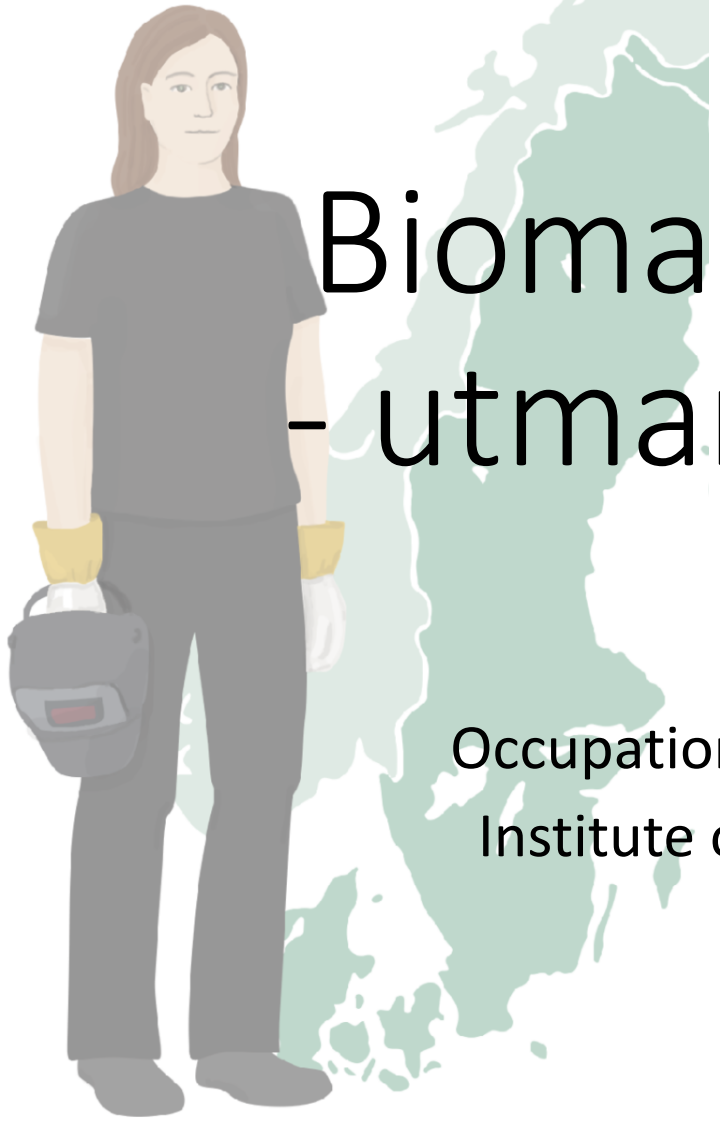


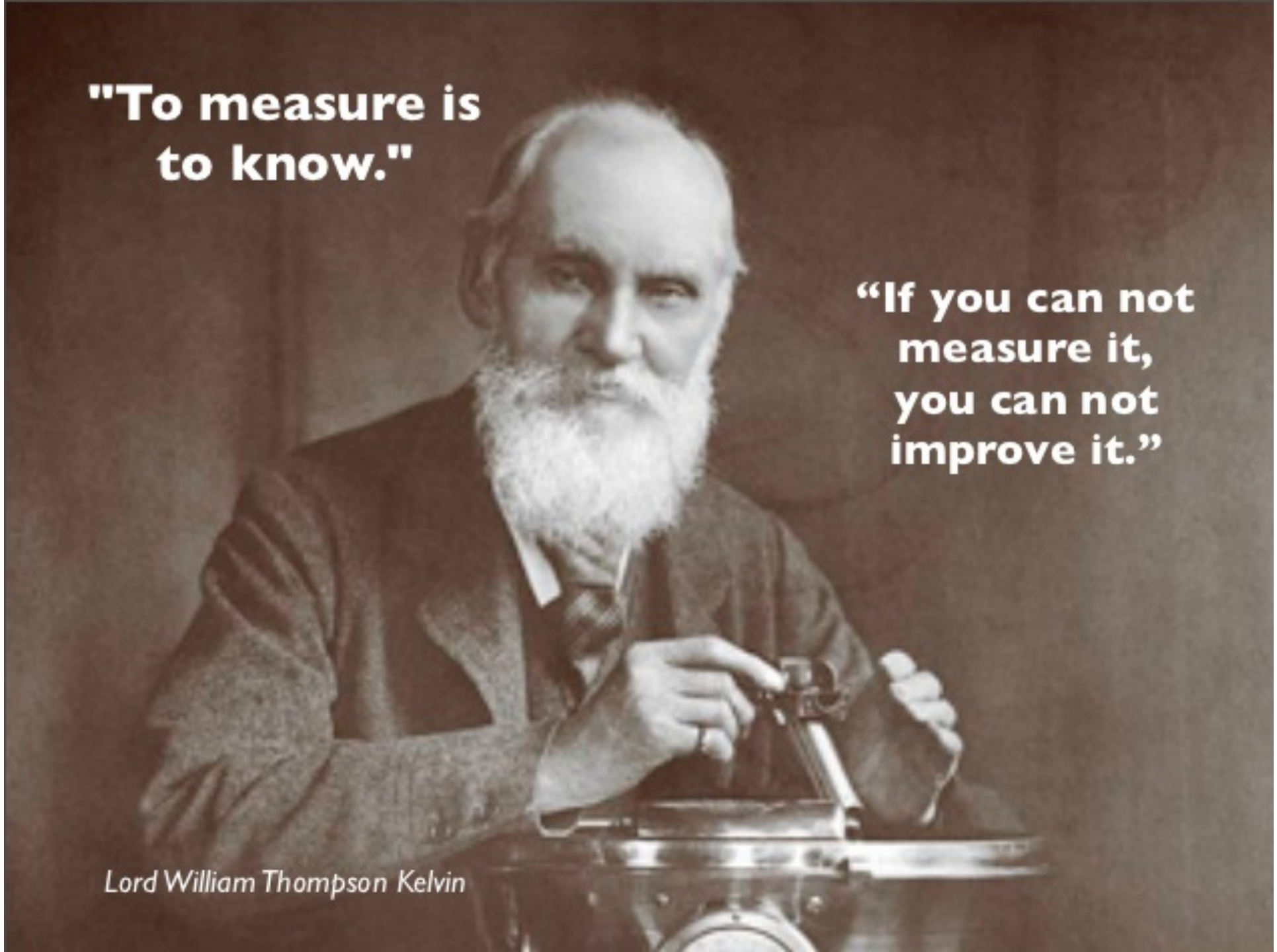
# Biomarkörer i arbetsmedicin - utmaningar och möjligheter

Sandbjerg gods, 24 november 2021

Karin Broberg, professor

Occupational and Environmental Medicine, Lund University

Institute of Environmental Medicine, Karolinska Institutet


"To measure is to know."
"If you can not measure it, you can not improve it."
Lord William Thompson Kelvin

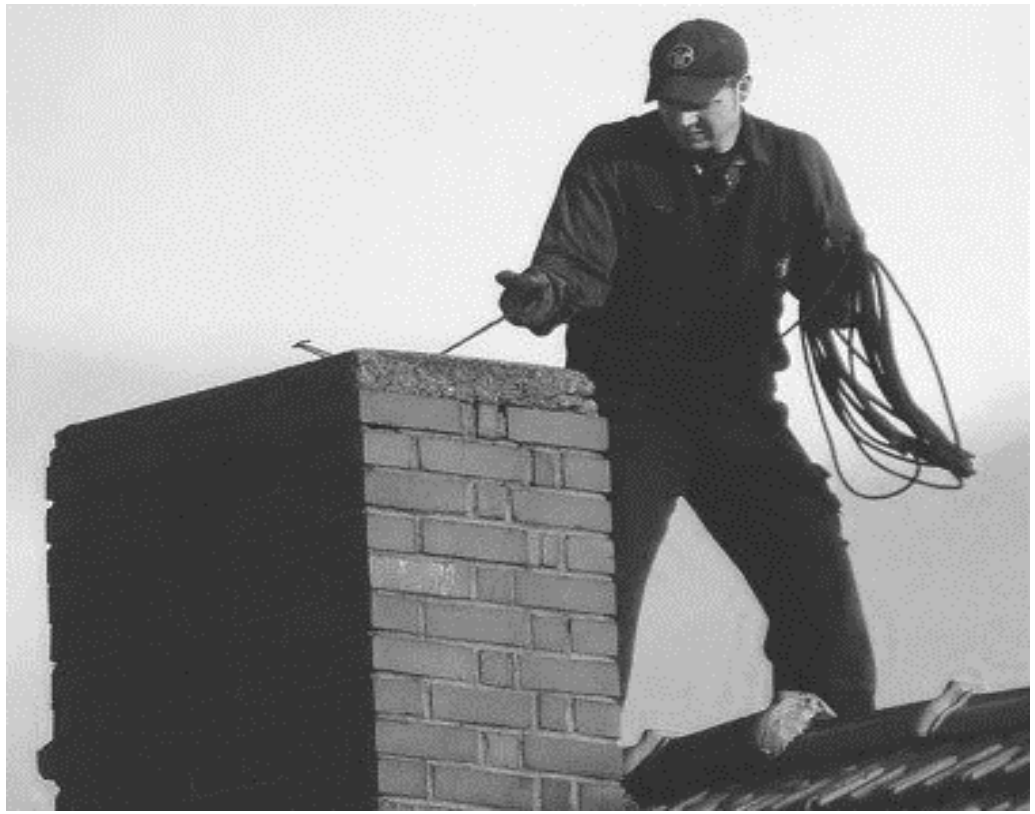
# Biomarkörer i arbetsmiljö

## Möjligheter - utmaningar

- Styrka orsakssamband
- Mekanistisk kunskap
- Tidiga effekter – prevention

- Krävs strikta protokoll för provtagning
- Hantera prover lika
- Ta hänsyn till dygnsvariation, konfounders, kinetik, lagring
- Djävulen sitter i detaljerna!

# Earlier project: Occupational exposure to polycyclic aromatic hydrocarbons and risk for cancer and CVD – a cross-sectional study



Work by Ayman Alhamdow

# Earlier epidemiological findings

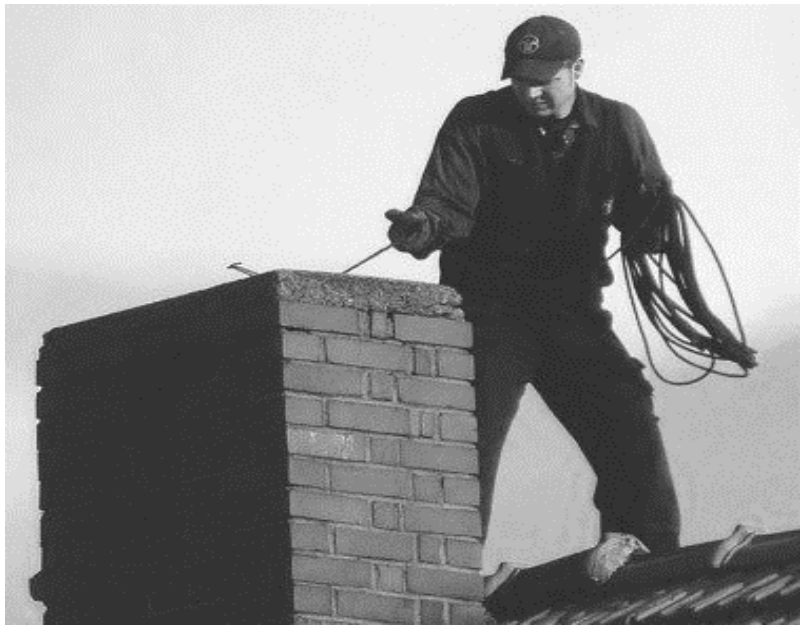
- Chimney sweeping is carcinogenic (class 1, IARC): increased risk for cancers from the esophagus, liver, lung, and bladder, and all types of blood cancers.
- Chimney sweeps showed an increased risk for myocardial infarction.
- Exposure to soot and its content of polycyclic aromatic hydrocarbons (PAH) considered as the main cause for cancer and cardiovascular diseases (CVD) among chimney sweeps.

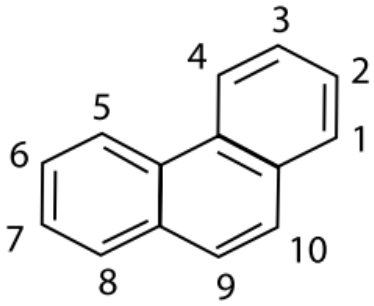
Gustavsson et al. 2013; Jansson et al. 2012; Evanoff B et al. (1993) Hogstedt C et al. (2013) Jansson C et al. (2012)

# Research questions:

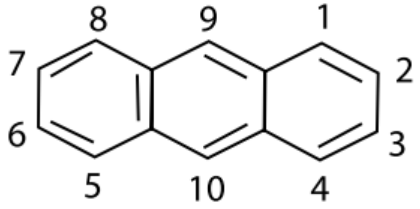
- How is the exposure to PAH among chimney sweeps today?

- How is the risks of cancer given the exposure levels among currents chimney sweeps?

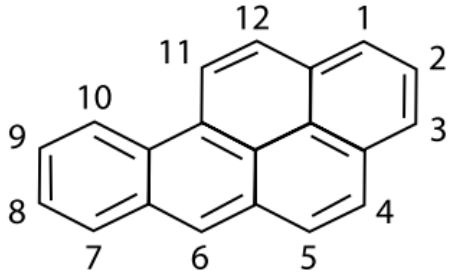




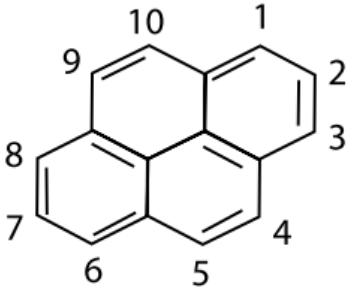

Phenanthrene



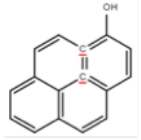

Anthracene




Benzo[*a*]pyrene

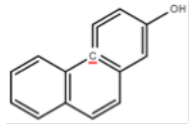



Pyrene



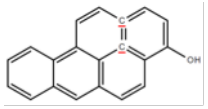
1-OH-pyrene

**Group 3 (IARC)**



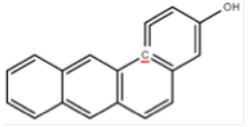
2-OH-phenanthrene

**Group 3 (IARC)**



3-OH-benzo[a]pyrene

**Group 1 (IARC)**



3-OH-benzo(a)anthracene

**Group 2B (IARC)**

## Study groups

151 chimney sweeps from southern Sweden

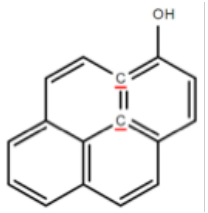
150 controls (not occupationally exposed to particles or PAH)

19 creosote -exposed workers

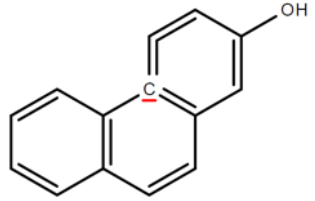
Smokers: 17%

## Markers of PAH exposure

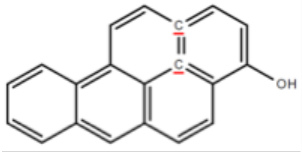
PAH metabolites in urine using (LC-MS/MS)



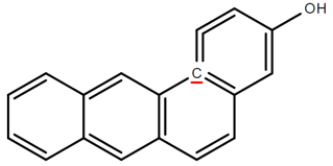

1-OH-pyrene




2-OH-phenanthrene



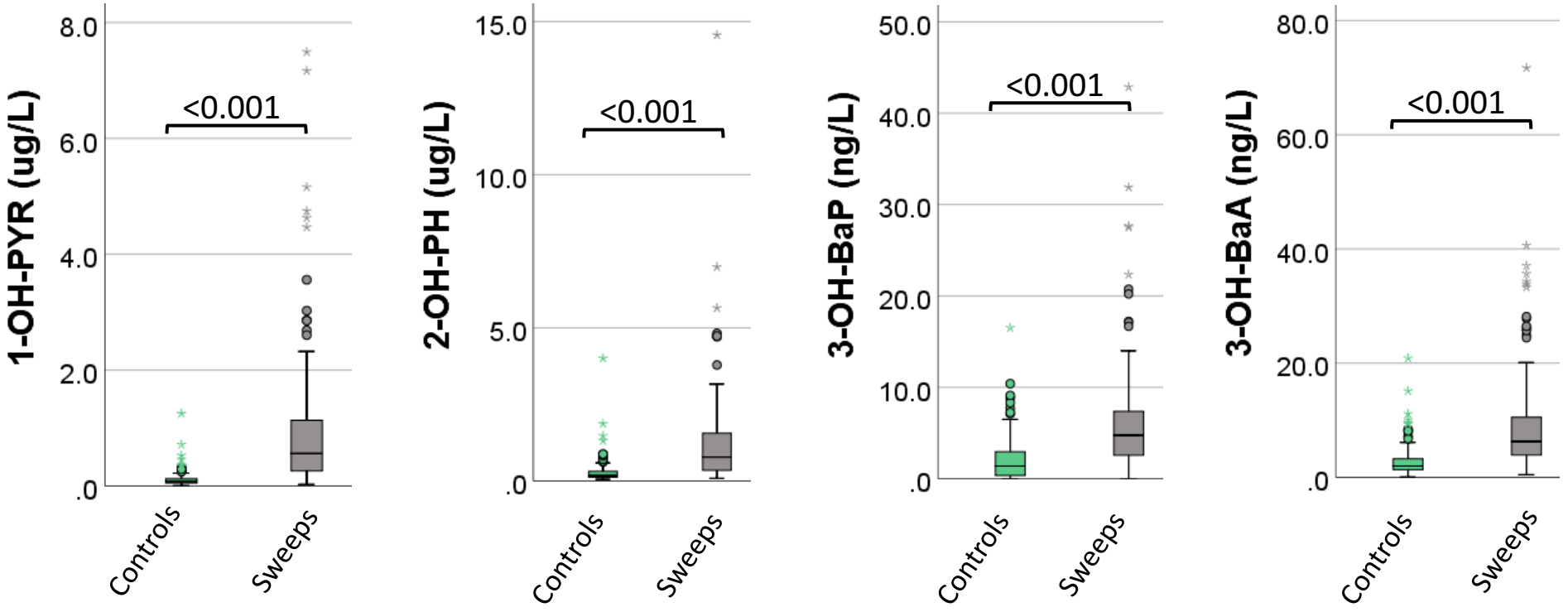

3-OH-benzo[a]pyrene




3-OH-benzo(a)anthracene

Alhamdow et al. 2017

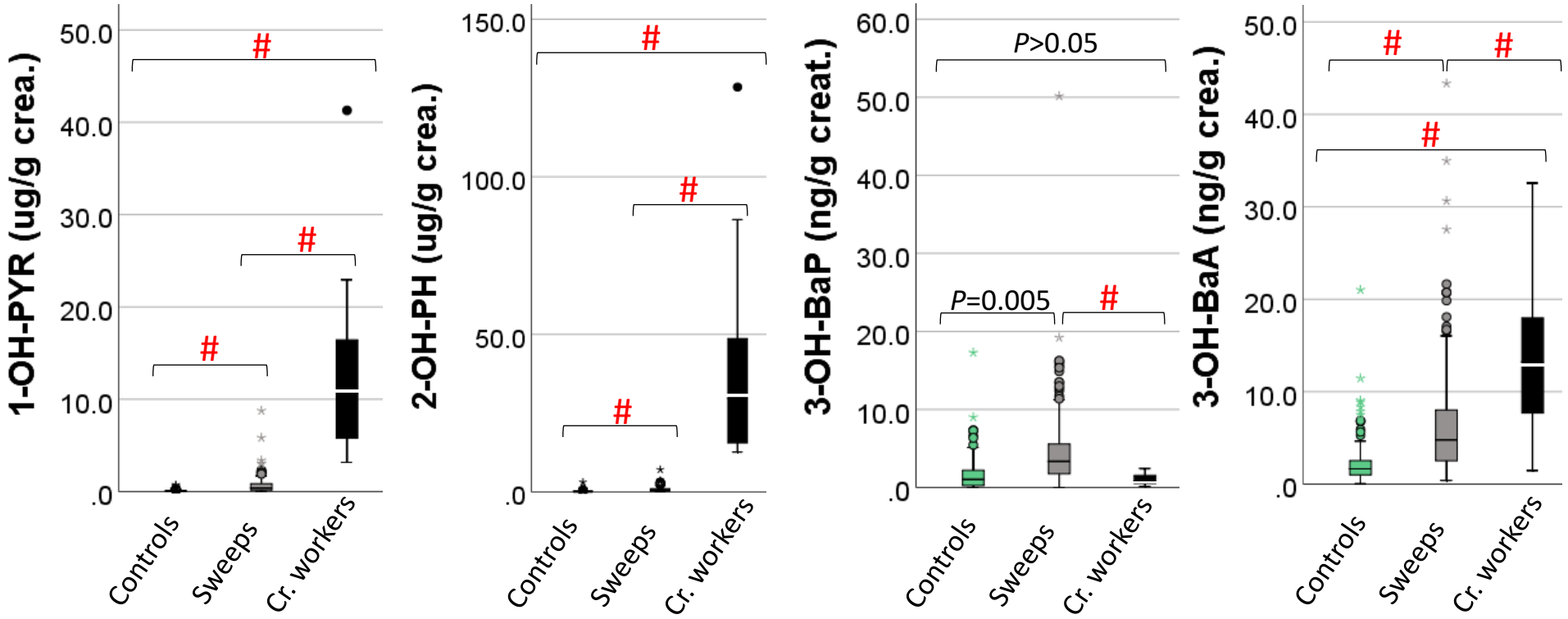
# Chimney sweeps - clear occupational exposure to PAH, up to 7 times higher PAH metabolite concentrations in urine compared with controls




* µg/g creatinine, ** ng/g creatinine

# General linear model adjusted for age, BMI, and smoking status

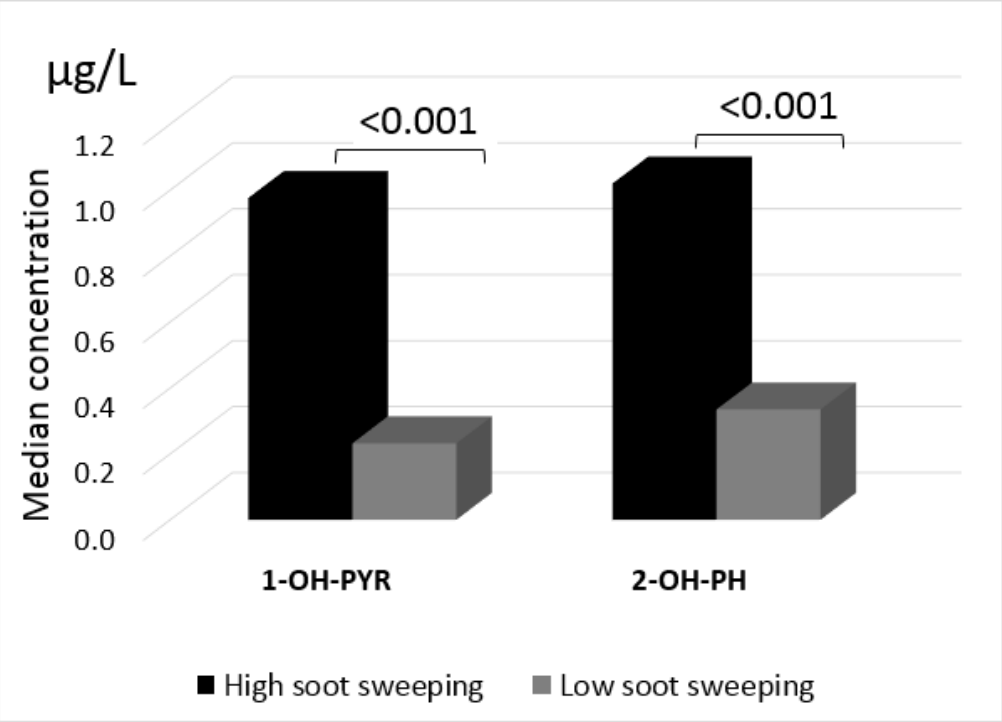
# Creosote-exposed workers higher concentrations of PAH metabolites 1-OH-PYR, 2-OH-PH, and 3-OH-BaA, but not 3-OH-BaP compared with both chimney sweeps and controls

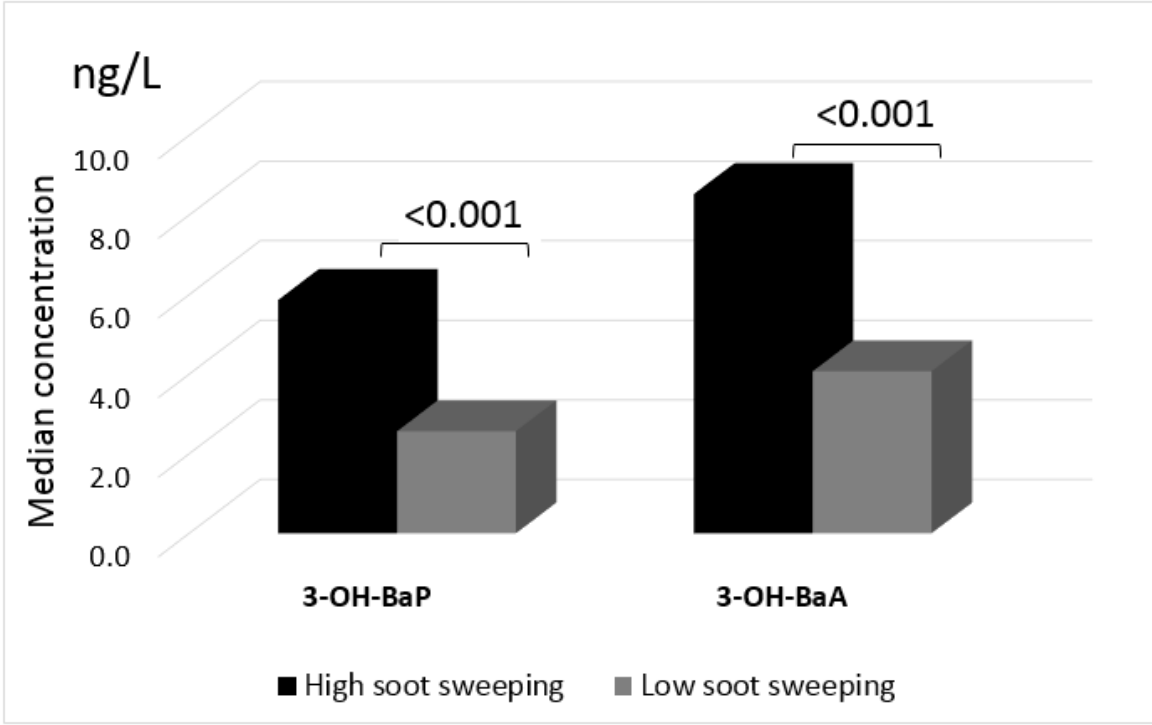



# P<0.001

Linear regression models adjusted for age and smoking

# Higher PAH metabolite concentrations in urine among chimney sweeps who performed more soot sweeping during the past 12 months




*P*-value from linear regression model adjusted for age, BMI and smoking status

**1-OH-PYR** (1-**h**ydroxy**pyr**ne), **2-OH-PH** (2-**h**ydroxy**ph**enanthrene), **3-OH-BaP** (3-**h**ydroxy**b**enzo[**a**]**p**yrene), **3-OH-BaA** (3-**h**ydroxy**b**enzo[**a**]**a**nthracene)

**Aim**: To explore early markers of lung cancer in relation to occupational exposure to PAH in chimney sweeps as well as in a small group of creosote workers

OXFORD

*Carcinogenesis*, 2018, Vol. 39, No. 7, 869–878

doi:10.1093/carcin/bgy059
Advance Access publication May 2, 2018
Original Article

ORIGINAL ARTICLE

## DNA methylation of the cancer-related genes *F2RL3* and *AHRR* is associated with occupational exposure to polycyclic aromatic hydrocarbons

Ayman Alhamdow[1], Christian Lindh[2], Jessika Hagberg[3,4], Pål Graff[4,5], Håkan Westberg[3,4], Annette M. Krais[2], Maria Alb
Per Gustavsson[1,6], Håkan Tinnerberg[2] and Karin Broberg[1,2,*]



OPEN ACCESS

ORIGINAL RESEARCH

## Fluorene exposure among PAH-exposed workers is associated with epigenetic markers related to lung cancer

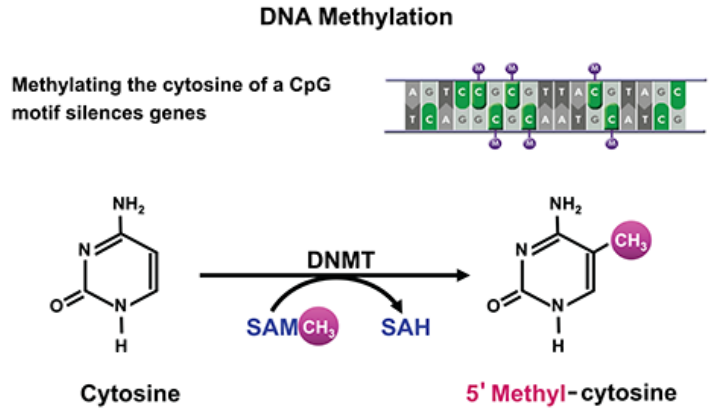
Ayman Alhamdow [1], Yona J Essig,[2] Annette M Krais,[2] Per Gustavsson [1,3]
Håkan Tinnerberg,[4] Christian H Lindh,[2] Jessika Hagberg,[5,6] Pål Graff [7]
Maria Albin,[1,2,3] Karin Broberg [1,2]

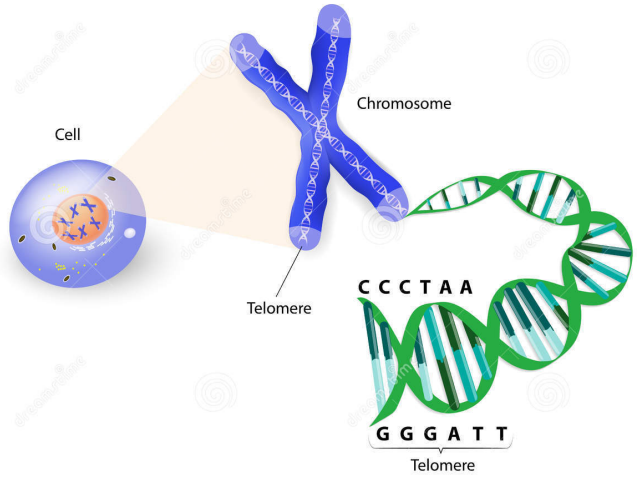
# Genetic markers

- *F2RL3*: Coagulation factor II (thrombin) recept (also known as PAR-4) encodes a protein invol inflammatory reactions and blood coagulation
- *AHRR*: Aryl-hydrocarbon receptor repressor a repressor and key regulator for metabolizing carcinogens from tobacco smoke.
- Low DNA methylation of *F2RL3* (cg03636183) and *AHRR* (cg05575921) associated with:

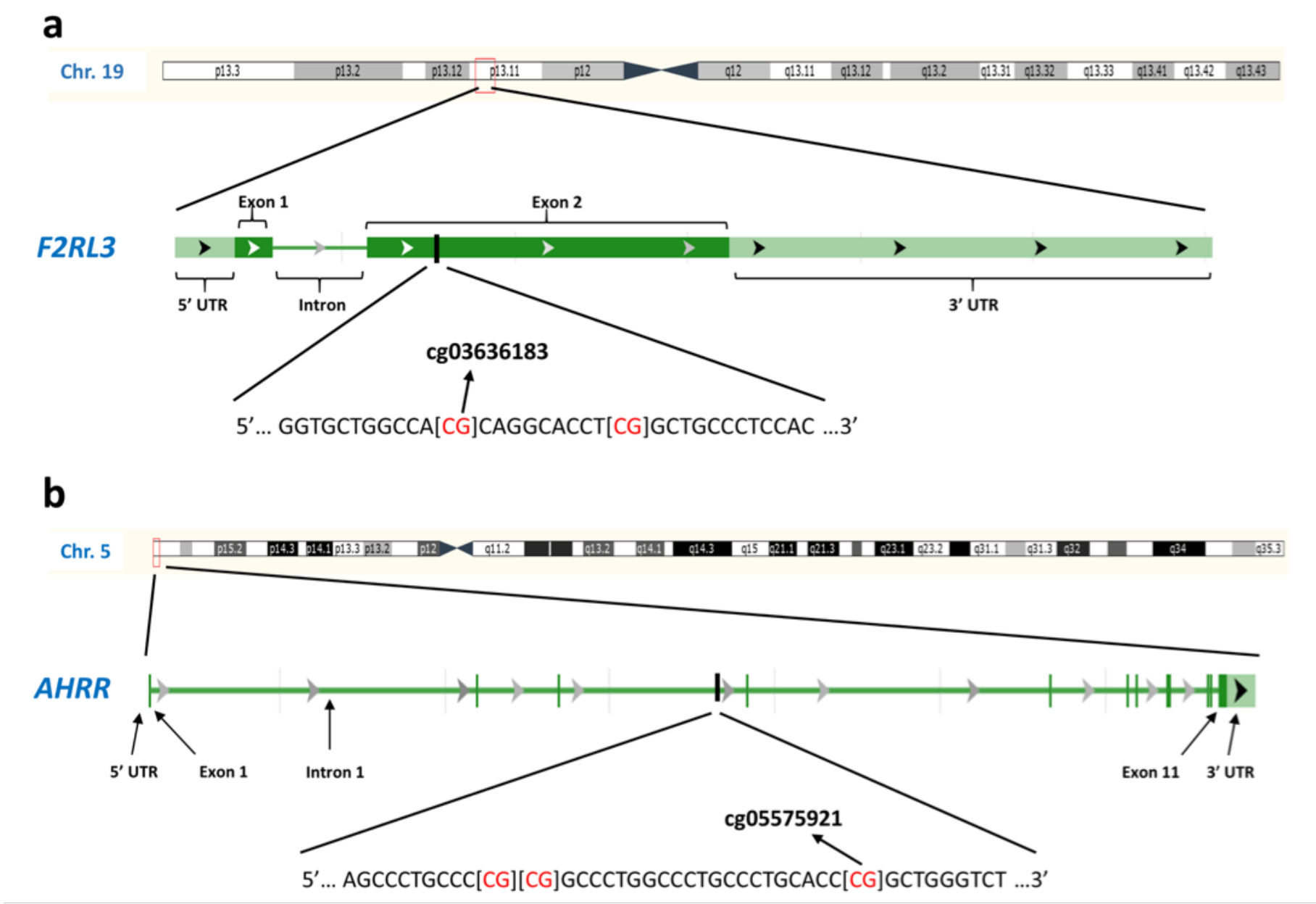
  I) smoking  II) lung cancer, CVD

Zhang et al. 2015, 2016; Reynolds et al. 2015; Fasanelli et al. 2015

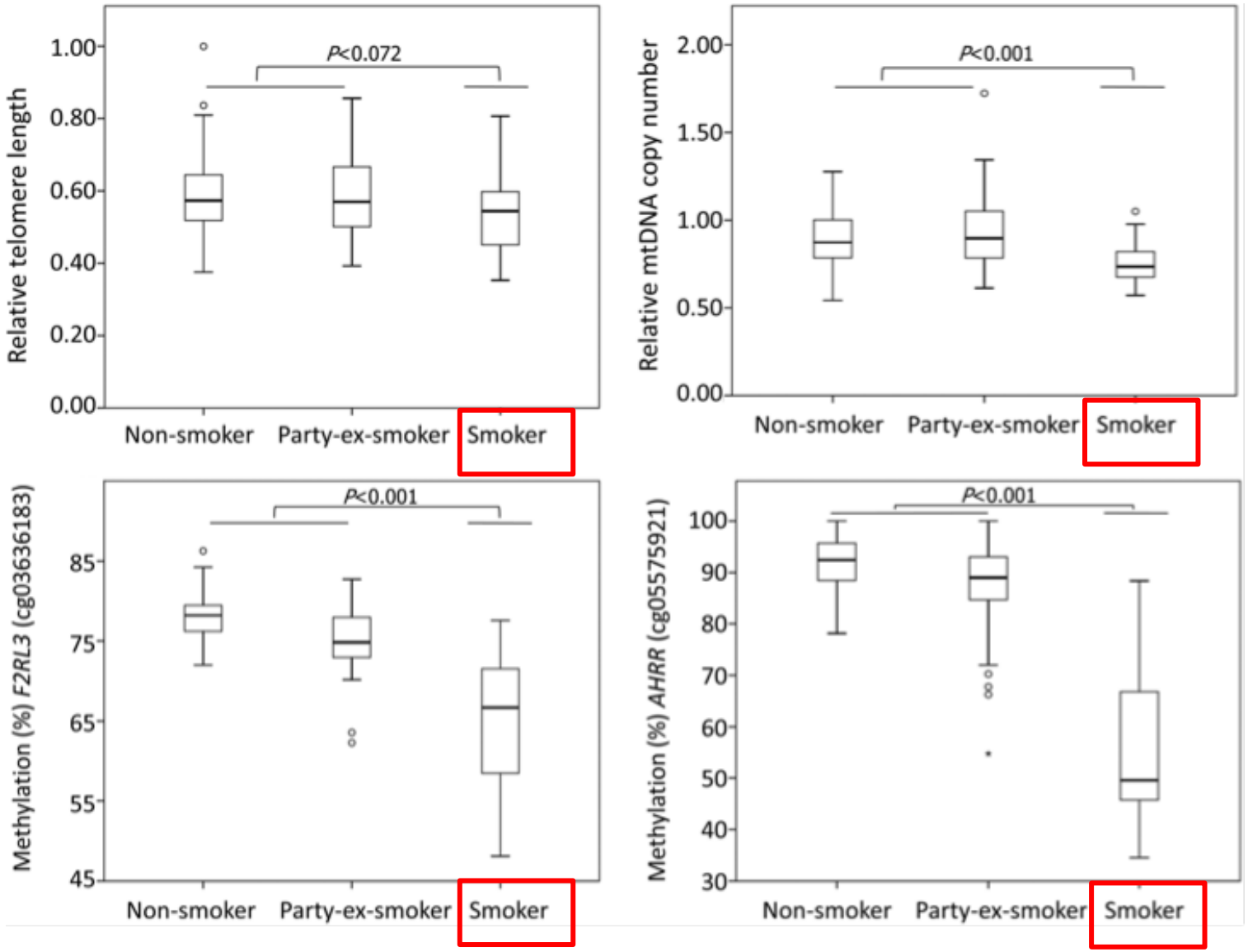


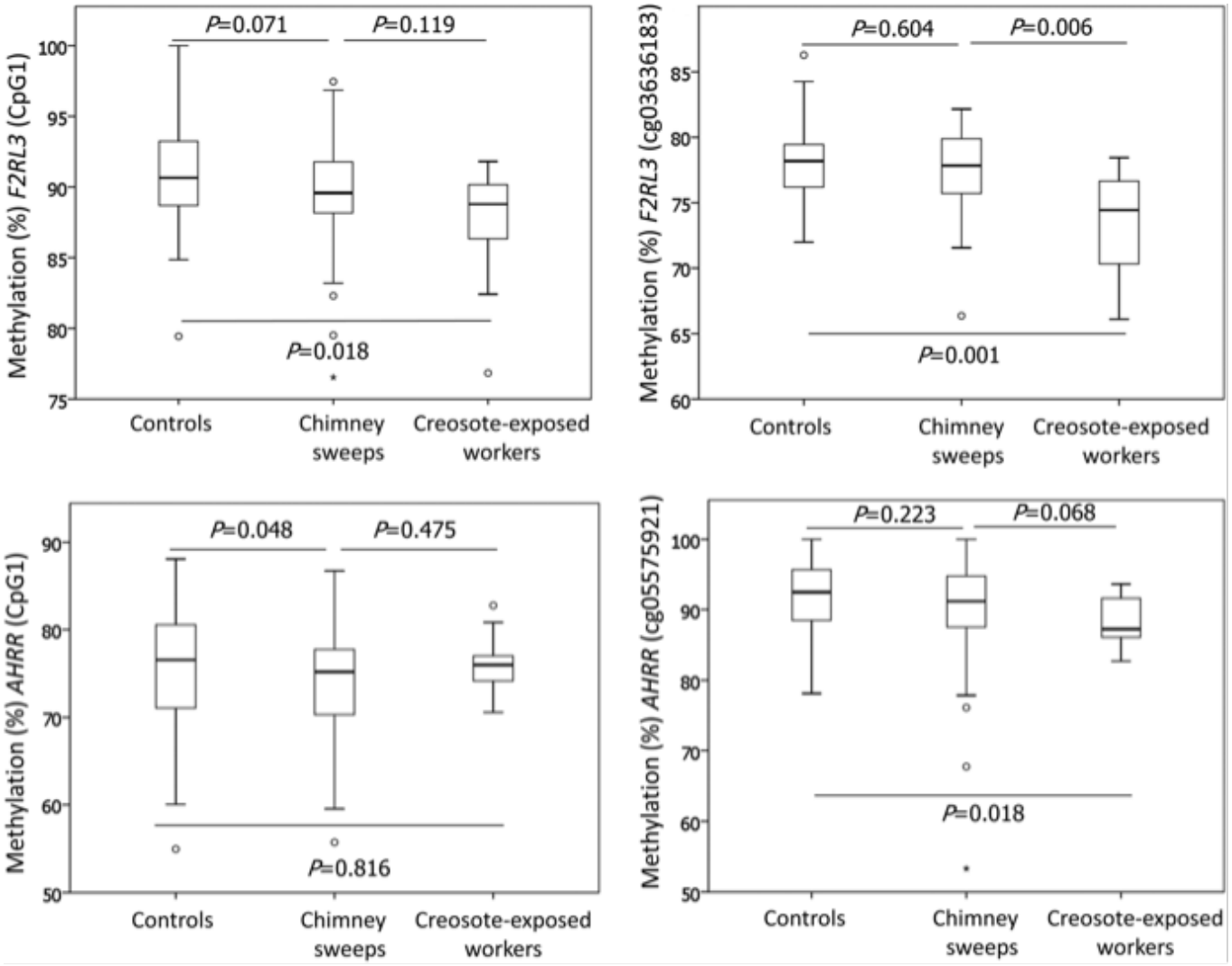

- Telomere length, mitochondrial DNA copy number


a
Chr. 19
p13.3
p13.2
p13.12
p13.11
p12
q12
q13.11
q13.12
q13.2
q13.31
q13.32
q13.33
q13.41
q13.42
q13.43
F2RL3
Exon 1
Exon 2
5' UTR
Intron
3' UTR
cg03636183
5'... GGTGCTGGCCA[CG]CAGGCACCT[CG]GCTGCCCTCCAC ...3'
b
Chr. 5
p15.2
p14.3
p14.1
p13.3
p13.2
p12
q11.2
q13.2
q14.1
q14.3
q15
q21.1
q21.3
q23.1
q23.2
q31.1
q31.3
q32
q34
q35.3
AHRR
5' UTR
Exon 1
Intron 1
Exon 11
3' UTR
cg05575921
5'... AGCCCTGCCC[CG][CG]GCCCTGGCCCTGCCCTGCACC[CG]GCTGGGTCT ...3'

# Smokers showed shorter telomeres, lower mitochondrial DNA and hypomethylation of both genes



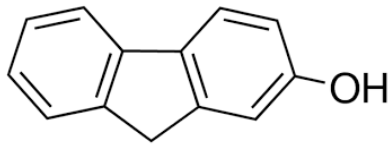
Occupational exposure to PAH was associated with hypomethylation of *F2RL3* (cg03636183) and *AHRR* (cg05575921) (linked to smoking and lung cancer).




Occupational exposure to PAH was not associated with telomere length or mtDNA copy number.

# Higher fluorene – lower methylation of cancer-related genes

**Table 3** Linear regression analyses for the associations between urinary concentrations of ∑OH-fluorene (µg/g creatinine) and cancer biomarkers among chimney sweeps, controls and creosote-exposed workers




| | Model 1 (unadjusted) | | Model 2 (age-adjusted and smoking-adjusted) | | Model 3 (age-adjusted and pack-years-adjusted among smokers) | |
|---|---|---|---|---|---|---|
| | B (95% CI) | P value | B (95% CI) | P value | B (95% CI) | P value |
| Chimney sweeps | *n=143** | | *n=142** | | *n=27†* | |
| Relative telomere length | −0.013 (−0.036 to 0.0096) | 0.25 | −0.0046 (−0.031 to 0.022) | 0.73 | −0.019 (−0.073 to 0.034) | 0.46 |
| Relative mtDNAcn | −0.0017 (−0.039 to 0.035) | 0.93 | 0.0081 (−0.038 to 0.054) | 0.73 | 0.028 (−0.097 to 0.15) | 0.65 |
| *F2RL3*_CpG1 | −6.0 (−7.1 to −4.9) | <0.001‡ | −4.0 (−5.3 to −2.7) | <0.001‡ | −5.9 (−9.5 to −2.3) | 0.003 |
| *F2RL3*_CpG2 (cg03636183) | −5.1 (−6.2 to −4.0) | <0.001‡ | −2.7 (−3.9 to −1.5) | <0.001‡ | −4.2 (−7.6 to −0.8) | 0.019 |
| *AHRR*_CpG1 | −10 (−12 to to 8.3) | <0.001‡ | −5.9 (−8.2 to −3.5) | <0.001‡ | −8.2 (−13 to −3.5) | 0.002 |
| *AHRR*_CpG2 | −10 (−12 to to 8.5) | <0.001‡ | −5.8 (−7.8 to −3.8) | <0.001‡ | −7.7 (−12 to −3.7) | 0.001 |
| *AHRR*_CpG3 (cg05575921) | −14 (−16 to to 12) | <0.001‡ | −7.1 (−9.6 to −4.7) | <0.001‡ | −10 (−16 to to 5.1) | 0.001 |
| Controls | n=*147** | | n=*147** | | n=*25*† | |
| Relative telomere length | −0.016 (−0.044 to 0.012) | 0.27 | 0.049 (−0.0014 to 0.099) | 0.06 | 0.060 (−0.0023 to 0.12) | 0.058 |
| Relative mtDNAcn | −0.069 (−0.12 to -0.021) | 0.005‡ | −0.0038 (−0.091 to 0.084) | 0.93 | −0.012 (−0.10 to 0.079) | 0.78 |
| *F2RL3*_CpG1 | −6.5 (−7.8 to −5.1) | <0.001‡ | −3.9 (−6.3 to −1.6) | 0.001‡ | −2.4 (−6.7 to 1.9) | 0.26 |
| *F2RL3*_CpG2 (cg03636183) | −6.8 (−8.0 to −5.6) | <0.001‡ | −3.7 (−5.7 to −1.8) | <0.001‡ | −2.6 (−6.3 to 1.1) | 0.15 |
| *AHRR*_CpG1 | −12 (−14 to to 9.9) | <0.001‡ | −6.8 (−11 to −2.7) | 0.001‡ | −3.0 (−9.2 to 3.2) | 0.32 |
| *AHRR*_CpG2 | −11 (−13 to to 8.9) | <0.001‡ | −5.7 (-9.3 to -2.0) | 0.003‡ | −3.3 (−8.9 to 2.3) | 0.23 |
| *AHRR*_CpG3 (cg05575921) | −18 (−20 to to 16) | <0.001‡ | −8.2 (−12 to −4.6) | <0.001‡ | −5.5 (−11 to 0.1) | 0.055 |

**Aim**: To elucidate current occupational exposure to PAH among chimney sweeps and early markers of CVD

SCIENTIFIC REPORTS

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## Early markers of cardiovascular disease are associated with occupational exposure to polycyclic aromatic hydrocarbons

Ayman Alhamdow[1], Christian Lindh[2], Maria Albin[1,2], Per Gustavsson[1], Håkan Tinnerberg[2] & Karin Broberg[1,2]

OXFORD

SOT | Society of Toxicology
www.toxsci.oxfordjournals.org

Tox Spotlight article

TOXICOLOGICAL SCIENCES, 171(1), 2019, 23

doi: 10.1093/toxsci/kfz142
Advance Access Publication Date: June 22, 2019
Research Article

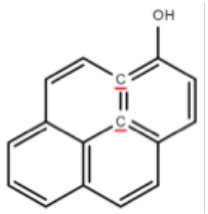
## Cardiovascular Disease-Related Serum Proteins in Workers Occupationally Exposed to Polycyclic Aromatic Hydrocarbons

Ayman Alhamdow,*,[1] Christian Lindh,[†] Maria Albin,*,†,‡ Per Gustavsson,*,‡ Håkan Tinnerberg,[§] and Karin Broberg*,†

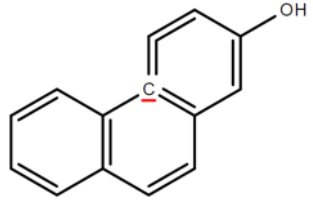
**Study groups:** 151 chimney sweeps from southern Sweden; 150 controls (not occupationally exposed to particles or PAH)

## Markers of PAH exposure

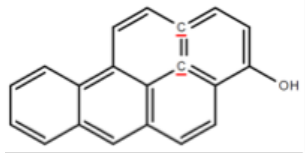
PAH metabolites in urine using (LC-MS/MS)



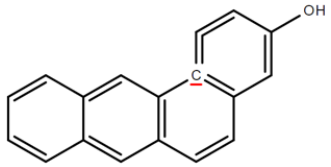

1-OH-pyrene




2-OH-phenanthrene




3-OH-benzo[a]pyrene

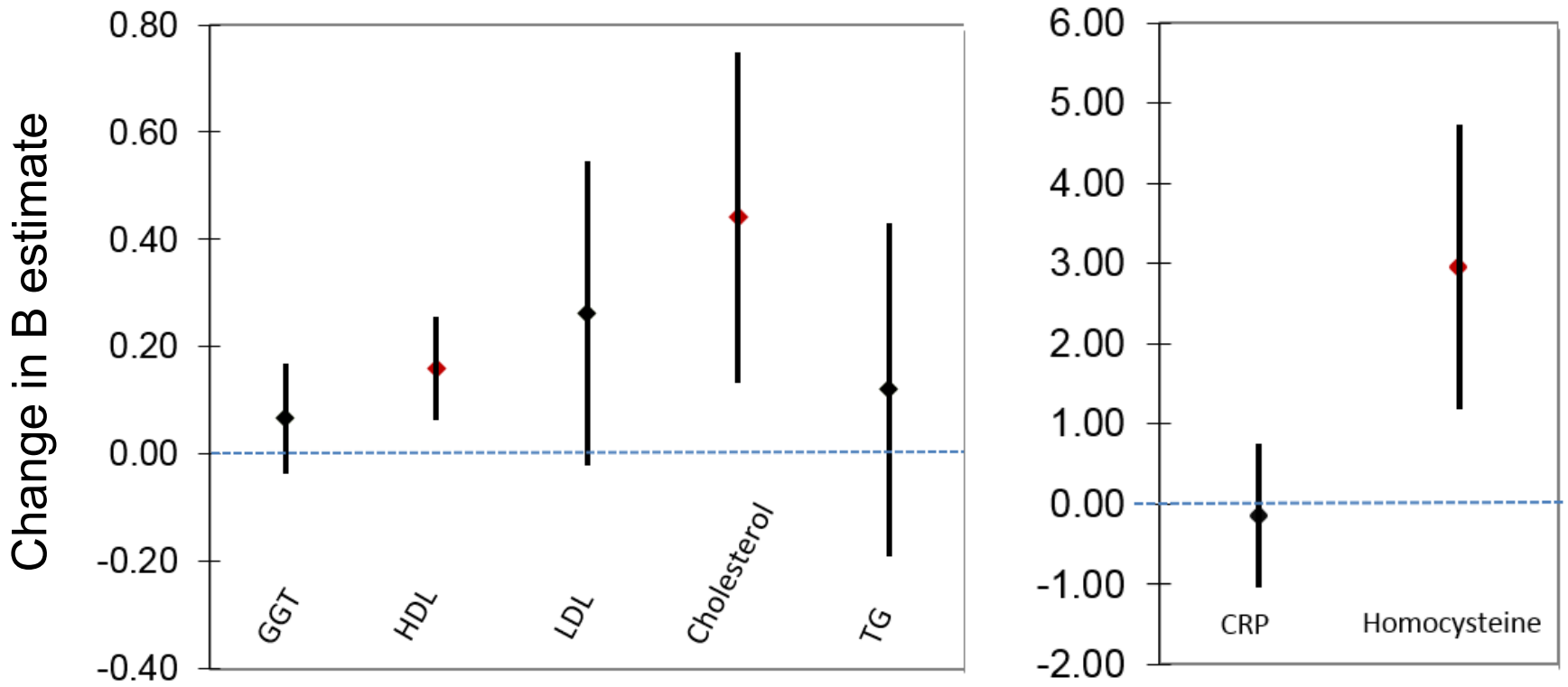



3-OH-benzo(a)anthracene

## Markers of CVD

Blood pressure
(chimney sweeps only)
C-reactive protein, CRP
Homocysteine
γ-glutamyltransferase
Low-Density Lipoprotein, LDL
High-Density Lipoprotein, HDL
Cholesterol
Triglycerides

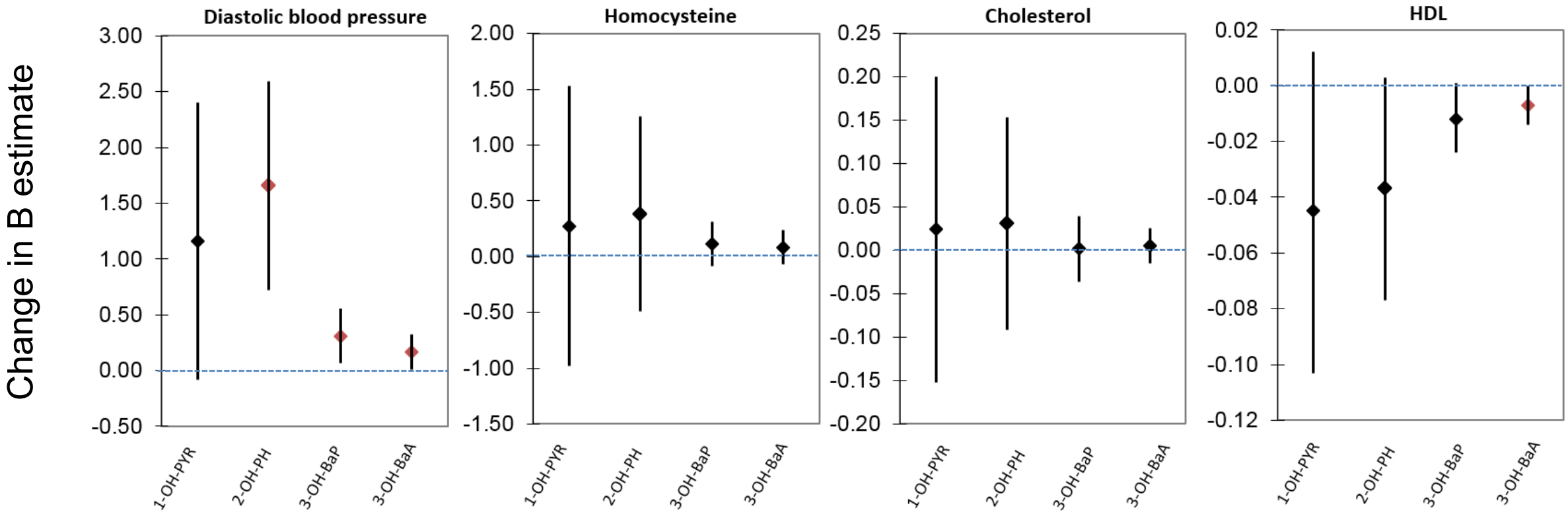
# Higher homocysteine, cholesterol, and HDL in serum among chimney sweeps compared with controls




Adjusted for: age, BMI, smoking status, use of snus, physical activity, passive smoking, residential area, education, family history of CVD, exposure to smoke from hobby

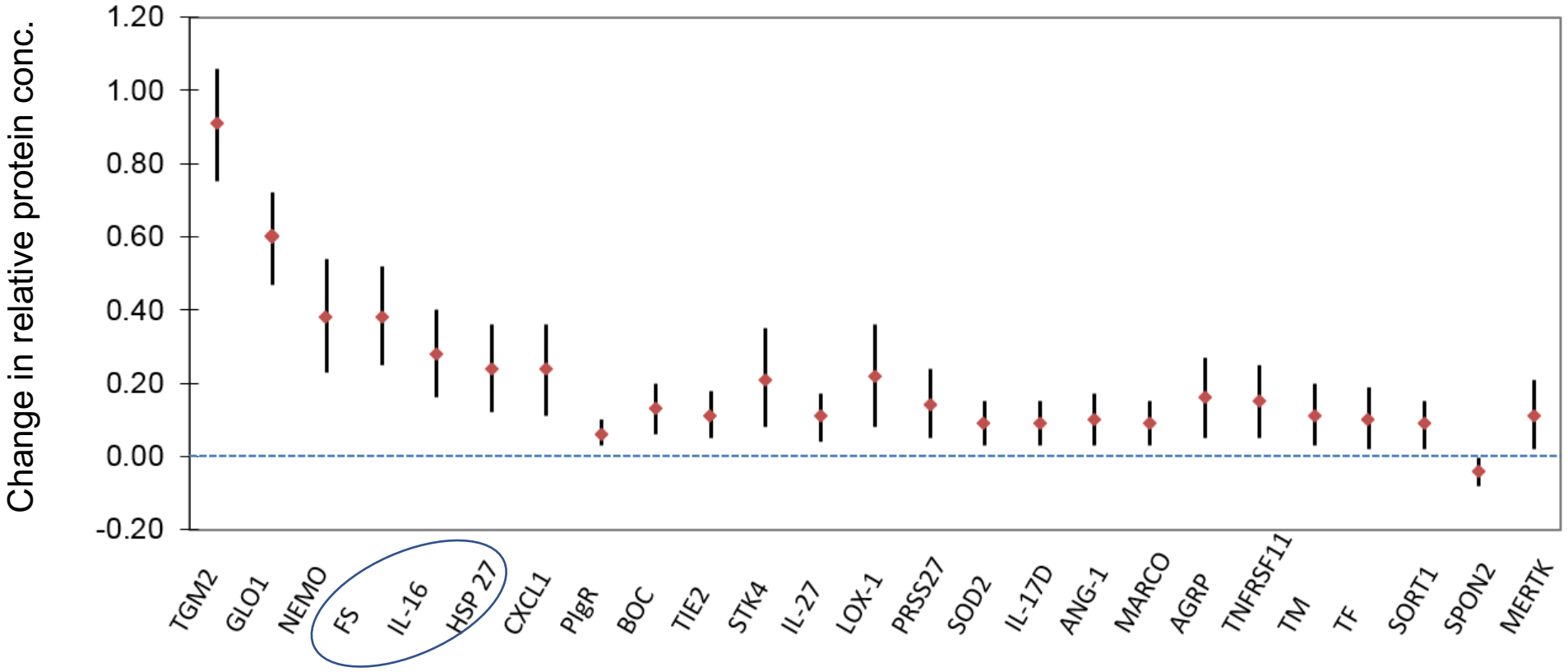
**GGT:** gamma-glutamyltransferase**, HDL:** high-density lipoprotein**, LDL:** low-density lipoprotein**, TG:** triglycerides**, CRP:** C-reactive protein

# Higher concentrations of PAH metabolites in urine were associated with higher diastolic blood pressure



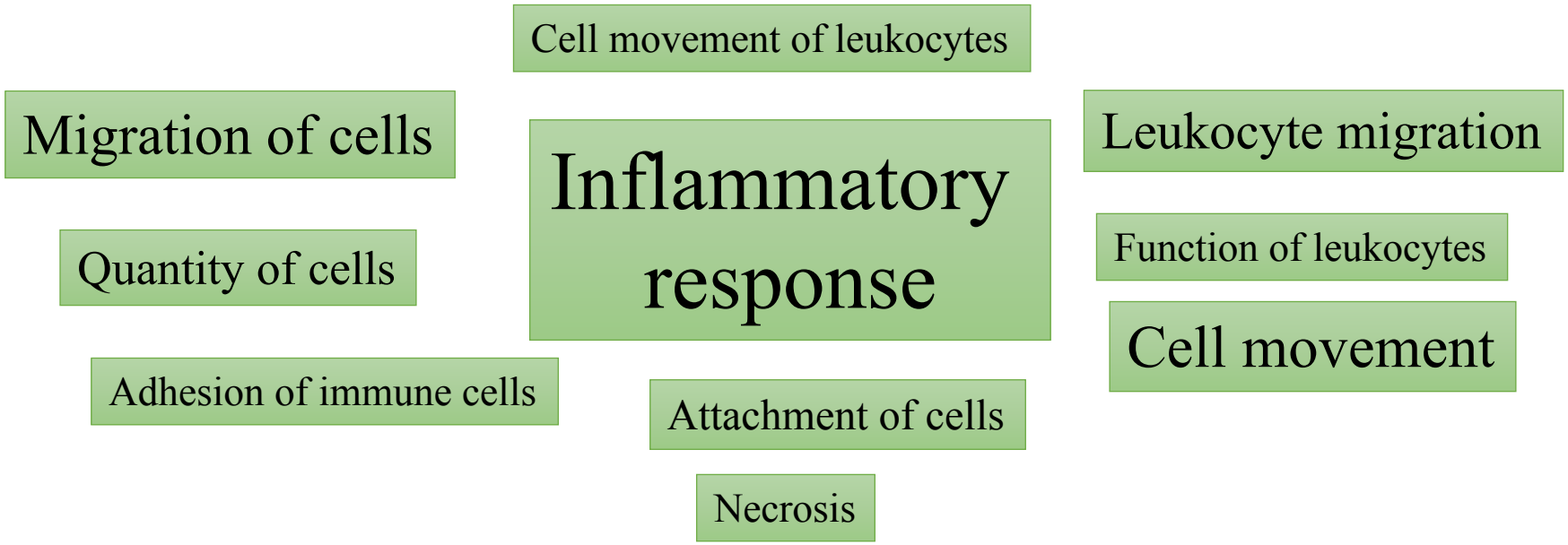

Only blood pressure in the sweeps. Linear regression analysis adjusted for age, BMI and smoking

# Targeted protein analysis of 92 CVD-related proteins in serum: 25 proteins were differentially expressed between chimney sweeps and controls



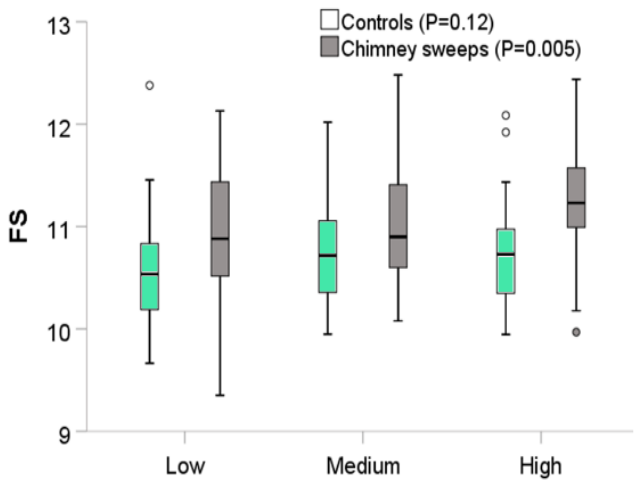

- Linear regression models adjusted for age, BMI, and multiple comparisons (FDR <0.05)

# Top pathways and functions related to the 25 proteins



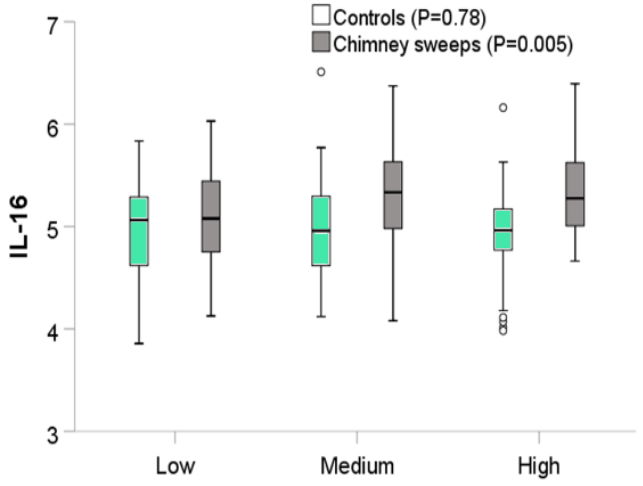
# Serum concentrations of FS, IL-16, and HSP 27 were associated with PAH metabolite concentrations in a dose-reponse manner

**Follistatin (FS)**



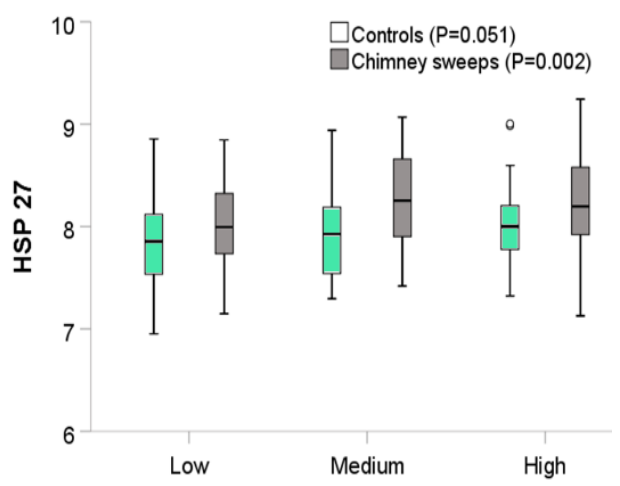

**3-OH-BaP**

**Pro-interleukin-16 (IL-16)**




**3-OH-BaP**

**Heat shock protein beta-1 (HSP 27)**




**3-OH-BaP**

# Summary

- Current chimney sweeps are at higher risk for cancer and CVD.
- PAH exposure from soot is associated with several of the biomarkers of effect and for some biomarkers a dose-response relationship has been found.
- Reduction of PAH exposure among workers is warranted.

# Pågående projekt: Hur ser Cr (VI)-exponeringen ut i Sverige?

Berlingske

NYHEDER OPINION BUSINESS AOK

SAMFUND

## Danske forskere advarer om regler for farligt stof: »Folk kan få kræft - det må sige noget om denne sags alvor«

Danskere kan ende med at blive alvorligt syge efter at have arbejdet med det kræftfremkaldende stof krom-6. Den danske

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## Ved et tilfælde opdagede forsker opsigtsvækkende kræftrisiko - nu er danske arbejdere i farezonen

Dette er historien om, hvordan en dansk forsker pludselig opdagede, at arbejdere kan være langt mere udsatte for kræft, end vi

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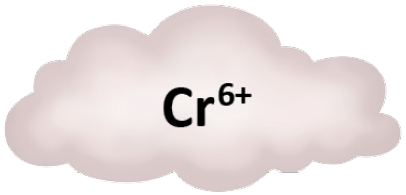
## Her er myndighedernes syv svigt i sagen om krom-6: »Jeg er blevet løjet lige op i mit åbne ansigt«

Beskæftigelsesminister Troels Lund Poulsen (V) kritiserer i kontante vendinger sine egne direktører, efter at han har modtaget en redegørelse om forløbet om krom-6.

# Cr(VI) - vad vet vi idag?

- Cr(VI) är cancerframkallande, ffa lungcancer. Mekanismer: oxidativ stress, oxidativ DNA-skada, telomerskada, DNA-addukter, epigenetiska förändringar och aneuploidi
- Det nuvarande gränsvärdet för sexvärt krom i Sverige är 5 µg/$m^3$ luft vilket skattas till 20 extra lungcancerfall per 1000 arbetare exponerade under 40 år.
- I arbetsmiljön ffa exponering via luftvägarna.
- De huvudsakliga källorna till Cr(VI) i europeisk arbetsmiljö är: produktion, bearbetning och gjutning av rostfritt stål och andra kromlegeringar; hårdförkromning, produktion av kromater
- 1990-talet: 10 000–21 000 arbetare yrkesmässigt exponerade för Cr(VI) i Sverige.
- Idag: ingen helhetsbild av exponeringsnivåer för (Cr(VI)) i Sverige - är alarmerande med tanke på relativt många cancerfall även vid existerande gränsvärde

# SafeChrom




Kartlägga exponering för sexvärt krom på arbetsplatser i Sverige genom mätning av inhalerbart sexvärt krom och sexvärt krom i röda blodkroppar.

Undersöka om nuvarande exponeringsnivåer för sexvärt krom ger DNA-skada.

Undersöka riskuppfattning och riskhantering för sexvärt krom på olika arbetsplatser.

Skapa ett nordiskt nätverk kring sexvärt krom i arbetsmiljö samt jämföra resultat från Sverige med danska och finska studier.

Utveckla riktlinjer för att minimera exponering för sexvärt krom.

**SafeChrom 2021-2023**

FORTE: Forskningsrådet för hälsa, arbetsliv och välfärd

afa FÖRSÄKRING

## Setting

**Focus**: 2–4 large and small recycling companies near each partner site: Umeå, Stockholm, Göteborg, Lund, approximately 50 subjects per site

## Exposure

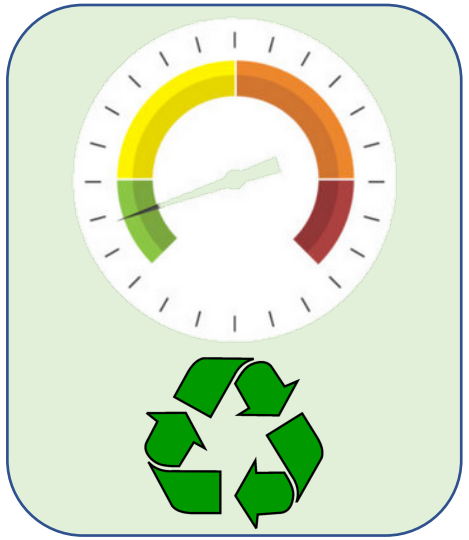

**Focus:** metals
**Screening**: dust, organic chemicals, body posture and movements

## Health Outcomes

**Inflammation**
**Oxidative stress**
Epigenetic changes
Lung function
Musculoskeletal symptoms
Accidents

**Focus:** inflammation and oxidative stress
**Screening**: epigenetics, lung function, musculoskeletal symptoms, accidents

## Risk mitigation



**Focus**: risks and work environment management

GreenMetalWaste 2022-2024 align with Genanvend

FORTE
Forskningsrådet för hälsa, arbetsliv och välfärd

# TACK!

FORTE:
Forskningsrådet för hälsa, arbetsliv och välfärd

afa FÖRSÄKRING