue et al. 2018). Further, epigenetic biomarkers, particularly DNA methylation, have been demonstrated to be associated with exposure to various toxicants (Leenen et al. 2016; Martin and Fry 2018; Meehan et al. 2018) and adverse health outcomes such as cancer and CVD (Kim et al. 2010; Koch et al. 2018; Kulis and Esteller 2010; Zhong et al. 2016). Other emerging biomarkers include, but not limited to, microRNAs, long non-coding RNAs, and various proteomic molecules. Taken together, these biomarkers can be promising tools for early detection of diseases in connection with environmental and occupational exposures.

2.6 RECAPITULATION

Exposure to PAH constitutes a major health concern for the general public and even, to a larger extent, for workers in many work environments. PAH are omnipresent pollutants to which humans are exposed through inhalation, oral intake, and dermal absorption. Humans are often exposed to a mixture of PAH, which adds an extra layer of complexity when trying to identify the toxic contribution of each PAH. *In vitro* and *in vivo* toxicological studies have shown tumorigenic effects of a number of individual PAH, of which BaP is the most studied carcinogen. Registry-based epidemiological studies on chimney sweeps have found increased incidence and mortality of not only cancer but also CVD. However, data linking individual exposure to PAH with these diseases or early disease-related markers are scarce. This prompts the need for studies evaluating today's PAH exposure among chimney sweeps as well as investigating early biomarkers linked to cancer and CVD. This project is trying to produce a unique piece of knowledge to advance the field of occupational research.

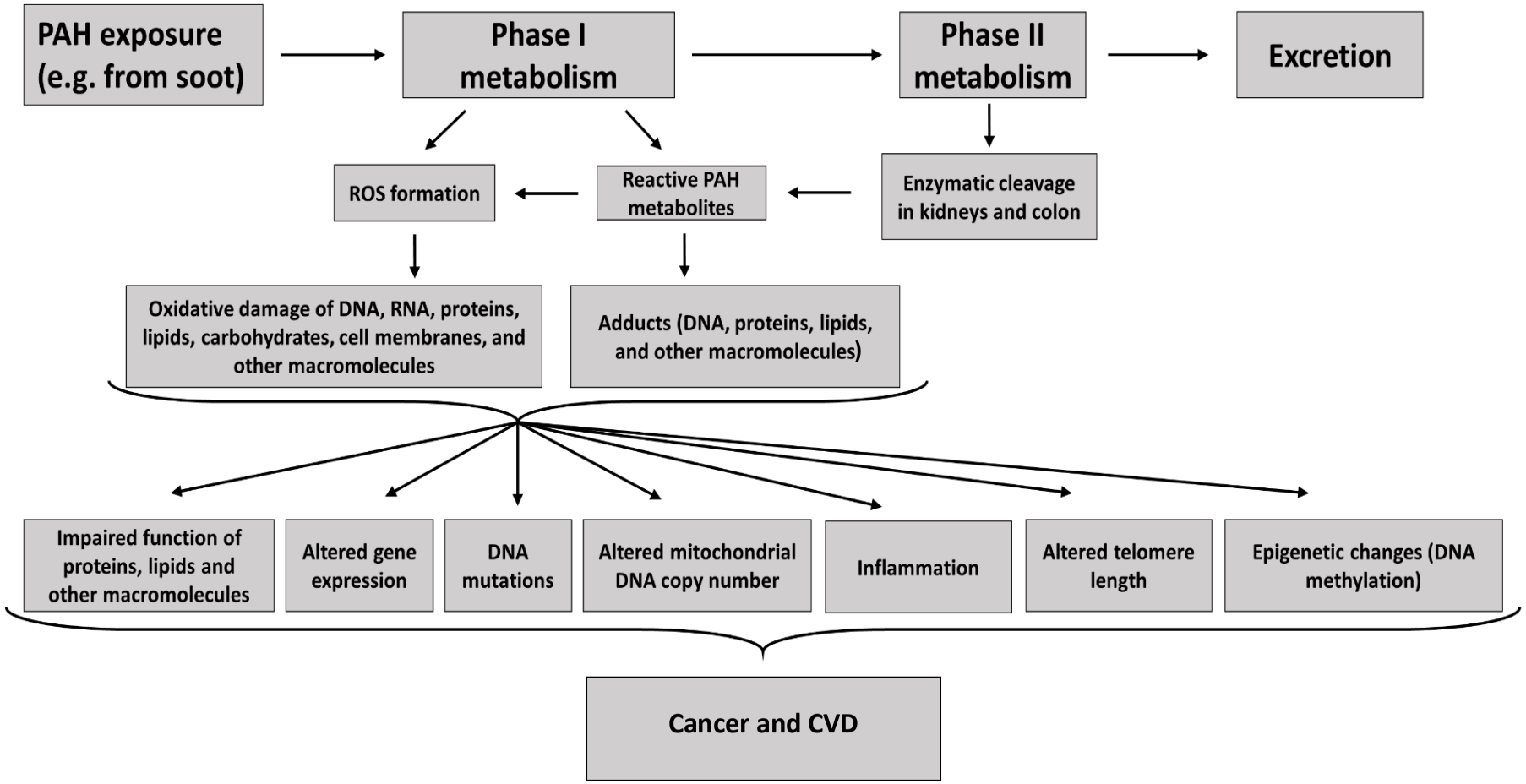

Figure 7. Potential biological effects resulted from PAH exposure.

Table 1. Classification of several PAH and occupational exposures according to IARC. http://monographs.iarc.fr/ENG/Classification/latest_classif.php [Accessed; 04 Jan 2019].

PAH (exposure)	IARC classification group*	Year of evaluation
Benzo[a]pyrene	1	2012
Dibenzo[a,l]pyrene	2A	2010
Cyclopenta[c,d]pyrene	2A	2010
Dibenz[a,h]anthracene	2A	2010
Benz[a]anthracene	2B	2010
Benzo[c]phenanthrene	2B	2010
Naphthalene	2B	2002
Dibenzo[a,i]pyrene	2B	2010
Dibenzo[a,h]pyrene	2B	2010
Benzo[b]fluoranthene	2B	2010
Benzo[a]fluoranthene	3	2010
Dibenzo[a,e]pyrene	3	2010
Pyrene	3	2010
Phenanthrene	3	2010
Anthracene	3	2010
Soot (as found in occupational exposure of chimney sweeps)	1	2012
Coal gasification	1	2012
Coal-tar distillation	1	2012
Coal-tar pitch	1	2012
Coke production	1	2012
Diesel engine exhaust	1	2013
Rubber manufacturing industry	1	2012
Aluminium production	1	2012
Indoor emissions from household combustion of coal	1	2012
Outdoor air pollution	1	2016

*Group 1=(*carcinogenic to humans);* Group 2A=(*probably carcinogenic to humans);* Group 2B=(*possibly carcinogenic to humans);* Group 3=(*Not classifiable as to its carcinogenicity to humans)*

Table 2. Concentration of 1-OH-PYR; a biomarker of total PAH exposure, in post-shift urine samples from workers in different occupations.

Occupational group	Workers' country	*n*	Measure	Concentration (µg/g creatinine)*	Reference
Chimney sweeps	Sweden	148	median	0.39	(Alhamdow et al. 2017b)
Chimney sweeps	Germany/ Poland	100	median	0.5	(Letzel et al. 1999)
Chimney sweeps	Italy	27	mean	1.1	(Pavanello et al. 2000)
Creosote-exposed workers	Netherlands	10	mean	4.5	(Van Rooij et al. 1993b)
Creosote-exposed workers	Finland	6	mean	123.5	(Elovaara et al. 1995)
Aluminium industry (potroom workers)	Surinam	8	mean	34.7	(Ny et al. 1993)
Aluminium industry (potroom workers)	Netherlands	6	mean	13	(Van schooten et al. 1995)
Aluminium industry (potroom workers)	Sweden	96	mean	8.3	(Carstensen et al. 1999)
Coke oven workers	Germany	87	mean	7.1	(Förster et al. 2008)
Coke oven workers	Poland	24	median	4.4	(Mielżyńska et al. 1997)
Coke oven workers	Belgium	33	mean	1.4	(Van Hummelen et al. 1993)
Coke oven workers	Belgium	54	mean	2.9	(Ferreira et al. 1994)
Coke oven workers	Italy	95	mean	2.5	(Clonfero et al. 1995)
Coke oven workers	Poland	55	median	10.5	(Campo et al. 2010)
Coke oven workers	Poland	50	median	17.4	(Siwińska et al. 2004)
Coke oven workers	Poland	49	mean	6	(Pavanello et al. 2008)
Workers in graphite electrode production	Belgium	15	mean	12	(Van Hummelen et al. 1993)
Workers in graphite electrode production	Belgium	92	mean	6.2	(Ferreira et al. 1994)
Workers in graphite electrode production	Germany	26	mean	4.8	(Förster et al. 2008)
Workers in graphite electrode production	Germany	67	mean	8.7	(Angerer et al. 1997)

*Different studies reported concentrations of 1-hydroxypyrene (1-OH-PYR) in different units. The reported values in this table (µg/g creatinine) were calculated based on the conversion factor reported in IARC 2010 as follows (1 µmol/mol creatinine = 1.93 µg/g creatinine = 2.84 µg/L)

3 AIMS

The overall aim of this thesis was to study chimney sweeps' exposure to PAH, elucidate the associations between working as a chimney sweep and early biomarkers related to development of CVD and cancer, and evaluate dose-response relationships with PAH exposure. The specific aims of the thesis were as follows:

- To elucidate whether currently working chimney sweeps are exposed to PAH and also, to elucidate the associations between chimney sweeping and concentrations of PAH metabolites in urine with classical risk markers for development of CVD.

- To explore putative CVD-related serum proteins in relation to chimney sweeping and concentrations of PAH metabolites in urine.

- To elucidate the associations between chimney sweeping and concentrations of PAH metabolites in urine with DNA methylation, telomere length, and mitochondrial DNA copy number; markers related to DNA damage and cancer development.

- To explore putative cancer-related serum proteins in relation to both chimney sweeping and concentrations of PAH metabolites in urine.

4 MATERIALS AND METHODS

4.1 STUDY DESIGN

The study design for all papers included in this thesis was cross-sectional.

4.2 PARTICIPANTS

We recruited 151 chimney sweeps (also called chimneysweepers or chimney sweepers) and 152 unexposed control individuals from southern Sweden (counties of Skåne, Öland, Blekinge, Halland, Småland, and Västergötland). These two groups of workers (or a subset of them) were included in all four studies. A third group of 19 creosote-exposed workers from Örebro County was included in **study III** (Figure 8).

All participants were males 19-66 years of age and all gave informed consent. We included only male participants because the majority of currently working chimney sweeps in Sweden are men.

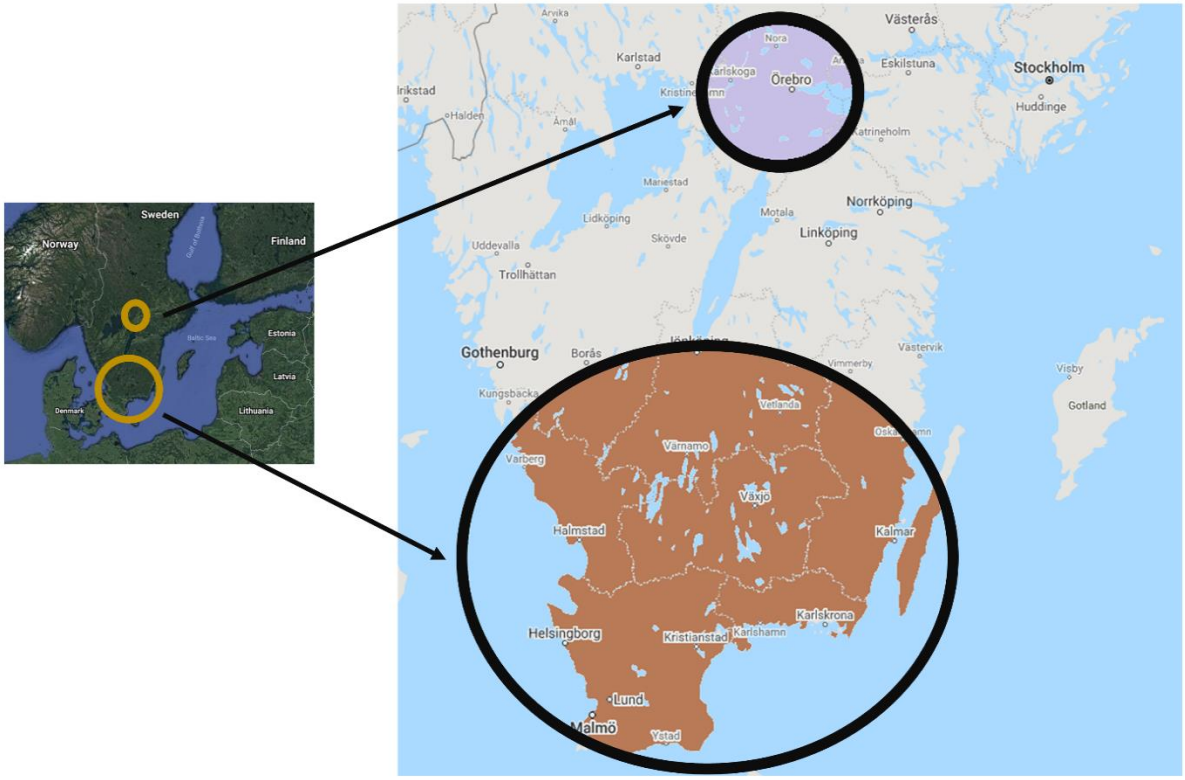

Figure 8. The geographical areas in Sweden from which the study participants were recruited (adapted from © Scribble Maps).

4.2.1 Chimney sweeps

Chimney sweeps usually perform multiple daily tasks i.e. soot sweeping in private homes, soot sweeping in industrial facilities, inspection of fire-safety systems, cleaning ventilation channels, mandatory inspection of ventilation channels, cleaning exhaust-ducts in restaurants, and administrative work. They start their work shift in the morning driving their cars to multiple

clients throughout the day. After the work shift is over, they return to their company to shower and report their activities.

We recruited chimney sweeps between 2013 and 2015 in co-ordination with chimney sweeps' trade union (Kommunal) and the employer organization of chimney sweeps (Sveriges Skorstensfejaremästares Riksförbund; SSR). We started by contacting the managers of chimney sweeping companies to ask for participation. The managers were instructed to invite their employees for participation. In total, we contacted 39 chimney sweeping companies of which, 34 were positive for participation. Due to the nature of the recruitment process, it was not possible to calculate the response rate for individuals. Instead, we calculated the response rate for companies, which was 87%. Out of the 34 companies, 4 were far-off located and one had only female chimney sweeps, therefore, these 5 companies were excluded (final number of companies *n*=29). We sent, via regular mail, a rationale for the study, an informed consent form, and a questionnaire to all chimney sweeps who had agreed to participate. We also booked a visit at the company facility to collect the questionnaires, measure blood pressure, weight, and height, and sample blood and urine. The visits were on Wednesdays or Thursdays after chimney sweeps had finished their work shift.

4.2.2 Unexposed controls

The controls were workers that had no known occupational exposure to PAH. During 2010–11, we contacted 12 companies/municipalities, of which 7 (6 companies and 1 municipality) agreed to participate, resulting in 58% company response rate. These companies were running warehouses or food storage facilities. We further recruited 25 currently smoking controls in 2015 from 3 companies and 4 municipalities to match the smoking status of chimney sweeps. To note, it was difficult to find smoking controls currently working in a PAH-free working environment. The scarcity of the smoking controls had made us contact a large number of companies, several of which did not have smoking employees. For this reason, we could not calculate the company response rate. Similar to chimney sweeps, the controls filled in a questionnaire, donated blood and urine samples and got their weight and height measured.

4.2.3 Creosote-exposed workers

We recruited workers from a factory where railway switches are produced. During the production process, the switches are connected to wooden ties impregnated with creosote oil, which provides protection against humidity. The workers are exposed to creosote from handling impregnated ties, dripping creosote oil, and disassembling and transporting the final products i.e. railway switches. The workers are exposed to PAH from the creosote oil. All workers were recruited from one company located in Örebro County with 100% participation rate. We collected questionnaires, blood and urine samples from the participants.

4.3 QUESTIONNAIRE

The questionnaire for all study groups included questions about age, personal history of disease, family history of disease, airway symptoms, level of education, prescribed medication, non-prescribed medication, tobacco smoking habit, use of snus, exposure to passive smoking, intake of fish, vegetables, and fruits, physical activity, exposure to dust, diesel exhaust, welding fumes or soldering smoke from hobbies, current residency, and employment history. The questionnaire

of chimney sweeps and creosote-exposed workers further explored occupational history, details about work environment and work conditions. For example, the extent to which chimney sweeps were involved in different work tasks (such as soot sweeping in private homes and soot sweeping in industrial facilities) was investigated for the periods 1963–72, 1973–82, 1983–92, 1993–2002, 2003–12, and for the past 12 months. The use of protective equipment (e.g. gloves, masks, and overall suits) was also explored among chimney sweeps throughout different periods.

4.4 BIOLOGICAL SAMPLES

We collected EDTA venous blood and urine samples from chimney sweeps, controls, and creosote-exposed workers. From chimney sweeps and controls, we further collected serum samples obtained by allowing blood coagulation in SST tubes for 10 min at room temperature and then centrifuging at 1800 ×g for 15 min. Blood and urine samples were stored at –20°C, while serum samples at –80°C.

4.5 EXPOSURE ASSESSMENT

We analysed the monohydroxylated metabolites of the PAH pyrene, BaP, phenanthrene and benz[a]anthracene (BaA). Pyrene (four benzene rings) and phenanthrene (three rings) are classified as "*not classifiable as to its carcinogenicity to humans"* (Group 3) by IARC (IARC 2010). BaP (five rings) and BaA (four rings) are classified as "*carcinogenic to humans*" (Group 1) and "*possibly carcinogenic to humans*" (Group 2B), respectively (IARC 2010). We used liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) for analysis. There could be several isomers for each monohydroxylated metabolite that were quantified against one pure standard of a specific monohydroxylated PAH metabolite. For instance, the peak for monohydroxylated metabolites of BaP included several isomers, but the quantification was carried out using a pure standard of 3-OH-BaP. Therefore, we measured 1-OH-PYR, 2-OH-PH, 3-OH-BaP, and 3-OH-BaA as proxy for exposure to pyrene, phenanthrene, BaP, and BaA, respectively. In short, the urine samples were treated with ß-glucuronidase in presence of ammonium acetate buffer. Vitamin C (for stabilization purposes) and internal standards were added. The samples were then injected onto an LC-MS/MS system. All samples were run in duplicates. Urinary creatinine and urine specific gravity were measured to correct for urine dilution. More details were provided in **Study I** (Alhamdow et al. 2017b). The exposure data were used in all four studies.

4.6 MARKERS RELATED TO CVD

4.6.1 Blood pressure

High blood pressure is a known risk factor for CVD (Chobanian et al. 2003). We measured blood pressure (both systolic and diastolic) for chimney sweeps, using a mercury sphygmomanometer in the upright (sitting) position, during the company visit. These data were used in **Study I**.

4.6.2 Classical serum markers for CVD risk

4.6.2.1 Homocysteine, C-reactive protein (CRP), gamma-glutamyltransferase (GGT)

Higher levels of these markers (risk markers) have been associated with CVD (Ganguly and Alam 2015; Ridker et al. 2002; Ruttmann et al. 2005). Serum aliquots from chimney sweeps and controls were used for analyses, which were carried out using standard methods at the Department of Clinical Chemistry, Lund University Hospital. Details of analyses were described in **Study I**.

4.6.2.2 Serum lipids

Total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglycerides (TG) were measured in serum for chimney sweeps and controls by the same laboratory at the Department of Clinical Chemistry (Lund University Hospital). More details were described in **Study I**.

4.6.3 Exploratory serum markers for CVD risk

Among current nonsmoking chimney sweeps and controls, we measured 92 different putative CVD-related proteins in serum. The analysis was performed by Olink Proteomics (CVD II panel; Olink, Uppsala, Sweden) based on the proximity extension assay (PEA). Aliquots of serum samples (40 μL) were randomized in 96-well plates and shipped on dry ice to the company. A pooled serum sample was collected and shipped along with the samples in order to be used for calculations of coefficient of variation (intra- and inter-plate). In this analysis, each protein is targeted by a unique pair of antibodies labelled with complementary DNA sequences (PEA probes). These probes can hybridize upon successful protein targeting and thereafter produce a short piece of double-stranded DNA specific for the protein. The resulted DNA sequence is then amplified by real-time PCR (qPCR) and quantified by microfluidic chip (96.96 Dynamic Array IFC, Fluidigm Biomark). For more details, please refer to **Study II** and Olink's website (olink.com).

4.7 MARKERS RELATED TO CANCER

4.7.1 Telomere length (TL) and mitochondrial DNA copy number (mtDNAcn)

We measured relative TL and mtDNAcn in DNA extracted from whole blood samples of chimney sweeps, controls, and creosote-exposed workers based on a previously described method (Cawthon 2002). The method was based on qPCR where the copy number of telomere repeats (T) and the copy number of the single copy gene hemoglobin beta (*HBB)* (S) were calculated, thereafter, the relative TL was calculated as T/S. For mtDNAcn, a similar method was employed where the absolute mtDNAcn (M) as well as the copy number of the single copy gene (*HBB*) (S) were obtained and thereafter, the relative mtDNAcn was calculated as M/S. All samples, including negative controls, were run in triplicates and 10% of randomly selected samples were rerun to ensure analysis quality. For more details, please refer to **Study III** (Alhamdow et al. 2018).

4.7.2 DNA methylation of the genes *F2RL3* and *AHRR*

F2R like thrombin or trypsin receptor 3 (*F2RL3*) and Aryl-Hydrocarbon Receptor Repressor (*AHRR*) are protein-coding genes consisting of 2 and 11 exons, respectively. Hypomethylation (low methylation) of two CpG sites (cytosine-phosphate-guanine) in these genes i.e. cg03636183 (*F2RL3*; based on Illumina beadchips 450K; Illumina Inc., CA, US) and cg05575921 (*AHRR*) were, in several studies, shown to be associated with smoking as well as lung cancer (Gao et al. 2015; Zhang et al. 2016b). Assays for the genomic regions spanning cg03636183 and cg05575921 were developed based on previously published literature (Hossain et al. 2015; Wu et al. 2014). Additional CpG sites that were in close proximity to cg03636183 and cg05575921 were also considered in the analysis (Figure 9).

We used the pyrosequencing technique to perform DNA methylation analysis for DNA extracted from whole blood samples of chimney sweeps, controls, and creosote-exposed workers. Briefly, DNA samples were treated with bisulfite, converting unmethylated cytosine (C) to uracil and subsequently to thymine (T), but keeping methylated C unchanged. The genomic sequence of interest was then amplified by PCR and thereafter, specific amplification was examined on agarose gel. The pyrosequencing was then carried out following a standard protocol using PyroMark Q96 ID platform (Qiagen). Negative and positive controls were included and 10% of the samples were randomly selected and rerun for quality control purposes. For more details, please refer to **Study III**.

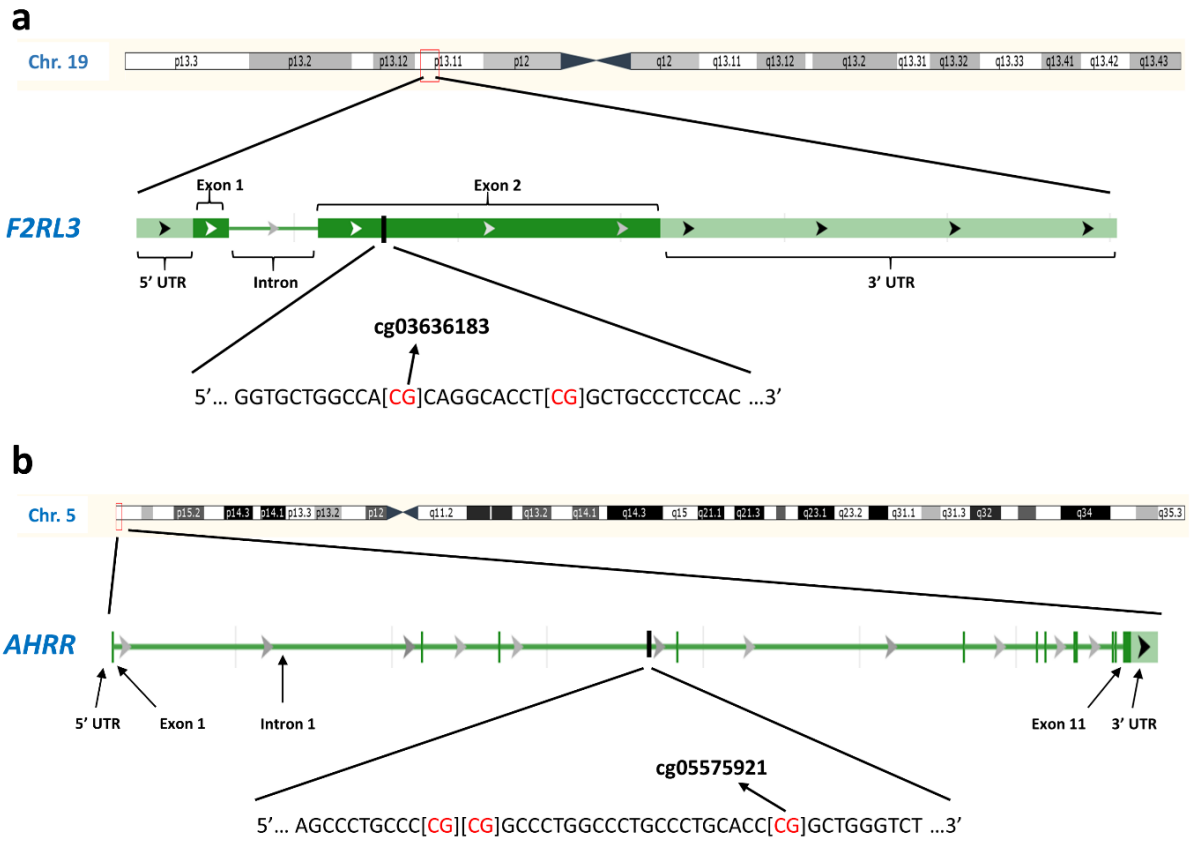

Figure 9. (**a**) Location of CpG1 and cg03636183 (*F2RL3* gene; chromosome 19). (**b**) Location of CpG1, CpG2, and cg05575921 (*AHRR* gene; chromosome 5).

4.7.3 Exploratory serum markers for cancer risk

We analysed a panel of 92 putative cancer-related proteins (Oncology panel II; Olink) in serum using the same approach i.e. PEA provided by Olink proteomics for the same current nonsmoking chimney sweeps and controls involved in **Study II**. The same aliquots of serum (i.e. 40 μL), used for analysis of CVD-related proteins, were used for this analysis. For more details, please refer to **Study IV** (Alhamdow et al. 2019).

4.8 STATISTICAL ANALYSIS

All statistical analyses were carried out using the statistical software SPSS (IBM SPSS Statistics, NY, USA) and R (version 3.5.1; R core team, 2018).

In all four studies, median value, a measure for central tendency, was calculated for continuous variables such as age and body mass index (BMI), while frequency was considered for categorical variables (smoking and use of snus). Spearman's correlations were used to examine crude correlations between continuous variables. Crude differences between study groups were evaluated by Kruskal–Wallis test or Mann-Whitney U test for continuous variables and Fisher's exact test for categorical variables. Linear regression analysis was used to investigate associations between study groups (binary variable) and PAH metabolites in urine (continuous variables), as well as between study groups and outcome markers (continuous variables) adjusting for confounders and covariates. Similar linear regression models were adopted to evaluate associations between exposure markers and outcome markers. Wilcoxon signed-rank test was used to explore trends over time for two related samples (e.g. extent of engagement in chimney sweeping tasks during two different time periods). Trend analysis using Jonckheere-Terpstra test or linear regression models was also performed to examine dose-response relationship between exposure markers and outcome markers.

For **Study II** and **Study IV**, principal component analysis was performed to examine clustering of study groups according to proteomics data. Principal component regression analysis followed by Kolmogorov-Smirnov test (KS test) was considered to explore potential confounders when examining the differences in serum protein concentrations between study groups. False discovery rate correction (FDR<0.05) was applied to adjust for multiple comparisons.

4.9 PATHWAY AND GENE ONTOLOGY ANALYSES

These analyses were employed in **Study II** and **Study IV** to explore relevant pathways and biological functions for the differentially expressed proteins between chimney sweeps and controls. Ingenuity Pathway Analysis (IPA) software (Qiagen, Hilden, Germany) and the online tool WebGestalt (www.webgestalt.org) were used to perform the analyses.

4.10 ETHICAL CONSIDERATIONS

In our studies, ethical permits were obtained from relevant ethical committees i.e. the Regional Ethics Committee at Lund University, Lund, Sweden and the Regional Ethics Committee at Uppsala University, Uppsala, Sweden. Written informed consent was obtained from all individuals prior to participation. The informed consent encompassed adequate and understandable background information (in Swedish language) about study rationales and

purposes, type and amount of samples needed, and method of handling confidential personal data and ensuring participants' privacy. Other aspects of the informed consent include access to the measurements (blood pressure, weight and height) and information about potential risk of discomfort due to sampling, estimated time of sampling, and handling of biological samples. It was also indicated that the findings would be presented at the group level not individual level. All participants voluntarily agreed to participate in our studies. It was clearly stated in the informed consent that participants have the right to withdraw from the study at any time without motivation and with no consequences. As authors, we have an ethical and moral responsibility to deliver the findings of our research to participants and, therefore, we have communicated our results, via written reports and smaller meetings as well as larger conferences, with the chimney sweeps' union (Kommunal) and the chimney sweeps' employer organization (SSR). Another ethical consideration is that all authors in all published or ongoing studies declare that they have no conflict of interest.

5 RESULTS

5.1 STUDY I

5.1.1 Characteristics of study groups

Chimney sweeps (n=151), compared with controls (n=152), had similar median age (43 y), lower BMI, and higher frequency of party smokers, snus users, and small-town residents (P<0.05). The two study groups did not differ in other lifestyle factors such as passive smoking, level of education, physical activity, and intake of different dietary items; however, more chimney sweeps were recruited in autumn compared with controls (P<0.05).

5.1.2 Characteristics of chimney sweeping profession

Although it has been decreasing over time, soot sweeping in private homes was the major task for chimney sweeps constituting more than 50% of their working time, compared with the other tasks (Figure 10). Comparing the periods 1975–2002 and 2002–2013, a decrease in the use of oil fuel by clients and an increase in the use of pellets and wood (for fireplaces) were observed (P<0.05). In addition, more than 50% of the chimney sweeps did not use mask during soot sweeping in private homes (Figure 11).

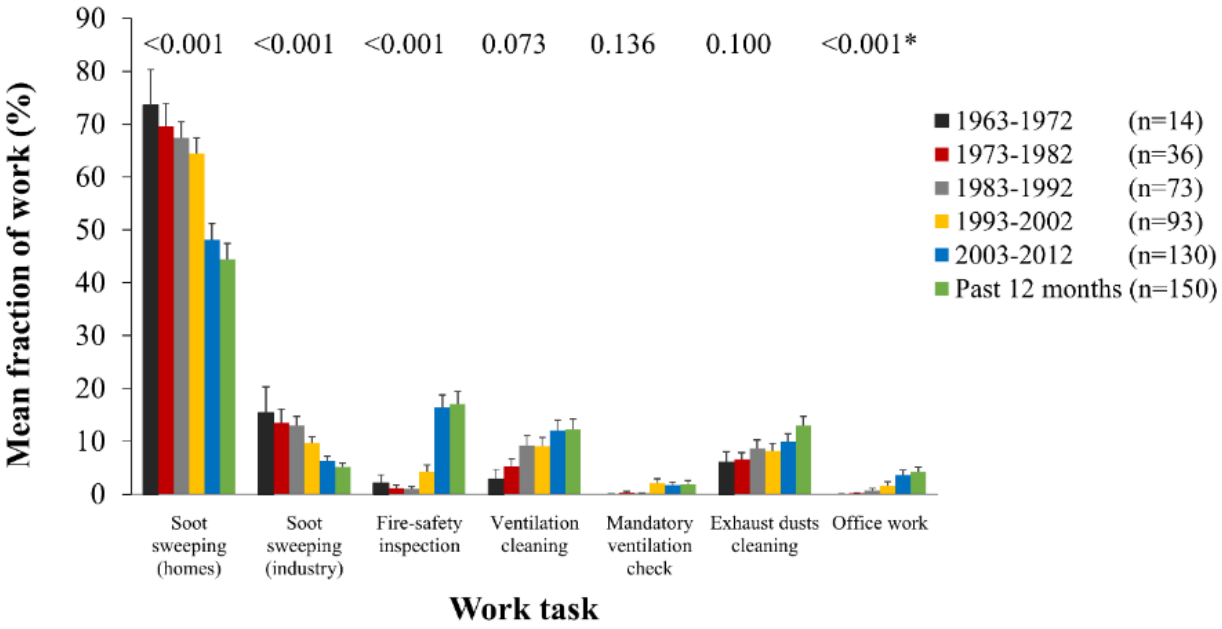

Figure 10. Trends for fraction of work (%) for different work tasks from 1963 to 2013. *Wilcoxon signed rank test for work tasks before/after the year 2002. Standard errors are represented by whiskers on top of each bar (adapted from **Study I**; Alhamdow et al. 2017b).

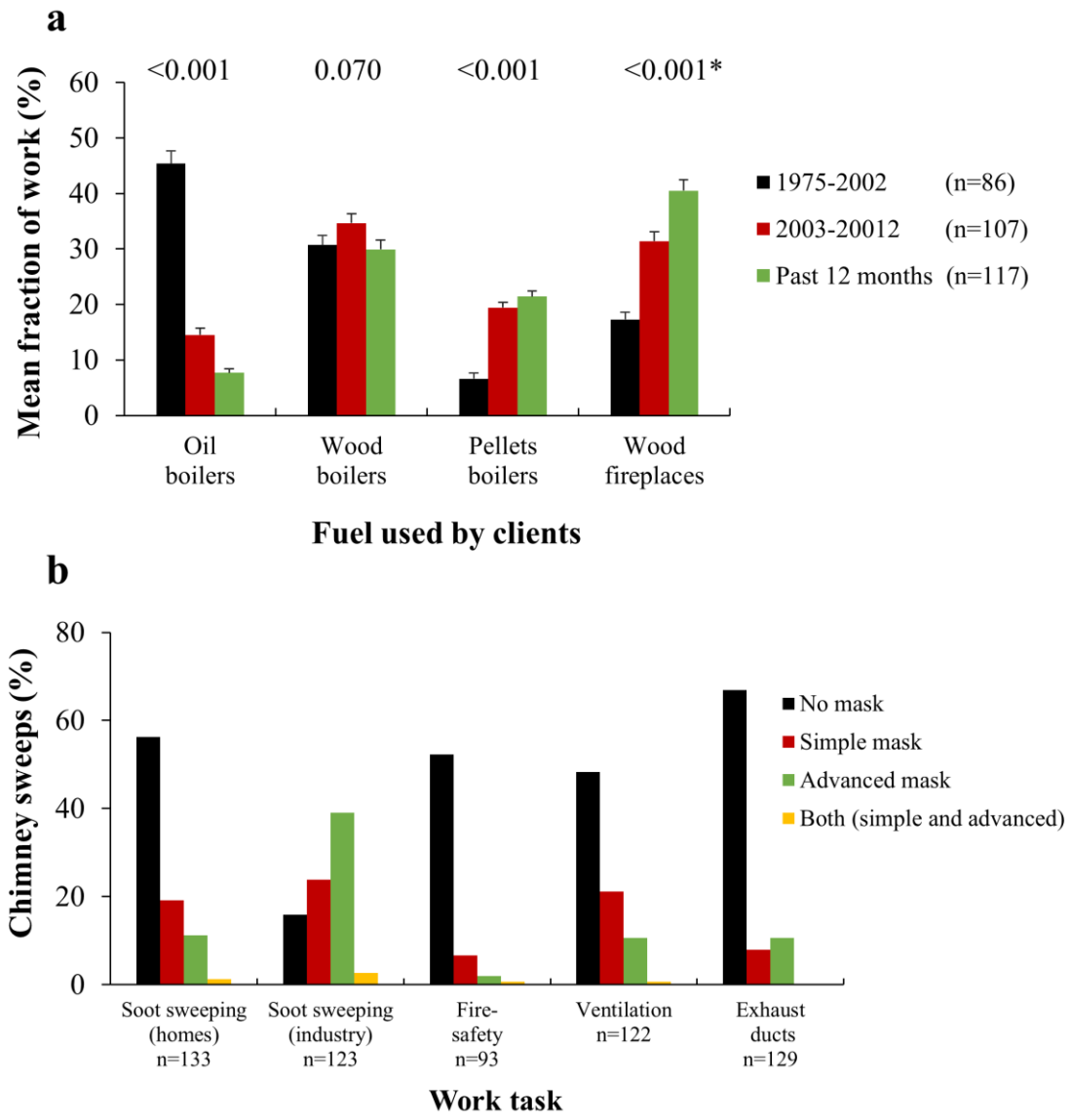

Figure 11. Trends for use of different fuel materials and use of masks reported by chimney sweeps. (**a**) Percentage of use of different types of fuel used by clients whenever chimney sweeps were doing soot sweeping for boilers or chimneys of fireplaces during the period (1975 to 2013). The whiskers represent standard errors. **P* value of Wilcoxon signed rank test for use of fuel by clients before/after the year 2002. (**b**) Percentage of chimney sweeps who used masks during different work tasks (adapted from **Study I**; Alhamdow et al. 2017b).

5.1.3 PAH exposure

Chimney sweeps had higher concentrations of all 4 monohydroxylated metabolites of PAH (1-OH-PYR, 2-OH-PH, 3-OH-BaP, and 3-OH-BaA) compared with controls (P<0.001; adjusted for age, BMI, and smoking) (Table 3). For example, the median value of 1-OH-PYR was 0.39 (µg/g creatinine) for nonsmoking chimney sweeps compared with 0.06 (µg/g creatinine) for nonsmoking controls (around 7 times difference). The 4 metabolites were intercorrelated (P<0.001; average r_S>0.79; for nonsmoking chimney sweeps). Further, soot sweeping (in the past 12 months) was positively associated with the concentrations of all 4 PAH metabolites (P<0.001; linear regression models adjusted for age, BMI, and smoking). It is worth mentioning that the use of gloves and masks did not correlate with the concentrations of PAH metabolites.

5.1.4 PAH exposure and classical markers of CVD risk

Chimney sweeps had higher serum concentrations of homocysteine, cholesterol, and HDL compared with controls ($P<0.05$; adjusted for age, BMI, and smoking; Table 3). Moreover, the PAH metabolites 2-OH-PH, 3-OH-BaP, and 3-OH-BaA were positively associated with diastolic blood pressure among chimney sweeps ($P<0.05$; adjusted for age, BMI, and smoking; Table 4). In addition, weak non-significant associations were observed between PAH metabolites and homocysteine (positive estimates) and HDL (negative estimates) ($P>0.05$; Table 4).

Table 3. Differences between chimney sweeps and controls in PAH exposure and traditional serum markers of cardiovascular disease (CVD), explored by linear regression analysis adjusted for age, BMI, and smoking.

	Controls		**Chimney sweeps**		
	n	**Median (min, max)**	***n***	**Median (min, max)**	***P***
Markers of PAH exposure					
1-OH-PYR (µg/g creatinine)	151	0.06 (0.0–0.73)	148	0.39 (0.02–8.8)	<0.001
2-OH-PH (µg/g creatinine)	151	0.14 (0.04–3.1)	148	0.57 (0.08–7.1)	<0.001
3-OH-BaP (ng/g creatinine)	130	1.03 (0.0–17.3)	132	3.35 (0.0–50.1)	<0.001
3-OH-BaA (ng/g creatinine)	144	1.66 (0.04–21.0)	142	4.78 (0.39–43.3)	<0.001
Markers of CVD					
C-reactive protein (mg/L)	148	1.05 (0.42–19.0)	146	0.7 (0.42–46.0)	0.71
Gamma-glutamyl transferase (µkat/L)	148	0.48 (0.11–2.1)	146	0.49 (0.19–3.2)	0.19
Homocysteine (µmol/L)	148	13 (4.0–63)	146	16 (5.4–76.0)	<0.001
Cholesterol (mmol/L)	148	5 (2.0–9.7)	146	5.47 (3.2–8.7)	0.003
High-density lipoprotein (mmol/L)	148	1.15 (0.44–2.6)	146	1.32 (0.7–3.04)	0.004
Low-density lipoprotein (mmol/L)	148	3.31 (1.2–7.75)	146	3.53 (1.5–6.5)	0.061
Triglycerides (mmol/L)	148	1.7 (0.41–7.4)	146	1.6 (0.53–8.4)	0.190

Table 4. Associations between urinary PAH metabolites and diastolic blood pressure, homocysteine, and high-density lipoprotein in chimney sweeps (*n*=151), explored by linear regression analysis adjusted for age, BMI, and smoking. [95% CI = 95% confidence interval].

	Diastolic blood pressure (mm Hg)		**Homocysteine (µmol/L)**		**High-density lipoprotein (mmol/L)**	
	P	**B (95% CI)**	***P***	**B (95% CI)**	***P***	**B (95% CI)**
1-OH-PYR (µg/L)	0.067	1.16 (−0.08, 2.40)	0.67	0.27 (−0.98, 1.53)	0.12	−0.05 (−0.10, 0.01)
2-OH-PH (µg/L)	0.001	1.66 (0.72, 2.60)	0.39	0.38 (−0.49, 1.25)	0.073	−0.04 (−0.08, 0.00)
3-OH-BaP (ng/L)	0.013	0.31 (0.07, 0.55)	0.26	0.11 (−0.09, 0.32)	0.067	−0.01 (−0.02, 0.00)
3-OH-BaA (ng/L)	0.044	0.16 (0.01, 0.32)	0.26	0.09 (−0.07, 0.24)	0.042	−0.01 (−0.01, 0.00)

5.2 STUDY II

5.2.1 Characteristics of study participants

This study included 116 chimney sweeps and 125 controls. All participants were current nonsmokers, for whom serum and urine samples, full questionnaire information and valid proteomics data were available. Study groups had similar age and BMI ($P>0.05$), but differed in physical activity, use of snus, and residential area ($P<0.05$).

5.2.2 PAH exposure and exploratory CVD-related serum proteins

We found 25 differentially expressed proteins (DEP) between chimney sweeps and controls ($P<0.05$; adjusted for age, BMI, and FDR). The list of the DEP includes, among others, protein-glutamine gamma-glutamyltransferase 2 (TGM2), lactoylglutathione lyase (GLO1), NF-kappa-B essential modulator (NEMO), follistatin (FS), pro-interleukin-16 (IL-16), and heat shock protein beta-1 (HSP 27; also called HSPB1). Twenty-four DEP showed higher levels in chimney sweeps, compared with controls, and one DEP (i.e. SPON2; spondin-2) showed lower levels (Table 5). Three proteins i.e. FS, IL-16, and HSP 27 showed positive associations with PAH metabolites in a dose-response manner ($P<0.05$; adjusted for age, BMI, and day of sampling). Pathway analysis showed predicted activation of a number of up-stream regulators, including TNF (tumor necrosis factor), that are involved in inflammation. The analysis also showed that inflammation and immune response were the top diseases and functions associated with the DEP.

Table 5. List of the differentially expressed proteins between chimney sweeps and controls (FDR<0.05). Data presented are *P* value, unstandardized beta (B) and 95% confidence interval (95% CI) for respective linear regression models.

	Model 1 (unadjusted)		**Model 2 (adjusted for age and BMI)**	
Protein	***P***	**B (95% CI)**	***P***	**B (95% CI)**
TGM2	$1.5E^{-24}$	0.90 (0.75, 1.05)	$1.4E^{-24}$	0.91 (0.75, 1.06)
GLO1	$6.6E^{-19}$	0.59 (0.47, 0.71)	$6.0E^{-19}$	0.60 (0.47, 0.72)
FS	$8.4E^{-7}$	0.37 (0.22, 0.51)	$1.1E^{-7}$	0.38 (0.25, 0.52)
NEMO	$9.8E^{-7}$	0.40 (0.24, 0.56)	$2.7E^{-6}$	0.38 (0.23, 0.54)
IL-16	$1.4E^{-5}$	0.27 (0.15, 0.39)	$1.1E^{-5}$	0.28 (0.16, 0.40)
HSP 27	0.0001	0.23 (0.12, 0.35)	$6.8E^{-5}$	0.24 (0.12, 0.36)
CXCL1	0.0004	0.23 (0.11, 0.36)	0.0003	0.24 (0.11, 0.36)
PIgR	0.0004	0.06 (0.03, 0.10)	0.0005	0.06 (0.03, 0.10)
BOC	0.0007	0.13 (0.06, 0.21)	0.0002	0.13 (0.06, 0.20)
TIE2	0.0007	0.12 (0.05, 0.18)	0.0008	0.11 (0.05, 0.18)
STK4	0.0009	0.23 (0.10, 0.37)	0.002	0.21 (0.08, 0.35)
IL-27	0.001	0.11 (0.05, 0.18)	0.002	0.11 (0.04, 0.17)
LOX-1	0.001	0.23 (0.09, 0.37)	0.002	0.22 (0.08, 0.36)
PRSS27	0.003	0.15 (0.05, 0.24)	0.004	0.14 (0.05, 0.24)
SOD2	0.003	0.09 (0.03, 0.15)	0.002	0.09 (0.03, 0.15)
IL-17D	0.003	0.09 (0.03, 0.16)	0.004	0.09 (0.03, 0.15)
ANG-1	0.003	0.10 (0.03, 0.17)	0.004	0.10 (0.03, 0.17)
MARCO	0.003	0.09 (0.03, 0.15)	0.003	0.09 (0.03, 0.15)
AGRP	0.005	0.17 (0.05, 0.28)	0.004	0.16 (0.05, 0.27)
TNFRSF11A	0.009	0.14 (0.04, 0.24)	0.004	0.15 (0.05, 0.25)
TM	0.011	0.11 (0.03, 0.20)	0.011	0.11 (0.03, 0.20)
TF	0.013	0.11 (0.02, 0.19)	0.020	0.10 (0.02, 0.19)
SORT1	0.013	0.08 (0.02, 0.14)	0.008	0.09 (0.02, 0.15)
SPON2	0.014	-0.05 (-0.09, -0.01)	0.033	-0.04 (-0.08, -0.004)
MERTK	0.014	0.12 (0.02, 0.21)	0.015	0.11 (0.02, 0.21)

5.3 STUDY III

5.3.1 Characteristics of study participants

In addition to the same participants in **Study I** (151 chimney sweeps and 152 controls), this study included creosote-exposed workers (*n*=19). Median age for creosote-exposed workers was non-significantly lower (32 y) than chimney sweeps (43 y) and controls (43 y). Use of snus and residential area significantly differed between the three study groups (*P*<0.05).

5.3.2 PAH exposure

As reported in **Study I**, chimney sweeps had up to 7 times higher concentrations of PAH metabolites in urine than controls (P<0.001). Creosote-exposed workers were highly exposed to PAH; their concentrations of 1-OH-PYR and 2-OH-PH were 10.7 and 29.6 (μg/g creatinine), respectively, compared with 0.06 and 0.14 (μg/g creatinine) for the controls (P<0.001; Table 6). However, the concentrations of 3-OH-BaP were non-significantly lower among creosote-exposed workers compared with controls (P=0.703), which might be due the European regulation of BaP content in the creosote oil manufactured for industrial use (EC 2001).

Table 6. Concentrations of monohydroxylated PAH metabolites in urine for study groups.

	Controls[e]		**Chimney sweeps**			**Creosote-exposed workers**		
	n	**Median (min-max)**	***n***	**Median (min-max)**	***P*[e]**	***n***	**Median (min-max)**	***P*[f]**
1-OH-PYR[a]	151	0.06 (0.00–0.73)	148	0.39 (0.02–8.77)	<0.001	19	10.7 (3.2–41.3)	<0.001
2-OH-PH[b]	151	0.14 (0.04–3.09)	148	0.57 (0.08–7.10)	<0.001	19	29.6 (12.7–128.5)	<0.001
3-OH-BaP[c]	130	1.03 (0.00–17.28)	132	3.35 (0.00–50.15)	<0.001	19	0.75 (0.15–2.45)	0.703
3-OH-BaA[d]	144	1.66 (0.04–21.02)	142	4.78 (0.39–43.35)	<0.001	19	13.2 (1.5–32.6)	<0.001

[a]1-hydroxypyrene (μg/g creatinine), [b]2-hydroxyphenanthrene (μg/g creatinine), [c]3-hydroxybenzo[a]pyrene (ng/g creatinine), [d]3-hydroxybenz[a]anthracene (ng/g creatinine). [e]Mann-Whitney U test for differences between chimney sweeps and controls. [f]Mann-Whitney U test for differences between creosote-exposed workers and controls.

5.3.3 PAH exposure and cancer-related markers

TL and mtDNAcn were not different between study groups (P>0.05). Chimney sweeps had lower methylation of all CpG sites of *F2RL3* and *AHRR* compared with controls, and significance was reached for all three CpG sites of *AHRR* (P<0.05; Table 7). Creosote-exposed workers had significantly lower methylation of *F2RL3* (CpG1 and cg03636183) and *AHRR* (cg05575921) compared with controls (P<0.05; Table 7). As well, creosote-exposed workers had significantly lower methylation of *F2RL3* (cg03636183) and *AHRR* (cg05575921) compared with chimney sweeps (P<0.05).

No significant associations between PAH metabolites and cancer-related markers were observed when evaluated by linear regression analysis among nonsmoking chimney sweeps and controls (P>0.05). The linear regression analysis was not performed for nonsmoking creosote-exposed workers due to small sample size (n=9).

Table 7. DNA methylation of *F2RL3* and *AHRR* for study groups.

	Controls *n*=147	**Chimney sweeps *n*=145-146**		**Creosote-exposed workers *n*=19**	
CpG site	**Median (min-max)**	**Median (min-max)**	***P*[a]**	**Median (min-max)**	***P*[b]**
F2RL3 CpG1	89.5 (58.1–100)	88.8 (54.7–98.6)	0.063	87.2 (64.9–96.4)	0.014
F2RL3 (cg03636183)	76.7 (48.1–86.3)	76.6 (44.8–82.5)	0.786	73.3 (49.5–78.4)	0.003
F2RL3 average	83.6 (53.8–89.9)	83.0 (50.8–88.2)	0.173	80.1 (57.2–87.1)	0.002
AHRR CpG1	75.0 (24.7–88.1)	73.0 (20.8–89.7)	0.030	74.1 (30.9–82.8)	0.345
AHRR CpG2	69.1 (22.7–80.4)	66.5 (20.3–76.6)	0.002	68.7 (30.9–81.7)	0.482
AHRR (cg05575921)	90 (34.5–100)	88.1 (29.3–100)	0.038	84.9 (37.3–93.6)	0.003
AHRR average	78.6 (27.3–87.2)	76.2 (23.5–87.5)	0.007	77 (33.7–82.9)	0.262

[a]Mann-Whitney U test for differences between chimney sweeps and controls.

[b]Mann-Whitney U test for differences between creosote-exposed workers and controls.

5.4 STUDY IV

5.4.1 Characteristics of study participants

This study included current nonsmoking chimney sweeps (*n*=118) and controls (*n*=126). Proteomics data, questionnaires, and serum and urine samples were available for all participants. Chimney sweeps did not differ from controls in age, BMI, and lifestyle factors such as passive smoking and intake of different dietary items ($P>0.05$). However, chimney sweeps differed from controls in physical activity, use of snus, and residential area ($P<0.05$).

5.4.2 PAH exposure and exploratory cancer-related serum proteins

We found 17 differentially expressed proteins (DEP) between chimney sweeps and controls (adjusted for age, BMI, and FDR; $P<0.05$; Table 8). All proteins were upregulated in chimney sweeps, except syndican-1 (SYND1). Moreover, most of the DEP showed weak positive correlations with PAH metabolites, particularly among chimney sweeps (Figure 12) and again, SYND1 showed inverse correlations. Kallikrein 13 (KLK13) was the only protein significantly associated with more than one PAH metabolite i.e. 3-OH-BaP and 3-OH-BaA ($P<0.05$; linear regression model adjusted for age, BMI, and sample storage time). KLK13 also showed a dose-response relationship with the aforementioned metabolites among chimney sweeps ($P<0.05$; Jonckheere trend test; Figure 12).

Pathway analysis including the 17 DEP showed that interferon gamma was an upstream regulator. Network analysis and disease and function analysis showed clear involvement of cell adhesion, cell movement, cell migration, and cell invasion. Likewise, gene ontology analysis showed cell adhesion and migration as the top biological processes.

Table 8. Differentially expressed proteins (DEP) between chimney sweeps and controls, explored by linear regression models adjusted for false discovery rate. Effect estimate is presented as B value. [95% CI= 95% confidence interval].

	Model I (unadjusted)		**Model II (adjusted for age and BMI)**	
Protein	***P***	**B (95% CI)**	***P***	**B (95% CI)**
S100A4	<0.001	0.31 (0.22, 0.39)	<0.001	0.31 (0.22, 0.39)
FADD	<0.001	0.31 (0.21, 0.40)	<0.001	0.30 (0.20, 0.39)
METAP2	<0.001	0.31 (0.21, 0.42)	<0.001	0.33 (0.22, 0.43)
ANXA1	<0.001	0.52 (0.36, 0.67)	<0.001	0.50 (0.35, 0.66)
S100A11	<0.001	0.22 (0.14, 0.30)	<0.001	0.21 (0.13, 0.30)
VIM	<0.001	0.47 (0.28, 0.66)	<0.001	0.46 (0.27, 0.64)
TXLNA	<0.001	0.26 (0.12, 0.40)	0.001	0.26 (0.11, 0.40)
LYN	<0.001	0.12 (0.06, 0.18)	<0.001	0.12 (0.06, 0.18)
SCAMP3	0.001	0.21 (0.09, 0.33)	0.001	0.21 (0.09, 0.33)
SYND1	0.001	-0.17 (-0.28, -0.07)	0.002	-0.17 (-0.27, -0.06)
KLK13	0.004	0.16 (0.05, 0.27)	0.005	0.16 (0.05, 0.26)
IGF1R	0.004	0.11 (0.03, 0.18)	0.004	0.11 (0.03, 0.18)
TCL1A	0.007	0.32 (0.09, 0.55)	0.008	0.31 (0.08, 0.53)
CXCL13	0.004	0.16 (0.05, 0.28)	0.004	0.17 (0.05, 0.28)
ITGB5	0.003	0.09 (0.03, 0.16)	0.002	0.09 (0.03, 0.15)
GPNMB	0.002	0.06 (0.02, 0.10)	0.003	0.06 (0.02, 0.10)
CEACAM5	0.005	0.26 (0.08, 0.44)	0.007	0.25 (0.07, 0.43)

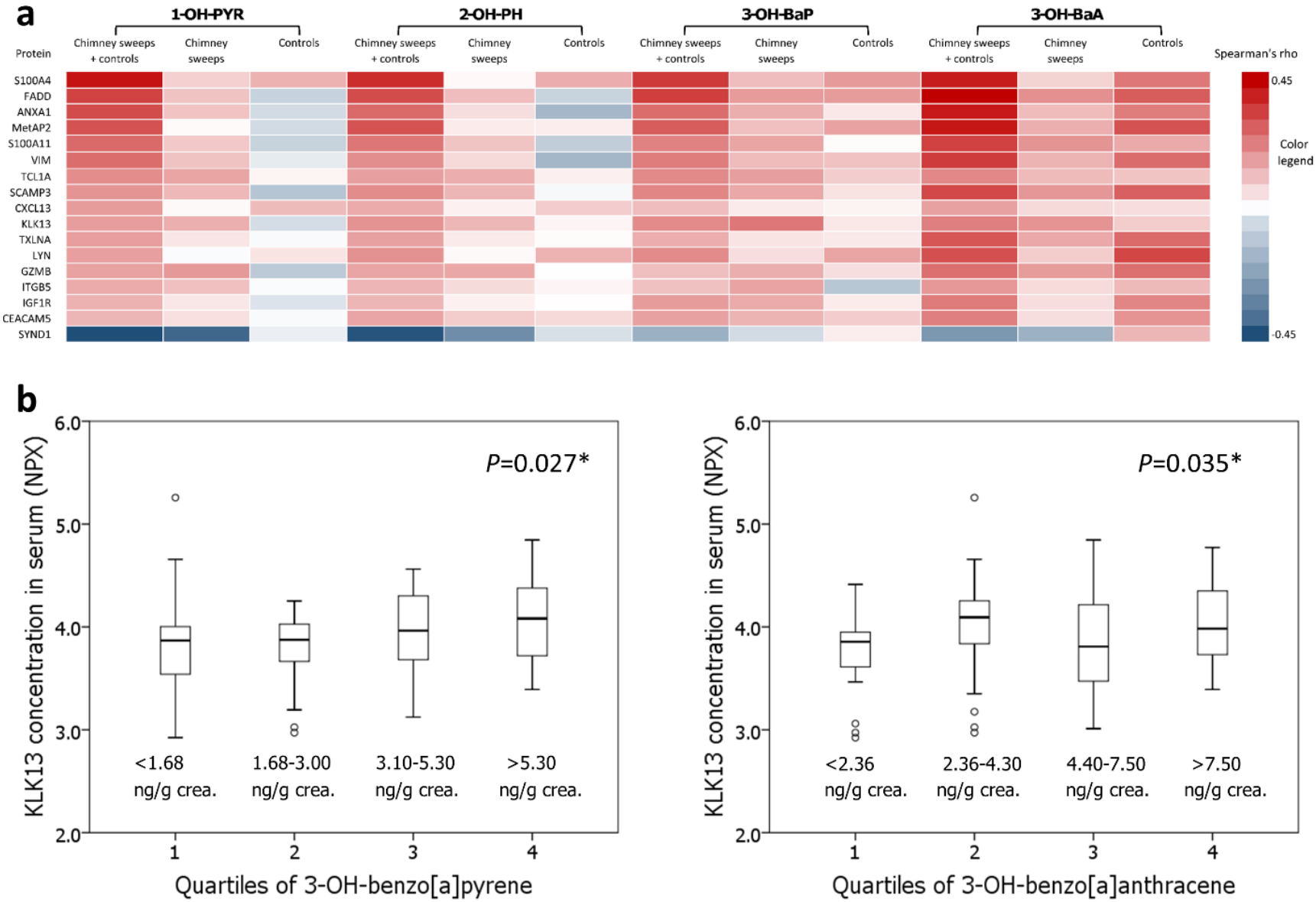

Figure 12. (a) Spearman's correlations between the differentially expressed proteins (DEP) and PAH metabolites. (b) Concentrations of kallikrien-13 (KLK13) across quartiles of 3-hydroxybenzo[a]pyrene and 3-hydroxybenzo[a]anthracene among chimney sweeps. *P value of Jonckheere-Terpstra trend test.

6 GENERAL DISCUSSION

6.1 CHIMNEY SWEEPING AND PAH EXPOSURE

We found that chimney sweeps working today are exposed to PAH as their urinary concentrations of monohydroxylated metabolites of PAH were up to 7 times higher compared with unexposed control workers. Our results indicated that chimney sweeps are exposed to PAH from soot sweeping as PAH metabolite concentrations were positively correlated with the amount of time spent with soot sweeping; a task that involves removing soot from chimneys. In addition, the use of gloves and masks (covering the nose and the mouth) was far from optimal.

Tobacco smoking is a main source of PAH exposure (CDC 2010; IARC 2004), hence we compared PAH metabolite concentrations between study groups stratifying by smoking status. The PAH metabolite concentrations were slightly lower in nonsmoking participants, yet, the differences between chimney sweeps and controls remained the same (up to 7 times). The four PAH metabolites were highly inter-correlated among chimney sweeps (average r_S=0.79; for nonsmokers), but not among the controls (average r_S=0.22). This indicates a common source and route of PAH exposure among chimney sweeps. The concentrations of urinary PAH metabolites have been measured in chimney sweeps in a limited number of studies. The median concentration of 1-OH-PYR in our study was 0.56 µg/L (0.39 µg/g creatinine), which is lower than the concentrations measured in chimney sweeps from Germany (median; 1.03 µg/L) (Göen et al. 1995), from Germany and Poland (median; 0.7 µg/L) (Letzel et al. 1999), and from Italy (mean; 1.6 µg/L; originally reported as µmol/mol creatinine). PAH exposure is common not only among chimney sweeps, but also among workers in various industries. It is important to mention that the level of PAH exposure may considerably vary between studies even for the same occupational group due to the variability in PAH mixtures and working conditions in different workplaces. For example, high concentrations of 1-OH-PYR were observed among workers in production of fireproof stones (*n*=19, median; 11.1 µg/g creatinine), converter infeed (*n*=29, median; 9.4 µg/g creatinine), production of refractories (*n*=84, median; 8.3 µg/g creatinine), production of graphite electrodes (*n*=92, median; 5.5 µg/g creatinine), creosote impregnation (*n*=19, median; 10.7 µg/g creatinine, **Study III**), and coke production (*n*=37, median; 3.8 µg/g creatinine) (Förster et al. 2008; Gündel et al. 2000; Rossbach et al. 2007). Further, urinary concentrations of 3-OH-BaP, a metabolite of the carcinogen BaP, among chimney sweeps in our study (median; 3.35 ng/g creatinine) appeared to be lower than those for workers in production of fireproof stones (*n*=19, median; 14 ng/g creatinine) (Gündel et al. 2000), but relatively higher compared with workers in converter infeed (*n*= 25, median; 1.2 ng/g creatinine), creosote impregnation (*n*=19, median; 0.75 ng/g creatinine, **Study III**), coke production (*n*=79, median; 0.5 ng/g creatinine), production of graphite electrodes (*n*=26, median; 1.3 ng/g creatinine), and production of refractories (*n*=86, median; 1.1 ng/g creatinine) (Förster et al. 2008). Taken together, chimney sweeps seem to be less exposed to PAH in comparison with other occupational groups when considering 1-OH-PYR, but more exposed when considering 3-OH-BaP concentrations. Therefore, evaluation of several biomarkers of exposure, e.g. urinary PAH metabolites, is of paramount importance for exposure assessment. It is worth mentioning that, in addition to PAH, chimney sweeps can also be exposed to particles, degreasing chemicals, asbestos, metals, and combustion gases (Andersson 1987; IARC 2010); however, these exposures are beyond the scope of this thesis.

6.2 PAH EXPOSURE AND CVD-RELATED MARKERS

In **Study I**, we found higher serum concentrations of homocysteine, cholesterol, and HDL in chimney sweeps compared with controls. Moreover, diastolic blood pressure showed positive associations with PAH metabolites in chimney sweeps. In **Study II**, we found 25 differentially expressed proteins (DEP) between chimney sweeps and controls. Of these proteins, FS, IL-16, and HSP 27 showed positive associations with PAH metabolites in a dose-response manner. Pathway analysis for the DEP suggested inflammation and immune response as the main molecular functions, which are involved in development of CVD (Mann Douglas and Kirschenbaum 2011; Tracy 1999; Willerson and Ridker 2004).

Earlier epidemiological studies have shown associations between chimney sweeping and CVD; both increased incidence and increased mortality of cardiovascular events were observed among chimney sweeps (Gustavsson et al. 1987; Gustavsson et al. 2013; Hansen 1983; Hansen et al. 1982; Hogstedt et al. 1982; Jansson et al. 2012). Results from an observational study including around one million individuals demonstrated that a difference of 10 mm Hg in diastolic blood pressure was associated with about 100% difference in mortality (hazard ratio) related to CVD (Lewington et al. 2002). Therefore, the associations between PAH metabolites and diastolic blood pressure in our study can be of concern. For example, an increase of 1 µg/L in urinary 2-OH-PH concentration was associated with an increase of 1.7 mm Hg in diastolic blood pressure. This is also consistent with the findings of a population-based study (n= 4765) where concentrations of 2-OH-PH were associated with higher odds of hypertension (Ranjbar et al. 2015).

Homocysteine is an amino acid involved in one-carbon metabolism cycle (Finkelstein 1998). Evidence from a vast array of observational studies demonstrated positive associations between plasma/serum levels of homocysteine and CVD (Casas et al. 2005; Eikelboom et al. 1999; Jacobsen et al. 2005; Khandanpour et al. 2009; Perk et al. 2012; Wang et al. 2005; Veeranna et al. 2011); however, randomized controlled trials did not support these findings as homocysteine-lowering supplementation showed no effect on CVD risk, particularly on myocardial infarction (Ebbing et al. 2008; Marti-Carvajal et al. 2017). These disparities might be explained by the differences in the follow-up periods (longer in observational studies) and presence of selection bias in the randomized controlled trials (Hannibal and Blom 2017; Marti-Carvajal et al. 2017; Veeranna et al. 2011). Higher levels of cholesterol or/and LDL and lower HDL levels are established risk factors for CVD (Pearson et al. 2002). In addition, researchers showed positive associations between urinary PAH metabolites and cholesterol levels in a population-based study (n=3640) in China (Ma et al. 2018). Given the aforementioned evidence, the higher concentrations of homocysteine and cholesterol in chimney sweeps might indicate higher risk for future CVD. Although not significant, the associations between homocysteine and PAH metabolites could possibly highlight an interaction between PAH exposure and homocysteine/one-carbon metabolism. Cholesterol, in turn, was not associated with PAH metabolites, which might suggests a role of other concurrent exposures, such as particles.

The advent of new technologies has enabled us to screen a large number of putative CVD-related proteins in serum, including new proteins that were not studied before in relation to PAH exposure. Our analysis showed that many of the DEP were involved in inflammation and immunological processes. PAH are capable of inducing oxidative stress (Eom et al. 2013; Kuang et al. 2013), which in turn can promote inflammation and development of CVD (Cervantes Gracia et al. 2017; Elahi et al. 2009; Elahi and Matata 2006). Given that, it is tempting to say that the

dysregulation of some of the DEP may be related to PAH exposure. This speculation is corroborated by the fact that many of the DEP, including FS and HSP 27, were shown to be implicated in oxidative stress, which can be induced by PAH exposure as mentioned before (Caccamo et al. 2012; Jo-Watanabe et al. 2014; Lin et al. 2016; Luedde et al. 2007; Wyttenbach et al. 2002).

6.3 PAH EXPOSURE AND CANCER-RELATED MARKERS

In **Study III**, we found lower DNA methylation (hypomethylation) of *F2RL3* and *AHRR* genes among workers occupationally exposed to PAH, compared with unexposed controls. Particularly, the occupational group with the highest PAH exposure (creosote-exposed workers), had the lowest DNA methylation compared with the occupational group with lower exposure (chimney sweeps) and the unexposed control group. These findings suggest a dose-response relationship between PAH exposure and DNA methylation of *F2RL3* and *AHRR* despite the lack of association between PAH metabolites and DNA methylation. In **Study IV**, we found 17 DEP between nonsmoking chimney sweeps and controls. The proteins were weakly correlated with PAH metabolite concentrations. In addition, one protein (kallikrein-13; KLK13) showed dose-response relationships with two metabolites of PAH known to have carcinogenic potential. Pathway analysis revealed that the DEP were involved in molecular functions related to cell movement, cell adhesion, cell invasion, and cell migration; cellular functions relevant for cancer (Bendas and Borsig 2012; Hanahan and Weinberg 2011).

Hypomethylation of *F2RL3* (cg03636183) and *AHRR* (cg05575921) has been strongly associated with cigarette smoking (Joehanes et al. 2016) as well as increased risk of lung cancer and CVD (Baglietto et al. 2017; Fasanelli et al. 2015; Zhang et al. 2016b; Zhang et al. 2015). In a population-based study including about 5000 participants, every 10% lower methylation of cg03636183 was associated with a 40% increase in lung cancer mortality and around 30% increase in lung cancer incidence (Zhang et al. 2015). Another study by the same research group including around 1500 participants aged 50-75 years found that every 5% lower methylation of cg03636183 and cg05575921 was associated with 2.3 and 1.6 times increased mortality of lung cancer, respectively (Zhang et al. 2016a). Similarly, a nested case-control study (n=600) showed that participants in the first quartile of cg03636183 and cg05575921 methylation had 10.5 and 15.9 times higher odds of developing lung cancer, respectively, compared with those in the fourth quartile (Zhang et al. 2016b). Interestingly, a study including four prospective cohorts suggested a mediating role of DNA methylation of *F2RL3* and *AHRR* for the association between smoking and lung cancer (Fasanelli et al. 2015). In our study, DNA methylation of *F2RL3* and *AHRR* was not associated with PAH metabolites. A likely explanation would be the profound differences in biological half-lives between PAH metabolites (4–35 h) and DNA methylation (up to years) (Buckley and Lioy 1992; Jongeneelen et al. 1990; Shenker et al. 2013).

F2RL3 participates in a wide range of biological processes including immune response, inflammation, and blood coagulation (Vergnolle et al. 2002). One can speculate that altered expression of *F2RL3* may give rise to imbalance in biological functions relevant for cancer development such as blood coagulation and inflammation (Coussens and Werb 2002; Ferrigno et al. 2001). AHRR is an essential regulator of aryl hydrocarbon receptor (AhR) and thus, plays a fundamental role in metabolism of xenobiotics such as PAH and dioxins (Hankinson 1995). AHRR is also capable of regulating inflammatory process and acting as a tumor suppressor

protein (Vogel and Haarmann-Stemmann 2017). Altered expression of *AHRR* was associated with both favorable and unfavorable outcomes in relation to cancer (Vogel and Haarmann-Stemmann 2017; Zudaire et al. 2008). Increased methylation of *AHRR* was associated with reduced mRNA expression in tumor tissues in humans suggesting that *AHRR* functions as a tumor suppressor gene (Zudaire et al. 2008). In contrast, *AHRR*-knockout mice showed development of skin cancer upon exposure to BaP (Vogel and Haarmann-Stemmann 2017). Taken together, the interactions between AHRR and AhR as well as between AHRR/F2RL3 and other molecular pathways are not well understood and warrant further research to comprehensively elucidate the potential role of AHRR/F2RL3 in PAH-induced carcinogenesis. The question of whether PAH exposure causes changes in DNA methylation of *F2RL3* and *AHRR* or whether DNA methylation mediates the carcinogenic potential of PAH is beyond the scope of this thesis.

The majority of the 16 putative cancer-related serum proteins that were significantly upregulated in chimney sweeps showed weak positive correlations with PAH metabolites. The weak correlations can be due to the drastic differences in biological half-lives between PAH metabolites (hours) and proteins (minutes to weeks) (Bachmair et al. 1986; Buckley and Lioy 1992; Doherty et al. 2009). KLK13 was upregulated in chimney sweeps and showed positive dose-response relationships with the metabolites of PAH that have carcinogenic effect (BaP and BaA). KLK13 is a member of the human kallikrein family and plays a role in various biological functions including degradation of different molecules in the extracellular matrix (Kapadia et al. 2004). KLK13 has also been suggested to play a role in cancer development, particularly during the metastatic stage (Borgono and Diamandis 2004). Similar to the scenario of *AHRR*, both increased and decreased expressions of *KLK13* (mRNA and/or protein) were observed in different cancers. Increased KLK13 expression was shown in serum from patients with non-small-cell lung cancer compared with healthy individuals (Planque et al. 2008). As well, increased *KLK13* expression was observed in tumor tissue samples from patients with colon cancer compared with the expression in nearby normal tissues (Talieri et al. 2009). On the other hand, decreased *KLK13* expression was found in specimens taken from bladders of cancer patients compared with samples of nearby normal tissues (Tokas et al. 2017). In the same study, the reduced expression of KLK13 was associated with cancer invasiveness. Likewise, reduced expression of KLK13 was observed in samples from patients of oral squamous cell carcinomas compared with the normal tissues (Ishige et al. 2014). *In vivo* and *in vitro* studies showed inconsistent expression profiles of KLK13 in relation to cancer aggressiveness and cell migration (Chou et al. 2011; Ishige et al. 2014). In addition to KLK13, the other DEP such as S100A4 (S100 calcium-binding protein A4), S100A11 (S100 calcium-binding protein A11), FADD (FAS-associated death domain protein), METAP2 (Methionine aminopeptidase 2), VIM (Vimentin), ANXA1 (Annexin A1), LYN (Tyrosine-protein kinase Lyn), TXLNA (Alpha-taxilin), SCAMP3 (Secretory carrier-associated membrane protein 3), and SYND1 showed dysregulated expression in association with cancer development (Cross et al. 2005; Fei et al. 2017; Mashidori et al. 2011; Moraes et al. 2017; Nguyen et al. 2016; Nikolova et al. 2009; Satelli and Li 2011; Tourneur et al. 2005; Tucker et al. 2008; Zhang et al. 2017a; Zhang et al. 2017b). Taken together, it can be speculated that the dysregulated expression of these proteins plays a role in physiological functions related to PAH-induced tumorigenesis i.e. cell migration and invasion. Thus, the role of KLK13 and other DEP in cancer development, particularly in relation to PAH exposure, requires further investigation.

6.4 STRENGTHS AND LIMITATIONS

The main strength of this thesis is the individual data on internal exposure to PAH and the individual data on a wide range of classical and newly emerging biomarkers related to CVD and cancer in occupational groups distinctly different in their workplace PAH exposure. This contrast in PAH exposure has enabled detection of subtle differences in the biomarkers between study groups and evaluation of dose-response relationships. In addition, we collected extensive information regarding lifestyle factors, disease history, and occupational details, which allowed controlling for potential confounders and covariates. Another strength is that chimney sweeps and controls were all males, similar in age, cigarette-smoking habit, and relatively similar in BMI.

On the other hand, some limitations should be commented upon. The design of all studies was cross-sectional, which does not permit inference of causality. The difference in company response rate between chimney sweeps (87%) and controls (58%) might have introduced a selection bias. However, the controls' health status is an unlikely reason for non-participating control companies. Some managers of these companies gave the reason that their employees could not leave their daily tasks and dedicate time for participation. Further, information about diet for the day or week of sampling was not available. Such data would be relevant for exposure assessment as the concentrations of PAH metabolites in urine can be influenced by the consumption of some food items such as grilled or smoked meat. Still, we did not observe differences between study groups in the intake of different dietary items as assessed by questionnaire. The LC-MS/MS analysis of the monohydroxylated metabolites of BaP and BaA encountered some challenges due to their low concentrations, compared with the metabolites of pyrene and phenanthrene. This was translated into a number of samples being "undetermined" after analysis of 3-OH-BaP and 3-OH-BaA, while almost all samples were successfully analysed for 1-OH-PYR and 2-OH-PH. It is worth mentioning that the 4 PAH metabolites were strongly inter-correlated among chimney sweeps. Even though chimney sweeps are mainly exposed to PAH from soot, they may concurrently be exposed to particles (that can adsorb PAH on their surface), metals, detergents or degreasing agents, and combustion gases. Therefore, there is a possibility that these unmeasured exposures could have contributed to the observed effects. Indeed, this is an issue in every human epidemiological study as it is impossible to measure all potential exposures. Further, the percentage of DNA methylation can vary between different types of leukocytes (Adalsteinsson et al. 2012). In our study, we extracted DNA from whole blood samples and we did not have information about white blood cell count to adjust for. Yet, adjustment for white blood cell count has been shown to be non-influential on DNA methylation of the CpG sites cg03636183 and cg05575921 (Tsaprouni et al. 2014).

7 CONCLUSIONS

The key findings of this thesis are (i) chimney sweeps working today are exposed to polycyclic aromatic hydrocarbons (PAH) and (ii) chimney sweeps appeared to exhibit early biological changes related to development of CVD and cancer. These molecular changes among chimney sweeps are summarized as follows:

- Increased diastolic blood pressure with increased PAH exposure
- Increased serum levels of homocysteine and cholesterol
- Altered serum profile of proteins involved in biological processes relevant for CVD development, i.e. inflammatory response and immune function
- DNA hypomethylation (low methylation) of the genes *F2RL3* and *AHRR;* relevant for risk of cancer and CVD
- Altered serum profile of proteins involved in biological processes relevant for cancer development, i.e. cell movement, cell migration, and cell invasion

We found dose-response relationships between PAH metabolite concentrations in urine (representing the PAH exposure) and some of the markers such as diastolic blood pressure and a number of serum protiens, which supports the notion that these associations were, at least partly, due to PAH exposure.

Our findings support the need for reducing PAH exposure among chimney sweeps and other occupational groups, which could be achieved by encouraging the use of protective equipment and introducing better work routines. Our findings also encourage further exploration of early markers related to PAH-induced CVD and cancer.

8 PUBLIC HEALTH IMPLICATIONS AND FUTURE PERSPECTIVES

Exposure to PAH is a global problem due to the omnipresent nature of these chemicals. In other words, not only chimney sweeps or other occupational groups are exposed to PAH, but also the general population; predominantly from cigarette smoking, wood burning, diesel exhaust emissions, air pollution, etc. Therefore, the findings of this thesis are of high relevance for individuals exposed to PAH from workplace as well as for the general public.

This thesis, by studying early markers of diseases, highlights mechanisms for occupational diseases and substantially contributes to the field of occupational medicine.

This thesis provides novel perspectives for future research, which should focus on:

- Investigating the effect of occupational PAH exposure using a longitudinal design, which can be achieved by following up the current group of workers
- Investigating the effect of occupational PAH exposure in a larger sample size including additional occupational groups
- Investigating additional markers of disease in association with PAH exposure and other potential exposures
- Analysing additional PAH metabolites in urine to obtain a more precise exposure assessment
- Analysing exposure biomarkers that can reflect long-term PAH exposure such as PAH-DNA adducts and PAH-protein adducts
- Investigating additional occupational exposures among chimney sweeps to address the question concerning the contribution of other unmeasured exposures to the effects observed in this thesis relative to PAH exposure

9 POPULAR SCIENCE SUMMARY (ENGLISH)

The journey from healthiness to illness may happen suddenly (e.g. car accident) or take place over years developing key changes ultimately leading to disease. In some scenarios, however, the long journey might be shortened when risk factor(s) – factors that increase the chance of getting the disease, are present; the more the risk factors, the higher the chance of getting the disease. For example, smoking and low physical activity are risk factors for cardiovascular diseases (or CVD for short), which means that smokers and those who do not exercise are more likely to get CVD as compared with nonsmoking and physically active people. However, that does not mean all smokers or physically inactive people will get the disease. The risk factor may also be an exposure to chemicals from work (getting in contact with chemicals by inhalation, skin contact or ingestion). Exposure from work may not only increase the chance of getting the disease, but also accelerate disease development. There are also disease-indicators (biomarkers), which can tell us whether the disease is approaching or already present. It is like when you enter your home with a welcoming smell of cake; an indicator that your mom is preparing a cake or she is already done with it.

Every time you burn wood in the fireplace in your cozy house, a black material so-called soot is formed and accumulated on the inner walls of the chimney; the fireplace lung. Soot mainly contains a group of chemicals known as polycyclic aromatic hydrocarbons (PAH). To maintain the chimney, soot must be removed by skilled workers i.e. chimney sweeps (chimney sweepers). However, this work historically comes with a cost on workers' health. Several studies have shown increased occurrence of CVD and cancer among chimney sweeps. Exposure to soot had been the main suspect (risk factor) for these health problems. In this thesis, we aimed to study the extent of soot exposure as well as explore potential mechanisms or disease-indicators (biomarkers) relevant for CVD and cancer in chimney sweeps recruited from southern Sweden. We found (i) high levels of PAH exposure (risk factor), (ii) higher levels of disease-indicators of CVD in blood (homocysteine and cholesterol), (iii) higher diastolic blood pressure associated with higher PAH exposure, and (iv) changes in blood levels of a number of CVD-related proteins mainly involved in inflammation; a molecular response highly relevant for CVD. For the sake of simplicity, the latter 3 factors (ii, iii, and iv) could be called disease-indicators (biomarkers) for CVD. We also found similar disease-indicators for cancer i.e. (i) changes in DNA methylation related to cancer and (ii) changes in blood levels of cancer-related proteins primarily involved in cellular functions relevant for cancer formation such as cell movement, cell migration, and cell invasion.

In conclusion, we found that chimney sweeps working today are exposed to PAH (risk factor). We also found a number of disease-indicators associated with CVD and cancer. Given the PAH exposure among chimney sweeps, our findings suggest that chimney sweeps might be at higher risk for CVD and cancer. Therefore, PAH exposure among chimney sweeps as well as other occupational groups should be reduced by applying more effective protective measures and promoting better work practices. In addition, further research exploring early markers of disease in PAH-exposed workers is encouraged.

10 POPULAR SCIENCE SUMMARY (SVENSKA)

Resan från hälsa till sjukdom kan hända plötsligt (t ex genom en bilolycka) eller ske över lång tid genom långsamma förändringar som slutligen leder till sjukdom. I vissa scenarier kan dock den långa resan bli avsevärt kortare. Det kan ske när riskfaktorer - faktorer som ökar risken att få en sjukdom - är närvarande. Ju fler riskfaktorerdesto högre är risken för att få sjukdomen. Till exempel är rökning och låg fysisk aktivitet riskfaktorer för hjärtkärlsjukdomar (kardiovaskulär sjukdom, förkortat CVD), vilket innebär att rökare och stillasittande personer är mer benägna att få CVD jämfört med icke-rökare och fysiskt aktiva personer. Men det betyder inte att alla rökare eller fysiskt inaktiva människor kommer att utveckla sjukdomen. En riskfaktor kan också vara exponering för kemikalier på arbetsplatsen (via inandning, hudkontakt eller intag via magtarmkanalen). Exponering på arbetet kan inte bara öka risken att få sjukdomen, utan också påskynda sjukdomsutveckling. Det finns sjukdomsindikatorer (biomarkörer) som kan berätta huruvida sjukdomen närmar sig eller redan är närvarande. Det är som när du kommer in i ditt hem med en välkomnande doft av kanelbullar; en indikator på att din mamma förbereder eller redan gjort en plåt.

Varje gång du eldar ved i eldstaden i ditt mysiga hus bildas ett svart material, så kallad svartsot, som ansamlas på skorstenens inre väggar, eldstadens lungor. Svartsot innehåller huvudsakligen en grupp av cancer-relaterade kemikalier som kallas polycykliska aromatiska kolväten (PAH). För att upprätthålla skorstenens funktion måste svartsot avlägsnas av kvalificerade yrkespersoner, dvs skorstenssvetsare (skorstensfejare). Men detta arbete kommer historiskt med en kostnad för arbetarnas hälsa. Flera tidigare studier har visat ökad förekomst av CVD och cancer hos skorstensfejare. Exponering för sot har varit den huvudmisstänkta riskfaktorn för dessa hälsoproblem. Syftet med denna avhandling har varit att studera omfattningen av sotexponering samt utforska potentiella mekanismer eller sjukdomsindikatorer relevanta för utveckling av CVD och cancer hos skorstensfejare i Sverige. Hos skorstensfejare fann vi (i) höga halter av PAH (analyserat i urin), ii) förhöjda blodnivåer av homocystein och kolesterol, iii) högre diastoliskt blodtryck med högre PAH-exponering, och (iv) förändringar i blodhalten av ett antal CVD-relaterade proteiner som huvudsakligen är involverade i inflammation. De senare 3 fynden (ii, iii och iv) kan ses som sjukdomsindikatorer för CVD. Vi hittade också liknande sjukdomsindikatorer för cancer, dvs (i) förändringar i DNA-metylering, som kopplats till risk för lung, i samband med cancer och (ii) förändringar i blodhalten av proteiner som huvudsakligen är involverade i cellulära funktioner som är relevanta för utveckling av cancer, såsom cellrörelse, cellmigration och cellinvasion.

Sammanfattningsvis fann vi att skorstensfejare idag utsätts för PAH i arbetet. Vi hittade också ett antal sjukdomsindikatorer förknippade med CVD och cancer. Dessa resultat tyder sammantaget på att skorstensfejare kan löpa högre risk för utveckling av CVD och cancer och att denna risk kan vara kopplad till PAH-exponering. Därför bör PAH-exponering bland skorstensfejare minimeras genom att man tillämpar effektivare skyddsåtgärder och bättre arbetsmetoder.

11 POPULAR SCIENCE SUMMARY (اللغة العربية)

قد يحدث التحول من حالة السلامة الصحية إلى الحالة المرضية بشكل مفاجئ كما هو الحال عند السقوط من علو شاهق أو في حوادث السيارات. ولكن في أغلب الأحيان يتطور المرض في جسم الإنسان عبر السنين وذلك لأن تشكل المرض يتطلب تظافر عدة مكونات وعوامل تؤدي في نهاية المطاف إلى وجود المرض بشكله الصريح كما هو الحال في الأمراض القلبية الوعائية والسرطان. بعض هذه العوامل يسمى "عوامل خطر" (risk factors). على سبيل المثال، التدخين وعدم ممارسة الرياضة هما عاملا خطر للإصابة بأمراض القلب. هذا يعني أن المدخنين والذين لا يمارسون الرياضة لديهم احتمال أعلى للإصابة بأمراض القلب مقارنة مع من هم غير مدخنين ويمارسون الرياضة. يجدر القول هنا بأن ليس كل مدخن وكل من هو غير رياضي سوف يصاب حتماً بالمرض، إنما الكلام هنا عن احتمالية أكبر مستنبطة من دراسات علمية معتبرة. من عوامل الخطر أيضا التعرض لمواد سامة خلال العمل اليومي سواء كان عن طريق التنفس, الجلد, أو حتى الفم. يمكن لهذا التعرض للمواد السامة من العمل أن يزيد ويسرع معدل الإصابة بالمرض بين العمال. هناك أيضاً ما يمسى واسمات المرض (biomarkers) وهي التي تخبرنا أن المرض قد حدث أو أنه في طريقه للحدوث, كما هو الحال عندما تدخل البيت وتشم رائحة "المحشي" والتي تعتبر في هذه الحال "واسمة" لطبختك المفضلة.

كل واحد منا لديه مدفأة "صوبة" في منزله والتي عادة ما تعمل بالديزل "المازوت" أو الخشب. ستلاحظ بعد فترة أن هناك مادة سوداء تدعى "السخام أو الشحوار" قد تشكلت من احتراق الديزل أو الخشب أو أي مادة أخرى قابلة للاحتراق. المكون الأساسي للسخام عبارة عن مركبات كربونية متعددة الحلقات وهي مركبات سامة وبعضها يؤدي للسرطان. هناك عمال مهمتهم الرئيسية هي إزالة السخام من المدافئ والمداخن ولذلك فهم معرضون (على تماس) لهذه المركبات الكربونية طوال فترة عملهم عن طريق استنشاق السخام أو ابتلاعه أو عن طريق الامتصاص عبر الجلد, فهم عادة يرجعون من عملهم وقد غطى السخام الأجزاء المكشوفة من أجسادهم. سبق للعديد من الدراسات العلمية أن أشارت إلى معدلات مرتفعة للأمراض القلبية الوعائية والسرطان بين عمال المداخن. لذلك هدفت هذه الأطروحة إلى معرفة مدى التعرض للمركبات الكربونية بين عمال المداخن وأيضا استكشاف الأليات الجزيئية المحتملة والتي قد تؤدي لأمراض القلب والأوعية والسرطان. لتحقيق هذه الأهداف قمنا بجمع عينات بول ودم من عمال مداخن وكذلك من عمال في مستودعات تخزين الأغذية (لا يتعرضون للسخام من العمل) للمقارنة. قمنا بقياس المركبات الكربونية في عينات البول وقياس الواسمات المرضية في عينات الدم. وجدنا بعد تحليل البيانات أن تركيز المركبات الكربونية كان أكبر بسبع مرات في عينات عمال المداخن مقارنة مع نظرائهم من عمال المستودعات مما يشير إلى تعرض واضح لهذه المركبات السامة لدى عمال المداخن. وجدنا أيضا تراكيز أعلى من الكوليسترول والحمض الأميني هوموسيستيين في دم عمال المداخن وكذلك أيضا علاقة طردية بين تراكيز المركبات الكربونية وضغط الدم الانبساطي- جميعها علامات أو واسمات للأمراض القلبية. ليس هذا فحسب بل وجدنا أيضا تغيرات في متللة بعض جينات المادة الوراثية (DNA methylation) لدى عمال المداخن مرتبطة بزيادة احتمال الإصابة بالسرطان. وأخيرا وجدنا اضطرابا في تراكيز مجموعة من البروتينات في الدم لدى عمال المداخن والتي لها علاقة مع تطور الأمراض القلبية الوعائية والسرطان.

وخلاصة القول هي أننا وجدنا أن عمال المداخن معرضين للمواد الكربونية وظهرت لديهم علامات قد تشير إلى إمكانية إصابتهم بالأمراض القلبية الوعائية والسرطان. وهذا يحثنا للقول بضرورة تقليل تعرض عمال المداخن للمركبات الكربونية وذلك باستخدام وسائل حماية ووقاية أو باستنباط طرق عمل جديدة تؤدي إلى حماية العمال من هذه المركبات السامة.

12 POPULAR SCIENCE SUMMARY (DEUTSCH)

Die Entwicklung von Gesundheit zum Krankheitsfall kann sich schlagartig vollziehen (z.B. Autounfall) oder sich über längere Zeit entwickeln, bleibende Veränderungen verursachen, bis schlussendlich zur Erkrankung führen. Jedoch kann sich so ein Ablauf in manchen Szenarien beschleunigen wenn gefährdende Umstände vorliegen, die die Erkrankungwahrscheinlichkeit fördert; je mehr solcher Umstände vorliegen, desto höher liegt diese Wahrscheinlichkeit. Raucher und unsportliche Personen unterliegen beispielsweise Risikofaktoren für Herzerkrankungen (Herz-Kreislauf-Erkrankungen, kurz HKL), das heißt sie erkranken wahrscheinlicher an HKL, als Nichtraucher und sportliche Personen. Das soll allerdings nicht bedeuten, dass die betreffenden Personengruppen daran erkranken werden müssen. Ein anderer Risikofaktor kann auch vorliegen, wenn man am Arbeitsplatz Chemikalien ausgesetzt ist und sie über die Atemwege, der Haut oder der Speiseröhre aufnimmt. Dieser Umstand kann nicht nur das Erkrankungsrisiko sondern auch den Krankheitsverlauf beschleunigen. Außerdem gibt es Krankheitsindikatoren (Biomarker), die verraten können, ob eine Krankheit sich anbahnt oder bereits ausgebrochen ist.

Jedesmal, wenn Holz in einem Kamin eines behaglichen Hauses brennt, bildet sich eine schwarze Substanz, der sogenannte Ruß, welcher sich an den Schornsteininnenwänden ansammelt; ”die Lunge“ des Kamins. Ruß enthält polyzyklische aromatische Kohlenwasserstoffe (PAK); eine Chemikaliengruppe, die den größten Anteil ausmacht. Zur Instandhaltung des Kamins muss der Ruß von einem Facharbeiter entfernt werden, einem Schornsteinfeger. Rückblickend in die Berufsgeschichte geht allerdings das Verrichten dieser Tätigkeit bis heute auf die Kosten der Gesundheit. Verschiedene Studien belegen ein erhöhtes Auftreten von HKL und Krebs unter Schornsteinfegern. Dem Ruß ausgesetzt worden zu sein ist dabei Hauptursache dieser Gesundheitsprobleme. Mit dieser Doktorarbeit untersuchten wir, bei dafür angeworbenen Schornsteinfegern in Südschweden, gezielt das Ausmaß der Rußaussetzung, sowie verdächtige Vorgänge oder Krankheitsindizien im Zusammenhang mit HKL und Krebs. Im untersuchten Urin dieser Personen stellten wir (I) hohe PAK-Werte fest, (II) höhere Werte von Krankheitsindikatoren zu HKL im Blut (Homocysteine und Cholesterin), (III) höherer diastolischer Blutdruck im Zusammenhang mit PAK-Belastung, und (IV) Änderungen der Blutwerte diverser HKL-relevanter Proteine, die mit Entzündungen beträchtlich zusammenhängen - Eine biologische Reaktion die HKL bedeutend begünstigt. Der verständlicheren Einfachheit halber: Die letzteren Faktoren (II, III, IV) dürften als Krankheitsindikatoren für HKL angesehen werden. Ähnliche Krankheitsindikatoren fanden wir auch für Krebs, d.h. (I) Änderungen an den Grundbausteinen der Erbsubstanz von Zellen (DNA-Methylierung), und (II) veränderte Blutwerte von Proteinen, die hauptsächlich an Krebsrelevanten Zellfunktionen beteiligt sind wie etwa sämtlichen Zellbewegungen (Zellmigration, Zellinvasion, etc).

Schlussfolgernd beobachteten wir nicht nur, dass Schornsteinfeger PAK ausgesetzt sind, sondern eine große Zahl an Krankheitsindikatoren für HKL und Krebs. Unter solcher PAK-Aussetzung lässt unsere Beobachtung darauf schließen, dass diese Berufsgruppe einem größeren Erkrankungsrisiko ausgesetzt ist. Daher sollte diese Gefahr durch effektivere Schutzmaßnahmen und bessere Arbeitsmethoden reduziert werden.

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