Development and evaluation of a wood smoke biomarker

Ontwikkeling en evaluatie van een biomerker om de mogelijke gezondheidsimpact van houtverbranding in te schatten (LNE/ OL201400014/ 15043/M&G)

Koppen Gudrun, Jacobs Griet, Spruyt Maarten, Daems Jef, Colles Ann, Lazarov Boris, van Poppel Martine, Voorspoels Stefan

Study accomplished under the authority of
2017/MRG/R/1147

May 2017

VITO NV
Boeretang 200 - 2400 MOL - BELGIE
Tel. + 32 14 33 55 11 - Fax + 32 14 33 55 99
vito@vito.be - www.vito.be

BTW BE-0244.195.916 RPR (Turnhout)
Bank 375-1117354-90 ING
BE34 3751 1173 5490 - BBRUBEBB

ABSTRACT

The current study aimed to evaluate different bio-indicators for human wood smoke exposure. Different techniques were tried out to assess their feasibility.

The current study consisted of method validation and optimization for the analysis of three main woud smoke exposure compounds, namely PAHs, levoglucosan and methoxyphenols. The analyses were applied on air filters, PDMS-Tenax cartridges for air sampling, silicone passive wristbands and scalp hair for personal 'dosimetry', and in urine for assessment of the internal levels. To assess the applicability of these monitoring tools/markers, they were applied in a controlled wood smoke experiment in which one field worker was exposed for 5h to an open wood fire in the open air. Followed by two feasibility studies to test the usefulness of the wood smoke marker analysis tools in daily life wood smoke exposure settings. The first feasibility study (pilot 1) consisted of analysis if wood smoke biomarkers in biobanked samples of a recent PAH/levoglucosan air quality study in Flanders. The second feasibility study (pilot 2) consisted of six voluntairs with and without indoor wood burning, where indoor and/or outdoor air concentrations of BC were measured at their homes using a multiwavelength aethalometer. During the time frame of the 7-days sampling, the participants collected urine samples on regular base. Furthermore, they wore a silicone wristband for passive sampling of PAHs during one month starting at the first day of the 7-days sampling period.

Samplers for monitoring wood smoke compounds

In the controlled wood smoke experiment, all applied samplers could trap enough wood smoke compounds during the time frame of the 5h exposure period. BC, a major wood burning compound can be sampled using an aethalometer (generating levels on minute base). The levels of BC were measured using a static aethalometer AE33, which was placed at a distance of about 1.5 m from the open fire were on average 11.4 ng/m^3, dropping to 2.4 µg/m^3 at 4 m distance from the fire. The latter being at higher end range of what is measured during winter in large city centres of Flanders. Air sampling during 5 hours was indeed enough to assess PAHs and methoxyphenols. This was done using PDMS-Tenax cartridges on which the 16 EPA PAHs and the methoxyphenols guaiacol and syringol were measured. At 4m distance of the fire, the concentration of PAHs was considerably higher (10-40x) than winter samples air analysed in a recent study performed in three Flemish regions (Koppen et al., 2016). The concentration of guaiacol and syringol were quantified semi-quantitativel, and were (at about 122-188 ng/m^3) above the range what was earlier reported in the breathing zone of cokes oven workers.

Using multiwavelength aethalometers, as was used in the pilot2 (either placed indoors and/or outdoors of the home), the BC fraction from wood burning (BC_WB) could be derived, allowing better estimation of the wood smoke exposure. Even if the levels of BC_WB were mostly lower indoors compared to outdoors, assessing indoor concentrations is very relevant, as people spent more hours inside than outside. The most optimal way to assess the complete wood smoke exposure, would be via personal personal multi-wavelength microaethalometer samplers (e.g. MA200). It was indeed observed in the current study, that spending several hours indoors at other locations with possible wood smoke exposure, had its impact on LEV and OH-PAH levels in the evening-next morning urine samples (e.g. visiting other wood stove residence).

Assessing of personal exposure was in the current study aimed for by applying the methodology of PAH and/or levoglucosan analysis in silicone wristbands or hair, passively collecting these compounds:
Analysing levoglucosan in hair was possible, but in some chromatograms, a high background was present. It did not appear to be a straightforward matrix for this analysis.
Levoglucosan collected on the silicone wristband, could not anymore being extracted from it, in other words the wristband was not a suitable sampler for levoglucosan.
The wristband was however suitable for PAH analysis (as published by (O'Connell, Kincl, & Anderson, 2014). A precleaning and LC-MS analysis methodology was tested and worked out. In the wood smoke experiment, it could be seen that mainly the low molecular weight PAHs (NAP, ACY, ACE, FLU, PHE, ANT, FLA) were dectectable on the wristband after exposure to the wood fire, and a gradient of decreasing concentrations away from the fire was observed. These lower molecular weigh compouds are known to be tracers for wood smoke exposure. In the feasibility pilot 2, the six participants were wearing a wristband during four weeks. A considerable amount of dermal compounds were accumulated on the wristband, and visible in the chromatogram. Within the frame of the current study no clean-up of the extract was done yet, but the crude extract allowed to compare individuals considering extend of (mainly lower molecular weight) PAH exposure. Mainly the two suspected higher wood smoke exposed individuals HV-02 (wood stove in neighborhood) and HV-06 (own wood stove), had levels of both FLU and PHE that were higher than the other paricipants. FLU and PHE may be interesting potential wood smoke biomarkers for comparative passive sampling of wood smoke exposure assessment via the wristband.

Assessment of internal exposure to wood burning compounds
The urinary markers (OH-PAHs and levoglucosan) that were measured in the current study were all indicators of wood smoke exposure. In the feasibility pilot 2 study with repeated analysis in six individuals, it was some of the days possible to pinpoint wood smoke exposure via the urinary markers, in a timeframe of 6-12h after exposure.
However, the analysed markers were not exclusively specific for wood smoke. Since all markers are also present in urine in case of dietary sources, smoking exposure, traffic exposure or other air concentrations, they are not that straight forward to use for general detection of daily-life (low-medium) personal exposure levels.
This was clear from the analysis of biobanked samples of the first pilot feasability study. Although on some days the winter levels of levoglucosan in the outdoor environment of the five inhabitants peaked to 500-700 ng/m^3, it was not possible to systematically discover this exposure in the inhabitants, and there was no similarity in biomarker levels among couples of the same household. PAH and levoglucosan sources, other than wood smoke, probably disturbed the exposure patterns. Detailed information on daily activities would have been needed to interpret the biomarker values, such as time spent at home, or in traffic, complaints/nuisance reporting due to burning, time spent indoors/outdoor (as was available in the pilot 2 study). It should also be remarked that the individuals of pilot 1 were exposed to woodsmoke only via outdoor sources, and the time frame for which outdoor accumulated exposure to woodsmoke was measured was broad, namely 48 hours, compared to the half-life of the biomarkers. Indeed, the pilot 2 feasibility study clearly showed that BC levels in the hours before collection of urine samples are needed to

allow precise assessment of inhalation exposure to wood smoke PAHs in relation to the measured urinay biomarkers.

Assessing pattern of wood smoke biomarkers in combination with air monitoring of a wood smoke compound

The pattern of all the assessed OH PAHs, was usefull for assessing the exposure source. In case there is consistency in the increased levels of the parameters (possibly in combination with increased urinary LEV), it is more plausible that wood smoke exposure appeared. 1OH PYR was not a suitable marker for wood smoke exposure. 2+3OH fluorene in combination with the unidentified OH-PAH metabolite were higher in individuals reporting daily exposure to traffic exhausts. Both 2+3OH FLU and 1OH PYR may therefore be helpfull in understanding that other PAH sources, aside from wood burning, were present.

Urinary Levoglucosan appeared to be elevated in case of wood smoke exposure, but not in a consistant way. In this context it is worth mentioning that it was stipulated that levoglucosan is not a suitable tracer for sophisticated appliances with automatically fired wood combustion in which high temperatures are reached (Schmidl et al., 2011). One participant of the pilot 2 study had (mostly) the highest BC_WB levels at the home environment, but had only temporarly higher levels of urinary levoglucosan. This compound tended to be mainly visible in case the air concentration of wood smoke compounds was clearly higher and if urine was sampled quickly after exposure (within the first hours). The increase in urinary levels of LEV, and also other markers in the controlled wood smoke exposure experiment was maximal a factor 2. Bergauff et al. (2010) reported a non-consistent response to a controlled campfire exposure (majority of participants between minor and 2-5 times increase, sometimes showing multiple peaks post exposure). It may be speculated that a urinary LEV increase of more than 5 to 10 times the background value of a person, may be due to a peak food exposure. The main dietary source of urinary LEV is food intake of caramelized products (as was clearly seen in current study with 100-fold increase of urinary LEV after consumption of pancakes with candy sugar by one of the participants).

Guaiacol and syringol were only measured in repeated urine samples during the controlled exposure study of 1 individual. Based on these limited amount of results, it was concluded that syringol, and guaiacol showed too variable levels (pre-woodfire exposure) in urine to allow detection of wood smoke exposure. They did not show a high increase in those extreme exposure conditions, that would only occur during unvented indoor fires or wild fire exposure, but likely not in ambient air of residential areas with wood burning.

Sampling strategies for wood smoke biomontoring

Overall, since the LEV and OH PAH biomarkers, may be to a variable larger or lesser extend influenced, by other than woodsmoke sources, such as e.g. diet, traffic and/or indoor sources, applying different biomarkers and/or personal samplers combined with a good sampling strategy to bypass confounding. Considering improving the biomarker sampling strategy, different approaches could be followed for urine sampling, such as:

- Season: Wood smoke exposure studies only need to be set up in the winter, assuring that a considerable proportion of the urinary OH PAHs originates from wood smoke. In case the aim

is to study the relationship between wood smoke exposure and measured or recorded health effect markers, a sampling in both seasons is favorable

- Since wood burning for domestic heating mainly occurs in the evenings, urine samples should be collected in the evening and ormorning. Sampling urine in the evening as well as at the first void in the morning, allows to assess the consistency of the exposure. Pooling of those samples could be a good option (not tested in the current study);
- Different individuals tend to have different levoglucosan background levels (most probably because of different basic diet intakes). Ideally it would be good to have this level assessed e.g. in the second void urine of the individual;
- Pooling from different individuals: Collection of urine in several individuals of a same close geographical area, and pooling them to assess small-regional levels of woodsmoke exposure, and at the same time randomizing the influence of peak exposure and/or dietary influences was suggested by Wallner et al. (2013b). This concept is interesting and should be further explored. However, we speculate that this allows mainly identification of high regional exposure. Pooling of the samples should be done according to the research question (e.g. individuals with/without indoor wood burning, urban/suburban areas, with/without mechanical ventilation)
- Food intake and other confounders:
    - There are some main confounders which are known to influence the urinary levels of the tested wood smoke biomarkers in the current study (Table 25~~Table 25~~).
    - In the pilot 2 feasibility study (of the current study) the participants were asked to fill out a diary table, which appeared very useful for interpretation of the data (See annex 2). Hourly reporting on time spent at home, or in traffic, complaints/nuisance due to burning, time spent indoors/outdoors, food intake, and cooking activity by the participant are needed for exposure estimation related to sources.
    - Considering food, for levoglucosan it is most feasible to ask participants to fill out a (diary) table indicating if they were eating one of those food items in the past 6-12 hours. There are a lot of food items, that can contribute to the intake. For OH-PAHs, Li et al. (2010) recommended that participants should be asked not to eat grilled, smoked and barbecued food 12 hours before urine collection, to avoid confounding.
- Collection conditions: There is no special requestment to sampling materials. Urine is collected in polypropylene tubes. After collection, urine can be left for 2 days at room temperature (Li et al., 2015a) and afterwards it is stored in the freezer at -20°C.
- The use of silicone wristbands for relative personal sampling in the context of wood burning is still further tested beyond the current project, including the pre-cleaning method (using SPE column) of the extracts. The wristbands need to be precleaned before use and stored at room temperature in airtight in PTFE (Teflon) bags with closure. After use they are again stored in those bags, and kept at room temperature.

Health interpretation methodology

In earlier studies, and from the current wood smoke project, 2OH NAP, OH FLU and OH ANT appeared to be the more specific biomarkers for wood smoke exposure. Health interpretation of the biomarkers is limited because of the lack of health guidance values for the OH PAH metabolites in urine. Based on a mass balance calculation done in the current study, during the controlled wood smoke exposure study, it could be seen that the amount of those compounds inhaled and

excreted in urine, is in the same magnitude of order. A possible approach for health interpretation, is to do a simplified calculation of the intake of PAHs via the airways, based on the biomarker values (using estimations of full metabolisation, absorption and assuming the total urine volume production of 1.5 L and inhalation volume levels of 16 m^3 per day). This calculation could be validated using PBPK modeling, such as IndusChemFate. Human biomonitoring health effect markers relevant to associate with the urinary markers studied in the current study are respiratory health outcome, inflammation, oxidative stress, cardiovascular and genotoxicity markers.

Conclusion

Different biomarkers and samplers were tested. Quantifying urinary LEV, 2OH NAP, 2+3OH FLU, 2+3OH PHE in urine, allows pattern analysis of the exposure and overcomes the difficulties of interpreting individual compound levels due to other exposure routes. The sensitivity of the markers appeared to be high enough. There was rather an issue on improving the specificity of the markers for wood smoke exposure. Assessing biomarkers in combination with ambient air black carbon levels (preferably BC_WB), was - aside from the urinary OH-PAH 'profiling' - an important key allowing more specific characterization of daily-life wood smoke exposure.

Overall, combining air quality concentrations in combination with human biomarkers gives certainly an effective tool for tackling the wood smoke exposure question in Flanders. Reliable internal exposure data allow (individual and population) follow-up of exposure reduction measures and this certainly stimulates individuals to apply reduction measures for controlling exposure to wood combustion products.

NEDERLANDSE SAMENVATTING

Deze studie was gericht op het evalueren van verschillende biomerkers voor blootstelling aan houtbranding. Verschillende technieken werden uitgetest om hun haalbaarheid te beoordelen in kader van eventuele humane biomonitoringscampagnes.

De huidige studie bestond uit een methode-validatie en analytische optimalisatie van drie belangrijkste componentengroepen die vrijkomen bij houtverbranding, namelijk PAK's, levoglucosan en methoxyfenolen. De analysen werden toegepast op luchtfilters, PDMS-Tenax cartridges voor luchtmonsterneming, silicone passieve polsbandjes en hoofdharen voor persoonlijke dosimetrie, en in urine voor de beoordeling van de interne niveaus.
Om de toepasbaarheid van deze samplers en biomerkers te beoordelen, werden ze toegepast in een gecontroleerd houtverbrandingsexperiment waarbij één veldwerker gedurende 5 uur aan een houtvuur in de open lucht blootgesteld werd. Daarna werden twee haalbaarheidsstudies uitgevoerd om de bruikbaarheid van de houtverbrandingsmerkers te testen bij niveaus van blootstelling die zich voordoen in Vlaanderen. De eerste haalbaarheidsstudie (piloot 1) bestond uit een analyse van houtverbrandingsbiomerkers in biobankmonsters van een recente PAK/levoglucosan luchtkwaliteitsstudie in Vlaanderen. De tweede haalbaarheidsstudie (piloot 2) werd uitgevoerd op zes vrijwilligers met en zonder binnenhuis houtverbranding. De binnenhuis- en/of buitenluchtconcentraties van black carbon (BC, zwarte koolstof) werd gemeten in/bij hun woning met behulp van een multiwavelength aethalometer. Gedurende de 7-dagen staalname verzamelden de deelnemers urinemonsters op regelmatige basis. Bovendien droegen ze een siliconen polsbandje voor passieve bemonstering van PAK's gedurende een maand, vanaf de eerste dag van de 7-dagen studieperiode.

Samplers voor het meten van houtvebrandingscomponenten

Het gecontroleerde houtverbrandingsexperiment duurde 5 uur. De lucht BC concentratie werd gemeten met behulp van een statische aethalometer AE33, die op een afstand van ongeveer 1,5 m en 4m van het open vuur was geplaatst. Gemiddeld bedroeg de BC concentratie op deze afstanden respectievelijk 11,4 ng/m^3, en 2,4 µg/m^3. Deze laatste lag in het hogere eindbereik van wat tijdens de winter wordt gemeten in grote stadscentra van Vlaanderen. Luchtmonsterneming gedurende dit 5 uur durende experiment was voldoende om PAK's en methoxyfenolen te meten. De sampling werd gedaan met behulp van PDMS-Tenax cartridges waarop de 16 EPA PAK's en de methoxyfenolen guaiacol en syringol werden gemeten. Op 4m afstand van het vuur was de concentratie van PAK's aanzienlijk hoger (10-40x) dan wat recent in de winterperiode werd gemeten in drie Vlaamse regio's (Koppen et al., 2016). De concentratie van guaiacol en syringol werd semi-kwantitatief gemeten en lag (met ongeveer 122-188 ng/m^3) boven de gehalten die eerder werd gemeten via persoonlijke samplers gedragen door coke ovenwerkers.

Met behulp van multiwavelength aethalometers, zoals gebruikt in de piloot2 haalbaarheidsstudie (binnen en/of buiten de woning geplaatst), kon de BC-fractie van houtverbranding (BC_WB) worden berekend. Dit liet toe een betere inschatting te hebben van de individuele blootstelling aan houtrook. De niveaus van BC_WB lagen (voor zover gemeten) binnenshuis lager, in vergelijking met buitenshuis. Echter het is belangrijk om overal te kunnen meten (binnenshuis, buitenshuis en op verplaatsing) We stelden inderdaad vast, dat OH-PAK's en levoglucosan

gemeten in de avond-en daaropvolgende ochtend urinemonsters hoger lagen bij een deelnemer die gedurende de dag meerdere uren op bezoek was bij mensen met een houtkachel. De meest optimale manier om de volledige blootstelling aan houtverbrandingscomponenten te beoordelen, is inderdaad via persoonlijke sampling, bv. via draagbare multiwavelength-microaethalometer-samplers (niet toegepast in de huidige studie, vanwege nog niet op de markt).

Urinaire metingen

De persoonlijke blootstelling aan houtverbrandingscomponenten (PAK's en/of levoglucosan) werd in de huidige studie geëxploreerd via het meten van die merkers in siliconen polsbandjes en in hoofdhaar.

Analyseren van levoglucosan in hoofdhaar was mogelijk, maar in sommige chromatogrammen was een hoge achtergrond aanwezig. Het was geen eenvoudige matrix voor deze analyse. De siliconen polsband was geen geschikte sampler voor levoglucosan. Levoglucosan verzameld op de siliconen polsbandjes, kon er niet meer uit worden geëxtraheerd.

De polsbandjes waren wel geschikt voor PAK analyse (zoals ook gepubliceerd door O'Connell, Kincl, Anderson, 2014). Een pre-cleaning en LC-MS analysemethode werd getest en uitgewerkt. Uit het gecontrolleerd houtverbrandingsexperiment bleek dat vnl. de PAK's met laag molecuulgewicht (NAP, ACY, ACE, FLU, PHE, ANT, FLA) detecteerbaar waren. Een gradiënt van afnemende concentraties werd waargenomen bij de siliconenbandjes die verder van het vuur werden gemonteerd. Deze lagere moleculaire PAK's zijn bekend als tracers voor blootstelling aan houtrook. In de haalbaarheidsstudie 2 droegen vijf deelnemers gedurende vier weken een polsbandje. Een aanzienlijke hoeveelheid verbindingen uit de huid werden tijdens die periode op de polsband geaccumuleerd en deze waren zichtbaar in het chromatogram. Dit verstoorde de meting van PAK's. Er wordt nog verder gewerkt aan het opzuiveren van de extracten. Toch kon nu al vastgesteld worden, dat het mogelijk was om blootstellng van de proefpersonen relatief te evalueren. Voornamelijk de twee individuen HV-02 (houtkachel bij de buren) en HV-06 (eigen houtkachel) die vermoedelijk aan meer houtverbrandingsrook blootgesteld waren, hadden zowel voor FLU als PHE, niveaus geaccumuleerd in het polsbandje, die hoger waren dan bij de andere deelnemers. FLU en PHE kunnen interessante potentiële verbrandingsmerkers zijn voor vergelijkende passieve bemonstering van blootstelling aan houtrook via zulke polsbandjes.

In de huidige studie werden urinaire merkers voor blootstelling aan houtverbrandingsrook gemeten (OH-PAK's en levoglucosan). In de haalbaarheidsstudie piloot 2 studie, waarin, door zes individuen urine werd gecollecteerd gedurende 7-dagen, waren sommige van de urinemetingen (6-12 uur na blootstelling) gelinkt aan houtrook blootstelling (BC_WB). De geanalyseerde merkers waren echter niet uitsluitend specifiek voor houtverbranding. De merkers kunnen ook verhoogd zijn in geval van voedingsbronnen, en/of andere luchtverontreiniging. Laag-medium blootstelling aan houtverbrandingsrook, was moeilijk te identificeren met de merkers. Dit bleek uit de analyse van biobankmonsters tijdens de eerste pilot-haalbaarheidsstudie. Hoewel bij de eerste pilootstudie op sommige dagen de winterniveaus van levoglucosan in het buitenmilieu van de vijf inwoners piekten tot 500-700 ng/m^3, was het niet mogelijk om deze blootstelling in urine steeds vast te stellen. Het niveau van biomerkers was ook niet gelijk bij koppels van hetzelfde huishouden. Gedetailleerde informatie over de dagelijkse activiteiten is nodig zijn om de biomerkerswaarden te interpreteren. Dit houdt in dat bv. geregistreerd wordt door het individu of deze binnenshuis/buitenshuis was, klachten/hindermeldingen had van houtverbranding, blootgesteld

werd aan uitlaatgassen/verkeer (info die wel beschikbaar was in de pilot 2 studie). De individuen van de eerste piloot studie werden ook enkel blootgesteld aan buitenhuisbronnen van houtverbranding (geen binnenhuis houtkachel/open haard). Levoglucosan werd bovendien gemeten in $PM_{2.5}$ gecollecteerd op filters in een tijdspanne van 48 uur voor de urinecollectie. Dit was een lange periode in vergelijking met de halfwaardetijd van de urinecomponenten (6-12h). In de tweede piloot haalbaarheidsstudie werd BC uitgemiddeld voor de 6 uren voorafgaand het verzamelen van urinemonsters. Deze korte-termijn informatie is nodig om een nauwkeurige beoordeling van de inhalatieblootstelling aan houtverbrandingscomponenten te kunnen linken aan urinemetingen van PAK's en levoglucosan.

Houtverbrandingsmerkers interpreteren in combinatie met luchtmetingen

Inschatten van houtverbrandingsblootstelling is het makkelijkst via evaluatie van het patroon van alle gemeten OH PAK's. Als alle OH-PAK's hoger zijn in combinatie met verhoogd urinair levoglucosan, is het waarschijnlijk dat er blootstelling aan houtrook was. 1-OH pyreen (1OH PYR) als dusdanig, was geen geschikte merker voor blootstelling aan houtrook. 2 + 3OH fluoreen (2+3OH FLU) in combinatie met een (in deze studie) niet-identificeerbare OH-PAK metaboliet waren hoger bij individuen die dagelijks blootstelling aan verkeersuitlaatingen rapporteerden. Zowel 2+3OH FLU als 1OH PYR kunnen bijgevolg gebruikt worden om andere dan houtverbradingsnbronnen te identificeren.

Urinair Levoglucosan bleek verhoogd te zijn na blootstelling aan houtverbrandingsrook maar de verhoging werd niet altijd consistent waargenomen. In die context moet aangegeven worden dat levoglucosan weinig voorkomt in houtverbrandingstoestellen waarbij hoge temperaturen worden bereikt (Schmidl et al., 2011).
Een deelnemer van de piloto 2 studie had (meestal) de hoogste BC_WB niveaus in de thuisomgeving, maar had slechts tijdelijk hogere niveaus van urine levoglucosan. Deze verbinding bleek pas verhoogd te zijn als de luchtconcentratie van houtrookverbindingen duidelijk hoger was en als de urine snel na de blootstelling (binnen de eerste uren) werd bemonsterd. Tijdens het gecontroleerd blootstellingsexperiment bij één veldwerker, namen levoglucosan en ook de andere merkers toe met maximaal een factor 2. Bergauff et al. (2010) meldde een niet-consistente respons bij individuene die werden blootgesteld aan een kampvuur (meerderheid van de deelnemers vertoonden een kleine tot 2-5 keer toename van de urinair gehalten, sommigen hadden meerdere kleinere pieken na blootstelling). Het kan worden gespeculeerd dat een urinaire levoglucosanverhoging van meer dan 5 tot 10 keer de achtergrondwaarde van een persoon, het gevolg is van een sterke blootstelling via de voeding. De belangrijkste voedingsbron van levoglucosan zijn gekarameliseerde producten (zoals duidelijk werd gezien bij een van de deelnemers in de huidige studie, waarbij een 100-voudige toename van urinair levoglucosan werd waargenomen na het consumeren van pannenkoeken met kandijsuiker).
Guaiacol en syringol werden enkel gemeten tijdens de gecontroleerde blootstellingsstudie bij één veldwerker. Op basis van deze beperkte hoeveelheid resultaten werd geconcludeerd dat de (pre-houtstook) urinaire concentraties van syringol en guaiacol te veel achtergrondvariatie vertoonden om blootstelling aan houtrook goed te kunnen detecteren. Tijdens deze hoge blootstellingsomstandigheden - die alleen zouden plaatsvinden tijdens extremere binnenhuis houtstook of bij blootstelling aan wildbranden, maar waarschijnlijk niet in de omgevingslucht van

woongebieden met houtbranden - werd **geen belangrijke toename gemeten van deze urinaire merkers**.

Samplingstrategieën voor biomonitoring

Aangezien levoglucosan en OH-PAK-biomarkers kunnen worden beïnvloed door andere dan houtverbrandingsbronnen, is het belangrijk om verschillende biomerkers en/of persoonlijke blootstellingsmetingen toe te passen, gecombineerd met een goede bemonsteringsstrategie. Verschillende aandachtspunten zijn van belang voor het opzetten van een biomonitoringsmeetcampagne:

- **Seizoen**: biomonitoring voor detectie van blootstelling aan houtverbrandingsproducen is enkel nuttig in de winter aangezien dan een aanzienlijk deel van de OH-PAK's in de urine daarvan afkomstig kunnen zijn. Als het doel is om een relatie te onderzoeken tussen blootstelling aan houtverbrandingsproducten en gezondheidseffectmerkers, dan is het interessant om de metingen in beide seizoenen te doen.
- Aangezien houtverbranding voor huishoudelijke verwarming vooral 's avonds gebeurt, worden **urinemonsters best 's avonds en/of in de ochtend verzameld**. Als ze beide worden verzameld, kan de consistentie van de blootstelling worden gemeten, maar op basis van de gegeven uit de huidige studie is het zeker een goede optie om **beide stalen te poolen** (niet getest in de huidige studie);
- Elk individu blijkt een persoonseigen achtergrond levoglucosangehalte te hebben (waarschijnlijk door de basis dieetinname). Het zou ideaal zijn om dit niveau te meten. Dit gehalte is meest waarschijnlijk te meten in de tweede urine van de dag (t.t.z. urinemonster na de eerste ochtendurine);
- **Urine-mengmonsters (poolen) van verschillende individuen**: Wallner et al. (2013b) vond verschillen in urinair levoglucosan van kleine rurale Oostenrijkse gebieden door het poolen van urine van individuen van hetzelfde geografisch gebied. Het laat toe tegelijkertijd de invloed van piekblootstelling en/of voedingsinvloeden te randomiseren. **Het poolen van de monsters moet uitgevoerd worden in functie van de onderzoeksvraag:** poolen van urine van individuen met vs. zonder binnenshuis houtverbranding, uit stedelijke vs. voorstedelijke gebieden, of met/zonder mechanische ventilatie in de woning.
- **Voedingsinname en andere confounders**:
    - Er zijn een aantal belangrijke confounders die houtverbrandingsbiomarkers beïnvloeden (Table 25~~Table 25~~).
    - In de tweede piloot haalbaarheidsstudie (van de huidige studie) werd aan de deelnemers gevraagd een beknopt dagboekje/sheet in te vullen, welke zeer nuttig bleek voor de interpretatie van de urinegegevens (zie bijlage 2). De deelnemers rapporteerden elk uur van de dag of thuis of in het verkeer doorbrachten, of ze klachten/hinder hadden gehad van verbrandingsreuk of -rook, of ze binnenshuis of buitenshuis waren, of ze hadden gegeten, en verder kan het ook interessant zijn na te vragen of ze zelf hebben gekookt (aan kookvuur hebben gestaan).
    - Er zijn veel voedingsbronnen van levoglucosan. Het meest haalbare is om aan de deelnemers te vragen of ze de afgelopen 6-12 uur een van die voedingsmiddelen hebben gegeten (opgelijst in Table 25~~Table 25~~). Voor OH-PAK's stelde Li et al. (2010) voor om aan de deelnemers te vragen 12 uur voor urinecollectie geen gegrilde, gerookte en barbecude voedingswaren te eten.

- Urinecollectie: Urine wordt verzameld in polypropyleenbuizen. Na het verzamelen kan de urine gedurende 2 dagen bij kamertemperatuur worden gehouden (Li et al., 2015a) en daarna bij -20°C worden bewaard.
- Het gebruik van siliconen polsbandjes voor vergelijkende persoonlijke blootstellingsinschatting wordt nog verder uitgetest buiten de tijdsperiode van het huidig project. De bandjes kunnen praktisch gezien gemakkelijk toegepast worden. Na reiniging en voor gebruik (pre-cleaning) moeten de polsbandjes worden verpakt in luchtdichte afsluitbare teflon (PTFE) zakjes. Na gebruik worden in deze zakjes op kamertemperatuur bewaard.

Interpretatie voor gezondheid

In eerdere studies en uit het huidige houtverbrandingsproject bleken 2OH NAP, OH FLU en OH ANT de meer specifieke biomerkers voor de blootstelling aan houtrook te zijn. De gezondheidsinterpretatie van deze biomerkers is beperkt door het gebrek aan gezondheidsrichtwaarden voor OH-PAK-metabolieten in urine. Op basis van een approximatieve massabalansberekening in de huidige studie, bleek (op basis van de blootstellings- en biomerkergegevens tijdens de gecontroleerde houtverbrandingsstudie), dat de hoeveelheid van deze PAK's die ingeademd werden en in urine als OH-PAK uitgescheiden werden, in dezelfde grootteorde lagen. Een mogelijke aanpak voor gezondheidsinterpretatie kan zijn om een approximatieve berekening te doen van PAK's inname via de luchtwegen, gebaseerd op de biomerkerwaarden (met de aannames dat de PAK volledig geabsorbeerd/gemetaboliseerd werd en gebruik makend van het totale theoretische urinevolume van 1,5 L en een inhalatievolume van 16 m^3 per dag). Deze berekening kan worden gevalideerd met behulp van PBPK-modellering, zoals het model 'IndusChemFate'. De gezondheidseffectmarkers die relevant zijn om bij humane biomonitoring te associëren met de houtverbrandingsurinemarkers zijn respiratoire parameters, inflammatie, oxidatieve stress, cardiovasculaire en genotoxiciteitsmerkers.

Conclusie

Verschillende biomarkers en samplers voor houtverbrandingscompenten werden getest. De gevoeligheid van de merkers is goed om blootstelling te meten. Het is van belang om alle urinaire merkers levoglucosan, 2OH NAP, 2+3OH FLU, 2+3OH PHE in urine te meten. Het laat toe patroonanalyse van de blootstelling te doen. Toch is het niet altijd eenduidig om specifiek de blootstelling aan houtverbrandingsrook vast te stellen. Het beoordelen van deze urinaire biomarkers in combinatie met BC gemeten in de lucht (bij voorkeur BC_WB) laat een meer specifieke inschatting toe van blootstelling aan houtverbrandingscomponenten.
Het is zeker zo, dat een combinatie van luchtkwaliteitsmetingen met biomerkermetingen zeker een effectief hulpmiddel zou kunnen zijn voor het aanpakken van de houtverbrandingsproblematiek in Vlaanderen.Betrouwbare interne blootstellingsgegevens maken het mogelijk om op individueel en populatieniveau blootstellingsreductie-maatregelen op te volgen. Het stimuleert particulieren om de maatregelen toe te passen en om op die manier de blootstelling aan houtverbrandingsproducten te verminderen.

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LIST OF ACRONYMS

ACY: acenaphthylene
ACE: acenaphthene
ANT: anthracene
BaA: benz(a)anthracene
BaP: benzo[a]pyrene
BghiP: benzo[g,h,i]perylene
BbF: benzo[b]fluoranthene
BeP: benzo[e]pyrene
BkF: benzo[k]fluoranthene
CHR: chrysene
CRT: creatinin
DahA:dibenzo[a,h]anthracene
FLA: fluroanthene
FLU: fluorene
2+3 OH FLU: 2+3 OH fluorenes urinary metabolites
IcdP: indeno(1,2,3-c,d)pyrene
NAP: naphthalene
2OH NAP: 2-OH naphthalene
PAH: polycyclic Aromatic Hydrocarbon
PIC: picene
PHE: phenanthrene
2+3 OH PHE: 2+3 OH phenanthrenes urinary metabolites
PYR: pyrene
1OH PYR: 1-OH pyrene urinary metabolite
Unidentified OH-PAH: unidentified hydroxyl-polyciclic aromatic hydrocarbon urinary metabolite which was visible on the chromatogram, just before 2OH NAP

CHAPTER 1 BACKGROUND

Wood burning is popular as a primary or secondary combustion source for households. During wood combustion, a significant amount of pollutants are released: $PM_{2.5}$, CO, NO_x, and a number of known carcinogens such as benzene and PAHs. Moreover, wood heating can cause nuisance in a residential area such as odors, irritating eyes and symptoms of difficulty breathing (VMM study wood firing, 2014; Bølling et al., 2009). In winter 'stable' weather can cause moderate emissions in residential areas to contribute to $PM_{2.5}$ in a similar amount as the contribution of traffic (Reis et al., 2009). The results of Chemkar 3 and 4 (VMM 2010, VMM, 2013) indicated a significant contribution from wood combustion to the total measured particulate matter concentrations (in certain locations) of Flanders. Campaigns in European cities showed the proportion of air pollutants from wood combustion in some cases comparable to contribution of traffic (Caseiro et al, 2009; Fuller et al, 2014). In recent measuring campaigns for VMM and LNE, the winter levoglucosan concentrations (January '16) showed a similar trend on all measuring locations on the same sampling days. For each of the measurements on all sampling locations, the $PM_{2.5}$ levoglucosan concentration was multiplied by 10.7, to calculate the local contribution of wood burning to the $PM_{2.5}$ levels. In summer-fall, as well as in winter, the contributions were relatively high (median levels less than 10% to up to 20.3%) (Koppen et al., 2016). These percentages were also observed during a levoglucosan measuring campaign performed during the time frame of 2010 on several locations in Flanders (Maenhaut et al., 2012). Recently Maenhaut et al. (2016) reported a for Flanders adapted biomass PM contribution factor of 22,6. Applying that factor to our data, the estimated contribution of biomass burning to $PM_{2.5}$ would be between lower than ca. 10% and about 40%. This means that (mainly during winter) humans are potentially exposed to rather high levels of particles (and associated pollutants) emitted from residential wood burning in Flanders.

For assessment of personal exposure to wood smoke some biomarkers exist, but still this field is open for some exploration of methods. The current project, aimed to identify a potential biomarker or personal sampling methodology that allows monitoring exposure to wood burning compounds.

CHAPTER 2 LITERATURE

2.1. TOXIC EMISSIONS FROM OPEN BURNING OF BIOMASS

Estrellan and Iino (2010) reviewed data collected on toxic emissions of open burning of biomass and anthropogenic sources. Anthropogenic materials burning released higher amounts of polycyclic aromatic hydrocarbons (PAHs), VOCs, PCDD/Fs, organic and inorganic ions, metals and other chemical species per unit mass of material burnt than biomass fuels, due to polymeric materials in anthropogenic wastes. For biomass open burning, rigid materials such as wood emit appreciably higher PAHs and PCDD/Fs concentrations compared to agricultural residues. Bhargava et al. (2002) demonstrated that while uncontaminated (untreated) wood resulted in lower levels of emissions, contaminated wood resulted in significantly higher level of emissions (see Table 1~~Table 1~~ on PAH and PCB levels). Dioxin and furan (PCDD/F) emissions from contaminated wood species were observed to exceed 0.1 ng/m^3, which is the emission limit for incinerator facilities in many countries.

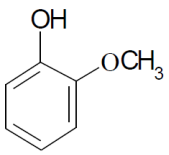
Table 1: PAH and PCB (Arochlor 1242) concentration (in µg/kg) in the emission samples from combustion of individual wood samples (after: Estrellan & Iino, 2010)

Compound name	Untreated pine	Camphor Laurel*	Medium Density Fiberboard (MDF)*	Chipboard*
Naphthalene	18 360	24 171	49 909	60 393
Acenaphthylene	636	843	2026	2466
Acenaphthene	50	64	197	212
Fluorene	297	200	640	841
Phenanthrene	379	482	1519	1379
Anthracene	98	150	499	581
Fluoranthene	296	389	1008	708
Pyrene	197	262	439	457
Benzo[a]anthracene	82	132	484	614
Chrysene	35	59	287	230
Benzo[b]fluoranthene	326	251	696	612
Benzo[k]fluoranthene	32	46	917	85
Benzo[a]pyrene	nd	nd	nd	nd
Indeno[1,2,3-cd]pyrene	nd	nd	81	113
Dibenz[ah]anthracene	nd	nd	nd	nd
Benzo[ghi]perylene	nd	nd	143	227
PAHs (total)	20 800	27 100	58 900	68 900
PCBs (Arochlor 1242)	nd	17	40	48

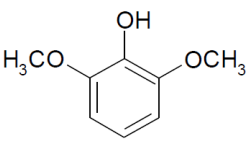
* **Camphor Laurel** is also a naturally occurring tree and contains camphor oil which is rich in methylenedioxybenzenes (safrole). From a chemical analysis of the wood, it was found that the wood chips contain a relatively small amount of chlorine. **MDF** is a wood-based composite and its main constituent is a softwood that has been broken down into wood fibres. These fibres are then formed into a board by processing under high temperature and pressure and using bonding agents such as phenolic (phenol, resorcin or cresol and formaldehyde) or amino compounds such as urea or melamine. **Chipboard** is manufactured in a similar fashion although rather than using the fibre from wood pulp, the wood chips or shavings are directly used for processing which involves sizing, bonding and hot pressing. Commonly used bonding agents for chipboard are phenolic and urea resins although bonding agents with a diisocyanate adhesive base are a relatively new development. Both MDF and chipboard contain up to 9% by weight of bonding agents. These timber products may also contain inorganic or organic additives and preservatives (Bhargava et al., 2002).

Woodsmoke is considered to contribute to the toxicity of PM outdoor as well as indoor. 'Indoor emissions from combustion of biomass fuel (primarily wood)' has been classified by the International Agency for Research on Cancer as a Group 2A carcinogen i.e. probably carcinogenic to humans (http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf). It has been reported that PM resulting from burning of plants (wood) has a higher oxidant capacity compared to particles from diesel exhaust (EPA, 2010; cited in Wallner et al., 2013a). Furthermore, recently, Manoli et al. (2016) reported that the wintertime carcinogenic and mutagenic potencies of particles and the PAH-induced inhalation cancer risk were almost equal at an urban and background site in Thessaloniki (northern Greece). Although traffic was the dominant contributor, also wood smoke contributed considerably namely 17 and 28 % to total ambient BaP at the traffic and at the urban background site, respectively.

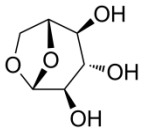
Major components included in vegetative biomass smoke are - aside from PM and PAH - carbon monoxide (CO), formaldehyde, acrolein, benzene, water-soluble potassium, levoglucosan, and methoxyphenols (for complete list see Annex A). Levoglucosan is not considered toxic and it is excreted unchanged in urine within relatively short time. Also methoxyphenols are excreted as such in urine, however they are toxic compounds, able to induce irritation and inflammation (http://old.ros.edu.pl/text/pp_2008_010.pdf). Levoglucosan and methoxyphenols, are respectively formed via pyrolysis of the wood polymers cellulose and lignin. Combustion of soft- and hardwoods give rise to respectively guaiacol (2-methoxyphenol), and syringol (4-hydroxy-3,5 methoxyphenol) compounds. Methoxyphenols, and especially levoglucosan have been widely used as marker for biomass burning in indoor/outdoor air samples. The transglycosylation process (cellulose degradation pathway) occurs at lower temperatures than previously assumed, between 150 and 350°C, with maximum yields at 250 °C, regardless of plant species (Kuo, Herbert, & Louchouarn, 2008). Therefore, levoglucosan is not a suitable tracer for sophisticated appliances with automatically fired wood combustion in which high temperatures are reached.
Levoglucosan, mannosan and galactosan have been used as tracers for residential wood combustion and wildfires (Fine et al.,2004; Engling et al., 2006; Vicente et al., 2012). The levoglucosan to mannosan ratio has been described to be wood type specific,with low ratios for softwoods and higher values for hardwoods (Engling et al., 2006; Schmidl et al., 2008). The use of levoglucosan as a quantitative tracer may be associated with large uncertainty since laboratory measurements of levoglucosan emissions have shown large variations depending on the type of stove, biofuel quality, and operator's behaviour (Hedberg et al., 2002). Schmidl et al. (2011) studied the influence of system (manually and automatically fired appliances) and wood type on anhydrosugars emissions. Automatically fired appliances did not emit detectable amounts of anhydrosugars in full- and part-load operation. Levoglucosan and mannosan were only detected during the start-up phase.



guaiacol



syringol



levoglucosan

Figure 1: Chemical structure of the methoxyphenols guaiacol (2-methoxyphenol) and syringol (4-hydroxy-3,5 methoxyphenols), and of the sugar compound levoglucosan (1,6-anhydro-ß-glucopyranose)

2.2. BIOMARKERS FOR WOOD SMOKE EXPOSURE

Table 2 gives an overview of the biomarkers used in wood smoke studies, which are discussed in the text below.

Urinary wood smoke biomarkers

Exposure to wood smoke is most easily measured via personal monitoring to airborn levoglucosan, methoxyphenols and polycyclic aromatic hydrocarbons. However, a biomarker of woodsmoke exposure may more accurately reflect the internal dose of those compounds.

A good biomarker is sensitive in a dose dependant way, specific for the targeted exposure, and biologically stable in a matrix. Considering the latter characteristic OH-metabolites of PAHs, levoglucosan and methoxyphenols are known to be stable compounds in urine (Li et al., 2015a). Considering specificity, all three compound groups were confounded by diet i.e. woodsmoke flavoring for metoxyphenols, caramelized sugar for levoglucosan, and smoked/grilled food in case of OH-PAHs (NB: Diet is also likely the primary source of 1-OH pyrene, in populations exposed to moderate to low PAH levels). The sensitivity of urinary markers is mainly influenced by their rather short half-life, which is for OH-PAHs around 6 hours for the smallest OH-naphthalenes, between 8-15 hours for fluorene and phenanthrene OH-metabolites, and on average 24 hours for 1-OH pyrene (Li et al., 2015a). Subjects showed a similar urinary elimination for several syringols and guaiacols, with a peak at 5-6 h postexposure (most apparent for syringols) (Dills et al., 2006). A large proportion of levoglucosan is excreted within a few hours (Moshammer, Weiss, & Neuberger, 2012). As it is difficult to capture a peak after uncontrolled exposure in a population, average concentrations of the individuals were suggested to be the best measure. Li et al. (2015) collected several urine samples during different time points after a generated wood smoke exposure. An average of urine samples collected within the first 12 hours, or pooling of the samples was suggested, to be a good strategy for overcoming the problem of peak exposure. Indeed, the correlation of a single urine level with personal $PM_{2.5}$ levels, was poor, whereas the peak exposure and the average of (mostly) three 0-12 hours post-exposure samples, showed a good correlation (except for 1-OH pyrene) (Li et al., 2015a). Another strategy could be considering pooling of different individuals living in a same environment and in this way excluding to some extend the influence of dietary intake, randomly of influence in the different individuals. Wallner et al. (2013) pooled first morning voids of 10 mothers and 10 children per region, and found a correlation between the urinary levoglucosan concentration (of each age group) and the agrarian quote of the respective communities (no correlation for urinary 1-OH pyrene). They suggested that, if one is not interested in the individual levels, levoglucosan could be a good marker to reflect regional exposure to woodsmoke (Wallner et al., 2013b). They therefore stated that human biomonitoring of levoglucosan may be suitable to detect differences in regional exposure to wood smoke.

Overall, urinary markers are the by far most used biomarkers in different woodsmoke exposure settings. In high exposure scenarios such as is the case for wildland firefighters, increased guaiacol or 4-OH phenanthrene levels were reported by respectively Neitzel et al. (2009) and Adetona et al. (2013), comparing pre- and post-shift collected urine samples. Dills et al. (2006) showed in a

controlled wood fire exposure experiment, that methoxyphenols can be used as biomarkers at moderate to high (>700µg/m^3) concentrations of $PM_{2.5}$ originating from woodsmoke. Nine non-smokers had to sit for 2 hours in the near of an indoor open wood fire. Urine was collected before and until 48h after exposure. The sum of five methoxyphenols (propylguaiacol, syringol, methylsyringol, ethylsyringol and propylsyringol) measured in urine samples collected in a 12-hour time frame after exposure, was found to correlated well with levoglucosan in airborn $PM_{2.5}$. Furthermore this methoxyphenol sum parameter seemed to show minimal/none confounding by diet (Russell L Dills et al., 2006). On the other hand, the urinary levoglucosan levels did not always increase after the controlled woodsmoke exposure in those voluntairs (Li et al., 2015a). The same variable response in urinary levoglucosan was observed in nine non-smokers after a controlled indoor exposure to an old-model wood stove (Bergauff et al., 2010c). Milgliaccio et al. (2009) reported non-significant higher levels of levoglucosan in Libbian children (7-10y) living in residences with a wood stove, compared to those having no stove.

Furthermore, Li et al. (2015) performed an analysis of urinary OH-PAHs in the individuals participanting in the above mentioned controlled woodsmoke exposure setting. Urine collected in a time frame of 12 hours after exposure, showed a clear increase in OH-PAHs, compared to pre-exposure levels. More specifically 2-OH naphthalene, followed by 1-OH naphthalene and 9-OH fluorene increased respectively, on average 7.2, 4.5, and 4.4 times post- vs. pre-exposure. Of those three OH-PAHs, 1-OH naphthalene is intrinsically least specific to biomass burning, as it is the main metabolite of the wide-spectrum carbamate insecticide carbaryl, the herbicide napropamide, and the widely used beta-blocker propanolol (refered to in Li et al. (2015)).

Generally, naphthalene (IARC group 2B), phenanthrene (IARC group3), and fluorene (IARC group3) are the most abundant PAHs in the gaseous phase of the environment (IARC 2002, 2010[1]). During wood burning, 0.24 to 1.6 g of naphthalene was released per kilogram of wood, compared to 8.10^{-4} to 3.1 10^{-2} g pyrene/kg wood (Larson & Koenig, 1994). Kato et al. (2004) indicated that charcoal workers were exposed to ca. 15 times higher levels of naphthalene than pyrene in the volatile air fraction, and 2-OH naphthalene rather than 1-OH pyrene appeared more appropriate for assessment of woodsmoke exposure in those workers (NB: urine sampled in middle of the workweek). In occupational and non-occupational settings, 2-OH naphthalene was also by others indicated as a more appropriate biomarker for inhalation exposure, compared to 1-OH pyrene (Kang, Cho, Kim, & Lee, 2002; Li et al., 2016a) and to 1-OH naphthalene - the latter being better associated with smoking (Kim et al., 1999). Kato et al. (2004) reported that the urinary levels of 2-OH naphthalene among charcoal workers were higher than shipyard workers or subjects occupationally exposed to jet fuel, but lower than the levels found in cokeoven workers.

Other possible wood smoke biomarkers in urine could be metabolites of aromatic amines of among others naphthalene. Indeed, urine of individuals exposed to PAH-containing combustion emissions i.e. wood smoke, cigarette smoke, and traffic combustions, contains OH-PAH metabolites, and metabolites of aromatic amines such as o- and p-toluidine, 1- and 2-aminonapthalene, and 4-aminobiphenyl. As for all other above metioned biomarkers, also these compounds are not specific for wood smoke exposure. Indeed, Neophytou et al. (2014) suggested that 1- and 2-aminonaphthalenes[2] might serve as a marker of vehicle exhaust in the general population (after adjustment for cigarette smoke exposure), and they further mentioned that 2-

[1] http://monographs.iarc.fr/ENG/Classification/

[2] Post-shift urinary 1- and 2-aminonaphthalene (and not 1-aminopyrene) concentrations of 82 male trucking industry workers (urine samples at beginning vs. end of workweek) were associated with the workweek $PM_{2.5}$ levels and with the urinary oxidative stress marker, 8-oxo-2'-deoxyguanosine (8-oxodG) (Neophytou et al., 2014)

aminonaphthalene and 4-aminobiphenyl detected in non-smokers, were diet-associated (Grimmer, Dettbarn, Seidel, & Jacob, 2000).

Another approach for studying the impact of wood smoke exposure is through assessment of its mutagenic capacity. Long et al. (2014) and Kato et al. (2004) studied the urinary mutagenic potency using the AMES test in individuals before and after exposure to woodsmoke, respectively via using a wood-fired steam bath (during ca. 30 min on average) or in occupationally exposed charcoal workers. They both showed that wood smoke exposure induced a higher urinary mutagenic potency. Although this approach is interesting, it has the disadvantage of being very unspecific for wood smoke exposure in case on non-controlled environmental wood smoke exposure.

Portable personal samplers for wood smoke compounds

In the context of wood smoke exposure, the most often used passive personal samplers are personal CO monitors (of the type Draeger Pac™III), for continuous data-logging, or the semi-quantitative CO color-stain passive-diffusion tubes. They were e.g. used in high-exposure scenarios of firefighters and in woodstove intervention studies. Riojas-Rodriguez (2011) commented that personal CO measurements were not a good surrogate for assessing PAH exposure, particularly in high-pollution conditions within an indoor stove intervention study in Mexico. On the other hand, CO levels from personal passive samplers worn 48h before urine collection were associated with urinary OH-PAH metabolites in a stove intervention study in Peru. Both Neitzel et al. (2009) and Adetona et al. (2013) used active personal CO samplers. Neitzel et al. (2009) reported a significant correlation between full-shift personal levoglucosan and CO levels in wildlife firefighters.

Recently silicone wristbands have been proposed as (comparative/semi-quantitative) passive sampler for different compounds, including PAHs (O'Connell, Kincl, & Anderson, 2014). In two asphalt occupational PAH exposure settings, 25 PAHs were sampled during 8 and 40 hours of exposure. The amount of PAHs sequestered differed between the workplaces. No reports were found about sampling of wood smoke exposure or of other major wood smoke compounds, using this type of wristbands.

Aside from the current aethalometers measuring Black Carbon (BC), very recently new types of portable and stationary multi-wavelength aethalometers (AE200, 300 and 350) are launched. These devices are of the type real-time 5-wavelength UV-IR Black Carbon monitors, which allow diversification of the BC source. This method assumes that elemental carbon may be differentiated based on their Angstrom coefficients (i.e. the wavelength dependence of the optical absorption coefficient). This makes them a potentially interesting device for wood smoke monitoring, although also here the methodology is not without confounding due to the presence of other similar UV-absorbing components such as coal smoke (Harrison, Beddows, Hu, & Yin, 2012).

Woodsmoke compounds in exhaled air

(Long et al., 2014) collected exhaled CO as biomarker for wood smoke exposure of 32 individuals taking wood-fired steam baths In Guatemala. The breath levels increased ~10 times the use of these baths.

Wood smoke compounds on the skin

Sampling on the skin is mainly performed for compounds having a low vapor pressure - i.e. tend not to become airborne through volatilization - that may be absorbed through the skin (Boeniger, Neumeister, & Booth-Jones, 2008).
Serdar et al. (2016) recently performed a study in which skin wipes were used to study dermal exposure of PAHs in roof workers. The collection method consisted of 1 minute rubbing hands together using 3mL of sunflower oil. Front, back of the the hands and between fingers were wiped off using wipes (DuPontTM Sontara®), stored in a glass vial on ice, and extracted using dichloromethane and analyzed for PAHs using GC/MS. Pre vs. post workshift the wipes showed no increase in (the more volatile) naphthalene, but did increase for (the partly particle-bound) pyrene. The PAH levels measured in the wipes and the corresponding OH-metabolites in urine were not correlated (Serdar et al., 2016).
Sampling of PAHs using dermal wipes has not yet been described in the context of wood smoke exposure. For levoglucosan or methoxyphenols, no reference to sampling with skin wipes was found. Potentially these latter compounds may be observed on the skin, as they are bound to fine particles.

Wood smoke compounds in hair
Non-invasive sampling and a time integration of exposure, seem the most attractive features of using hair for monitoring of pollutants. Indeed, incorporation of chemicals from blood into hair is a progressive and continuous process that smoothes variability in the concentrations measured in hair. In other words the levels are not influenced by short-time variations (intra- and inter-day) but may still be representative of changes in the exposure occurring on a monthly basis (Appenzeller, Mathon, Schummer, Alkerwi, & Lair, 2012). Storage is easy, usually done at room temperature (if the target compounds are not volatile) and for longtime period, as the matrix stability is higher than for liquid matrices.
There was not reference found on analysis of levoglucosan and/or methoxyphenols in hair. However, both PAHs and OH-PAHs have been so far measured in hair. Toriba et al. (2003) identified 10 different PAHs in hair, of which the concentration tended to increase with decreasing number of benzene rings. The effect of washing with organic solvents and shampoo was observed in that a part of the target molecules was removed by washing (most likely the external chemicals) and a remaining part seemed to be unaffected (most likely the inner chemicals). It is generally accepted that biologically incorporated molecules are located inside hair shafts (cortex and medulla), while external contamination is likely to remain on the surface of hair scales (i.e. cuticle) (Figure 2~~Figure 2~~).
Appenzeller et al. (2012) determined OH-PAHs in 105 hair samples of smokers and non-smokers. In 1/3 of these samples, no OH-PAH was detected. Most of the other samples contained one or two different OH-PAHs. Recently Grova et al. (2016) optimized a method to measure benzo[a]pyrene metabolites in hair. Two on eight hair samples analyzed from smokers were above the LOQ for benzo[a]pyrene-r-7,t-8,t-9,c-10-tetrahydrotetrol and benzo[a]pyrene-r-7,t-8,c-9,t-10-tetrahydrotetrol.
Considering the washing procedure of hair analysed for pollutants, no standardized procedure is followed: mostly hair is washed with organic solvents, deionized water or water with shampoo. Some authors consider that external deposition also represents chemicals to which individuals have been exposed, and should not be removed before analysis (Ostrea et al., 2009). This was also refered to as 'sampling of the personal cloud' using hair as a passive sampler (Kucharska, 2015).

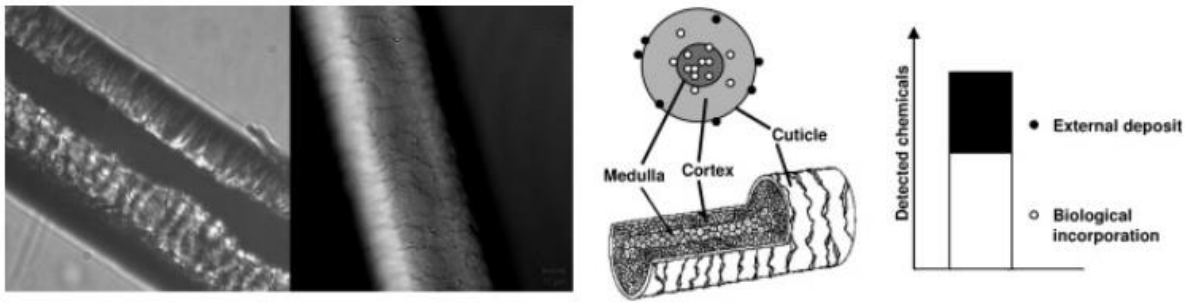


Figure 2: Hair structure and localization of chemicals in this structure. Left picture (transmitted light microscopy) shows inner hair (cortex and medulla). Right picture (confocal microscopy) shows scales composing hair surface (cuticle). Drawing: hair shaft cross-section with theoretical distribution of molecules '●' due to external deposition (i.e. contamination), and 'O' incorporated from biological pathway (after exposure and diffusion in the body) among cuticle, cortex and medulla; Graph: presents the corresponding theoretical representation of the total amount of chemicals detected in hair (determined in unwashed hair) and composed of both external contamination (possibly removed by hair washing) and incorporated molecules (not removed by washing). Taken from Appenzeller and Tsatsakis (2012)

Some thoughts after the literature screening…

Overall, since all biomarkers, may be to a variable larger or lesser extend influenced, by other than woodsmoke sources, such as e.g. diet, traffic and/or indoor sources, it may be fortunate to apply different biomarkers and/or personal samplers combined with a good sampling strategy to bypass confounding. In the current work, this will be aimed for as follows:

Different biomarkers and samplers will be tested, and a good combination of markers will be selected. Quantifying a suite of compounds as a screen for exposure to woodsmoke, overcomes the difficulties of interpretation for individual compounds (Dills et al., 2001). Furthermore it was also stipulated that assessing biomarkers in combination with ambient air quality measurements, could allow characterization of background wood smoke exposure (Dills, Zhu, & Kalman, 2001). Considering improving the biomarker sampling strategy, different approaches could be followed for urine sampling, such as: (1) Pooling of e.g. 3 urine samples spread over the day (Dills et al., 2006; Li et al., 2015); (2) Disposing the first morning void (exposure of day before), followed by collection of the second morning void (= reference concentration) and the end-of-day urine (=exposure). This would allow to relatively evaluate the short-term daytime exposure e.g. by deviding both concentrations to calculate the level of increase; (3) Collection of urine in several individuals of a same close geographical area, and pooling them to assess small-regional levels of woodsmoke exposure, and at the same time randomizing the influence of peak exposure and/or dietary influences (Wallner et al., 2013b).

Table 3: Overview of studies including wood smoke biomarkers

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
Environmental + background exposure						
Austria	N=10 mothers and N=10 children recruited in same families in **5 communities**. Urine samples were pooled per community and age group	urinary 1-OH pyrene, levoglucosan (first morning voids)	none	Levoglucosan higher in children vs. mothers **Correlation levoglucosan in urine pools with 'agrarian quota'** (GIS variable form Statistik Austria)	Intra-individual variability over time and inter-individual differences need to be studied. Short-term influence of diet	Wallner et al., 2015
Canada	83 non-smokers: 54 from **traffic**, 29 **woodsmoke regions**	IL-6, CRP, band cell count, reactive hyperemia peripheral arterial tonometry at end of each 7-day period	Indoor Harvard PM2.5 impactors during each of two consecutive 7-day periods with and without HEPA filtration device in living room and bedroom (10L/min): PM2.5, levoglucosan, absorbance	HEPA filtration reduced indoor PM2.5 by ca. 36% (traffic) and 48% (woodsmoke homes). Association only between CRP and PM2.5 exposure in traffic-impacted locations .	Areas with traffic or high woodsmoke were selected using spatial LUR models of NO, light absorption coefficient ("absorbance"), and woodsmoke	Kajbafzadeh et al., 2015
USA	21 adults, **background concentrations in urine**	urinary methoxyphenols	none	Guaiacol, 4-Meguaiacol, eugenol and vanillin detected in all individuals Ratio	Quantifying suite of methoxyphenols as screen for exposure of	Dills et al., 2001

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
				guaiacols/syringols = +/-8 Vanillin, eugenol are common flavorings	wood smoke allows better interpretation of exposure	
Indoor exposure, stoves, steam bath						
Libby	14 children, 7-10y	urinary levoglucosan	On day of urine collection: $PM_{2.5}$ outdoor: 5.9 µg/m^3; $PM_{2.5}$ inside school: 41.1 µg /m^3; levoglucosan in particulates indoor: 98.5 ng/m^3	**Levoglucosan slightly higher** in children living in houses with woodstoves (not significant)		Miggliacio et al., 2009
Guatemala	N= 32 people exposed to **wood-fired steam bath** (temazcal): first morning urine void on day of temazcal use + first morning urine void on day following temazcal exposure,	Exhaled breath CO measured immediately before and after temazcal use; mutagenic potency of urine extracts using AMES test	none	Exhaled CO ~10 times increased after temazcal use. Urinary mutagenecity ~1.7 times higher. CO level and time spent in temazcal positively associated with urinary mutagenic potency.	Effect of wood smoke exposure on urinary mutagenicity, was not persistent, and without consecutive daily exposures it returned to background.	Long et al., 2014

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
Peru	**Stove intervention study**: N=155 intervention group with chimney-equipped stoves vs. N= 179 controls with open-fire stoves. All women. Measurements 6-8 months after installation of new stoves	10 OH-PAHs recent eye consitions, respiratory conditions, headache	Passive personal CO monitoring using CO diffusion tube (Dräger), during 48h before urine collection	2-OH naphthalene lower in intervention group, weak correlation between OH-PAHs and CO. No difference in 1-OH pyrene	Even with improved stoves, biomarker concentrations exceeded those of general population	Li et al., 2016
Mexico	**Stove intervention study:** open fire (pre intervention) and installation of improved stove (post intervention), N=47 women. Morning urine collection after 1 day use of stove	urinary OH-PAHs	personal 8h CO monitoring: - continuous data-logging electrochemical monitors (Draeger Pac™III), with Draeger XS R CO sensors and a model D3T filter - CO color-stain passive-diffusion tubes	Average reduction of 34% for hydroxylated metabolites of naphthalene, fluorene, phenanthrene, and pyrene on a creatinine-adjusted basis.	The CO passive sampler (color stain tube) is easy-to-use indicator of personal CO and biomass smoke exposure but is not effective in determining its specific constituents such as PAHs	Riojas-Rodriguez et al., 2011

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
USA	**Controlled exposure to open wood fire**, 9 adults (20-65y). Urine collection 24h prior until 48h post exposure	urinary methoxyphenols: propylguaiacol+syringol+methylsyringol+ethylsyringol+propyl syringol	Harvard personal PM2.5 monitor, 1 person monitored with nephelometer(continuous PM, CO, CO2, temp). Individual exposure betwen 0.84 adn 2.99 mg/m3	12-h average of sum of selected **methoxyphenols** is best measure (to exclude dietary exposure). Useful for moderate to high short-term exposures (>700µg/m3) woodsmoke.	Highly variable background in urine.	Dills et al., 2006
		urinary OH-PAHs		**2-NAP most increased, followed by 1-NAP and 9-FLU**	1-NAP also main metabolite of carbamate pesticide carbaryl, herbicide npropamide, beta-blicker propanolol	Li et al., 2015
		urinary levoglucosan		no consistent response after exposure		Bergauff et al., 2010

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
USA	**Controlled exposure to old-model wood stove**: 4 non-smokers (18-65y, 1 urine sample pre, and 4 post exposure), 5 non-smokers (18-65y, 1 urine sample pre and 5 post exposure).	urinary levoglucosan	TSI Dust Track: in room + personal PM2.5: individual exposure between 1.15-1.97 mg/m3 PM2.5	**variable response in urinary levoglucosan**, no change or multiple peaks	ratio levoglucosan/PM depends on: type of wood, moisture, combustion conditions, combustion device. Correction for cotinine needed (smoking)	Bergauff et al., 2010
USA	**Campfire inhalation experiment:** 1 adult	urinary methoxyphenols	personal cascade impactor(6-stag) with PUF tube. Particle exposure 30-4000µg/m3	Increased excretion ratios in guaiacols and syringols after exposure. Better than concentration compairison	fortunate to use individual as own control (pre vs. post exposure)	Dills et al., 2001
Occupational exposure: fire workers, coke workers						

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
Australia	12 adults, pre-and post-exposure **fire training** exercise, 1 urine sample post exposure	urinary levoglucosan, methoxyphenols	no	No significant increase post-exposure to smoke from fire	Quantification of different methoxyphenols might be better, 12-24h collection of urine is better as metabolisation is rapid	Hinwood et al. 2008
USA	14 **wildland firefighters,** 16 work shifts of ca. 7.6h	urinary OH PAHs: 1NAP, 2NAP, 2FLU, 3FLU, 1PHE, 2PHE, 3PHE, 4PHE	personal monitoring of levoglucosan, and CO (Dräger)	All OH-PAHs increased. **4-OH phenanthrene** strongest correlated with levoglucosan		Adetona et al., 2015
USA	13 non-smoking **wildland firefighters**, pre-and postshift urine samples, 20 work shifts in winter of ca. 11.6h	22 methoxyphenols: guaiacol, methylguaiacol, 2,3-dimethoxyphenol, ethylguaiacol, syringol, eugenol, propylguaiacol, vanillin, cis-isoeugenol, methylsyringol, trans-isoeugenol, acetovanillone, ethylsyringol, guaiacylacetone, allylsyringol, propylsyringol, syringaldehyde, acetosyringone, coniferylaldehyde, propionylsyringone, butyrylsyringone, sinapylaldehyde	- breathing zone measurements of CO (Dräger PACIII single gas meter) - PM2.5 cyclone for personal sampling+ levoglucosan analysis of filters	Cross-shift increase of 3 syringols and 4 guaiacols. Best correlated of **guaiacol** (soft wood) with CO and to lesser extend with levoglucosan. Urinary methoxyphenols able to measure high exposure to wood smoke.	Consumption of grilled food caused increased urinary syringol compounds and vanillin (observation in 1 individual)	Neitzel et al., 2009

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
Brazil	250 male **charcoal workers** (19 -65 y), half of them were nonsmokers , and only 10 workers (7%) smoked more than 10 cigarettes/day. Urine samples collected after third day of workweek.	Urinary 2-naphthalene and 1-OHpyrene. Mutagenic potency of urine extracts using AMES test.	none	**Urinary mutagenicity** increased significantly with exposure to wood smoke (modified by smoking). **Urinary 2-naphthol** was most sensitive indicator of wood smoke exposure.	PAH metabolites were significantly elevated in wood smoke exposed individuals with GSTM1 null genotype	Kato et al., 2004
Controlled oral intake						
Austria	**Kinetics experiment** 2 adults- oral administration of levoglucosan	urinary levoglucosan	none	short T1/2 of 4-5hours		Moshammer et al. 2012
USA	**Caramel study**: 9 non-smokers: consumption of 5 cubes of appr 40g. Urine sampling just before, and at time 2, 6, 12 and 24h	urinary levoglucosan	none	Intake of ca. 26.5 mg levoglucosan (high!). At least 2x increase in urinary levels post-exposure		Bergauff et al., 2010
USA	**Wood smoke flavoring ingestion:** 3 adults feeding experiment: urine collected for 7-10 days, on day 6 ingestion of 7 mg total methoxyphenols	urinary methoxyphenols	none	Urinary levels eleveated within 90min of ingestion. Peak conc. and excretion rates within 4h, returned to		Dills et al., 2001

Country	Individuals/sampling	Biomarkers	Air measurements	Results/sources	Remarks	Ref
	(wood smoke flavoring)			background in approximately 18h. Episodic exposures only detectable for 1-2days afterwards		

CHAPTER 3 OPTIMIZATION OF MEASURING TECHNIQUES FOR ANALYSIS OF WOOD SMOKE COMPONENTENTS IN AND ON HUMAN

This chapter describes the optimization of measuring techniques for the wood smoke components levoglucosan, methoxyphenols and polyaromatic cyclic hydrocarbons (PAHs) in/on human matrices and via passive personal sampling methods.
Assessed in the body, none of the above mentioned compounds has a 100% specificity to woodsmoke exposure. Therefore it was speculated that measuring several markers simultaneously in and on the body, may allow tracing of woodsmoke exposure.

Table 4: Samplers and biomarkers tested and/or optimized to assess woodsmoke compounds

Samplers/Biomarkers	Exposure
Passive sampling/biomarker	
Silicone wristband	Levoglucosan external
Scalp hair	Levoglucosan, methoxyphenols external PAHs external+internal
Dermal wipes	Levoglucosan external PAH external
Urine	PAH, levoglucosan, methoxyphenols internal
Radiello (Tenax) personal sampler	PAH, (methoxyphenols?) external
Active sampling	
Personal $PM_{2.5}$ sampler	Levoglucosan external

3.1. WRISTBAND PERSONAL PASSIVE SAMPLERS

The first focus of the work was to combine a combination of a specific woodsmoke tracer measured via a passive sampler on the body, in combination with PAHs in urine, indicators of internal exposure. Active devices that pump air through filters and are extracted for target compounds, are the optimal sampler types for quantitative and accurate assessment of the targeted compounds. However, active personal devices are relatively expensive, require energy, and limited to implement on a large scale. Recently silicone wristbands have been proposed as passive sampler for different compounds, including PAHs (O'Connell, Kincl, & Anderson, 2014).

Figure 3: silicone wristband used as passive sampler for environmental contaminants

Precleaning of silicon passive samplers was done in order to remove compounds interfering with in gas chromatograph (GC-MS) chromatogram (siloxanes), using soxhlet extraction with 250 mL ethylacetate, during 48 hours.

Various methods for extraction and measurement of PAHs in silicon passive samplers were tested:
(a) Extraction with ethylacetate of wristband cut into pieces of 0.5 cm. The pieces were extracted once in an Erlenmeyer with a glass stopper with 50 mL ethylacetate during 2 hours. When starting the extraction (directly after adding the ethylacetate), internal standard was added. One extraction step was sufficient for an extraction recovery of 92%. After the extraction the extract was transferred to a flask and the ethylacetate was evaporated under a very gentle nitrogen stream (important: if evaporated too quickly, the volatile PAHs, especially naphthalene, will also be evaporated). The extract wa spiked with a recovery standard (300-500 ng per vial, the amount has to be comparable with the amount of internal standard) before injection of samples into the GC-MS.
(b) Thermal desorption in microchamber followed by GC-MS: The wristband was brought into a microchamber and heated during 2 hours to 60°C. The air of the microchamber was collected on a PDMS-Tenax cartridge They were spiked with internal standard solution (the solvent used is methanol: D8-naphtalene, D10-fluorene, D10-fluoranthene, D12-benzo(a)pyrene, D12-benzo(g,h,i)perylene. Several (non-exposed) cartridges were prepared for a calibration curve by spiking them with both native PAHs and internal standard. The cartridges were placed in the thermal desorper, which desorbs the volatiles and injectd into the TD-GC-MS.
(c) Direct Thermal Desorption GC-MS:wristband was cut into pieces of 0.5 cm and around 0.5 g was put into an empty cartridge. The cartridges were spiked with internal standard solution (the solvent used was methanol). The cartridges were placed in the thermal desorper, which desorbed the volatiles and then injected into GC-MS.

➔ Comparison of the methods

Wristbands were exposed to a mixture of PAHs (16 EPA-PAHs & retene) in a petridish sealed off with parafilm, without direct contact between the spiking solution and the wristband. The wristbands were precleaned with different methods and for each method two wristbands were available (four halves). The different cleaning methods were: soxhlet extraction using ethylacetate as described above (EA), precleaned wristbands bought as such (MyExposome) and heating in an oven with venting of the air every 10 minutes (heated for1-3h) (Table 5~~Table 5~~). After exposure the wristbands PAHs were extracted via solvernt extraction using ethylacetate (method a, done in duplo). The recovery was calculated as the fraction of the extracted amount to the amount of PAHs that was spiked. The PAHs from the spiked wristbands were also extracted via two different thermal

desorption extraction methods (method b and c). Per precleaning method, both the microchamber PAH extraction as well as the cartridge direct thermal desorption method was used (Table 7).

Table 5: Extraction via ethylacetate (EA) solvent extraction of wristbands (in duplo, 1&2) that were spiked with PAHs: results in ng per halve of a wristband (upper table) and expressed as % recovery of the spike (lower table). Wristbands were cleaned either via: soxhlet extraction as described above (EA), or purchased precleaned wristbands (MyExposome) or heated in an oven with venting of oven air every 10 mins (Heated 1h-3h)

ng

	EA		Exposoom		Heated 1h		Heated 2h		Heated 3h	
	1	2	1	2	1	2	1	2	1	2
Naftaleen	367.5	326.0	303.2	378.9	-683.0	354.6	296.0	193.1	234.4	99.0
Acenaftyleen	289.8	269.8	219.9	289.9	238.3	268.3	240.0	230.0	319.8	219.8
Acenafteen	430.0	390.0	350.0	430.0	360.0	380.0	320.0	350.0	470.0	320.0
Fluoreen	452.0	392.0	445.0	475.0	394.0	484.0	300.0	380.0	450.0	400.0
Fenanthreen	611.0	461.0	630.0	650.0	480.0	610.0	400.0	510.0	570.0	600.0
Anthraceen	13.5	23.5	13.3	11.3	35.2	12.2	28.0	5.0	36.4	21.4
Fluorantheen	559.8	169.8	506.6	576.6	417.5	287.5	206.5	216.5	477.0	487.0
Pyreen	445.0	135.0	373.0	433.0	372.0	222.0	173.0	163.0	361.0	401.0
Benzo(a)anthraceen	0.6	-0.1	1.0	1.4	1.3	0.0	20.0	-0.5	1.2	0.6
Chryseen	0.0	-0.6	0.4	0.3	0.9	1.7	29.3	0.2	-0.2	0.1
Benzo(b)fluorantheen	-0.9	-0.7	-0.7	-0.5	-0.2	0.9	26.3	0.9	0.0	0.1
Benzo(k)fluorantheen	-1.0	-1.0	-0.3	-0.4	-0.2	0.0	25.8	0.1	0.0	-0.3
Benzo(e)pyreen	0.2	0.1	-0.1	0.0	0.1	0.3	74.7	1.1	-1.0	-1.1
Benzo(a)pyreen	-1.7	-2.5	-0.3	-0.1	-0.3	-0.4	0.0	0.0	0.5	-0.1
Indeno(1,2,3,c,d)pyreen	-1.6	-1.6	-0.2	-0.1	-0.3	-0.3	19.0	0.3	0.0	0.1
Dibenzo(a,h)anthraceen	-1.1	-0.3	-0.1	-0.1	0.3	0.1	17.0	0.4	-0.3	-0.3
Benzo(g,h,i)peryleen	-0.4	-0.2	-0.6	-0.6	-0.5	-0.5	15.8	0.0	-0.1	-0.1

%

	EA		Exposoom		Heated 1h		Heated 2h		Heated 3h	
	1	2	1	2	1	2	1	2	1	2
Naftaleen	36.7	32.6	30.3	37.9	-68.3	35.5	29.6	19.3	23.4	9.9
Acenaftyleen	29.0	27.0	22.0	29.0	23.8	26.8	24.0	23.0	32.0	22.0
Acenafteen	43.0	39.0	35.0	43.0	36.0	38.0	32.0	35.0	47.0	32.0
Fluoreen	45.2	39.2	44.5	47.5	39.4	48.4	30.0	38.0	45.0	40.0
Fenanthreen	61.1	46.1	63.0	65.0	48.0	61.0	40.0	51.0	57.0	60.0
Anthraceen	1.4	2.4	1.3	1.1	3.5	1.2	2.8	0.5	3.6	2.1
Fluorantheen	56.0	17.0	50.7	57.7	41.8	28.8	20.7	21.7	47.7	48.7
Pyreen	44.5	13.5	37.3	43.3	37.2	22.2	17.3	16.3	36.1	40.1
Benzo(a)anthraceen	0.1	0.0	0.1	0.1	0.1	0.0	2.0	-0.1	0.1	0.1
Chryseen	0.0	-0.1	0.0	0.0	0.1	0.2	2.9	0.0	0.0	0.0
Benzo(b)fluorantheen	-0.1	-0.1	-0.1	-0.1	0.0	0.1	2.6	0.1	0.0	0.0
Benzo(k)fluorantheen	-0.1	-0.1	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0
Benzo(e)pyreen	0.0	0.0	0.0	0.0	0.0	0.0	7.5	0.1	-0.1	-0.1
Benzo(a)pyreen	-0.2	-0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0
Indeno(1,2,3,c,d)pyreen	-0.2	-0.2	0.0	0.0	0.0	0.0	1.9	0.0	0.0	0.0
Dibenzo(a,h)anthraceen	-0.1	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0
Benzo(g,h,i)peryleen	0.0	0.0	-0.1	-0.1	0.0	0.0	1.6	0.0	0.0	0.0

EA: wristbands pre-cleaned using ethylacetate, Exposoom: pre-cleaned wristbands purchased from MyExposome

Table 6: Extraction of PAHs from writstband via microchamber heating (1) or direct thermal desorption of wristband pieces in a cartridge (2). Grey cells indicate compounds that were not detected, green cells indicate internal standard peaks that were not detected. Benzo(e)pyrene was not determined via the

thermodesorption methods. Upper table: amount of PAH expressed in ng per halve of a wristband. Lower table: recovery of the spiked PAHs in %

ng

	Original		EA		Exposoom		Heated 1h		Heated 2h		Heated 3h	
	1	2	1	2	1	2	1	2	1	2	1	2
Naftaleen	-1.2		313.3	-0.9	253.6	-86.8	268.0	-60.9	261.6	-85.3	196.8	-95.4
Acenaftyleen	532.5	1343.3	119.9	1308.7	109.6	600.2	142.5	180.3	108.5	231.9	71.8	1392.6
Acenafteen	5.9	3.9E+17	53.1	4092.3	47.6	1.5	53.2	7.9	52.1	119.9	33.4	-28.0
Fluoreen	47.0		39.1	165.4	35.0	2937.9	37.1	64.1	39.0	79.5	25.1	2746.5
Fenanthreen	12.0		6.6	14768.1	8.4	243.6	6.6	262.9	6.7	1464.7	5.3	142.1
Anthraceen	15.9	10952.6	6.0	39.5	6.8	108.4	5.5	107.2	8.4	70.1	4.3	122.9
Fluorantheen	36.6		14.0	144.6	5.3	289.0	9.6	55.3	6.5	191.5	5.0	233.8
Pyreen	34.7		13.7	133.2	4.2	318.1	9.3	317.1	6.4	169.5	4.7	259.8
Benzo(a)anthraceen	8.6		5.1	-1.0	1.7		2.8	-0.1	2.1	-0.9	1.2	
Chryseen	13.3		3.8	37.0	0.7		2.9	-8.3	1.4	-5.9	1.4	
Benzo(b)fluorantheen	2.9		1.3	0.3	0.3	240.5	0.9		0.5	-3.1	0.4	21.7
Benzo(k)fluorantheen	1.3	-2.8	0.7	-3.2	0.2		0.3		0.3	-3.7	0.0	
Benzo(e)pyreen												
Benzo(a)pyreen	1.3		0.5	-3.2	0.2		0.3	-3.7	0.2	-4.0	0.2	
Indeno(1,2,3,c,d)pyreen	0.1	-5.3	-0.3	-2.6	-0.1	-4.9	-0.2	-4.3	-0.2	-4.1	-0.1	-5.4
Dibenzo(a,h)anthraceen												
Benzo(g,h,i)peryleen	0.3	-2.5	-0.3	-2.9	-0.1	-4.4	-0.2	-4.0	-0.2	-3.8	-0.2	-4.4

%

	Original		EA		Exposoom		Heated 1h		Heated 2h		Heated 3h	
	1	2	1	2	1	2	1	2	1	2	1	2
Naftaleen	-0.1		26.1	-2.4	21.1	-238.4	22.3	-167.2	21.8	-234.2	16.4	-261.9
Acenaftyleen	12.4	7671.0	2.8	7473.4	2.5	3427.4	3.3	1029.5	2.5	1324.4	1.7	7952.6
Acenafteen	1.4	4.4E+17	13.0	4578.6	11.6	1.7	13.0	8.9	12.8	134.2	8.2	-31.3
Fluoreen	6.2		5.1	28818.0	4.6	511810.1	4.9	11174.1	5.1	13849.1	3.3	478463.9
Fenanthreen	0.3		0.1	9667.8	0.2	159.5	0.1	172.1	0.1	958.8	0.1	93.0
Anthraceen	2.9	10400.2	1.1	37.5	1.2	102.9	1.0	101.8	1.5	66.5	0.8	116.7
Fluorantheen	1.0		0.4	2703.4	0.2	5403.2	0.3	1033.8	0.2	3580.7	0.1	4371.6
Pyreen	1.0		0.4	5804.0	0.1	13862.5	0.3	13818.2	0.2	7384.7	0.1	11319.7
Benzo(a)anthraceen	0.4		0.3	-6.4	0.1		0.1	-0.5	0.1	-5.9	0.1	
Chryseen	6.5		1.9	88.6	0.3		1.4	-19.9	0.7	-14.2	0.7	
Benzo(b)fluorantheen	6.7		3.0	-107.3	0.8	-88961.0	2.1		1.1	1137.8	1.0	-8029.0
Benzo(k)fluorantheen	3.6	239.6	1.9	277.7	0.6	0.0	0.9		0.7	316.5	0.1	
Benzo(e)pyreen												
Benzo(a)pyreen	3.9		1.5	2007.8	0.5		0.9	2288.3	0.7	2502.4	0.6	
Indeno(1,2,3,c,d)pyreen	11.3	1206.0	-29.6	593.1	-12.2	1111.8	-19.6	975.5	-15.4	927.1	-11.1	1230.7
Dibenzo(a,h)anthraceen												
Benzo(g,h,i)peryleen	25.2	344.3	-28.8	394.9	-12.0	600.8	-14.8	539.7	-15.6	514.7	-16.6	590.7

EA: wristbands pre-cleaned using ethylacetate, Exposoom: pre-cleaned wristbands purchased from MyExposome

The direct thermal desorption of wristband in a cartridge method (indicated with '2' in Table 6~~Table 6~~), was not optimal. The results from this method were nog comparable to the other extraction methods. For the uncleaned wristband ('original'), almost no peaks were found. This could be because of an inherent higher amount of interfering compounds in the uncleaned wristbands and because the wristbands were heated for a shorter time at a higher temperature than in the microchamber, and thus possibly releasing more interfering compounds in the GC-MS chromatogram.

The microchamber thermal desorption method (indicated with '1' in Table 7), gave relatively comparable PAH levels in comparison to the liquid extraction method (Table 5~~Table 5~~), i.e. almost the same PAHs are found and their amounts are comparable when considering them as a fraction of total found PAHs. The microchamber method took a day, whereas the liquid extraction method was slower because of the time consuming step of gentle evaporation of the solvent. However, the thermal desorption caused more interfering compounds to occur in the chromatogram. The liquid extraction method showed good chromatographic peaks. NB: Interfering compounds from environmental exposure or skin compounds were not present, as they were not worn.

Liquid extraction was seen as the most optimal method for extraction, and further experiments on extraction efficiency an extract cleaning are continuing. Furthermore, the optimal conditions for storing the wristbands for a longer period are not yet known, and further tests are ongoing.

3.2. ANALYSIS OF WOOD SMOKE COMPOUNDS IN/ON SCALP HAIR

For the experiments below a pool of non-exposed hair sample (from one male adult individual) was collected during a few weeks, and stored at room temperature. This sample was used for the method development of all analyses in hair: PAHs, levoglucosan and methoxyphenols.
It is speculated that levoglucosan, which is a highly polar compound, will not circulate long in the blood stream, and therefore will hardly appear in hair. Therefore, washing of hair with diionised water will be tested. The main reason why hair is washed prior to analysis is to remove external contamination that can interfere with the analysis of interest. For example dust is know to be a carrier for certain non-polar organic molecules, such as legacy POPs and flame retardants. This might be also of relevance for PAHs, and even the 'sticky' component levoglucosan which can be loaded onto dust. Our experience learned that prior washing using ultrapure water to remove dust and particles is sufficient to remove irrelevant external contamination, as more aggressive washing procedures will affect recovery of analytes as extraction might already occur during that washing precedure.

3.2.1. PAHs IN SCALP HAIR

Protocol
The method used consisted of cutting 100 mg of the pooled hair sample into small pieces. The samples were weighted in the extraction vials and spiked with deuteriorated internal standards (IS) of 16 PAHs (all, except benzo(j)fluoranthene, listed in *Table 7*~~*Table 7*~~). Dichloromethane was added and the samples were vortexed and sonicated for 20 minutes. After sonication, the extract was transferred to acollection vial and the extraction was repeated twice. Thereafter, the volume in the collection vial was reduced, via solvent evaporation under a nitrogen stream, and transferred to hexane. The extract was transferred to a silica-alumina column for clean-up. The compounds of interest (17 PAH compounds, see *Table 7*~~*Table 7*~~) were elueted with hexane/dichloromethane (1/1 v/v). The extract was concentrated by evaporation of the solvent under a nitrogen stream to a volume of 1 mL. Thereafter it was transferred to toluene, further evaporated to a final volume of 1 mL, and injected for GC-MS analysis. The PAH compounds were measured using an in-house validated method
(https://esites.vito.be/sites/reflabos/2016/Online%20documenten/WAC_IV_A_002.pdf).

Figure 4: Nylon gauze (left) and glass syringe (right) filled with scalp hair (and glass wool in case of syringhe), as used in the experiments for testing of hair sampling method for wood smoke components

First explorative experiment

In a first stage, three glass syringhes, filled with a filter, hair and glass wool (Figure 4~~Figure 4~~). The syringe was directly exposed to woodsmoke of soft wood shavings burned in a barbecue. For each of the syringhes, one full aluminium box filled with wood shavings was burned (during ca. 30'). The woodsmoke was collected through the syringe. This was repeated three times. Extraction of 100 mg hair, was in this early phase of the experiments, done with 5 mL acetonitrile during 5 minutes in an ultrasonic bath (the method was later further optimized, see protocol above). On the hair samples following high levels of levoglucosan were measured: 197 and 233 µg levoglucosan/g hair. However, in the third sample only 11 µg levoglucosan/g hair was analysed. After this experiment the hair extraction procedure was optimized by using dichloromethane and longer vortexing and sonication for 20 minutes.

Figure 5: Wood smoke was generated in a barbecue via burning of wood shavings in small aluminium boxes (left). The woodsmoke was actively (using a pump) sucked through a glas syringe filled with scalp hair

Extraction efficiency

To check the extraction efficiency of the method, four hair samples were measured: two non-spiked samples and two spiked with a standard concentration of all 17 PAHs. The extraction recoveries of the internal standards were acceptable, and ranged for all PAH compounds (except D8-naphthalene) between 70 and 120%. The D8-naphthalene was around 50%, but still acceptable according to the WAC procedure (WAC/IV/A/002 - https://emis.vito.be/nl/wac-2016). Naphthalene is a more volatile PAH, which evaporates more easily and therefore its recovery is lower in spiking experiments. This was corrected for using the internal standard.

Trueness

Trueness refers to the closeness of agreement between the true value of the analyte concentration and the (mean) result that is obtained by applying the experimental procedure. Thereto, hair samples (duplicate) were fortified with the compounds of interest at a high (10 µg/g) concentration level. The recoveries ranged between 90 and 116% (*Table 7~~Table 7~~*). These recoveries were acceptable and within the range of the internal spike 70% - 120% recoveries. The experiment will be repeated using a lower concentration spike, to check if the procedure is suitable for low levels of PAHs.

In the pooled non-exposed hair samples, 17 PAHs were analysed (*Table 8~~Table 8~~*). The concentrations of the PAHs in that hair sample were almost all below the LOQ. Only low levels of phenanthrene, fluoranthene and chrysene could be quantified in the hair.

Table 7: Recoveries of the spiked hair sample (duplicate, n=2) in %

Compounds	Hair sample 1	Hair sample 2
Naphthalene	107%	114%
Acenaphthylene	108%	115%
Acenaphthene	107%	116%
Fluorene	106%	114%
Phenanthrene	103%	112%
Anthracene	104%	110%
Fluoranthene	104%	110%
Pyrene	105%	111%
Benzo(a)anthracene	98%	105%
Chrysene	98%	107%
Benzo(b)fluoranthene	95%	101%
Benzo(k)fluoranthene	107%	115%
Benzo(j)fluoranthene		
Benzo(a)pyrene	105%	113%
Indeno(1,2,3,c,d)pyrene	90%	97%
Dibenzo(a,h)anthracene	101%	107%
Benzo(g,h,i)perylene	100%	109%

Table 8: measured concentrations in the pooled non-woodsmoke-exposed hair sample (duplicate analysis, n=2)

Compounds	Hair sample 1	Hair sample 2
	□g/g	□g/g
Naphthalene	< 0.03	< 0.01
Acenaphthylene	< 0.01	< 0.01
Acenaphthene	< 0.01	< 0.01
Fluorene	< 0.01	< 0.01
Phenanthrene	0.0184	0.0207
Anthracene	< 0.01	< 0.01
Fluoranthene	0.0129	0.0124
Pyrene	< 0.01	0.0107
Benzo(a)anthracene	< 0.01	< 0.01
Chrysene	0.0365	0.0338

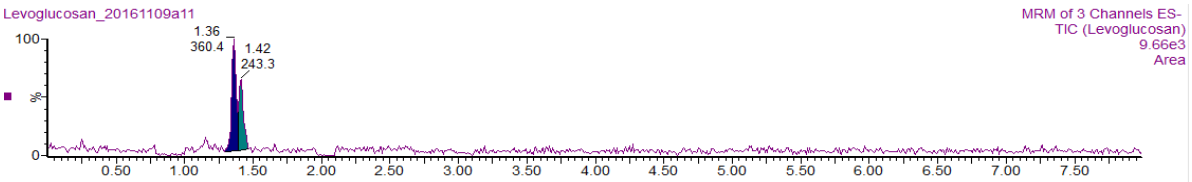
Compounds	Hair sample 1	Hair sample 2
Benzo(b)fluoranthene	0.0254	< 0.02
Benzo(k)fluoranthene	< 0.01	< 0.01
Benzo(j)fluoranthene	< 0.5	< 0.5
Benzo(a)pyrene	< 0.01	< 0.01
Indeno(1,2,3,c,d)pyrene	< 0.01	< 0.01
Dibenzo(a,h)anthracene	< 0.01	< 0.01
Benzo(g,h,i)perylene	< 0.01	< 0.01

3.2.2. LEVOGLUCOSAN IN SCALP HAIR

100 mg of the pooled hair sample was used and cut into small pieces. The samples were weighted in the extraction vials and spiked with levoglucosan (non-deuterioreated internal standard). An amount of acetonitril was added and the samples were vortexed and sonicated for 15 minutes. After sonication, the extract was transferred to a collection vial and the extraction was repeated twice. The solvent from the extract was evaporated under nitrogen to 250 µL. Samples were analyzed using ultra-performance liquid chromatography system combined with triple tandem quadrupole mass smectrometry (UPLC-MS/MS) as described by You et al. (2016). They developed a sensititive method which was used for assessment of trace concentrations levels of levoglucsoan in snow and ice.

The method appeared sensitive enough to detect levoglucosan in the hair sample (Figure 3, peak at retention time 1.36min). The isomers of levoglucosan (mannosan and/or galactosan) were not completely separated with the LC-method (*Figure 6*~~*Figure 6*~~, peak at retention time 1.42min).

Figure 6: Total ion current chromatogram of a pooled non-exposed hair sample. Levoglucosan was visible at a retention time of 1.36 min



3.3. ANALYSIS OF WOOD SMOKE COMPOUNDS IN DERMAL WIPES

Sampling of compounds on the skin is mainly performed for compounds having a low vapor pressure - i.e. tend not to become airborne through volatilization - that may be absorbed through the skin (Boeniger et al., 2008). Sampling of PAHs using dermal wipes has not yet been described in the context of wood smoke exposure. For levoglucosan or methoxyphenols, no reference to sampling with skin wipes was found. Potentially these latter compounds may be observed on the skin, as they are bound to fine particles.

Polypropylene wipes presaturated with 70% isopropyl alcohol and 30% deionised water (PROSAT PS-911, 23x28 cm, were used. The wipes were evaporated to dryness in a fume for 24 hours. Afterwards, they were spiked with an IS mixture of 16 of the PAHs. Dichloromethane was then added and the wipes were shaked for 15 minutes, followed by 30 minutes sonication. After sonication, the extract was transferred to a collection vial and the extraction was repeated twice. The extract volume in the collection vial was reduced to 1 mL by evaporation of the solvent under nitrogen and then transferred to toluene for further evaporation until a final volume of 1 mL. In case of floating particles (form the tissue) in the extract, they were removed by centrifugation. The extract was reconstituted and injected for GC-MS analysis. The GC-MS methodology was an in-house validated method (https://esites.vito.be/sites/reflabos/2016/Online%20documenten/WAC_IV_A_002.pdf).

The trueness of the method was assessed via spike recovery experiments. The recovery was calculated as the ratio between the experimentally observed concentration and the nominal concentration in (*Table 9~~Table 9~~*). Two wipes were fortified with 17 PAHs at a high concentration level of 1 µg of each PAH, per wipe.

Table 9: Recoveries of the spiked wipe samples (duplicate, n=2) in %

Compounds	Wipe sample 1	Wipe sample 2
Naphthalene	114%	90%
Acenaphthylene	125%	73%
Acenaphthene	123%	19%
Fluorene	120%	86%
Phenanthrene	119%	102%
Anthracene	144%	137%
Fluoranthene	115%	91%
Pyrene	117%	87%
Benzo(a)anthracene	120%	112%
Chrysene	109%	139%
Benzo(b)fluoranthene	109%	89%
Benzo(k)fluoranthene	122%	133%
Benzo(j)fluoranthene		
Benzo(a)pyrene	126%	112%
Indeno(1,2,3,c,d)pyrene	102%	95%
Dibenzo(a,h)anthracene	112%	133%
Benzo(g,h,i)perylene	118%	109%

Only for a few PAH compounds the recoveries ranged between 70% and 120% (*Table 9~~Table 9~~*). This was acceptable and within the range of the internal standards (70% - 120%). However, the recoveries were not sufficient for several of the PAHs (compounds marked in red in *Table 9~~Table 9~~*). Since it was a first try-out, it was decided to explore the method further. To check the sensitivity, five wipes were collected by rubbing 10x on the front and backside of both hands . A non-used wipe was used as a procedure blank. Furthermore, a wipe was taken from smoker hands (fingers) before smoking, another wipe was collected from the smoker's fingers after smoking a cigarette, one wipe was used to collect the exhaled smoke of a smoker during the time of smoking one cigarette, and another wipe was taken in the exhaust of a diesel car.

In *Table 10~~Table 10~~* the concentrations (µg/g wipe) are listed for the different samples. All PAH concentrations measured in the samples were low. Only naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene and anthracene were detected in the samples. However, no distuinguish could be made in PAH levels on the hands, before and after smoking. We expected high concentrations of PAH in the 'smoker's exhale' wipe, of which the yellow color of smoke exposure was visible after sampling. However the measured concentrations were rather low, even sometimes lower than the PAH levels in the finger wipe of the smoker. The wipe held before the car exhaust had a black color. PAHs were expected to be found. However none of the PAHs could be detected in the sample. It was clear that the method was not sensitive enough for measuring PAHs in the wipe samples. We decided not to proceed with the method-optimization for wipe sampling of PAHs, as environmental woodsmoke exposure would result in lower PAH exposure as the scenarios tested out.

Table 10: Concentration of PAHs (µg/g wipe) in the exposed wipes

µg/g wipe	Blank	Hands (before smoking)	Hands (after smoking)	Exhaling (blow in the wipe during smoking)	Exhaust car
Naphthalene	0.040	0.091	< 0.06	0.115	< 0.01
Acenaphthylene	< 0.01	0.335	0.200	0.105	< 0.01
Acenaphthene	< 0.01	0.020	0.018	< 0.01	< 0.01
Fluorene	< 0.01	< 0.01	< 0.01	0.014	< 0.01
Phenanthrene	< 0.01	0.010	< 0.01	0.011	< 0.01
Anthracene	0.018	0.029	0.024	< 0.04	< 0.02
Fluoranthene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(a)anthracene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Chrysene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(b)fluoranthene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(k)fluoranthene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(j)fluoranthene	< 0.02	< 0.02	< 0.01	< 0.01	< 0.01
Benzo(a)pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Indeno(1,2,3,c,d)pyrene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Dibenzo(a,h)anthracene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Benzo(g,h,i)perylene	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01

3.4. ANALYSIS OF WOOD SMOKE COMPOUNDS IN URINE

Urine is probably the human matrix mostly used for environmental or occupational biomonitoring of substances with short biological half-lives. PAHs, levoglucosan and methoxyphenols, are compounds with an estimated half-life of respectively on average 6-24, 4-5 and 5-6 hours. The timing of the collection of the urine samples is therefore one of the concerns for the use of this matrix in human biomonitoring. Furthermore, the urinary concentration of all three compound (groups) is known to be unspecific for woodsmoke, being biased via diet, cigarette smoking, and for PAHs also exposure such as traffic exhausts, incense/candle burning or asphalt emissions.

3.4.1. LEVOGLUCOSAN IN URINE

In a first stage no clean-up was performed of the urine samples. The samples were only enzymatic pretreated with urease, and acetonitrile was added before injection on the UPLC-MS/MS (Bergauff et al., 2010a).
You et al. (2016) indicated that levoglucosan could not be sufficiently retained on alkyl-bonded silica columns or reversed phase columns due to its strong hydrophilic character. Polar compounds have been proven to achieve higher retention and selectivity on a Bridget Ethylene Hybrid (BEH) Amide stationary phase compared with BEH Hydrophilic Interaction Chromatography (HILIC) stationary phase in the UPLC system. Therefore, the Acquity BEH Amide column was chosen for analysis of levoglucosan.
Nevertheless, injection of the none-cleaned-up urine samples into the UPLC-MS/MS sytem showed poor peak shapes which made the integration difficult for quantification. Different amounts of acetonitrile were added to improve the retention on the HILIC column, but these efforts didn't result in better peak shapes. It was clear that the urine matrix contained a lot of interferences (polar compounds) that contributed to the poor peak shape of levoglucosan (Figure 7~~Figure 7~~). Based on a publication of Hinwood et al. (2008) it was decided to pretreat the urine samples via solid phase extraction (SPE amino-propyl cartridges) before injection into the UPLC-MS/MS. From first experiments it was clear that the peak shape improved (Figure 7: Chromatogram of levoglucosan in urine without SPE *clean-up*

~~Figure 7: Chromatogram of levoglucosan in urine without SPE *clean-up*~~

The use of an internal standard or standard addition was further used to compensate for losses during clean-up and compensate for matrix effects.

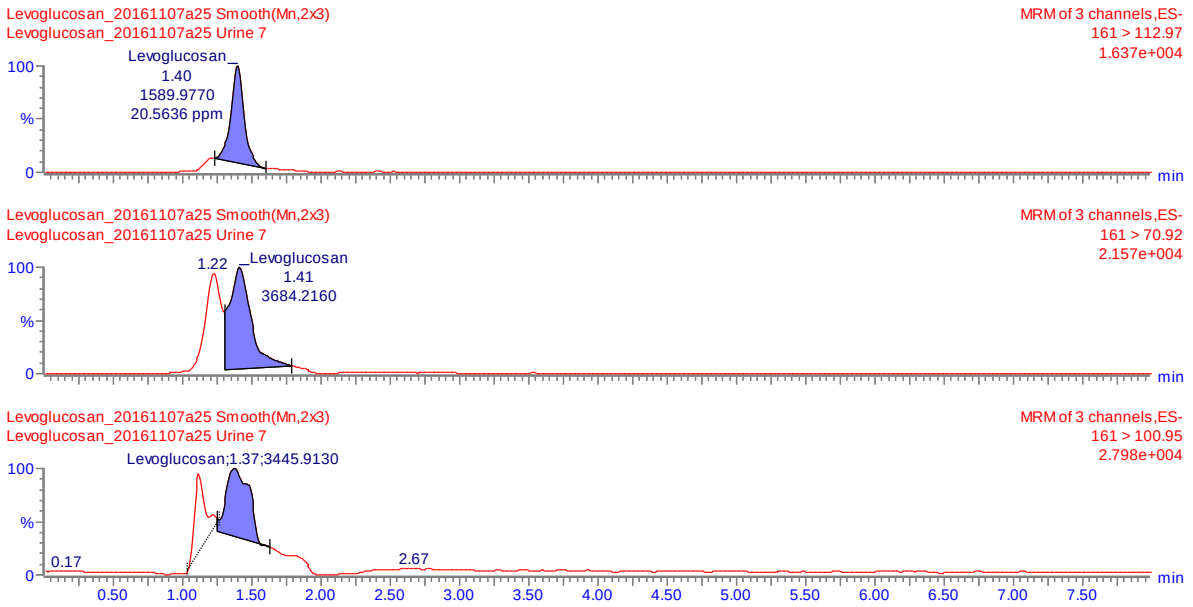


Figure 7: Chromatogram of levoglucosan in urine without SPE clean-up

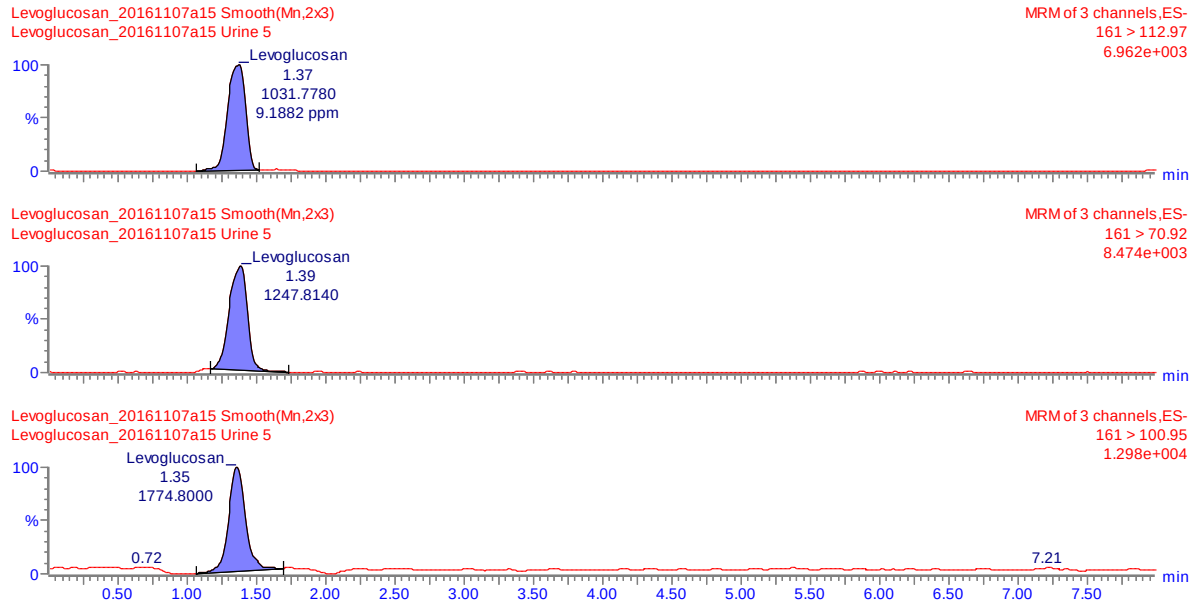


Figure 8: Chromatogram of levoglucosan in urine with SPE clean-up

3.4.2. METHOXYPHENOLS IN URINE

Urine samples were stored at -20°C until analysed. The samples were allowed to thaw and 500 µL aliquots were taken for enzymatic hydrolysis with β-glucuronidase/aryl sulfatase (pH= 5). After overnight incubation at 37 °C a solid phase extraction was performed for clean-up of the samples. The metoxyphenols were eluated with ethylacetate and evaporated under nitrogen to a volume of 1 mL (Hinwood et al., 2008). Ten µL was injected into the GC-MS for analysis. An in-house developed GC-MS method for measurement of degradation products from lignine was used. D8-O-cresol was used as an internal standard during the analysis of the metoxyphenols in urine. (O-cresol is predominant because nonwoody plants have lignine primarily composed of hydoxyphenyl units (Dills et al., 2001)).

In a first stage, an experiment was performed to see if we are able to detect guaiacol and syringol in a pooled urine sample after clean-up of the samples. Guaiacol and syringol were chosen as most favorable biomarkers for woodburning, as they are potentially less influenced by food intake as is the case for eugenol and vanilla (Russell L Dills et al., 2006). In *Figure 9*~~*Figure 9*~~ and *Figure 10*~~*Figure 10*~~, Selected Ion Mode (SIM) chromatograms are shown for guaiacol and syringol in unspiked urine samples. Guaicol and syringol were detectable in the urine sample. The method was sensitive enough for quantification of these urinary methoxyphenols. In a next step, spiking experiments (trueness of the method) were performed and the samples of the feasibility study were measured.

Figure 9 : SIM chromatogram (mass 124) for guaiacol (retention time 4.65 min) in an unspiked urine sample

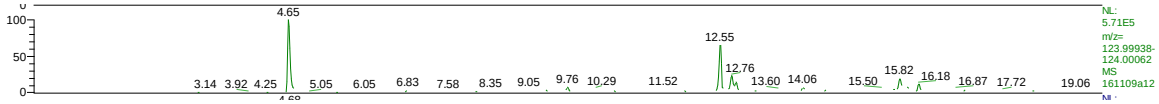
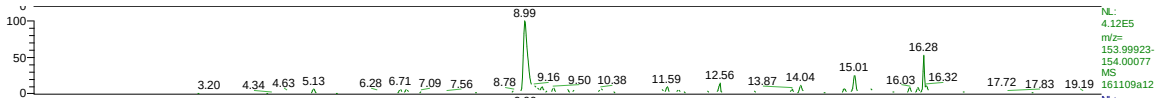


Figure 10 : SIM chromatogram (mass 154) for syringol (retention time 8.99 min) in an unspiked urine sample



3.4.3. HYDROXY-PAHS IN URINE

Based on an in house developed and validated method for 1-hydroxypyrene, the method was extended to the following hydroxyl-compounds: 1-naphtol (1-OH naphthalene), 2-naphtol (2-OH naphthalene), 3-hydroxyphenanthrene, 2-hydroxyfluorene. The method development was based on the publications of Ramsauer et al. (2011) and Onyemauwa et al. (2009). The other isomers of hydroxyphenantrene (1-,2-, 4- and 9- hydroxyphenantrene) and 3-hydroxyfluorene are respectively semi-quantitatively analysed using 3-hydroxyphenantrene and 2-hydroyfluorene.

Urine samples (0.5 mL) were pipetted into a glass tube. 50 µl of 1M acetate buffer with enzyme was added together with 30 µL of internal standards. After overnight incubation at 37°C, acetonitril was added to the samples. Afterwards the samples were centrifuged at 4000 rpm for 10 min. 500 µL of the sample was placed in a vial and a UPLC-MS/MS (reversed phase) analyses was performed. No clean-up of the samples was needed, which reduces the method variability. Small changes to the LC solvent gradient of the UPLC-MS/MS method had to be made to improve separate and retention of 1-naphthol and 2-naphthol. The latter compounds are more polar than 1-hydroxypyrene and the starting conditions of the gradient was therefore adjusted: instead of the starting the gradient with 60% water, 70% of water was added.

The trueness of the method was assessed by means of recovery of a spike. Thereto, five urine samples were fortified with the OH-PAHs at a concentration level of 1µg/L (NB: lower concentrations of 0.05 and 0.1 µg/L were also tested, but were too low in comparison with the concentration present in the urine). The recoveries of 1-OHpyrene and 3-OHphenanthrene, were rangeing respectively between 76 and 103% and 83% and 96% (*Table 11*~~*Table 11*~~), which was within the range of 70-120%. For 1 and 2-naphthol and 2-OHfluorene, the recoveries were outside the acceptance range. For 2-naphtol this could be explained by the low spike levels, compared to the high levels present in the urine samples without spike (*Table 12*~~*Table 12*~~).
Aside from the five above mentioned urine samples, G-EQUAS urine samples from a ring trial in 2014[3] were analysed to get a first idea of the methodology. Not the absolute levels, but rather the magnitude of the levels were the main interest, since the G-EQUAS samples were a few years old and thawed and frozen for multiple times imparing the OH-PAH stability during this time frame.

[3] The results of the ringtrial are online (http://www.g-equas.de/info.htm) available

In a next step more spike concentrations were used and a certified reference material (SRM 3672 – organic contaminants in smoker's urine) were analysed.

Table 11: Recoveries for the OH-PAHs in different urine samples spiked with 1 µg/L of each OH-PAH

recoveries (%)	1-OH-PYR	2-OH-NAP	1-OH-NAP	3-OH-PHEN	2-OH-FLU
urine 1 + 1 µg/L	76%	125%	76%	90%	82%
urine 2 + 1 µg/L	79%	10%	54%	90%	63%
urine 3 + 1 µg/L	76%	82%	88%	84%	8%
urine 4 + 1 µg/L	79%	-104%	57%	83%	79%
urine 5 + 1 µg/L	82%	67%	53%	90%	67%
EQUAS 54/2014-14/15A+ 1 µg/L	87%	-87%	87%	96%	41%
EQUAS 54/2014-14/15B + 1 µg/L	103%	467%	-5%	84%	77%

Table 12: Concentrations of the OH-PAHs present in the different urine samples without spike

measured conc (µg/L)	1-OH-PYR	2-OH-NAP	1-OH-NAP	3-OH-PHEN	2-OH-FLU
urine 1	0.16	1.71	0.26	0.21	0.33
urine 2	0.21	4.79	0.00	0.27	0.40
urine 3	0.18	4.03	0.16	0.31	0.32
urine 4	0.27	16.55	0.09	0.51	0.48
urine 5	0.16	2.30	0.15	0.28	0.23
EQUAS 54/2014-14/15A	0.21	22.78	6.94	0.36	0.42
acceptance range	0.06-0.12	12.72-20.52	8.48-12.44		
EQUAS 54/2014-14/15B	0.37	48.13	28.45	0.32	0.34
acceptance range	0.13-0.31	30.87-45.75	35.33-50.57		

3.5. WOOD SMOKE COMPOUNDS SAMPLING WITH RADIELLO PASSIVE SAMPLER

The Radiello® is a radial diffusive passive sampler, which consists of a ca. 6 cm long cylindric diffusion chamber containing an adsorption cartridge filled with Carbograph 4. It has a weight of 9 gramm inclusive holder, with which it can be attached to clothes (

Figure 11~~*Figure 11*~~). To our knowledge, it has so far not yet been used for sampling of methoxyphenols. Very recently McAlary et al. (2015) tested four passive diffusive samplers (such as Radiello) for assessing indoor air concentrations of some VOCs and the PAH naphthalene. They concluded for Radiello samplers filled with charcoal, that preferably field calibration is needed to estimate the specific uptake rate for naphthalene in each condition (McAlary et al., 2015).
In the current project we did some explorative experiments during the controlled wood smoke exposure experiment (see further). For sampling of PAHs and methoxyphenols, we used the thermal Radiello (membrane type 145) filled with Carbograph4. For the PAHs and methoxyphenols, an aproximative virtual uptake rate was calculated, namely the uptake rate for naphthalene on solvent Radiello divided by three. This was deduced from the fact that the uptake rate was on average 3 times lower for a thermal desoroption Radiello (membrane type 145) compared to a solvent Radiello (membrane type 120) (based on information from benzene, toluene, limonene, p-xlyene, and 1,2,4-trimethylbenzene).
Gaseous molecules diffuse through the diffusive surface of the sampler as a result of a concentration gradient across a diffusive barrier. Diffusing molecules are captured by an adsorbing surface that has a specific uptake rate for each compound[4]: e.g. benzene has an uptake rate of 27.8 mL/min. At the start of the sampling the absorption cartridge is taken out of a glass cartridge holder and placed in the diffusion cylinder. After sampling the absorption cartridge is disassembled, put back into the glass cartridge holder, and stored under nitrogen atmosphere. Date and time are noted at the start and end of the sampling period, to calculate the cumulative adsorbed amount of gaseous molecules (µg/m^3). This was done as follows: the amount of gaseous compounds adsorbed (µg) per number of minutes the sampler was used (min) is divided by the uptake rate of the gas into the absorption cartridge (mL/min) (x 10^6). The Carbograh4 adsorbens was thermally desorbed and analysed using GC/MS. Samples were injected once, following the analysis sequence: blanc control, low concentrated standard, high concentrated standard, control standard, samples.

Figure 11: Radiello diffusive passive sampler: gaseous molecules diffuse through the yellow membraneous cylinder (diffusive surface) from all sides, and are adsorbed onto the adsorbing

[4] Uptake rates for a particular compound and sampler can vary by sorbent type, sample duration and air velocity

surface (protruding metal cylinder on right hand photo), which was filled with a Tenax sorbent in the current study

Table 13: Limit of detection (LOD) and limit of quantification (LOQ) of the PAHs for the Radiello samplers. Calculated based on the theoretical sampling volume of 0.0027 m³ assumed to pass over the Radiello sampler in the time frame of 5 hours as it was used in the fire wood experiment of the current study (feasibility study)

	Radiello	
	LOD(ng/m³)	LOQ(ng/m³)
	ng/m³	ng/m³
Naphthalene	0,057	0,191
Acenaphthylene	0,206	0,688
Acenaphthene	0,120	0,400
Fluorene	0,190	0,635
Phenanthrene	0,202	0,675
Anthracene	0,518	1,728
Fluoranthene	0,092	0,305
Pyrene	0,125	0,417
Benzo(a)anthracene	6,520	21,733
Chrysene	0,473	1,578
Benzo(b)fluoranthene	8,215	27,384
Benzo(k)fluoranthene	25,602	85,340
Benzo(a)pyrene	25,930	86,434
Indeno(1,2,3,c,d)pyrene	0,810	2,700
Benzo(g,h,i)perylene	9,979	33,264

3.6. PAH ANALYSIS VIA PDMS/TENAX CARTRIDGES

The cartridges for sampling and thermal desorption were stainless-steel tubes (Markes International Ltd.) with the following dimensions: 31/2 inch length, ¼ inch o.d. and packed with PDMS/Tenax sorbent material. Prior to each use, the sampling tubes were conditioned by thermal cleaning under a nitrogen flow rate of 75 mL/min at 300°C for 60 min, sealed with end caps and stored under nitrogen atmosphere to prevent any contamination of the sorbent. After loading, the samples were immediately sealed again with the end caps and stored under nitrogen atmosphere until analysis. The samples were analyzed within seven days after collection.

Analysis

The 16 EPA-PAHs (except DahA) were measured in the air samples. All the analyses were performed on a TD-GC-MS system, which consisted of a TD100 Thermal desorber (TD, Markes International Ltd.) and coupled to a gas chromatograph Thermo Trace GC Ultra and a mass selective detector Thermo DSQII (Thermo Fisher Scientific Inc.). Thermal desorption of the sampling tubes was carried out at 300°C with a flow rate of 20 mL/min for 12 min, followed by cold trap (10 °C) focusing. Thermal desorber system control was performed using Thermal Desorber System control Program version 4.4.1 (Markes International Ltd.). The GC-MS system control, data logging and data handling were performed using Xcalibur 2.0 software (Thermo Fisher Scientific Inc.). The chromatographic separation of the PAHs was conducted on Rxi-5Sil MS capillary column (Restek) 30m x 0.25mmi.d. x 0.25 mmfilm thickness. Helium was used as a carrier

gas. All compounds were analyzed by using time scheduled Selected Ion Monitoring (SIM) of the most intensive ion fragment of each compound. The MS ion source and the transfer line from the GC to the MS were kept at 250 °C and 320°C respectively.
The LOD and LOQ of the PAHs for the PDM-Tenax samplers was as indictad below. In this calculation a sampling volume 0.1026 m^3 has been used (volume of air passed over cartridge in 308 minutes experiment, with pump flow of 333 mL/min).

Table 14: PAH compounds measured in the air samples collected via PDMS/Tenax cartridges

Abbreviation	Compounds	Number of rings	Molecular weight	IARC, vol.92, 2010	LOD (ng/m^3)	LOQ (ng/m^3)
NAP	naphthalene	2	128.2	2B	0,001	0,005
ACY	acenaphthylene	2	152.2	-	0,005	0,018
ACE	acenaphthene	2	154.2	3	0,003	0,010
FLU	fluorene	2	166.2	3	0,005	0,017
PHE	phenanthrene	3	178.2	3	0,005	0,018
ANT	anthracene	3	178.2	3	0,014	0,045
FLA	fluroanthene	3	202.3	3	0,002	0,008
PYR	pyrene	4	202.1	3	0,003	0,011
BaA	benz(a)anthracene	4	228.3	2B	0,170	0,566
CHR	chrysene	4	228.3	2B	0,012	0,041
BbF	benzo[b]fluoranthene	4	252.3	2B	0,214	0,713
BkF	benzo[k]fluoranthene	4	252.3	2B	0,667	2,223
BaP	benzo[a]pyrene	5	252.3	1	0,675	2,251
IcdP	indeno(1,2,3-c,d)pyrene	5	276.3	2B	0,021	0,070
DahA	dibenzo[a,h]anthracene	5	278.3	2A		
BghiP	benzo[g,h,i]perylene	6	276.4	3	0,260	0,866

3.7. LEVOGLUCOSAN ANALYSIS IN ACTIVELY SAMPLED PM OR BC FILTERS

Blank quarz tissue filters or were spiked with levoglucosan to check the extraction efficiency. The filters were threated in the same manner as described for the scalp hair samples. After spiking, an acetonitrile was added and the samples were vortexed and sonicated for 30 minutes. After sonication, the extract was transferred to a collection vial and the extraction was repeated twice. The extract was evaporated under nitrogen to 250 µL. Samples were analyzed using ultra-performance liquid chromatography combined with triple tandem quadrupole mass spectrometry (UPLC-MS/MS) as described by You et al. (2016). The methodology was also used for the Teflon coated glass fibre BC filters from the microaehtlometer, analysed for levoglucosan in the controlled exposure study (see furtherFout! Verwijzingsbron niet gevonden. ~~4.3.1.~~).

CHAPTER 4 CONTROLLED WOOD SMOKE EXPOSURE STUDY

4.1. AIM

The aim of this phase of the study, was to determine whether concentrations of levoglucosan. PAHs and methoxyphenols - emitted during controlled combustion of wood - are measurable using personal samplers and urinary biomarkers.

4.2. MATERIALS AND METHODS

Study design

One voluntair field worker, non-smoker, not occupationally exposed to PAHs, performed a controlled wood smoke experiment. Birch wood was burned in a basket-like type of woodburning device in the open air. During the controlled exposure period the field worker stayed withing four meters diameter of the wood fire. The wood burning took place from 10:52 in the morning until 16:00 in the afternoon of 8/9/16 (5.13h). During this period the field worker was wearing gloves.

The day before the wood burning experiment, background measurements were done for all samplers used in the experiment (Figure 12~~Figure 12~~).

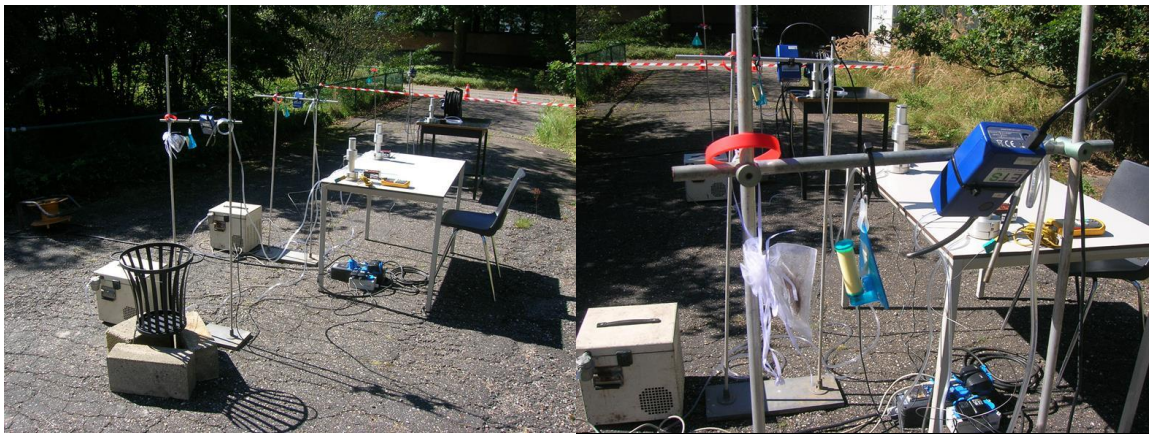


Figure 12: Sampling equipment installed on the day before the wood burning experiment (13:56 on 7/9/16 until 10:45 on 8/9/16)

Figure 13 Sampling equipment sampling on the day before the wood burning experiment (10:52 on 8/9/16 until 16:00 on 8/9/16)

Passive and active (personal) samplers

Respectively near to the wood basket (0m), at 1.5m and 4m away from the basket, active and passive samplers were placed. The compounds analysed using these samplers are listed in Table 15~~Table 15~~.

As passive sampler, a sampler of the type Radiëllo filled with Carbograph4, a wristband and a nylon gauze filled with human hair were mounted on a frame at a hight of 1.5m above the ground. The active samplers used were the $PM_{2.5}$ Harvard impactor (foreseen with Whatman Grade QMA 37mm filter) connected to a Tecora pump with a flow rate of 10 L/min. Furthermore, the following active samplers were used: aethalometer type 51 (flow rate 150 mL/min) and a PDMS-Tenax cartridge connected with a Gillian pump with flow rate of 333 mL/min were mounted on a frame at a hight of 1.5m from the ground. The field worker, who was mainly operating at an average distance of about 1.5m from the fire, also wore a personal $PM_{2.5}$ sampler (Buck-VSS) which sampled air (with a flow of 2 L/min) at the breathing zone.

Urine sampling

All urine voids were collected by the field worker from 15h before the wood burning experiment (i.e. 19:15 on 7/9/16) until 24h after the start of the wood fire (i.e. 10:30 on 9/9/16). The urine was collected in a pre-labeled polyprobylene 500mL container. The samples were stored in -20°C. Urine sampling was approved by the ethical committee approval of the Antwerp University Hospital (Belgian Registration number: B300201316329.

Table 15: Samplers used and sampling frequency before and during the wood burning experiment

Samplers	Compounds analysed	Position relative relative to fire	Sampling frequency	
			During wood burning	Before wood burning (background)
Active sampling				

Samplers	Compounds analysed	Position relative relative to fire	Sampling frequency During wood burning	Sampling frequency Before wood burning (background)
Micro Aethalometer type 51[a] (T60=Teflon coated glass fiber filter)	Black Carbon (on-line), levoglucosan off-line in filter (UPLC-MS/MS)	0. 1.5. 4m	Sampling every minute	Sampling every minute
$PM_{2.5}$ Harvard Impactor (Quarz: Whatman QMA 37mm)	Levoglucosan (UPLC-MS/MS)	0. 1.5. 4m	N=1 sample	N=1 sample
Personal $PM_{2.5}$ sampler Buck-VSS (Quarz: Pall TissueQuartz 25mm)	Levoglucosan (UPLC-MS/MS)	breathing zone	N=1 sample	N=1 sample
PDMS-Tenax cartridges	PAHs, methoxy-phenols(?) (TD-GC-MS)	0. 1.5. 4m	N=1 sample	N=1 sample
Passive sampling				
Urine	PAHs, levoglucosan, methoxyphenols (UPLC-MS/MS)	urine	N=2 during and N=4 after end of experiment	N=4 before experiment
Scalp hair mounted in gauze	Levoglucosan, methoxyphenols (UPLC-MS/MS)	0. 1.5. 4m	N=1 sample	N=1 sample
Silicone wristband mounted on frame	PAHs (LC-MS)[5]	0. 1.5. 4m	N=1 sample	N=1 sample
Radiëllo - Carbograph	PAHs, methoxyphenols(?) (TD-GC-MS)	0. 1.5. 4m	N=1 sample	N=1 sample

[a]: dimensions of microaethalometer type 51: 117x66x38mm

4.3. RESULTS

4.3.1. LEVELS OF BLACK CARBON, LEVOGLUCOSAN AND PAHS IN AIR NEAR TO THE FIRE

The fire was lit in the morning at 10:52 and ceased at 16:00. The peak temperature in the basket was between 600 and 1000 degrees, which is a normal temperature for a wood fire (inlet of Figure 14~~Figure 14~~).

→ Black carbon in air (microaethalometer)

As expected, near to the fire, at a distance of 0m, the levels of BC were very high and lowered further away from the fire: on average 32 647 ng/m^3 at 0m, 11 358 ng/m^3 at 1.5m, and 2433 ng/m^3 at 4m distance. The background BC levels measured the day before the experiment at this sampling locations were on average 742 ng/m^3, or about three times lower than the levels at a distance of 4m of the fire during it was burning (Figure 14~~Figure 14~~). As a comparison, in the Flemish HEAPS study, in which BC was measured at 42 locations in the urban environment of

[5] TD-GC-MS analysis was also tested on the wristband samples

Antwerp (year 2011), the median (P_{25}-P_{75}) 24h-levels of BC in summer and in winter were respectively 1292 (917-2000) ng/m^3 and 3662 (1152-4710) ng/m^3 (Dons et al., 2014).

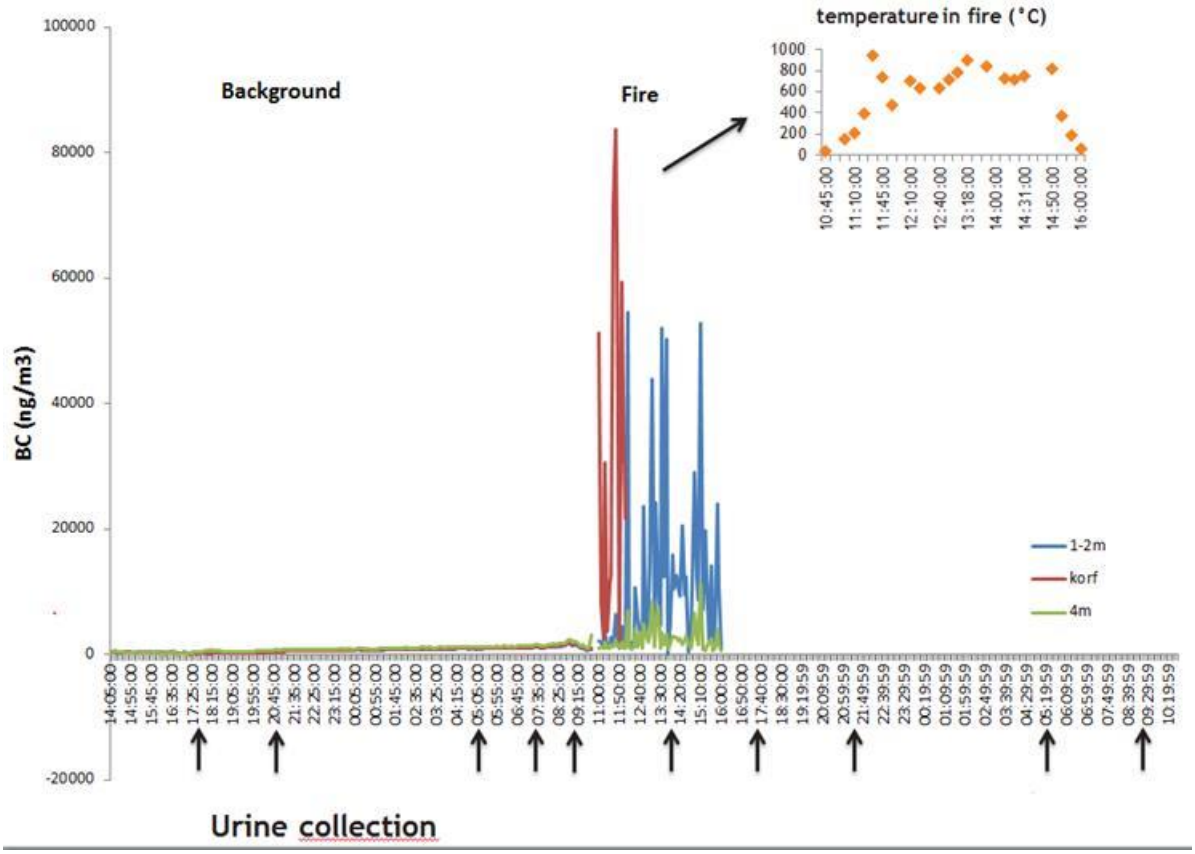


Figure 14: Concentrations of BC at the wood basket, and at a distance of 4m. During the 5.13 hours woodfire experiment the temperature of the fire was measured in the basket (see inlet). Before, during and after the fire, urine was collected from the field worker which was operating the fire and stayed within a distance of 4m of it during the whole wood burning experiment

→ Levoglucosan in filters

Levoglucosan was analysed in $PM_{2.5}$ filters of a static Harvard impactor, and as a try-out also in the disks of the micro-aethalometer. On each of the distance of the fire, a sampler of the different types was mounted. The concentrations of both samplers differed, with the levoglucosan concentration (NB expressed in µg because of the high levels) measured on the filters of the Harvard impactor being higher compared to those collected on the microethalometer disk: at 0m respectively 462.9 and 123.4 µg/m^3 at 1.5m distance respectively 19.4 and 9.3 µg/m^3 and respectively 2.2 µg/m^3 and below LOQ at 4m distance from the fire. This difference may be due to the fact that the samplers did not get exactly the same fumes from the fire, which was plausible as the fire was close to the samplers. They sample another fraction of PM, and it might be that there are some more losses within the aetholometer compared to the Harvard impactor. The personal $PM_{2.5}$ sampler levels of levoglucosan in the breathing zone of the field worker were 20.9 µg levoglucosan/m^3, which was at the higher end range of the values measured on the static samplers at a distance of 1.5m of the fire.

The concentrations of levoglucosan in the air, in the time frame of 20 hours before the fire was started, was 17.7 ng/m3 on the personal sampler (left on the sampling location during that period). With the Harvard impactor and microaethalometer, each time three measurements were done being: 2.06, 2.15 and 18.7 ng/m^3 on a distance of 0, 1.5 and 4m of the fire inactive fire. The microaethalometer levoglucosan levels were on those distances all below the quantification limit of 20 ng/m^3. For comparison, the median levels of levoglucosan measured (using $PM_{2.5}$ Harvard impactor) in Menen, Genk and Houtem in the period of September 2015 were between 24.3-44.6 ng/m^3 (Koppen et al., 2016). Levoglucosan measurements done in the Teflon coated glass fiber filter disks of a microaethalometer, which is used to measure BC, deviated to some extend from levels measured in the PM fraction (e.g. collected using an Harvard Impactor filter set-up with Quartz filter). This needs further examination in a repetitive sampling set-up.

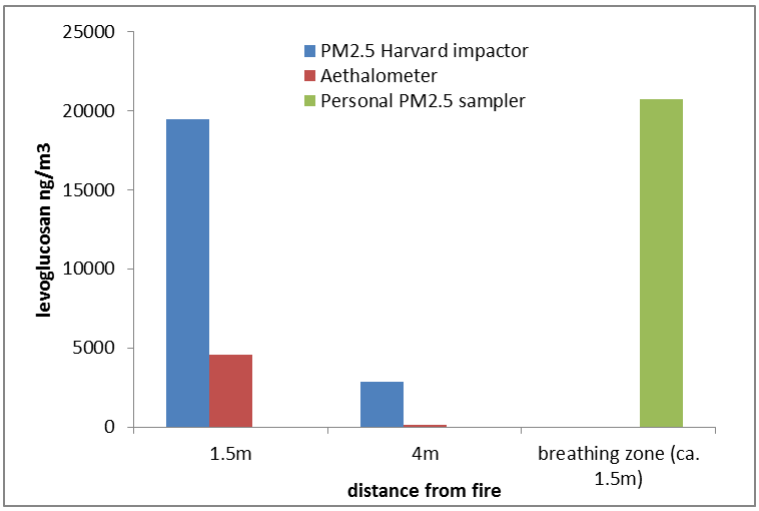


Figure 15: Concentrations of levoglucosan measured at 1.5 and 4m distance of the fire. The levels were measured in the $PM_{2.5}$ fraction collected using an Harvard impactor (quartz filter) or a Buck-VSS Personal Sampler(quartz filter), or using a microaethalometer for BC analysis (Teflon coated flass fibre filter). NB: 'breathing zone' = breathing zone of the field worker, mainly operating at about an average distance of 1.5 m from the fire

→ Methoxyphenols in air (PDMS-Tenax)

The methoxyfenols guaiacol (2-methoxy phenol) and syringol (2,6-dimethoxy phenol) were determined semi-quantitatively[6] in the GC-MS chromatograms of the PDMS-Tenax cartridges (d8-naphthalene was used as internal standard to relay to). The levels determined in this way are therefore indicative, i.e. semi-quantitative. The air sampling was done only during the 5 hours fire experiment. This period was long enough to measure detectable levels of these methoxyphenols. Near to the fire at 1.5m, the concentration of guaiacol and syringol was 122 ng/m^3 and 188 ng/m^3. At a distance of 4 meters the levels were dropped considerably to a level of respectively 21 and 33 ng/m^3. For comparison, Bieniek & Stepien (2011) measured a guaiacol concentration of 9.9 ng/m^3, in the breathing zone of coke oven workers, during the workshift.

[6] The ions with m/z= 109 and 154 at a retention time of 8.9 and 11.9 min, were selected as quantitation ions of guaiacol and syringol respectively

Guaiacols are expected to be predominant when coniferous wood (softwood) is burned, syringols when burning nonconiferous (hardwood). In the current experiment softwood was used. Both guaiacol and syringol were measured semi-quantitatively in the air around the fire via the PDMS-Tenax cartridges. The semi-quantitatively assessed levels of both compounds were in the same order of magnitude.

→ PAHs in air (PDMS-Tenax)

As expected, the PAH concentrations measured using PDMS-Tenax cartridges, were decreasing away from the fire. PAHs being more apparent in wood smoke are known to be NAP PHE, FLA and PYR. At 4m distance the levels of these latter PAHs were present in higher levels compared to the other PAHs, and as expected considerably higher (10-40x) than winter (Jan-Feb2016) PAH levels observed in a Flemish PAH measuring campaign in the Flemish regions Genk, Menen, Houtem (Figure 16~~Figure 16~~) (Koppen et al., 2016; same sampling technique as used in the current study).

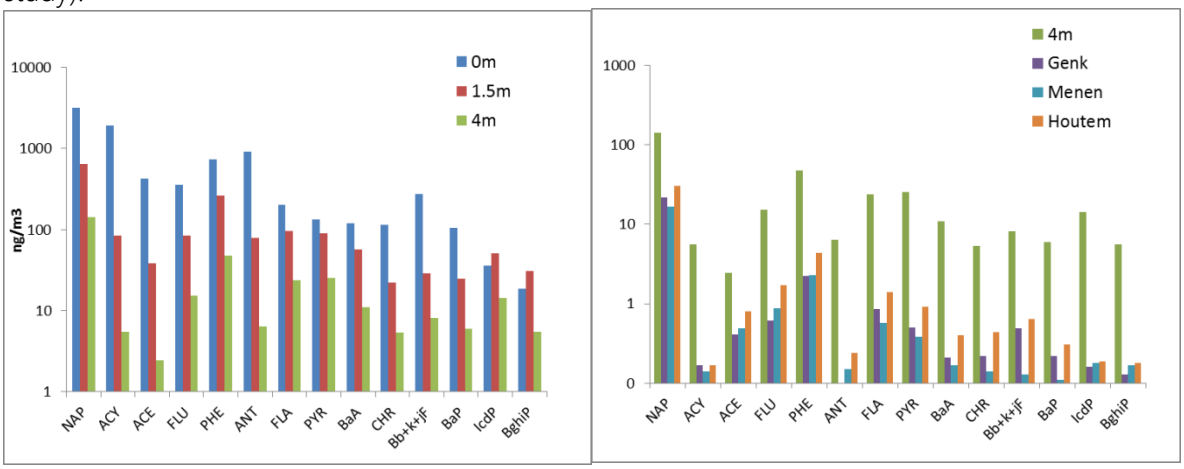


Figure 16: Air concentrations of PAHs measured at a distance of 0m, 1.5m and 4m of the fire, during the 5 hours sampling period (left). Comparison of the levels at 4 m distance to air concentrations measured in the winter during a measuring campaign in 3 regions of Flanders (average levels in Genk, Menen, Houtem for period jan-feb 2016; 48h-samples collected using PDMS-Tenax at respectively N=6, 6 and 1 sampling location(s) in residential areas) (right). Both graphs are on log-schale.

→ PAH levels in wristband

During 20h before the fire and during the time frame of the fire, wristbands were mounted at the different distances of the wood basket (0, 1.5 and 4m). The wristband concentrations of PAHs in those wristbands were all below the quantification limit. For the wristbands mounted for only 5.13h in the near of the fire, a gradient could be observed away of the fire. During this short sampling period, the higher molecular weight PAHs (from BaA to BghiP) were measurable above the fire, but not at distance further away from the fire. The lower molecular weight PAHs, namely mainly

ACY, ACE, FLU, PHE, ANT, FLA and PYR seem to be compounds feasible to trap and sample with the wristbands. For these compounds, the decrease in concentration at distance 1.5m compared to 0m, and 4m compared to 1.5m, was similar to what was observed in the air measured via the PDMS-Tenax cartridges (Figure 17~~Figure 17~~). Aside from the classical PAHs, also retene was analysed in the wristbands. That compound, was only detectable near to the fire (58.3 ng/wristband). (NB: In the PDMS-Tenax cartridges the compound could not be identified, because of a high background and lack of an internal standard). Based on these limited amount of results, it seems that wristband sampling can be used for relative comparison of exposure to the more volatile PAHs via the air.

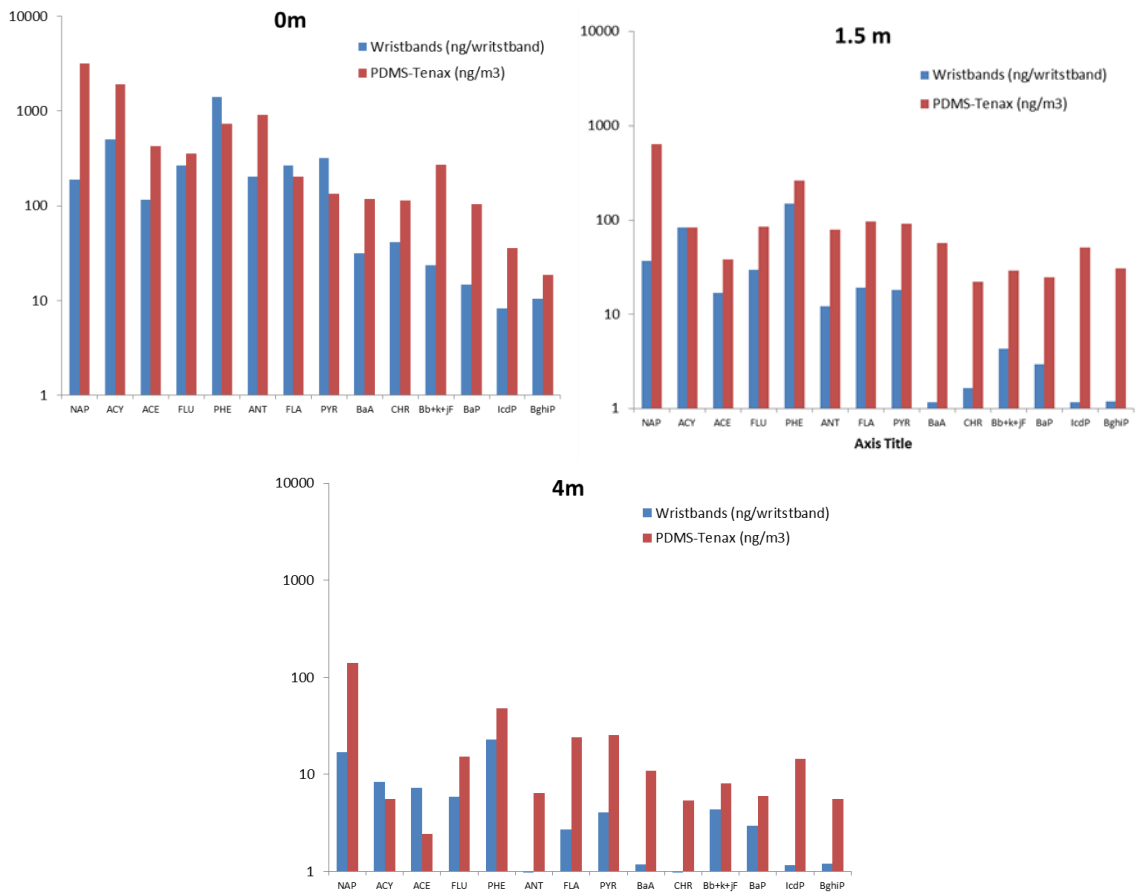


Figure 17: Profile of accumulated PAHs measured on the wristbands (in ng/wristband) mounted at the wood basket, and at 1.5m and 4m distance from the fire burning during the 5-hours woodsmoke experiment. For comparison, the profile of PAHs measured on the PDMS-Tenax cartidges was also included. Graphs on log-scale

→ PAH passive sampling with Radiello

In the current project we did some explorative experiments with the Radiello passive sampler - for sampling of PAHs and methoxyphenols - (membrane type 145) filled with Carbograph4. These experiments were purely explorative, as the uptake rate of those compounds through the membrane of the sampler was not known. or the PAHs and methoxyphenols, an aproximative

virtual uptake rate was calculated, namely the uptake rate for naphthalene on solvent Radiello divided by three. This was deduced from the fact that the uptake rate was on average 3 times lower for a thermal desoroption Radiello (membrane type 145) compared to a solvent Radiello (membrane type 120) (based on information from benzene, toluene, limonene, p-xlyene, and 1,2,4-trimethylbenzene).
It was possible during the 5h experiment to trap the PAHs using the passive Radiello. It showed to have potential to be used for PAH sampling. Even when the concentrations were determined semi-quantitatively, they were in the range of what was seen using PDMS-Tenax active sampling. The higher molecular weight PAHs tended to deviate more from the PDM-Tenax concentration, but that was to expected, as they are mainly bound to the particle phase of the air. Guaiacol and Syringol were not detected via the 5-hours passive sampling with the Radiello.

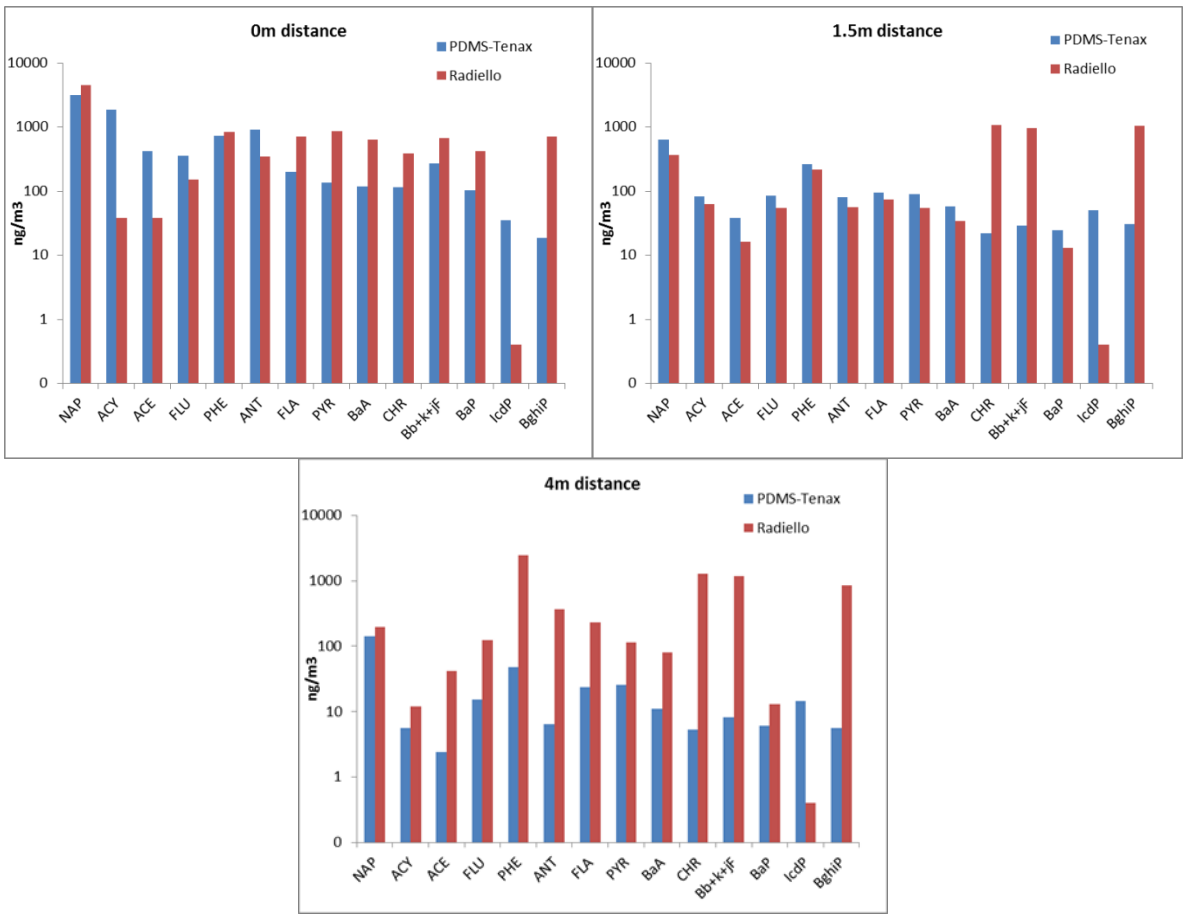


Figure 18: Profile of accumulated PAHs measured via Radiello passive sampler (in ng/m³) mounted at the wood basket, and at 1.5m and 4m distance from the fire burning during the 5-hours woodsmoke experiment. For comparison, the profile of PAHs measured on the PDMS-Tenax cartidges was also included. Graphs on log-scale

4.3.2. URINARY LEVELS OF POTENTIAL WOOD SMOKE TRACERS OVER THE TIME SPAN OF THE FIRE EXPERIMENT

The field worker collected ten consecutive urine samples the day before, the day itself and the day after the wood smoke experiment. The creatinine levels in the urine were rather high, especially those in the afternoon-evening of the wood smoke experiment, being 253, 261 and 333 mg/dL (Figure 19 ~~Figure 19~~). As a comparison the median (P_{25}-P_{75}) creatinine levels of morning urine collected in 209 adults (age 49-65y) participating in the FLEHS-3 human biomonitoring was 70 (42-128) mg/dL.[7]

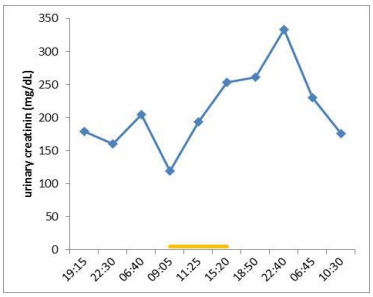


Figure 19: Urinary creatinine levels of the field worker collecting all consecutive urine samples the day before the wood smoke experiment (at time points 19:15, 22:30), the day itself (6:40, 9:05, 11:25, 15:20, 18:50, 22:40), and the next day (6:45 and 10:30). The yellow line indicates the time frame of the wood smoke experiment (10:52-16:00)

During the 3 days of the urine collection in the current study (7-9/9/2016), the maximum outdoor temperature was respectively 27, 28 and 24°C. On the day of the fire experiment (8/9/16), the field worker reported a temperature of 30.1°C near to the basket, before the fire was lit. It was therefore plausible, that correction for (the high levels of) creatinine, caused an overcorrection (because of high creatinine levels in the nominator). Therefore, the urinary excretion rate (mass eliminated/hour) was also calculated, to correct for a changing water content in urine. For that calculation the metabolite concentration in urine is multiplied by the volume of the void and divided by the duration of time the void was accumulating in the bladder (time point of void minus time point of previous void).

Aside from levoglucosan (LEV), guaiacol (GUA) and syringol (SYR), the OH-PAHs 2OH-naphthalene (2OH NAP), 2+3OH fluorene (2+3OH FLU), 2+3OH phenanthrene (2+3OH PHEN) and 1-OH pyrene (1OH PYR) were analysed in urine. A clear peak, which had a retention time just before 2OH NAP, was clearly present, but could not be identified. It was further reported as 'unidentified OH PAH'.

*Table 16: Urinary compounds in 10 samples from one individual, collected before, during (indicated in yellow), and after the woodfire experiment. The fire was lit in the morning at 10:52 and ceased at 16:00. The maximum concentration observed after starting of the fire was indicated in red (NB: not necessarily the maximum of all measurements done). The coefficient of variation of the pre-exposure urinary levels was calculated ($CV_{pre\text{-}exposure}$). The median urinary concentration was calculated for 12h time periods pre and post exposure (12h before, 0-12h, 12-24h). Furthermore, the urinary excretion of the compounds was calculated for the urine samples collected in those time intervals (= concentration *urinary volume/time frame in which the urine was collected)*

Time	LEV	GUA	SYR	2OH-NAP	unidentified OH PAH	2+3OH-FLU	2+3OH-PHE	1OH-PYR
Concentration	µg/L	µg/L	µg/L	ng/L	ng/L	ng/L	ng/L	ng/L
07/09/16 19:15	6160.0	260.5	84.6	5203.0	3424.6	379.0	227.0	91.0
07/09/16 22:30	5230.0	107.1	28.1	4654.0	3585.9	330.0	200.0	96.0

[7] http://www.milieu-en-gezondheid.be/sites/default/files/atoms/files/Samenvatting%20volwassenen%20ref%20steunpunt%203.pdf

Time	LEV	GUA	SYR	2OH-NAP	unidentified OH PAH	2+3OH-FLU	2+3OH-PHE	1OH-PYR
08/09/16 06:40	4660.0	229.5	53.6	5198.0	3725.1	468.0	343.0	190.0
08/09/16 09:05	2690.0	37.6	24.3	5142.0	3847.3	25.0	324.0	175.0
08/09/16 11:25	4190.0	92.8	26.7	2680.0	2220.8	335.0	178.0	73.0
08/09/16 15:20	7200.0	102.2	46.2	25.0		25.0	429.0	201.0
08/09/16 18:50	8140.0	84.4	47.7	10692.0		25.0	572.0	253.0
08/09/16 22:40	8020.0	63.5	43.6	5195.0	6411.8	25.0	583.0	264.0
09/09/16 06:45	4030.0	54.9	28.9	5579.0	1822.0	613.0	453.0	226.0
09/09/16 10:30	2980.0	36.1	16.7	4732.0	3389.0	454.0	223.0	126.0
$CV_{pre-exposure}$ (%)	31.3	65.8	58.5	5.2	5.0	64.0	25.8	37.5
12h-median	µg/L	µg/L	ng/L	ng/L	ng/L	ng/L	ng/L	ng/L
12h before	4660.0	107.1	28.1	5142.0	3725.1	330.0	324.0	175.0
0-12h	7610.0	88.6	44.9	3937.5	4316.3	25.0	500.5	227.0
12-24h	3505.0	45.5	22.8	5155.5	2605.5	533.5	338.0	176.0
Post/pre exposure ratio of 12h-median concentration								
0-12h/12h before	1.63	0.83	1.60	0.77	1.16	0.08	1.54	1.30
12-24h/12h before	0.75	0.42	0.81	1.00	0.70	1.62	1.04	1.01
Excretion rate	µg/h	µg/h	µg/h	ng/h	ng/h	ng/h	ng/h	ng/h
12h before	625.2	18.6	5.3	745.2	554.6	40.9	43.1	22.9
0-12h	1045.7	13.0	6.2	705.7	308.0	15.6	66.9	30.0
12-24h	684.2	8.9	4.5	1006.4	508.6	104.1	66.0	34.4
Post/pre exposure ratio of 12h-urinary excretion rate								
0-12h/12h before	1.67	0.70	1.18	0.95	0.56	0.38	1.55	1.31
12-24h/12h before	1.09	0.48	0.85	1.35	0.92	2.55	1.53	1.50
Total excretion								
12h before	6617	197	55.7	7886.8	5869.2	432.9	456.0	242.5
0-12h	11763.9	146.4	70.1	7938.8	3686.1	175.1	752.4	337.8
12-24h	2565.7	33.3	16.7	3773.8	1907.2	390.5	247.4	128.8
Post/pre total exposure ratio of 12h-total urinary excretion								
0-12h/12h before	1.78	0.74	1.26	1.01	0.63	0.40	1.65	1.39
12-24h/12h before	0.39	0.17	0.30	0.48	0.32	0.90	0.54	0.53

It should be marked that the background OH-PAH levels and LEV levels observed in the field worker (later also participating as HV-03 in the pilot2 feasibility study) were rather high, due to (PAH/wood burning?) exposure in daily life of the field worker (see pilot 2 feasability study furtheron). Four urine samples were collected before the start of the wood burning experiment, two in the evening before (19:15 and 22:30 of 7/9/16), and two morning urine samples (6:40 and 9:05 of 8/9/16). The variability in concentrations among those samples was seen as the background variation in urine levels. Based on these limited results it was observed that the levels of guaiacol, syringol and 2+3OH-fluorene showed a high variability, with a CV between 58.5 and 65.8%. The variability in urinary levoglucosan and 2+3OH-phenanthrene was acceptable, being 31.3 and 25.8%, in case one considers about 30% as a possible technical variability among repeated measurements. 2-OH naphthalene and the unidentified OH-PAH component, showed a small variation among the four pre-exposure urine samples of about 5%, but the background levels were relatively high (about 5000 ng/L for 2OH-NAP). Both the variation and/or high background levels indicate that also other exposure sources present in the daily life of the field worker, had an influence on the urinary levels.

In the current experiment, the increase of urinary markers caused by the wood burning, was most clear in case the concentration of the urinary markers was expressed as mass per volume (µg/L or ng/L). After exposure, the levels of the field worker's urinary markers showed the following trend (Table 16~~Table 16~~):

- For levoglucosan a 50% increase in the urinary levels above background levels, was observed between 5h and 12h after litting the fire, with a peak at 8h after the start of the fire. The background level in the urine samples collected before the fire experiment (12h before) was 4660.0 µg/L.
- Syringol showed an increase in urinary levels, less than 6h after the start of the experiment, however the level was not above the background concentration measured the day before the fire (the median of the latter being 28.1 µg/L). The guaiacol urinary levels were not affected by the wood burning activities (being 107 µg/L before the fire and 88 µg/L in the first 12h after the start of it). This was unlike the observation made by Dills et al. (2001), who reported an increase of guaiacols and syringols between respectively 1.7-4.7 and 1.8-18 fold post vs. prior exposure. Dills et al. (2006) indicated that the threshold for detection of an acute exposure event (using methoxyphenols) would be approximately 760 µg/m^3 particulate matter from woodsmoke, as was not reached in the current experiment.
- 2-OH naphthalene showed a short, but clear peak of 100% increase 8h after after litting the fire, which was visible despite rather high background levels observed on the day before the experiment, of 5142.0 ng/L. Li et al. (2015a) reported earlier in a controlled wood smoke exposure experiment, that the peak of urinary 2OH NAP was most increased compared to the other OH-PAHs (variable increase among individuals of a factor 2 to almost 10).
- 2+3OH-fluorene showed increased exposure levels 20 h after the start of the wood burning experiment, which was however not above background levels observed before the experiment.
- 2+3OH phenanthrene in urine was observable 5h after the exposure and the (60-70%) increase in metabolite lasted until 20h. The peak of almost 100% increase could be seen 12h after the start of the experiment.
- 1-OH pyrene urinary levels showed a more or less similar pattern as the 2+3OH phenanthrenes, with an increase starting 8h after litting the fire, a peak at 12h, and elevated levels visible until 20h after the start of the experiment.

Based on these limited amount of results, it was concluded that syringol, guaiacol showed too variable levels in urine to allow detection of wood smoke exposure, even in the current extreme exposure conditions, that would only occur during unvented indoor fires or wild fire exposure, but likely not in ambient air of residential areas with wood burning. The variable background 2+3 OH fluorene levels were most probably due to exposures in daily life of the field worker (examined in pilot2 feasability study furtheron), and therefore it was decided to keep this compound for further analysis in the feasibility studies, dispite some variability in the levels.

It is impossible to collect a single urine sample at the peak excretion of a person. Li et al. (2015a) suggested to collect multiple urine samples, and possibly pooling them to obtain an estimate of the average exposure. Therefore, in the current experiment 12-h median concentrations were calculated for the time frame of 12h before the start of the experiment, within 12h, and between 12 and 24h after the start. Comparing the median levels of the first 12 hours vs. those 12h before exposure, an increase of levoglucosan, syringol, the unidentified OH-PAH, 2+3OH phenanthrene and 1-OH pyrene of respectively 1.63, 1.60, 1.16, 1.54 and 1.30 was observed. For FLU increased with 1.62, only later in the time frame of 12-24h post litting the fire. This means, that averageing or pooling several urine samples, would allow to observe the increased levels, except for 2-OH

naphthalene, where the peak increase was not anymore visible when averageing over a 12h period. The excretion rate during 12h periods was also calculated (mass of compound excreted per h). It appeared to allow to observe the exposure peak of levoglucosan within the first 12h and of 2OH-naphthalene, 2+3OH-phenanthrene and 1OH pyrene in either the second or both 12h periods after the exposure.

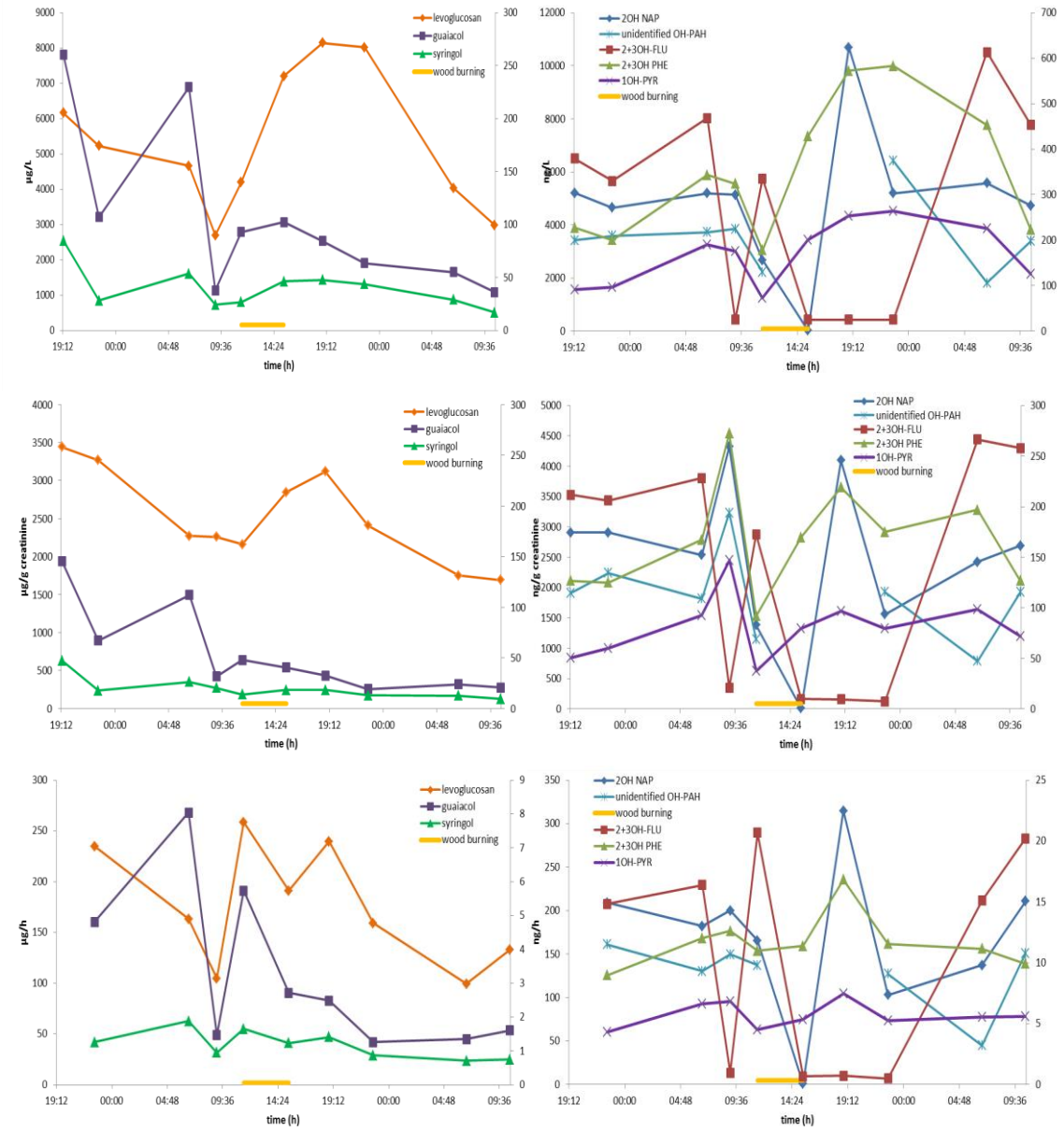


Figure 20: Concentration of levoglucosan, guaiacol and syringol and OH-PAH metabolites, from a field worker exposed for 5 hours to a wood fire (that time period indicated by yellow line in the graphs). The field worker collected urine before, during and after the fire. The urinary levels were either expressed in mass per liter urine (2 graphs on top row), mass per gram creatinine (middle row), and mass per hour i.e. urinary excretion rate (2 lower graphs. NB: levoglucosan values indicated on left axis, guaiacol and syringol values to be read from the right axis. 2OH NAP and unidentified OH-PAH metabolite numbers need to be read on the left axis, the other PAH levels are indicated on the right axis of the graphs

The concentrations of the chemicals measured at 1.5m distance were assumed to reflect the personal exposure of the field worker. The inhaled concentration of the compounds were calculated for 5.13h, the period of the wood fire burning, taking a theoretical inhalation volume of 16 m^3/day. Based on this theoretical approach the ratio of the amount of the compound or metabolite excreted via the urine in the 0-12h and 12-24h period after litting the fire (nmol/12h) over the inhaled amount in that period. For the OH-PAH metabolites, the levels in urine were in the range of the parent compounds that were inhaled. This means that the levels found in urine were most probably mainly originating from what was inhaled.
On the other hand, the amount of levoglucosan, guaiacol, syringol excreted in urine was considerably higher than the inhaled dose during the experiment. This indicates that the background intake was higher, than what was taken up during the fire experiment. Furthermore, it was earlier reported, that exposure to the higher volatile methoxyphenols guaiacol and syringol may be underestimated by measuring only the particle phase of the smoke (Russell L Dills et al., 2006). Using the PDMS-Tenax sorbent, both particle and gas phase of the smoke were trapped, but the compounds were measured semi-quantitatively for this experiment. For levoglucosan the argument of higher exposure may be valuable, as that compound was only measured on PM filters, i.e. in the particle phase.
Despite the possible confounding of these urinary parameters, they clearly showed decrease in urinary excretion (nmol excretion/12h) diminishing with time after exposure, being indicative for a peak exposure.

Table 17: Ingested dose of the analysed woodsmoke compounds during the wood smoke experiment, calculated based on the levels measured in the environment of the field worker, and compared with the levels of the compound excreted in urine. For the PAHs, the number or mol of the OH-metabolites (2OH-NAP, 2+3OH-FLU, 2+3OH-PHE, 1OH-PYR) was compared to the number of mol of the parent compound inhaled.

Compound	Concentration in personal air space at 1.5m (ng/m^3)	Concentration in personal air space at 1.5m ($nmol/m^3$)	Sampling technique	Estimated inhaled dose (nmol/exposure period of 5.13h)	Urinary excretion of metabolite (ng/12h)		Urinary excretion of metabolite (nmol/12h)		ratio excreted/inhaled	
					0-12h	12-24h	0-12h	12-24h	0-12h	12-24h
Levoglucosan	19448.4	119.9	Harvard Imp.	410.2	11763850	2565660	72554	15824	**166**	**36.2**
	9301.9	57.4	AE51	196.2						
	20740.0	127.9	Pers. Sampler	437.5						
Guaiacol	122.2	0.984	PDMS-Tenax	3.37	146441	33294	1180	268	**350**	**79.7**
Syringol	187.6	1.217	PDMS-Tenax	4.16	70139	16710	455.0	108.4	**109.3**	**26.0**
NAP	639.2	4.986	PDMS-Tenax	17.1	7939	3774	54.7	26.0	**3.21**	**1.52**
ACY	83.4	0.548	PDMS-Tenax	1.87						
ACE	38.1	0.247	PDMS-Tenax	0.84						
FLU	84.5	0.508	PDMS-Tenax	1.74	175.1	390.5	0.96	2.13	**0.55**	**1.23**
PHE	261.4	1.467	PDMS-Tenax	5.02	752.4	247.4	3.85	1.27	**0.77**	**0.25**
ANT	79.6	0.447	PDMS-Tenax	1.53						
FLA	96.3	0.476	PDMS-Tenax	1.63						
PYR	90.8	0.449	PDMS-Tenax	1.54	337.8	128.8	1.54	0.59	**1.00**	**0.38**
BaA	57.1	0.250	PDMS-Tenax	0.855						
CHR	22.0	0.097	PDMS-Tenax	0.330						
BbF	28.6	0.114	PDMS-Tenax	0.388						
BkF	0.33	0.001	PDMS-Tenax	0.005						
BaP	24.9	0.099	PDMS-Tenax	0.337						

Compound	Concentration in personal air space at 1.5m (ng/m³)	Concentration in personal air space at 1.5m (nmol/m³)	Sampling technique	Estimated inhaled dose (nmol/exposure period of 5.13h)	Urinary excretion of metabolite (ng/12h)		Urinary excretion of metabolite (nmol/12h)		ratio excreted/inhaled	
					0-12h	12-24h	0-12h	12-24h	0-12h	12-24h
IcdP	50.9	0.184	PDMS-Tenax	0.630						
BghiP	30.7	0.111	PDMS-Tenax	0.380						

→ Levoglucosan and methoxyphenols in scalp hair

Scalp hair samples mounted at 0, 1.5 and 4m distance of the fire, were analysed for the typical wood smoke compounds, levoglucosan and the methoxyphenols guaiacol (2-methoxy phenol) and syringol (2,6-dimethoxy phenol). The latter compounds were not quantifiable using the current method. Levoglucosan was clearly quantifiable in the hair mounted at the fire (44.9 µg/g hair) and at 4m distance (2.57 µg/g hair). However the level of levoglucosan analysed at 1.5m distance of the fire (0.28 µg/g hair) was in the same range of the field background level of 0.46 µg/g hair (mounted for 20h before the fire). The lab blancs were about in the same range of the field background hair samples: 0.10 and 0.33 µg levoglucosan/g hair.

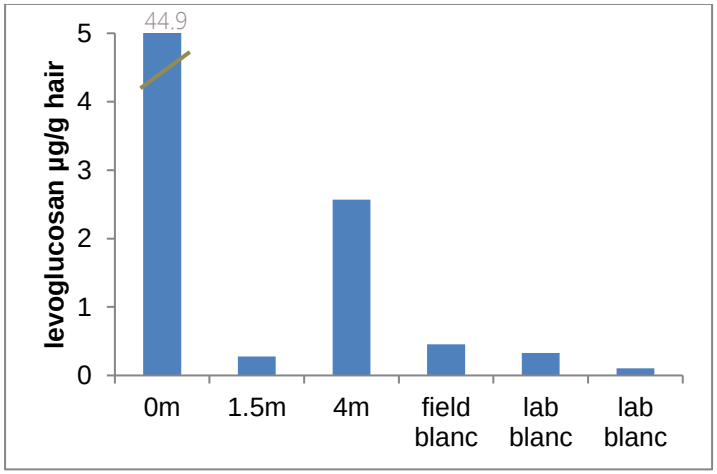


Figure 21 Levoglucosan levels (µg/g hair) measured in scalp hair mounted in a gauze at a distance of 0, 2.5 and 4m from the fire. The field blanc was mounted at the sampling location, during 20 hours before the fire was started. Two lab blancs i.e. identical hair samples kept in the lab, were also analysed.

CHAPTER 5 PILOT FEASABILITY STUDIES

5.1. AIM

The aim of the pilot studies was to assess the levels of the potential wood smoke biomarkers in a population of individuals exposed to daily-life wood smoke chemicals in Flanders.
In a first pilot, biobanked urine samples of five individuals participating in a PAH/levoglucosan measurement campaign performed in 2016, were used for analysis of wood smoke biomarkers (Koppen et al., 2016). In a second pilot, six participants were recruited and wood smoke biomarkers, as well as multiwavelength aethalometer black carbon concentrations were measured during a period of 7 days.

5.2. PILOT STUDY 1: URINE ANALYSIS FROM BIOBANKED SAMPLES

5.2.1. STUDY POPULATION

Biobank samples of five participants of a former study on PAH and levoglucosan environmental exposure were used to compose this pilot study. The five individuals were selected from three residences (

Table 18~~Table 18~~), based on following criteria: (i) not (anymore) performing any professional activity, (ii) (nearly) complete sampling of air PAH, and levoglucosan at their home address during each time, four consecutive weeks in respectively the Summer 2015 and Winter 2016 and (iii) urine collection, at the end of the 48h air sampling intervals in both seasons. This means that from the five individuals, each time 4 urine samples from respectively the Winter and Summer time were available; collected in polypropylene urine containers, and further stored at -80°C until analysis, about 1-1.5 years after collection.
The urine of the Summer/Fall and Winter period were analysed for levoglucosan and the OH-PAH, 1-OHnaphthalene, 2-OH naphthalene, 1-OH pyrene, 2-OH fluorene and 3-OH phenantrene. Ethical approval was observed from the University Hospital of the Antwerp University (Belgian Registration number: B300201525280) for analysis of PAH and wood smoke metabolites in urine.

The measured levoglucosan levels in the home environments of the selected individuals varied between 12 and 769 ng/m^3. The levels of levoglucosan earlier measured in Flanders, were in winter between 138-640 ng/m3 (year 2010-2011) on seven monitoring locations (Maenhaut et al., 2012); 122-192 ng/m3 on four urban locations in the winter of 2011-2012 (Maenhaut et al., 2016); between 200-300 and less than 100 ng/m3 average levels in Lille (France), Antwerp (Belgium), Leicester (UK), and Amsterdam (The Netherlands) during the JOAQUIN project (Cordell et al., 2016); 263 ng/m3 and less in four European regions measured within the EU project ESCAPE (Jedynska et al., 2015).

Table 18: Selected individuals living at five residences where air PAH and levoglucosan measurements were performed during a measuring campaign done in 13 measuring locations in 3 Flemish regions (6 Menen/6 Genk/1Houtem), in Sept'15 and Jan-Feb'16 (LNE project) (Koppen et al., 2016)

Individual	Sex	Area	subtype	Measuring unit	Number of Urine samples collected	
					Summer/Fall '15	Winter '16
1-G-PAK-03	male	Genk	industry	G03	4	4
1-G-PAK-11	female	Genk	industry	G03	4	4
1-G-PAK-05	male	Genk	background	G05	4	4
1-M-PAK-05	male	Menen	traffic	M05	4	3
1-M-PAK-17	female	Menen	traffic	M05	4	4

5.2.2. WOOD SMOKE BIOMARKER LEVELS IN BIOBANKED URINE SAMPLES

The seasonal changes in biomarker levels of the five individuals were tested, but there was no statistical significant difference between the summer and winter data, except for 2+3OH PHE, which was higher in summer-fall (200.6 ng/g CRT) compared to winter (143.4 ng/g CRT). However, it could be observed, that levoglucosan, and even more 2OH NAP were slightly higer in the winter season compared to the summer-fall period: respectively 3233.3 vs. 2859.2 ng/g CRT for levoglucosan and 2048.4 vs. 1605.2 ng/g CRT for 2OH NAP (Figure 22~~Figure 22~~). Looking more into detail, to the repeated measurements in the individuals (over both seasons), there was no clear indication of a relationship between the levels of levoglucosan measured in the air, and the levels of the biomarkers measured in the urine samples (Table 19~~Table 19~~). There was also no similarity in biomarker levels among couples of the same household (G-PAK-03 and G-PAK-11, M-PAK-05 and M-PAK-17). Most probably, the time frame of 48h sampling of $PM_{2.5}$ for analysis of levoglucosan in air was too broad to allow comparison with the urinary markers, having a shorter half life of a 5-12 hours. Furthermore, the individuals of this population were selected for having no indoor wood smoke exposure, meaning that the exposure to wood burning only occurred outdoors. PAH and levoglucosan sources, other than wood smoke, probably disturbed the exposure patterns. Indeed, also in summer-fall samples, higher levels of these biomarkers were observed in these participants (e.g. high summer levels of G-PAK-11 participant, Table 19~~Table 19~~). We did not have detailed information on daily activities, which made the interpretation difficult (time spent at home, or in traffic, complaints/nuisance reporting due to burning, time spent indoors/outdoor as was collected in pilot study2; see further).

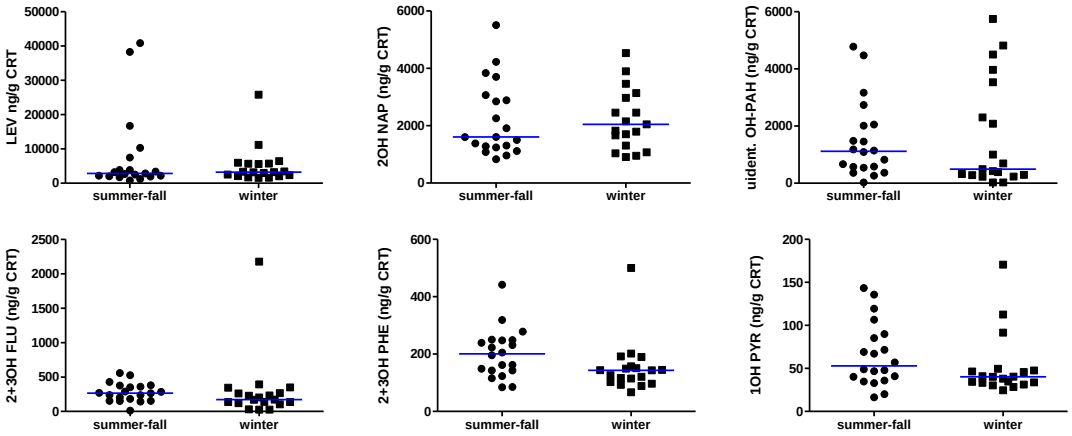


Figure 22: Scatter plots of the biobanked urine samples. Comparison of summer-fall vs. winter levels. The blue lines indicate the median levels for the biomarker

Table 19: Overview of concentrations of levoglucosan in air measured in $PM_{2.5}$ collected during 48h before urine collection. Urinary levels of OH PAHs. Gradual color scale of which green indicates the lowest, yellow the P_{50} and red the P_{95} value. The color scale was applied for each parameter individually over all participants.

	ID	Date	LEV air (ng/m3)	LEV (µg/L)	unid OH-PAH (ng/L)	2OH NAP (ng/L)	2+3OH FLU (ng/L)	2+3OH PHE (ng/L)	1OH PYR (ng/L)	LEV (µg/g CRT)	unid OH-PAH (ng/L)	2OH NAP (ng/g CRT)	2+3OH FLU (ng/g CRT)	2+3OH PHE (ng/CRT)	1OH PYR (ng/g CRT)	CRT (mg/dL)
summer -fall	1-G-PAK-03	01/09/2015	19.09		360.3	5395.0	258.0	140.0	47.0		367.7	5505.1	263.3	142.9	48.0	98
	1-G-PAK-03	08/09/2015	26.27	2050.0	581.8	1644.0	217.0	275.0	107.0	1722.7	488.9	1381.5	182.4	231.1	89.9	119
	1-G-PAK-03	15/09/2015	31.11	36360.0	262.7	2007.0	137.0	76.0	32.0	40853.9	295.1	2255.1	153.9	85.4	36.0	89
	1-G-PAK-03	22/09/2015	67.73	63900.0	25.0	2680.0	257.0	193.0	55.0	38263.5	15.0	1604.8	153.9	115.6	32.9	167
winter	2-G-PAK-03	19/01/2016	638.69	10520.0	801.1	3509.0	278.0	187.0	62.0	6454.0	491.5	2152.8	170.6	114.7	38.0	163
	2-G-PAK-03	26/01/2016	84.86	3250.0	538.2	4133.0	484.0	168.0	47.0	2338.1	387.2	2973.4	348.2	120.9	33.8	139
	2-G-PAK-03	02/02/2016	98.16	18010.0	369.9	2888.0	223.0	149.0	56.0	11186.3	229.7	1793.8	138.5	92.5	34.8	161
	2-G-PAK-03	11/02/2016	184.81	5220.0	527.6	2777.0	224.0	167.0	40.0	3202.5	323.7	1703.7	137.4	102.5	24.5	163
summer -fall	1-G-PAK-11	01/09/2015	19.09	1190.0	571.9	1189.0	163.0	137.0	37.0	3838.7	1844.9	3835.5	525.8	441.9	119.4	31
	1-G-PAK-11	08/09/2015	26.27	1840.0	820.2	1966.0	260.0	220.0	99.0	2666.7	1188.8	2849.3	376.8	318.8	143.5	69
	1-G-PAK-11	15/09/2015	31.11	2890.0	369.0	1184.0	120.0	70.0	38.0	10321.4	1317.9	4228.6	428.6	250.0	135.7	28
	1-G-PAK-11	22/09/2015	67.73	2340.0	667.7	2259.0	219.0	151.0	65.0	3836.1	1094.6	3703.3	359.0	247.5	106.6	61
winter	2-G-PAK-11	19/01/2016	638.69	970.0	691.2	1360.0	118.0	35.0	10.0	3233.3	2304.2	4533.3	393.3	116.7	33.3	30
	2-G-PAK-11	26/01/2016	84.86	570.0	170.1	1749.0	370.0	85.0	29.0	3352.9	1000.8	10288.2	2176.5	500.0	170.6	17
	2-G-PAK-11	02/02/2016	98.16	1210.0	1498.2	2806.0	188.0	91.0	81.0	1680.6	2080.9	3897.2	261.1	126.4	112.5	72
	2-G-PAK-11	11/02/2016	184.81	4650.0	2931.2	2873.0	25.0	120.0	76.0	5602.4	3531.5	3461.4	30.1	144.6	91.6	83
summer -fall	1-G-PAK-05	04/09/2015	39.90	3270.0	1182.6	4567.0	431.0	241.0	60.0	2194.6	793.7	3065.1	289.3	161.7	40.3	149
	1-G-PAK-05	08/09/2015	23.27	18240.0	1481.7	3148.0	258.0	162.0	51.0	16733.9	1359.3	2888.1	236.7	148.6	46.8	109
	1-G-PAK-05	15/09/2015	50.19	5360.0	2012.3	3019.0	226.0	195.0	55.0	3392.4	1273.6	1910.8	143.0	123.4	34.8	158
	1-G-PAK-05	22/09/2015	85.83	4830.0	540.3	1958.0	225.0	126.0	30.0	3220.0	360.2	1305.3	150.0	84.0	20.0	150
winter	2-G-PAK-05	19/01/2016	769.08	27610.0	248.6	2630.0	368.0	205.0	53.0	25803.7	232.3	2457.9	343.9	191.6	49.5	107
	2-G-PAK-05	26/01/2016	118.60	9810.0	1139.4	4034.0	283.0	258.0	67.0	5981.7	694.7	2459.8	172.6	157.3	40.9	164
	2-G-PAK-05	02/02/2016	93.25	2900.0	25.0	1773.0	101.0	86.0	39.0	2989.7	25.8	1827.8	104.1	88.7	40.2	97
	2-G-PAK-05	09/02/2016		1870.0	25.0	1905.0	216.0	139.0	32.0	2010.8	26.9	2048.4	232.3	149.5	34.4	93
summer -fall	1-M-PAK-05	01/09/2015	20.02	1150.0	2051.1	2122.0	536.0	392.0	101.0	815.6	1454.7	1505.0	380.1	278.0	71.6	141
	1-M-PAK-05	08/09/2015	27.70	5050.0	4470.8	1685.0	25.0	483.0	115.0	2500.0	2213.2	834.2	12.4	239.1	56.9	202
	1-M-PAK-05	15/09/2015	46.92	1330.0	1091.2	654.0	183.0	169.0	58.0	1955.9	1604.6	961.8	269.1	248.5	85.3	68
	1-M-PAK-05	22/09/2015	39.57	1660.0	1456.7	1678.0	274.0	219.0	66.0	1229.6	1079.1	1243.0	203.0	162.2	48.9	135
winter	2-M-PAK-05	26/01/2016	79.41	340.0	59.4	225.0	25.0	14.0	10.0	1619.0	283.1	1071.4	119.0	66.7	47.6	21
	2-M-PAK-05	02/02/2016	139.82	830.0	138.8	299.0	88.0	32.0	10.0	2515.2	420.7	906.1	266.7	97.0	30.3	33
	2-M-PAK-05	09/02/2016	246.28	2480.0	350.0	1145.0	163.0	242.0	55.0	2066.7	291.7	954.2	135.8	201.7	45.8	120
summer -fall	1-M-PAK-17	01/09/2015	20.02	2030.0	4775.5	1140.0	396.0	158.0	49.0	2859.2	6726.1	1605.6	557.7	222.5	69.0	71
	1-M-PAK-17	08/09/2015	27.70	5470.0	1144.1	936.0	257.0	150.0	49.0	7493.2	1567.2	1282.2	352.1	205.5	67.1	73
	1-M-PAK-17	15/09/2015	46.92	2050.0	2738.0	1007.0	264.0	182.0	38.0	2204.3	2944.0	1082.8	283.9	195.7	40.9	93
	1-M-PAK-17	22/09/2015	39.57	1220.0	3166.5	681.0	146.0	87.0	10.0	2000.0	5191.0	1116.4	239.3	142.6	16.4	61
winter	2-M-PAK-17	21/01/2016	534.99	4640.0	3646.0	1059.0	165.0	122.0	23.0	5728.4	4501.2	1307.4	203.7	150.6	28.4	81
	2-M-PAK-17	26/01/2016	79.41	3340.0	3848.2	1614.0	25.0	184.0	45.0	3443.3	3967.2	1663.9	25.8	189.7	46.4	97
	2-M-PAK-17	02/02/2016	139.82	5340.0	5402.7	975.0	25.0	135.0	38.0	5680.9	5747.6	1037.2	26.6	143.6	40.4	94
	2-M-PAK-17	09/02/2016	246.28	1300.0	3998.6	2605.0	190.0	119.0	26.0	1566.3	4817.6	3138.6	228.9	143.4	31.3	83

5.3. PILOT STUDY 2: FOLLOW-UP STUDY ON WOOD SMOKE EXPOSURE

5.3.1. STUDY POPULATION AND STUDY DESIGN

Six individuals participated in a measuring campaign on wood smoke exposure at and/or in their homes. The participants were all non-smokers, three men and three women, living in non-smoker residences, and had no professional exposure to PAHs. The age range was between 38y and 72y old (average 49y).
For each participant, the sampling week started on a Wednesday (middle of the week), and ran for 7-days. Urine was collected by them at at least two time periods per day in which they were mostly home: in the morning period before possibly leaving the house, and in the evening before sleeping. Hair was collected at the end of the sampling week, by cutting a string of 100 mg from near to the scalp. The participants were wearing four Passive Sampling Wristbands conditioned and individually packaged in PTFE bags with closures, before use (MyExposome Inc, USA). At the end of the week, two wristbands were again packed and stored at room temperature in the PTFE bags, and two wristbands were further worn during a continuous period of 1 month before being packed and stored in the same way (as the other wristbands) until analysis. BC monitoring was performed outdoor and/or indoors using an AE33 multi-wavelength aethalometer, during 1 week. The apparatus collects particles on a filter tape (total sample flow of 5 L/min) and transmission of light is measured every minute and compared with an unloaded reference filter spot. The instrument uses the DualSpot™ measurement to compensate for loadi
ng effects. BC is calculated based on the attenuation of light and measured at different wavelengths. BC originating from wood smoke can be calculated based on absorption at different wavelengths. The following calculations were used:
Percentage biomass burning (BB%) = babs_950_WB/Babs_950

$$b_{abs}(\lambda) = b_{abs}(\lambda)_{FF} + b_{abs}(\lambda)_{WB}$$

$$\frac{b_{abs\ 470nm,FF}}{b_{abs\ 950nm,FF}} = \left(\frac{470nm}{950nm}\right)^{-\alpha(FF)}$$

$$\frac{b_{abs\ 470nm,WB}}{b_{abs\ 950nm,WB}} = \left(\frac{470nm}{950nm}\right)^{-\alpha(WB)}$$

With: α(FF) = 1 en α(WB) = 2

BC from wood burning (BC_WB) = $BC_{\_at950nm}$ * BB%/100
BC originating from fossil fuel[8] (BC_FF) = $BC_{\_at\ 950nm}$ – BC_WB
Total Particle Mass originating from wood burning (PM_WB) = C_2 x $B_{abs\_WB\_at470}$ (the factor C_2 was empirically defined by Van Poppel et al., 2016 in a study on impact of wood burning in Flanders). Note that there is some uncertainty on this factor.

In the current study, we used two different instruments. Therefore calculated exposure can be partly due to inter-instumental differences. Whereas extensive comparison between instruments was not the focus of the study, instruments were co-located during 2 days to check if reported values were similar. Results (based on 30 min averages) showed a good agreement between both

[8] Fossil fuel: petroleum, coal, gas

instruments for different BC channels with a slope close to 1: BC_470 (R^2 = 0,95, y=0,94x) , BC_950 (R^2 = 0,90, y=0,95x). Whereas calculated %BB and BC_WB showed larger differences between instruments and especially a lower correlation (R^2 = 0,49, y=0,90x). This is due to the fact that a logarithmic function is used to calculated BC_WB from BC_470 and BC_950.

The differences between instruments are more plausible to occur at high filter loading, just before filter tape change or due to oscillations in instrument temperature. In both cases unrealistic k-values that are used for the filter loading compensation algorithm, result in deviations in BC concentrations. In some occasions during this campaign, short periods of observed high concentrations were probably due to these issues. These issues are currently looked at by the manufacturer but could not be corrected for within the timing of this project.

Within this project however, we only used larger (6h) averaging times in which short periods of differences will be averaged out resulting in better correspondence. During the intercomparison period of 2 days, 6-hours averaged BC_950 values showed a difference between -22% and +17%, whereas the difference for BC_WB was between -18% and 36%. In future studies, when using different instruments, care should be taken in the underlying algoritms and parameters used by different instruments, especially if shorter time resolution is used.

Figure 23: wristbands conditoned and individually packed in PTFE bags, before use (left), and as they were worn by the participants during sampling period (right)

During the sampling week, the participants filled out a logbook in which they indicated on hourly basis activities, such as: spending time outdoor/indoors, use of indoor wood stove/open fire, suffering from complaints of own wood stove or from the neighborhood (on scale from 1-10: light- high hindrance/nuisance), having meal/snack, being exposed to traffic exhaust gasses, exposure to cigarette smoke, window of home open for at least 30min, outdoor sports activities. The study was approved by the ethical committee of the University Hospital of the Antwerp University (UZA, Belgian Registration number: B300201316329).

5.3.2. SAMPLING LOCATIONS

The sampling of the six participants was done in a time frame of seven weeks, between December 2016 and February 2017. The participants were chosen, so that they had a variety of exposure

conditions considering wood smoke exposure of the environment and/or by an own indoor wood fire/stove (Table 20~~Table 20~~).

Table 20: Overview of participants (activities), details on localization of sampling equipment, and wood burning activities in pilot2-feasability study on wood smoke exposure biomarkers

ID	Sex	Date	Community /city	Wood smoke conditions	BC multiwave length aethalometer	Max day temp. (7d average °C)	Total rain (mm)
HV-01	M	22-28/12/16	Mechelen	Indoor open fire place for wood	indoor	7.7	11mm
HV-02	F	18-24/01/17	Vlimmeren	No wood stove use , important complaints of burning smells and black deposition from neighboring residential chimney	indoor + outdoor	2.9	0mm
HV-03	M	25/1-1/02/17	Balen	No wood stove, and no important complaints about burning smells	outdoor	5.5	8mm
HV-04	F	01-08/02/17	Olmen	No wood stove use, and only very occasionally complaints about burning activities in neighborhood	indoor + outdoor	8.7	8mm
HV-05	M	08-15/02/17	Dessel	Indoor wood stove	outdoor	4.7	3mm
HV-06	F	08-15/02/17	Dessel	Indoor wood stove	outdoor	4.7	3mm

Details on sampling period/localization of sampling equipment at each location	
HV-01 indoor sampling	
	Sampling period During the Christmas holidays. Participant HV-01 was home, and indoors for most of the time of sampling week. On two days HV-01 was indoors, but not home during the afternoon-early evening. Cooking activity 50% of the time. Localization measurement equipment Sampling in living room. A tubing from microaethalometer was guided from near of wood fire (1.5 meters away from the fire) to basement were AE33 aetholometer equipment was put Wood burning during sampling The living room had an open wood fire, which was used on two of 7-d sampling period. Complaints of score=4 on 7th sampling day
HV-02 outdoor and indoor sampling	
	Sampling period During normal working week/weekend. On weekdays participant HV-02 was home until 8:00 and again from 16:00 or 17:00 on. Use of car to go to work (20' each way). During the sampling weekend HV-02 was mostly home, spending some time outside also. HV-02 is mostly cooking the meals at home (except on Wednesdays and Fridays). Localization measurement equipment Outdoor: sampling on terras in garden, a few meters from the neighbors chimney (visible on the photo) Indoor: sampling in open living room/hall area of house

	**Wood burning during sampling** No indoor wood stove. However, complaints of wood burning by neighbors: Weekdays: r neighbor's wood stove in operation 16:00 to 23:00, except on 6th day. In weekend: neighbor's wood stove in operation most of the day Regular complaints of score= 6-10 the first 3 days of the sampling, which was bit less in the weekend, except for Sunday, and minor to less complaints on 6th and 7th sampling day.
HV-03 outdoor sampling	
	**Sampling period** During normal working week/weekend. On weekdays participant HV-03 was home until 7:00 and again from 18:00 on. Use of bike from/to work (ca. 12 km). During the sampling weekend HV-03 was home in the early morning, and from 19:00 or 21:00 on. No cooking activity by HV-03. **Localization measurement equipment** Outdoor: sampling on terras in garden **Wood burning during sampling** No indoor wood stove or open fire No complaints about burning-related smells or nuisance
HV-04: outdoor and indoor sampling	
 	**Sampling period** During normal working week/weekend.On weekdays participant HV-04 was home until 8:00 and again from 19:00 on. Use of the car to drive to and from work, and sometimes during work. During the sampling weekend HV-04 was home during most of the time, and being in traffic in afternoon for short periods. Bedroom windows (upstairs) open during daytime. Cooking mostly done by HV-04. **Localization measurement equipment** Outdoor: sampling on terras in garden Indoor: sampling in open living room area of house **Wood burning during sampling** No indoor wood stove or fire No or very minor (score=1) complaints about burning-related smells or nuisance Reporting of indoor candle burning on 7th sampling day (7/2/17)
HV-05 outdoor sampling	

	Sampling period Participant HV-05 was retired and spent some days most of the time home, and other days not at home during the day Localization measurement equipment Outdoor: sampling on terras in garden Wood burning during sampling Indoor wood stove, which was used every day between 17:00 and 22:00
HV-06 outdoor sampling	
	Sampling period During normal working week/weekend. On weekdays participant HV-06 was home until 7:30 and again from about 17:00 on. Use of the car to drive to and from work (15' each way). During the sampling weekend HV-06 was home until 11:00 and back later in the evening, spending several hours in car/traffic. Bedroom window open during most of day. Cooking mostly done by HV-06. Localization measurement equipment Outdoor: sampling on terras in garden Wood burning during sampling Indoor wood stove, which was used every day between 19:00 and 24:00 on weekdays, and a bit less in weekends.

Legend: M=male, F=female

5.3.3. BLACK CARBON (BC) LEVELS AT THE SIX DIFFERENT MEASURING LOCATIONS

For each participant, the sampling week started on a Wednesday (middle of the week), and ran for 7-days, this means that the sampling was each time done during 2.5 week days, followed by a weekend and again 2.5 week days. This pattern allowed to assess the influence of possible changing living conditions in these parts of the week.
BC was sampled either indoor or outdoor of the six locations using the multiwavelength aethalometer AE33. The sampling was not done simultaneously, but in successive weeks, except for location HV05 and HV-06, where outdoor BC was sampled in the same week (Table 20~~Table 20~~). Both black carbon originating from wood burning (BC_WB) and from fossil fuels (BC_FF) were reported as 6h-averages for the period before each urine collection. The sum of BC_WB and BC_FF equals the total BC concentration measured at each time point.

Based on the median concentrations for the 7-d sampling period, it was observed that during that study period, the participant at location HV-02 (with serious complaints about neighbors with wood stove) was most affected by wood smoke BC, especially if considering the indoor concentrations (0.82 µg/m^3) in comparison to the two other locations, where BC was measured indoors (HV-01 and HV-04 with respectively BC_WB indoors being 0.20 and 0.19 µg/m^3). Also, the

home outdoor median BC_WB levels were the highest for participant HV-02 (1.36 µg/m^3), although more or less in the same range as for participant HV-05 (1.25 µg/m^3).
Base on these data, one could speculate that during the study the participants HV-02 > HV-05 > HV-06 were theoretically more exposed to BC_WB than the participants HV-03, HV-01 and HV-04.

Table 21: Indoor (_in) and outdoor (_out) black carbon (BC) concentrations to which the participants were potentially exposed during the 7-days urine sampling. BC was measured for each participant during seven successive days, using the multiwavelength aethalometer AE33 (generating minute levels). Based on the minute levels, average concentrations were calculated for a 6-hours period before each urine collection (N=xx indicates the number of urine samples collected by each participant). The median(P_{25}-P_{75}) for all calculated 6-h values of each measuring location/participant was given in the table

Air concentration	HV-01 (N=13)			HV-02 (N=23)			HV-03 (N=19)			HV-04 (N=28)			HV-05 (N=23)			HV-06 (N=21)		
µg/m^3	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}
INDOOR																		
BC_in	0.81	0.48	1.71	2.29	1.99	3.18	-	-	-	0.65	0.59	0.94	-	-	-	-	-	-
BC_WB_in	0.20	0.15	0.34	0.82	0.61	1.30	-	-	-	0.19	0.14	0.26	-	-	-	-	-	-
BC_FF_in	0.63	0.32	1.11	1.46	1.26	2.12	-	-	-	0.50	0.40	0.68	-	-	-	-	-	-
PM_WB_in	2.17	1.66	3.90	8.78	6.95	12.30	-	-	-	2.21	1.64	3.03	-	-	-	-	-	-
OUTDOOR																		
BC_out	-	-	-	4.01	2.85	5.21	1.43	1.05	2.03	1.07	0.85	1.43	3.98	3.24	5.66	2.86	2.38	3.59
BC_WB_out	-	-	-	1.36	0.77	2.38	0.45	0.12	0.87	0.31	0.16	0.51	1.25	0.74	1.71	0.72	0.57	1.08
BC_FF_out	-	-	-	2.60	2.00	3.60	0.93	0.70	1.43	0.81	0.51	1.00	3.24	2.18	4.05	2.16	1.84	2.77
PM_WB_out	-	-	-	15.40	8.25	27.61	5.65	1.39	10.01	3.57	1.83	5.95	14.26	8.42	19.54	7.73	4.91	10.62

BC: total black carbon, BC_WB: black carbon originating from wood smoke, BC_FF: black carbon originating from fossil fuels (coal, petroleum, gas), PM_WB: particle concentration originating from wood burning (all expressed in µg/m3).

On location HV-01, the owner lit the indoor open wood fire, two times during the 7-weak sampling period from 22-28/12/16 (two peaks in Figure 24~~Figure 24~~). At the first occasion the fire was burning for 9 hours (15:00-23:00), at the second occasion the wood fire was on only for 4 hours (17:00-20:00). The second BC_WB peak was less visible (Figure 24~~Figure 24~~).

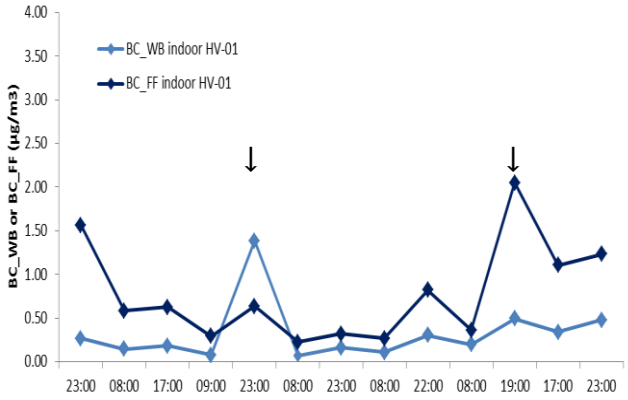


Figure 24: 6h-averages of indoor BC_WB and BC_FF at location HV-01. This residence had an indoor open wood fire, which was active at two occasions during the 7-days sampling period (indicated with arrow)

At location HV-02, the wood stove of the neighbors was operative every day (except on 23/1/17) from about 16:00 in the afternoon, and in the weekend (21-22/1/17) during the whole day. The

inhabitants had serious complaints about excessive burning smells and black deposition from the neighboring residential chimney. The BC_WB concentrations were peaking mainly in the evenings, and were higher outdoors than indoors. Outdoor BC_WB levels were a sum of the own and neighborhood emissions.

Simultaneous outdoor and indoor measurments were only done at this location (HV-02) as well as on location HV-04. The BC levels were clearly higher at location HV-02, outdoors, as well as indoors (Figure 25~~Figure 25~~). At location HV-04 (sampled from 1-8/2/17), the inhabitants had no complaints, and did not have an indoor wood stove or open fire. During the 7-days sampling the average maximum temperature was on laction HV-04 slightly higher compared to HV-02 (8.7 vs. 9.2°C). On both locations it was visible that BC_WB (and also BC_FF) was mostly higher outdoors than indoors (on average a factor of about 1.6 times higher outdoors compared to indoors, see Table 21~~Table 21~~). Furthermore, the concentrations raised in the evening hours (both indoors and outdoors), i.e. in the time frame in which domestic wood burning mostly takes place. This indicates that BC originating from wood burning contributed to both indoor and outdoor exposure, even in residences where there was no indoor wood burning source.

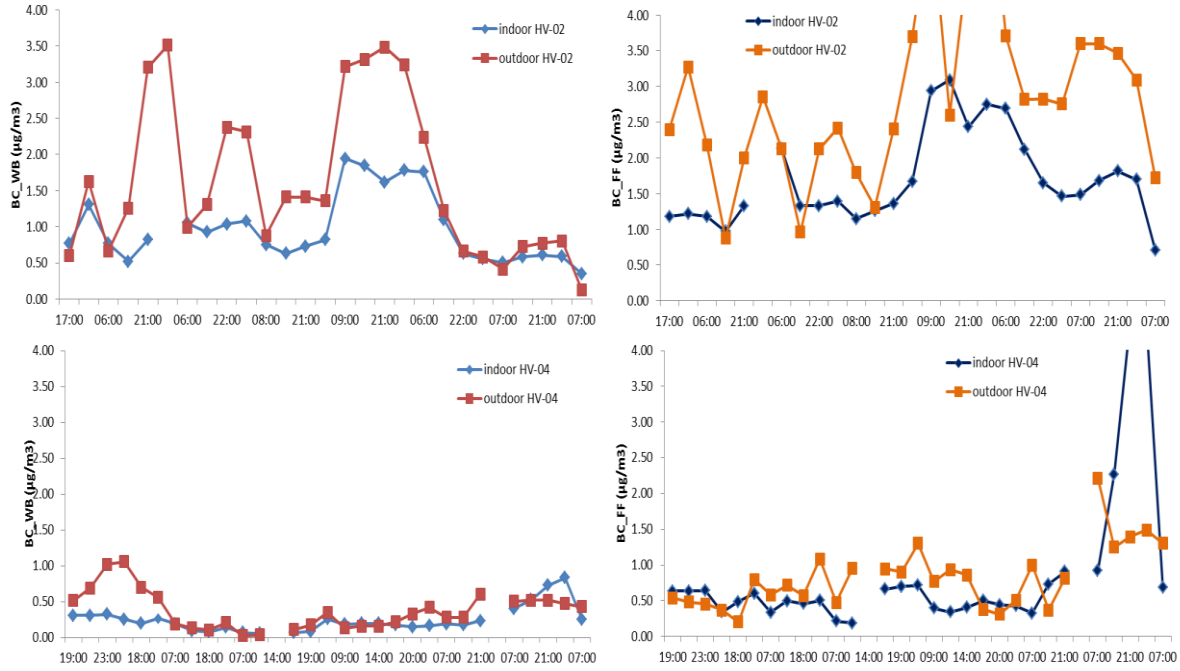


Figure 25: 6h-averages of BC_WB (left) and BC_FF (right) measured on two different home locations (HV-02 and HV-04), where outdoor and indoor measurements were done using a multiwavelength aethalometer AE33. The upper two curves were from location HV-02. The inhabitants had no wood stove, but had strong complaints about wood burning in the neighborhood. The 7d-average of the maximum air temperature was 2.9°C without any precipitation during the sampling period (18-24/1/2017). The residence of location HV-04 had no wood stove, and the inhabitants had hardly complaints about wood burning in the neighborhood. The 7d-average of the maximum air temperature was 8.7°C, and very limited precipitation of 6mm during the 7-days sampling period (1-8/2/2017).

At location HV-03 only outdoor measurements were done in the period of 26/1-1/2/17. The owners of this residence had no indoor wood stove/fire and no complaints about fire smells or related inconveniences from the neighborhood. The first 2 days (26+27/1/17), the 6h-averaged

BC levels were rather high (5.59 µg/m^3), but the contribution was mainly due to fossil fuel burning (BC_FF) (Figure 26~~Figure 26~~).

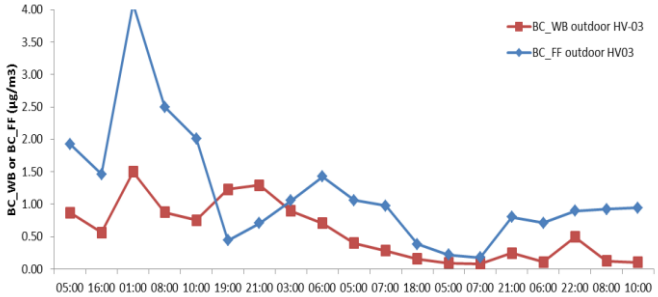


Figure 26: 6h-averages of indoor BC_WB and BC_FF at location HV-03 (samples in period 26/1/17-1/2/17). This residence had no indoor wood fire/stove, and there were no complaints about fire smells or related invonveniences from the neighborhood.

At locations HV-05 and HV-06 were located 2 km away from each other, situated both about 1 km either West or East from the centre of the community Dessel, outdoor black carbon measurements were done simultaneously in the same sampling week. Both residences had a wood stove indoors, which was used every day from 17:00- about 22:00 at location HV-05, and mostly from 19:00 or 20:00 to 24:00 at location HV-06. The outdoor BC_WB and BC_FF levels were systematically higher at location HV-06 compared to HV-05, following some kind of similar time trend tendency during the 7d-sampling period. BC_WB 6h-average concentrations on both locations (measured in the same week) were correlated with spearman rank correlation coefficient of r=0.62. The BC_FF levels were even better correlated with r=0.84.

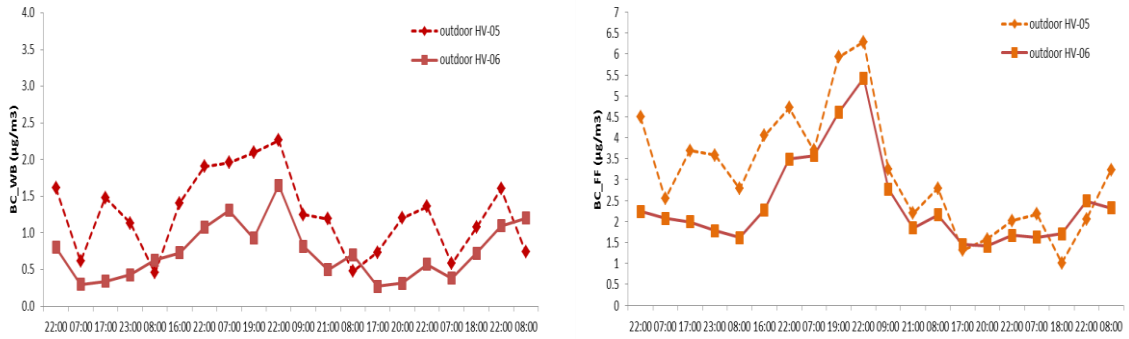


Figure 27: 6-h averages of outdoor BC_WB (µg/m^3, left) and BC_FF (µg/m^3, right) measurements using multiwavelength aethalometer AE33, at locations HV-05 and HV-06 simultaneously in the week of 8-15/2/2017 (7d-average maximum temperature 4.7°C, total of 3mm precipitation during the 7-days sampling period)

5.3.4. WRIST BAND PASSIVE SAMPLERS

The participants were wearing four wristbands, two during the individual's 7-days sampling period, and the other two during one month, starting on the first day of the sampling week. The two wristbands of each time frame, were from different origine, either bought precleaned via MyExposome(O'Connell et al., 2014), or bought as normal silocone wristband via the internet and precleaned in the lab using Soxhlet extraction with ethylacetate during 2hours.

As a try-out of the analysis technique, firstly halve of each of the wristbands worn during one month were analysed, and this was reported here. The extract of the wristbands contained a lot of background compounds, mainly fatty acids, which were most probably originating from the skin of the individual wearing the wristband (for in this case 1 month). This means that the peaks that were quantifiable are present, and those not observed, were either not detected, or not visible among the high background. The clean-up of the extract is further worked on to optimize the quantification of the PAH compounds.

As was also seen in the first wood burning experiment, mainly the compounds NAP, ACE, FLU, PHE and PYR were detectable on the wristband. Furthermore BeP and IcdP were above the quantification limit. There was some difference between both wristbands with respect to quantity in which it was measured, but some similar conclusions could be made:

Two of the three individuals, that were daily exposed to woodsmoke (either via wood burning activities in neighborhood, HV-02; or by own wood buring activities, HV-06) in the period of sampling, had detectable levels of lower molecular weight PAHs in the wristband of one or more of the PAHs that were clearly higher than what was measured in all other individuals (Figure 28~~Figure 28~~):

- HV-02: NAP[9], FLU, PHE, FLU, PYR clearly visible above background, and had for the urinary markers measured in the 7-days sampling period relatively high levels of 2OH NAP (Figure 29~~Figure 29~~).
- HV-06: NAP, ACE, FLU, PHE, FLA, BeP and IcdP which were mostly higher than the other individuals. The higher exposure of HV-06 was also confirmed with the biomarkers measured in urine during the 7-days measuring campaign (mainly urinary 2OH NAP, 2+3 PHE, but also 2+3 OH FLU, Figure 29~~Figure 29~~).

Also, the two individuals, HV-03 and HV-04, that were in theory 'not' exposed to wood smoke components at their homes, showed to have been collecting NAP, PHE, PYR, BeP (HV-03) or NAP, ACE, FLA and BeP (HV-04) on their wristband. HV03 showed during the 7-days sampling week mostly relatively higher levels of LEV and all other OH PAHs (except of the unidentified OH-PAH metabolite). This may have been due to a temporarly high outdoor BC_WB episode and/or to exposure to wood smoke possibly during the in total daily 2 hours biking to and from work. HV-04 had overall relatively high urinary levels of the unideintified NAP, 2+3OH FLU and 1OH PYR (Figure 29~~Figure 29~~). It could be speculated that this latter may be due daily traffic exposure (at least in total 40 minutes in car from/to work).

[9] On one of both wristbands analysed

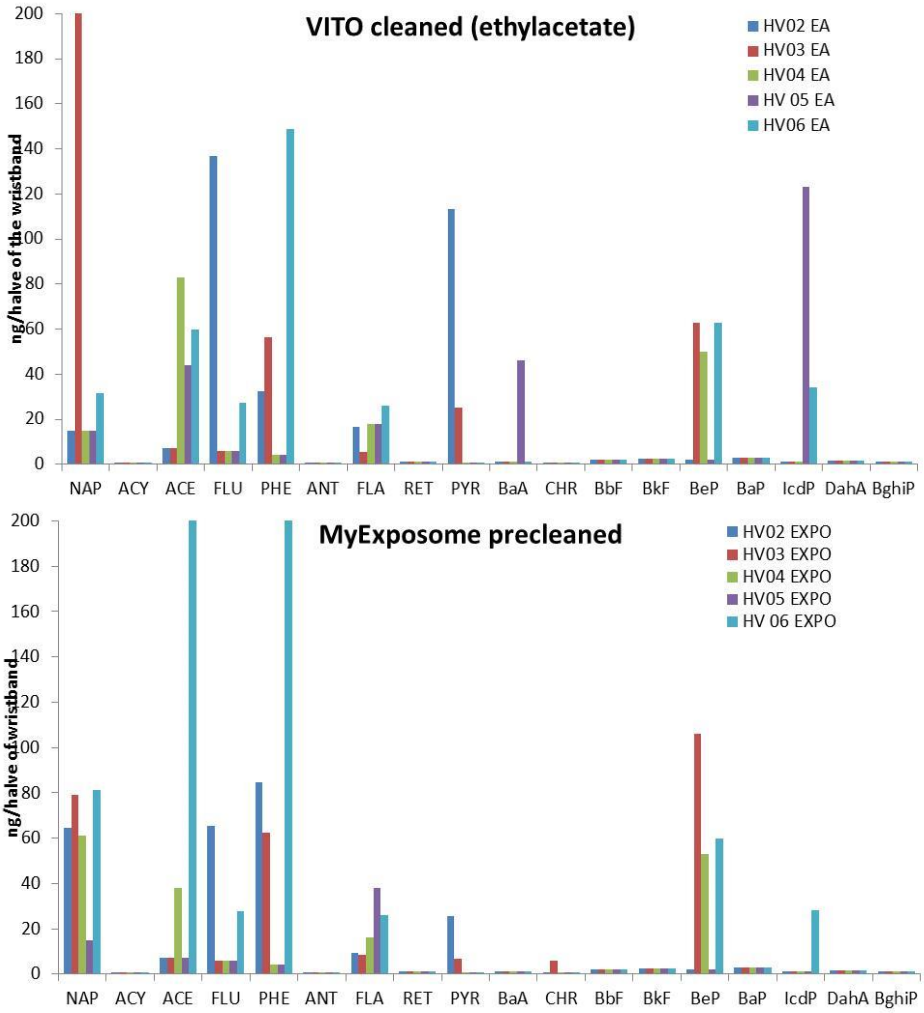


Figure 28: PAH profile in wristbands worn by five of the participants of the pilot2 study.Only halve of the wristband was used to perform a first try-out of the PAH analysis analysis method. NB: method needs to be further optimized. The visible peaks were present, the non-visible peaks, were either not present, or were not detectable because of high background peaks (from skin compounds) in the chromatogram

5.3.5. PASSIVE SAMPLING OF LEVOGLUCOSAN USING HAIR

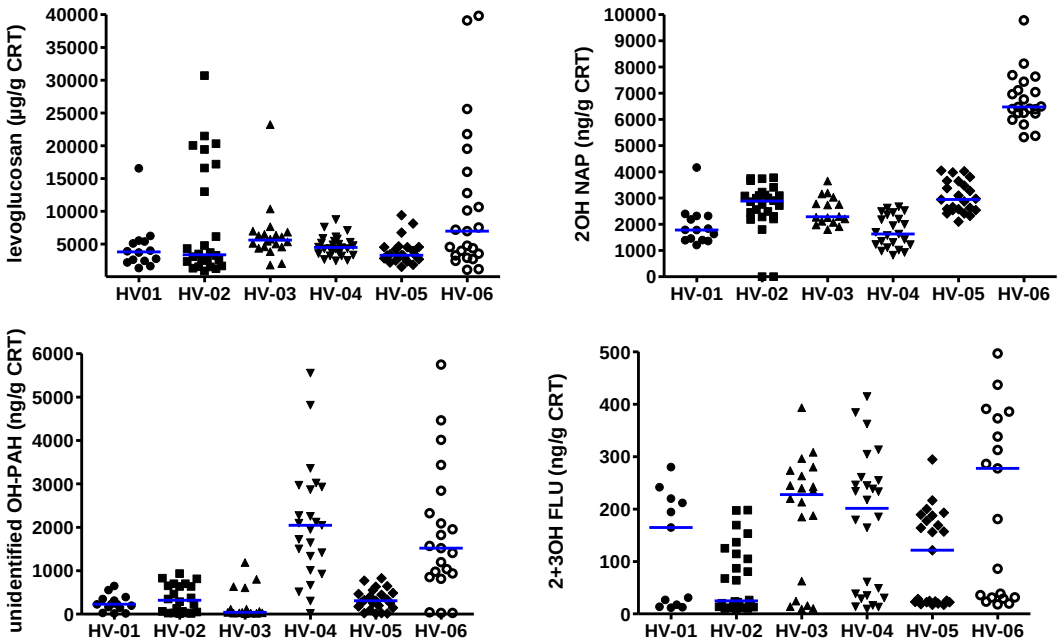
From the six individuals hair samples were collected at the end of the 7-days sampling. Levoglucosan was analysed in the hair extract, which was not further cleaned. The chromatograms were not completely clear, meaning that all results are to be interpreated as semi-quantitative results. There was especially a problem with the HV-05 and HV-06 samples, of which the chromatogram appeared overloaded, not allowing to quantify levoglucosan. These are a set of very limited results, allowing no further conclusion, than that levoglucosan analysis in hair can be done for the levels present in the indoor/outdoor air. The exposure of HV-02 and HV-04, was comparable, and in the range of what was detected in hair samples mounted at 4m distance during the earlier controlled wood smoke experiment (Figure 21~~Figure 21~~).

Table 22: results of levoglucosan measured in hair of th participants of the pilot2 feasiblity study

Inidividual	Levoglucosan in hair (µg/g hair)
HV02	4.67
HV03	0.28
HV04	5.97
HV05	Overloaded chromatogram
HV06	Overloaded chromatogram

5.3.6. OVERALL BIOMARKER LEVELS IN THE SIX PARTICIPANTS

In total 142 urine samples were collected and analysed from the six participants. Five samples were excluded, as the urine was too diluted (creatinine < 30 mg/dL). Looking overall at the biomarker results, it could be seen that the individuals with the highest wood smoke levels at the home location (HV-02, HV-05 and HV-06) had overall the higest levels in 2-OH naphthalene. The urinary levels of levoglucosan were not higher in those individuals, but HV-02 and HV-06 showed some high levels in periods after which wood smoke exposure had occurred (see further for detailed discussion on the time patterns in each individual). On first view 2+3OH fluorene in combination with the unidentified OH-PAH metabolite were higher in individuals reporting daily exposure to traffic exhausts (HV-04 and HV-06). 2+3OH phenanthrenes were not consistently high in case of wood smoke exposed individuals.



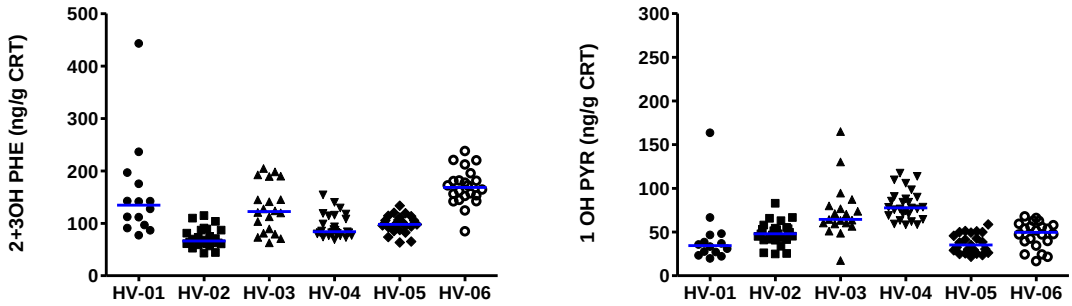


Figure 29: scatter plots of urinary wood smoke biomarker concentrations measured in the the during the 7-days sampling period, repeatedly collected urine samples of all participants (HV-01 to HV-06). The blue bar represents the median value of all collected urine samples by the individuals (cf. 'all samples' in Table 23~~Table 23~~). For 2+3 OH FLU there was a clear dual clustering of the concentrations, a result of a large part of the samples having values under the limit of quantitification for each of the individuals (LOQ=50 ng/L)

As a first comparison, the median (P_{25}-P_{75}) concentrations of the repeated urine samples collected by the six different participants during each of the sampling weeks were calculated (Figure 29~~Figure 29~~). For each individual and each biomarker the median was calculated for either all urine samples collected (i.e. with creatinine level of at least 30 mg/dL); for only the first morning void samples; or for the samples collected in the evening after 19:00; or for only those samples collected on a time point that the individual spent at least 6h at home before the urine was collected. The latter was done, as the plausibility of exposure to wood smoke would in theory fit better with the home-environment related BC_WB concentrations.

Due to the low sample numbers and some variability in the results, statistically there was only a difference in the levels of the unidentified OH-PAH urinary compound, which were on average higher in evening compared to the morning urine samples, considering all days and measuring locations (Kruskal wallis ANOVA, p=0.026). However, one could observe, that in general the urinary levoglucosan levels were higher in the evening samples compared to first void morning samples. For the other markers there was not clear tendency. Certainly the best sampling time point very much depends on the appearance of the wood smoke exposure peak, and possible confounding exposures present in daily life. Overall the first urine sample and/or urine collected after 19:00 may reflect the short-living wood burning compounds in the best way.

Table 23: the median (P_{25}-P_{75}) concentrations of the repeated urine samples collected by the six different participants during each of the sampling weeks were calculated. Results of levoglucosan expressed in µg/g CRT, all OH-PAHs were expressed in ng/g CRT. All samples = results of all collected urine samples by each of the individuals (for which creatinine levels were > 30 mg/dL), first void= first void urine samples collected in the morning, after 19:00 = samples collected after 19:00 in the evening, home last 6h = levels in urine samples for which, in a time frame of 6h before collection, the individual was present at home.

µg or ng/g CRT	HV-01			HV-02			HV-03			HV-04			HV-05			HV-06		
	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}	Med	P_{25}	P_{75}
All samples	N=13			N=26			N=20			N=30			N=23			N=25		
LEV	3799.1	2421.3	5390.9	3363.9	2336.4	16596.2	5587.0	4566.7	6640.5	4652.7	3823.5	5872.1	3284.9	2700.0	4527.8	5285.7	3297.3	12760.9
2OH-NAP	1781.3	1401.0	2188.1	2885.7	2302.5	3117.8	2287.3	2095.8	2908.6	1698.8	1235.8	2453.9	2946.7	2548.0	3651.0	6494.3	6245.2	7691.4
Unid OH-PAH	224.9	148.0	312.1	318.5	50.0	653.6	42.8	27.9	363.0	2097.0	1335.7	2971.5	307.7	149.1	489.1	1411.3	812.4	2090.1
2+3OH-FLU	164.8	17.4	220.0	25.0	13.7	114.4	227.6	43.5	268.5	225.4	36.8	282.8	121.5	22.5	187.5	277.6	31.6	390.9
2+3OH-PHE	127.7	96.9	175.6	66.4	61.0	86.9	122.3	85.3	167.4	85.6	80.8	114.6	97.9	87.2	111.2	172.4	154.7	203.9
1OH-PYR	33.3	27.1	46.7	48.0	41.5	54.4	64.4	59.4	80.0	78.4	70.7	90.1	35.2	26.4	49.5	47.5	29.9	57.1
First void	N=5			N=7			N=7			N=7			N=7			N=7		
LEV	3991.8	3700.5	5088.1	1650.3	1313.1	17180.4	6092.1	3850.0	6810.3	3823.5	2644.2	4781.0	3181.8	2179.8	4527.8	4556.8	3297.3	6975.6
2OH-NAP	1461.8	1401.0	1786.8	2996.2	2563.4	3228.3	2210.2	1979.6	3196.0	2000.0	1235.8	2369.5	2631.4	2543.9	3279.9	6968.3	6227.3	8137.1
Unid OH-PAH	233.8	196.8	349.7	288.9	23.6	637.3	41.7	19.7	117.0	1010.6	518.7	2932.6	197.9	149.1	307.7	852.5	41.7	1905.3
2+3OH-FLU	26.6	17.4	164.8	67.5	13.7	87.1	187.5	62.5	239.7	238.5	49.0	362.4	25.5	18.7	177.5	390.9	67.6	543.9
2+3OH-PHE	111.8	96.9	127.7	61.2	59.1	68.0	112.5	70.9	145.4	82.1	76.5	93.4	94.0	82.5	105.7	172.3	124.4	224.3
1OH-PYR	27.7	27.1	31.0	49.7	41.5	52.7	64.4	59.4	80.0	84.3	78.5	106.0	32.6	23.6	50.0	27.0	24.4	39.8
After 19:00	N=4			N=13			N=4			N=10			N=11			N=10		
LEV	5390.9	2753.1	5532.3	3477.8	2544.3	16596.2	6028.7	4827.5	7297.1	4950.5	3555.6	5273.8	3295.2	2700.0	4642.2	7356.7	3517.2	12760.9
2OH-NAP	1618.1	1302.8	2014.4	2717.9	2204.2	3094.7	2711.3	2261.3	3161.3	1356.3	931.3	2453.9	2662.4	2418.8	3651.0	6444.6	6192.8	6628.8
Unid OH-PAH	268.5	194.2	434.0	580.5	28.4	814.9	320.1	31.3	609.0	1951.3	1418.3	2254.3	324.0	92.9	489.1	1949.4	1464.8	4238.9
2+3OH-FLU	230.8	125.4	260.8	22.3	11.7	114.4	231.3	115.3	269.5	209.2	99.9	309.0	121.5	22.5	164.3	156.7	25.7	299.4
2+3OH-PHE	158.6	127.0	206.0	73.3	64.7	91.1	125.7	101.6	166.1	108.5	85.6	117.9	95.5	87.2	105.6	181.4	164.4	220.3
1OH-PYR	29.630	21.012	42.609	53.2	45.3	58.0	71.3	60.5	73.9	90.1	76.6	98.5	29.5	25.7	38.7	55.8	53.8	59.5
Home last 6h	N=10			N=18			N=11			N=10			N=14			N=7		
LEV	3944.8	2753.1	5390.9	2572.2	1516.7	12978.9	5421.1	3850.0	6470.6	4050.2	2644.2	5273.8	2863.5	2346.9	3295.2	4556.8	1160.3	7523.8
2OH-NAP	1786.8	1401.0	2317.6	2810.1	2302.5	3094.7	2287.3	2116.7	2761.1	1634.6	1231.7	2177.8	2585.7	2418.8	2946.7	6323.7	6227.3	6968.3
Unid OH-PAH	196.8	28.4	312.1	375.3	26.3	699.3	37.3	22.3	634.9	1258.7	669.3	2932.6	263.8	176.6	447.5	914.7	41.7	3438.4
2+3OH-FLU	164.8	26.6	211.8	25.0	13.7	104.9	213.4	15.9	263.3	246.6	217.5	313.2	75.0	23.8	164.3	364.6	38.5	543.9
2+3OH-PHE	127.7	111.8	196.7	69.0	60.7	86.9	103.6	73.2	145.4	84.5	76.5	117.9	94.6	82.5	100.0	147.7	124.4	172.3
1OH-PYR	37.0	27.7	48.2	50.6	45.3	55.4	63.4	51.1	76.7	84.9	78.5	98.5	29.3	25.4	38.1	32.1	24.4	47.6

5.3.7. TIME AND ACTIVITY RELATED PATTERNS IN BIOMARKERS

For all participants a heat map of the individual urine samples was made, indicating for each of the biomarkers to which extend the urine sample was relatively high-low compared to all other collected samples in the study (Table 24~~Table 24~~). The air concentrations of BC (originiating from wood smoke or fossil fuel), the number of hours being not at home, home outside, reporting wood burning indoor, or having indicated of being exposed to traffic, were calculated for a 6-hours time period before the urine was collected. The complain scores (1-10) of each of the 6h previous to the urine collection were summed up.

The indoor or outdoor BC_WB air levels were in general higher at the locations were the participants reported own wood burning activities (HV-01 to lesser extend, but mainly locations HV-05 and HV-06) or serious complaints of wood burning in neighborhood (HV-02).

HV-01 was at home most of the time of the 7-days sampling period (Christmass holiday). HV-01 reported the indoor open wood fire was on in the evening of 24/12/16. This was visible in the indoor 6h-averaged BC_WB levels (increasing from below 0.5 to 1.38 µg/m^3). The exposure was measurabe in the late evening urine sample of that day and and first morning void of the following day, mainly for the urinary compounds 2OH NAP, 2+3OH PHE and 2+3OH FLU. On 27/12/16, the indoor open fire was on for a shorter time period, and again (this time) a slight increase in mainly 2+3OH PHE and 2+3OH FLU urinary compounds was seen. No increase of urinary levoglucosan was visible on those days. HV-01 had also relatively high levels of these compounds in the evening of two other days (23/12/16 and 25/12/16), whereas there was no indication of indoor home particle exposure. On the 23/12/16 HV-01 did outside acivities at home. On 25/12/16 afternoon-evening the participant had been not at home, but visiting people which had a closed indoor woodstove, and possibly explaining the higher levels of the OH-PAHs and LEV at that time point (Table 24~~Table 24~~).

HV-02 had no indoor wood stove, but nevertheless the highest levels of WB_BC indoors and outdoors of all participants. The complaint score was high, almost every day of the 7-d sampling period (hindrance of neighboring wood stove use). In the evening urine samples of 18/1/17, 22/1/17, 23/1/17 the urinary levels of levoglucosan were high, coinciding with high levels of indoor and outdoor BC_WB (above 1-1.5 µg/m^3 up to almost 3.5 µg/m^3 outdoors). On the other days, this urine LEV rise was not seen. The main urinary factor that was fairly always relatively and consistently medium to higher, was the concentration of 2OH NAP (Table 24~~Table 24~~).

HV-05 and HV-06, both had an indoor wood stove used only for wood burning. In both houses the wood stove was used every day between respectively 17:00-22:00 or 19:00-24:00 (bit less in weekends for HV-06). For HV-05 the increase in urinary levels, was mainly visible for 2OH NAP which was on average slightly higher. On those days, the 6h-average outdoor BC_WB concentration was above or around 1.5 and 2 ng/m^3 the urine collection. It was to some extend visible in the urinary levels of LEV, that raised slightly in the evening of 9/2/17 and were clearly higher in the evening of 11/2/17 and to some extend in the first void of the day after.
HV-06 showed in general the highest exposure pattern for all urinary markers except OH PYR. Mainly 2OH NAP, and 2+3 OH PHE were consistently higher in all collected urine samples (Table

20). It would have been interesting if indoor BC_WB had been monitored at the home of this participant. Unlike the outdoor concentrations of HV-06 were similar or lower than for HV-05, it may be speculated that the indoor concentrations might have been higher. Also the wristband worn by HV-06 suggested the highest PAH exposure among all participants. Aside from the possible differences in indoor BC_WB levels compared to HV-05, there was also an important difference in living conditions, as HV-06 (and not HV-05) reported a regular use of the car or being in traffic.

The other individuals HV03 and HV-04 had theoretically 'no' wood smoke exposure at home. Indeed, the outdoor/indoor BC_WB were almost always in the range below 0.30 ng/m^3. However, for HV-03, in the evenings of 26/1/17, 27/1/17 and morning of 28/1/17, the 6-h average outdoor BC_WB was higher, respectively 1.5 and 1.29 µg/m^3, and this was visible in the evening and next-morning urine samples of those days (LEV and all OH-PAH metabolites, except the unidentified NAP). A same increase of these urinary compounds was observed in the evening and next morning urine of 29-30/1/2017, although the outdoor BC_WB levels were low at that time period. However, the participant had not been home during the daytime, possibly leading to exposure away from the home environment. Surprisingly the same pattern was also seen in the evening and morning urine samples of 30-31/1/2017. As mentioned before, it could be speculated that this may have been due to a temporarly high outdoor BC_WB episode and/or to exposure to wood smoke possibly during the in total daily 2 hours biking to and from work (biking route via densely inhabited and city centre areas mainly). This individual also showed rather high levels of low molecular weight PAHs sampled on the wristband.

HV-04 had low indoor and outdoor BC_WB concentrations during the 7-days sampling period. Only in the evening of 7/2/17 the indoor BC levels were high up to 5.25 ng/m3 for 6-h average concentration. The participant reported candle burning that evening. Indeed the BC_WB level, was increased (up to 0.84 µg/m^3 6-h average), but the major compound was calculated as BC_FF (up to 4.41 µg/m^3 6-h average). This peak exposure was visible in the 2+3 OH FLU and PYR levels of that evening's urine samples. Overall, HV-04 had high levels of the unidentified OH-PAH metabolite in urine. Furthermore, this participant had relatively high OH PYR levels most of the time. HV-04 reported to be regularly underway with the car (in total 40 min/day). On 3/2/17 HV-04 mentioned extra car driving during the afternoon and evening (in total ca. 1h 40 min driving), which did not really lead a rise in the urine unidentified OH-PAH metabolite or OH PYR levels. One interesting observation was the exceptional high peak (in the evening of 4/2/17) of ca. 100x higher urinary levoglucosan observed i.e. 477 381.0 µg/g CRT vs. about 4600 µg/g CRT measured in all other urine samples collected by HV-04. HV-04 reported bacon and pancake baking in the afternoon of that day. The participant consumed pancakes with sugarcane, which caused this enormous levoglucosan peak in the urine.

Table 24: Overview of the concentrations of wood smoke compounds measured in indoor/outdoor air and in urine of the participants. The air concentrations and the number of hours being not at home, home outside, reporting wood burning (WB) indoor(*), or having indicated of being exposed to traffic, were calculated for a 6-hours time period before the urine was collected. The complain scores of the 6h previous to the urine collection were summed up. Gradual color scale of which green indicates the lowest, yellow the P_{50} and red the P_{95} value. The color scale was applied for each parameter individually over all participants. The black rectangulars indicate the weekend days. (*) WB indoor for participant HV-02 = number of hours wood stove of neighbors was operative (no own wood stove)

	Indoor air (µg/ m³)				outdoor air (µg/ m³)										urine (per volume)						urine (per creatinine)						CRT
Time point	BC_in	BC_WB	BC_FF	PM_WB	BC_out	BC_WB	BC_FF	PM_WB	not at home (h)	home outside (h)	WB indoor	com-plaint score	food	ex-haust	LEV (µg/ L)	unid. OH-PAH (ng/ L)	2OH-NAP (ng/ L)	2+3OH-FLU (ng/ L)	2+3OH-PHE (ng/ L)	1OH-PYR (ng/ L)	LEV (µg/ g CRT)	unid. OH-PAH (ng/ g CRT)	2OH-NAP (ng/ g CRT)	2+3OH-FLU (ng/ g CRT)	2+3OH-PHE (ng/ CRT)	1OH-PYR (ng/ g CRT)	(mg/ dL)
HV-01																											
22/ 12/ 2016 18:00											0	0	0	0	5230.0	495.7	3522.0	25.0	167.0	72.0	2421.3	229.5	1630.6	11.6	77.3	33.3	216
22/ 12/ 2016 23:00	1.84	0.27	1.57	3.07					0	0	0	0	0	0	2230.0	132.4	1491.0	25.0	91.0	30.0	2753.1	163.5	1840.7	30.9	112.3	37.0	81
23/ 12/ 2016 08:00	0.73	0.15	0.59	1.66					0	0	0	0	0	0	7290.0	460.5	2760.0	25.0	179.0	61.0	3700.5	233.8	1401.0	12.7	90.9	31.0	197
23/ 12/ 2016 17:00	0.81	0.18	0.63	2.08					0	**2**	0	0	1	0	3430.0	25.0	3669.0	887.0	390.0	144.0	3897.7	28.4	4169.3	1008.0	443.2	163.6	88
24/ 12/ 2016 09:00	0.37	0.08	0.29	0.89					0	0	0	0	1	0	8090.0	312.9	2841.0	309.0	154.0	44.0	5088.1	196.8	1786.8	194.3	96.9	27.7	159
24/ 12/ 2016 23:00	2.02	1.38	0.64	18.62					0	0	6	0	3	0	5930.0	343.3	1331.0	242.0	260.0	53.0	5390.9	312.1	1210.0	220.0	236.4	48.2	110
25/ 12/ 2016 08:00	0.30	0.07	0.22	0.79					0	0	0	0	0	0	4870.0	426.6	1664.0	201.0	240.0	57.0	3991.8	349.7	1363.9	164.8	196.7	46.7	122
25/ 12/ 2016 23:00	0.48	0.16	0.32	1.83					**4**	0	0	0	2	1	7450.0	250.2	628.0	126.0	79.0	10.0	16555.6	555.9	1395.6	280.0	175.6	22.2	45
26/ 12/ 2016 08:00	0.37	0.11	0.26	1.22					0	0	0	0	0	0	5860.0	612.1	2255.0	25.0	120.0	22.0	6234.0	651.2	2398.9	26.6	127.7	23.4	94
26/ 12/ 2016 22:00	1.12	0.30	0.82	3.47					**4**	0	0	0	2	1	2620.0	227.1	2210.0	244.0	143.0	20.0	2594.1	224.9	2188.1	241.6	141.6	19.8	101
27/ 12/ 2016 08:00	0.56	0.20	0.36	2.26					0	0	0	0	0	0	2330.0	25.0	2105.0	25.0	161.0	39.0	1618.1	17.4	1461.8	17.4	111.8	27.1	144
27/ 12/ 2016 19:00	2.55	0.50	2.05	5.65					0	**2**	3	0	0	2	2030.0	25.0	3546.0	324.0	218.0	102.0	1326.8	16.3	2317.6	211.8	142.5	66.7	153
28/ 12/ 2016 17:00	1.45	0.34	1.11	3.90					**6**	0	0	8	1	0	3960.0	269.3	3242.0	25.0	158.0	65.0	2175.8	148.0	1781.3	13.7	86.8	35.7	182
28/ 12/ 2016 23:00	1.71	0.48	1.23	5.48					0	0	0	0	1	1	3430.0						5532.3						62
HV-02																											
18/ 01/ 2017 17:00	1.95	0.77	1.18	8.78	3.00	0.60	2.40	6.81	0	**2**	3	23	1	0	12280.0	25.0	1510.0	79.0	25.0	10.0	30700.0	62.5	3775.0	197.5	62.5	25.0	40
18/ 01/ 2017 23:00	2.52	1.30	1.22	14.87	4.90	1.63	3.27	18.57	0	0	3	19	1	0	12330.0	25.0	2940.0	25.0	54.0	43.0	12978.9	26.3	3094.7	26.3	56.8	45.3	95
19/ 01/ 2017 06:00	1.95	0.77	1.18	8.77	2.85	0.67	2.18	7.59	0	0	0	0	0	0	6500.0	25.0	3176.0	25.0	47.0	44.0	6132.1	23.6	2996.2	23.6	44.3	41.5	106
19/ 01/ 2017 18:00	1.49	0.52	0.97	5.94	2.13	1.26	0.87	14.45	**3**	**2**	3	23	2	1	5660.0	205.2	3811.0	25.0	115.0	69.0	3699.3	134.1	2490.8	16.3	75.2	45.1	153
19/ 01/ 2017 21:00	2.15	0.82	1.33	9.33	5.21	3.20	2.00	43.55	0	**2**	6	41	1	0	4050.0	25.0	3742.0	172.0	103.0	71.0	2469.5	15.2	2281.7	104.9	62.8	43.3	164
19/ 01/ 2017 23:00					6.37	3.51	2.86	47.00	0	0	6	39	1	0	1160.0	25.0	1421.0	52.0	20.0	10.0	3052.6	65.8	3739.5	136.8	52.6	26.3	38
20/ 01/ 2017 06:00	3.18	1.06	2.12	12.03	3.12	1.00	2.13	11.37	0	0	0	0	0	0	2800.0	698.1	6345.0	162.0	110.0	98.0	1505.4	375.3	3411.3	87.1	59.1	52.7	186
20/ 01/ 2017 17:00	2.25	0.93	1.33	10.57	2.26	1.30	0.96	15.32	**4**	**1**	2	6	2	1	4400.0	1010.8	5922.0	25.0	141.0	86.0	2784.8	639.8	3748.1	15.8	89.2	54.4	158
20/ 01/ 2017 22:00	2.36	1.03	1.33	11.80	4.50	2.38	2.13	27.61	0	**1**	6	47	2	0	5000.0		10.0	25.0	186.0	135.0	2336.4		4.7	11.7	86.9	63.1	214
20/ 01/ 2017 23:00	2.47	1.08	1.39	12.30	4.74	2.32	2.42	26.88	0	0	5	41	2	0	3640.0		5250.0	25.0	168.0	129.0	1516.7		2187.5	10.4	70.0	53.8	240
21/ 01/ 2017 08:00	1.90	0.75	1.15	8.55	2.67	0.88	1.79	10.02	0	0	0	0	0	0	2050.0	25.0	7000.0	183.0	146.0	109.0	903.1	11.0	3083.7	80.6	64.3	48.0	227
21/ 01/ 2017 19:00	1.90	0.63	1.26	7.21	2.72	1.41	1.31	16.12	0	**4**	5	6	3	2	5300.0	1017.2	3343.0	25.0	100.0	59.0	4308.9	827.0	2717.9	20.3	81.3	48.0	123
21/ 01/ 2017 21:00	2.09	0.73	1.36	8.31	3.81	1.41	2.40	16.08	0	**2**	5	2	2	2	3130.0	841.7	2806.0	103.0	66.0	60.0	3477.8	935.2	3117.8	114.4	73.3	66.7	90
21/ 01/ 2017 23:00	2.49	0.82	1.67	9.35	5.06	1.36	3.70	15.47	0	**1**	6	4	1	1	4020.0	1287.6	4355.0	25.0	112.0	84.0	2544.3	814.9	2756.3	15.8	70.9	53.2	158
22/ 01/ 2017 09:00	4.88	1.95	2.93	22.20	8.67	3.21	5.46	36.65	0	0	1	6	0	0	3020.0	528.6	5321.0	25.0	111.0	91.0	1650.3	288.9	2907.7	13.7	60.7	49.7	183
22/ 01/ 2017 16:00	4.94	1.84	3.09	21.01	5.92	3.32	2.60	38.38	0	**2**	4	10	2	0	3680.0		10.0	25.0	129.0	101.0	1230.8		3.3	8.4	43.1	33.8	299
22/ 01/ 2017 21:00	4.05	1.62	2.43	18.43	8.32	3.49	4.83	40.38	**2**	0	4	29	3	2	35350.0	1493.9	4695.0	25.0	130.0	89.0	16596.2	701.4	2204.2	11.7	61.0	41.8	213
22/ 01/ 2017 23:00	4.53	1.78	2.75	20.31	9.53	3.24	6.29	36.97	**1**	0	5	32	2	1	47660.0		4289.0	25.0	154.0	97.0	20025.2		1802.1	10.5	64.7	40.8	238
23/ 01/ 2017 06:00	4.45	1.76	2.69	20.09	5.95	2.24	3.70	25.57	0	0	0	0	0	0	33330.0	1267.9	4973.0	25.0	132.0	79.0	17180.4	653.6	2563.4	12.9	68.0	40.7	194
23/ 01/ 2017 22:00	2.28	0.63	1.65	7.17	3.49	0.66	2.82	7.56	0	0	0	0	2	0	20400.0	484.8	3007.0	161.0	109.0	49.0	19428.6	461.8	2863.8	153.3	103.8	46.7	105
23/ 01/ 2017 23:00	2.02	0.56	1.46	6.35	3.33	0.58	2.75	6.62	0	0	0	0	1	0	24060.0	783.2	2749.0	25.0	102.0	62.0	21482.1	699.3	2454.5	22.3	91.1	55.4	112
24/ 01/ 2017 07:00	1.99	0.50	1.48	5.76	4.01	0.41	3.60	4.70	0	0	0	0	1	0	48120.0	1510.5	5457.0	160.0	145.0	122.0	20303.8	637.3	2302.5	67.5	61.2	51.5	237
24/ 01/ 2017 17:00	2.27	0.58	1.68	6.65	4.32	0.72	3.60	8.25	**5**	0	1	2	2	1	1860.0	135.8	1173.0	25.0	25.0	10.0	4769.2	348.2	3007.7	64.1	64.1	25.6	39
24/ 01/ 2017 21:00	2.42	0.61	1.81	6.95	4.24	0.77	3.47	8.83	**1**	0	5	10	2	1	2860.0	25.0	3229.0	149.0	101.0	73.0	3250.0	28.4	3669.3	169.3	114.8	83.0	88
24/ 01/ 2017 23:00	2.29	0.59	1.70	6.72	3.90	0.81	3.09	9.19	0	0	4	8	2	0	1300.0	25.0	1549.0	99.0	55.0	29.0	2600.0	50.0	3098.0	198.0	110.0	58.0	50
25/ 01/ 2017 07:00	1.06	0.35	0.71	3.98	1.86	0.13	1.73	1.50	0	0	0	0	0	0	1300.0	221.5	3196.0	124.0	86.0	65.0	1313.1	223.7	3228.3	125.3	86.9	65.7	99

	Indoor air (µg/ m³)				outdoor air (µg/ m³)				not at home (h)	home outside (h)	WB indoor	com-plaint score	food	ex-haust	urine (per volume)						urine (per creatinine)						CRT
Time point	BC_in	BC_WB	BC_FF	PM_WB	BC_out	BC_WB	BC_FF	PM_WB							LEV (µg/ L)	unid. OH-PAH (ng/ L)	2OH-NAP (ng/ L)	2+3OH-FLU (ng/ L)	2+3OH-PHE (ng/ L)	1OH-PYR (ng/ L)	LEV (µg/ g CRT)	unid. OH-PAH (ng/ g CRT)	2OH-NAP (ng/ g CRT)	2+3OH-FLU (ng/ g CRT)	2+3OH-PHE (ng/ CRT)	1OH-PYR (ng/ g CRT)	(mg/ dL)
HV-03																											
26/01/2017 05:00					2.80	0.87	1.93	9.90	0	0	0	0	0	0	29490.0	25.0	2807.0	235.0	90.0	62.0	23220.5	19.7	2210.2	185.0	70.9	48.8	127
26/01/2017 16:00					2.03	0.56	1.46	6.43	**6**	0	0	0	0	0	10980.0			327.0	128.0	60.0	10358.5			308.5	120.8	56.6	106
27/01/2017 01:00					5.59	1.50	4.09	17.08	0	0	0	0	0	0	6600.0	25.0	2159.0	25.0	194.0	89.0	6470.6	24.5	2116.7	24.5	190.2	87.3	102
27/01/2017 08:00					3.38	0.88	2.50	10.01	**2**	0	0	0	0	0	2510.0	25.0	1461.0	25.0	77.0	32.0	6275.0	62.5	3652.5	62.5	192.5	80.0	40
27/01/2017 10:00					2.77	0.75	2.01	8.57	**4**	0	0	0	0	0	3490.0	93.6	1442.0	196.0	113.0	51.0	4362.5	117.0	1802.5	245.0	141.3	63.8	80
27/01/2017 19:00					1.67	1.23	0.44	15.48	**4**	0	0	0	1	0	3640.0	25.0	2529.0	194.0	102.0	57.0	4550.0	31.3	3161.3	242.5	127.5	71.3	80
27/01/2017 21:00					2.00	1.29	0.70	16.17	**2**	0	0	0	1	0	5840.0			249.0	172.0		6952.4			296.4	204.8		84
28/01/2017 03:00					1.95	0.89	1.06	10.20	0	0	0	0	0	0	4120.0	25.0	2307.0	208.0	144.0	99.0	5421.1	32.9	3035.5	273.7	189.5	130.3	76
28/01/2017 06:00					2.13	0.71	1.43	8.06	0	0	0	0	0	0	2310.0	25.0	1645.0	158.0	87.0	46.0	3850.0	41.7	2741.7	263.3	145.0	76.7	60
29/01/2017 05:00					1.46	0.40	1.06	4.56	0	0	0	0	0	0	2250.0	25.0	2324.0	239.0	116.0	71.0	2008.9	22.3	2075.0	213.4	103.6	63.4	112
29/01/2017 07:00					1.26	0.28	0.97	3.21	0	0	0	0	0	0	1020.0	25.0	1313.0	134.0	51.0	10.0	1789.5	43.9	2303.5	235.1	89.5	17.5	57
29/01/2017 18:00					0.54	0.16	0.38	1.78	**6**	0	0	0	0	0	2750.0	25.0	1669.0	236.0	119.0	99.0	4583.3	41.7	2781.7	393.3	198.3	165.0	60
30/01/2017 05:00					0.31	0.09	0.22	1.07	0	0	0	0	0	0	4890.0			238.0	102.0	60.0	5752.9			280.0	120.0	70.6	85
30/01/2017 07:00					0.26	0.08	0.18	0.90	**1**	0	0	0	0	0	9260.0	177.9	3009.0	285.0	171.0	144.0	6092.1	117.0	1979.6	187.5	112.5	94.7	152
30/01/2017 21:00					1.05	0.25	0.80	2.80	**2**	0	0	0	1	0	10240.0			295.0	166.0	99.0	7641.8			220.1	123.9	73.9	134
31/01/2017 06:00					0.82	0.11	0.71	1.21	0	0	0	0	0	0	11850.0	25.0	5561.0	417.0	253.0	112.0	6810.3	14.4	3196.0	239.7	145.4	64.4	174
31/01/2017 22:00					1.39	0.50	0.90	5.65	**1**	0	0	0	0	0	12150.0	1449.3	5382.0	25.0	189.0	144.0	5105.0	609.0	2261.3	10.5	79.4	60.5	238
01/02/2017 08:00					1.05	0.12	0.92	1.39	0	0	0	0	0	0	14470.0	1720.7	5182.0	25.0	171.0	161.0	5339.5	634.9	1912.2	9.2	63.1	59.4	271
01/02/2017 10:00					1.05	0.10	0.94	1.16	0	0	0	0	0	0	11450.0	1445.9	4088.0	25.0	146.0	92.0	6361.1	803.3	2271.1	13.9	81.1	51.1	180
01/02/2017 13:00									0	0	0	0	0	0	7860.0	1871.1	4335.0	25.0	115.0	95.0	5006.4	1191.8	2761.1	15.9	73.2	60.5	157
HV-04																											
01/02/2017 17:00					0.79	0.37	0.42	4.52			0	0	0	0	7170.0	2755.7	836.0	202.0	94.0	62.0	8743.9	3360.7	1019.5	246.3	114.6	75.6	82
01/02/2017 19:00	0.94	0.31	0.63	3.52	1.05	0.51	0.54	6.03	0	**1**	0	0	0	2	5170.0	3777.1	560.0	213.0	88.0	67.0	7602.9	5554.5	823.5	313.2	129.4	98.5	68
01/02/2017 21:00	0.94	0.31	0.63	3.52	1.17	0.69	0.48	8.00	0	**1**	0	0	1	2	4430.0	778.9	1104.0	196.0	99.0	74.0	5273.8	927.3	1314.3	233.3	117.9	88.1	84
01/02/2017 23:00	0.97	0.33	0.64	3.71	1.47	1.02	0.45	11.63			0	0	1	2	1940.0	1109.9	966.0	103.0	42.0	29.0	6258.1	3580.4	3116.1	332.3	135.5	93.5	31
02/02/2017 06:00	0.59	0.25	0.34	2.91	1.43	1.06	0.37	13.31	0	0	0	0	1	2	4000.0	3323.0	1380.0	180.0	60.0	59.0	5797.1	4815.9	2000.0	260.9	87.0	85.5	69
02/02/2017 22:00	0.87	0.27	0.61	3.03	1.35	0.56	0.80	6.37	**2**	0	0	0	1	1	4950.0				156.0	91.0	4901.0				154.5	90.1	101
03/02/2017 07:00	0.53	0.19	0.34	2.21	0.77	0.19	0.58	2.18	0	0	0	0	1	0	3900.0	25.0	2548.0	424.0	164.0	124.0	3333.3	21.4	2177.8	362.4	140.2	106.0	117
03/02/2017 15:00	0.60	0.10	0.50	1.14	0.85	0.14	0.72	1.56	**6**	0	0	0	0	1	3940.0	1985.0	1530.0	227.0	96.0	76.0	4061.9	2046.4	1577.3	234.0	99.0	78.4	97
03/02/2017 18:00	0.54	0.08	0.46	0.95	0.68	0.11	0.57	1.24	**5**	0	0	0	0	1	7620.0	4072.6	1989.0	25.0	213.0	105.0	4257.0	2275.2	1111.2	14.0	119.0	58.7	179
03/02/2017 23:00	0.65	0.14	0.50	1.64	1.29	0.21	1.09	2.35	**6**	0	0	0	0	1	4090.0	2885.6	3141.0	237.0	113.0	98.0	3195.3	2254.3	2453.9	185.2	88.3	76.6	128
04/02/2017 07:00	0.29	0.07	0.22	0.80	0.50	0.03	0.48	0.17	0	0	0	0	0	0	2330.0	492.8	2251.0	394.0	78.0	80.0	2452.6	518.7	2369.5	414.7	82.1	84.2	95
04/02/2017 12:00	0.25	0.06	0.19	0.66	0.99	0.04	0.95	0.27	0	0	0	0	0	0	4630.0	3837.4	1803.0	466.0	140.0	142.0	2530.1	2097.0	985.2	254.6	76.5	77.6	183
04/02/2017 14:00									**2**	0	0	0	0	0	3710.0	2436.6	1393.0	25.0	69.0	52.0	4524.4	2971.5	1698.8	30.5	84.1	63.4	82
04/02/2017 17:00	0.74	0.07	0.66	-0.02	1.06	0.11	0.95	1.27	**3**	0	0	0	0	0	12400.0	5020.2	4425.0	314.0	144.0	134.0	7085.7	2868.7	2528.6	179.4	82.3	76.6	175
04/02/2017 19:00	0.79	0.08	0.70	0.12	1.08	0.17	0.90	1.98	**3**	0	0	0	0	0	200500.0	819.5	1124.0	128.0	35.0	29.0	477381.0	1951.3	2676.2	304.8	83.3	69.0	42
05/02/2017 03:00	0.98	0.26	0.71	3.00	1.65	0.35	1.30	4.01	**6**	0	0	0	0	0	2510.0	1239.6	1075.0	25.0	34.0	29.0	6122.0	3023.5	2622.0	61.0	82.9	70.7	41
05/02/2017 09:00	0.59	0.19	0.40	2.17	0.91	0.14	0.77	1.54	0	0	0	0	0	0	2780.0	1906.2	1619.0	25.0	52.0	51.0	4276.9	2932.6	2490.8	38.5	80.0	78.5	65
05/02/2017 13:00	0.54	0.20	0.34	2.25	1.10	0.16	0.93	1.82	**1**	0	0	0	0	0	7350.0	521.0	2434.0	419.0	125.0	100.0	4298.2	304.7	1423.4	245.0	73.1	58.5	171
05/02/2017 14:00	0.61	0.20	0.41	2.28	1.02	0.16	0.86	1.85	**2**	0	0	0	1	0	10180.0	17011.9		25.0	138.0	121.0	5443.9	9097.2		13.4	73.8	64.7	187
05/02/2017 17:00	0.68	0.18	0.50	2.04	0.60	0.22	0.38	2.61	**3**	0	0	1	1	0	11620.0	4502.7		25.0	201.0	155.0	4452.1	1725.2		9.6	77.0	59.4	261
05/02/2017 20:00	0.59	0.15	0.44	1.70	0.64	0.33	0.31	3.81	**1**	0	0	1	1	0	6600.0	2170.0	3371.0	25.0	131.0	95.0	4313.7	1418.3	2203.3	16.3	85.6	62.1	153
05/02/2017 22:00	0.59	0.16	0.43	1.88	0.93	0.42	0.51	4.81	**1**	0	0	1	1	0	4540.0						3197.2						142
06/02/2017 07:00	0.52	0.19	0.33	2.17	1.29	0.29	1.00	3.25	0	0	0	1	1	0	6550.0	1384.6	1693.0	298.0	128.0	156.0	4781.0	1010.6	1235.8	217.5	93.4	113.9	137
06/02/2017 18:00	0.91	0.17	0.73	1.99	0.65	0.28	0.37	3.32	**6**	0	0	1	1	0	2630.0	956.7	936.0	142.0	34.0	30.0	6921.1	2517.6	2463.2	373.7	89.5	78.9	38
06/02/2017 21:00	1.14	0.24	0.91	2.71	1.43	0.61	0.82	6.93	**3**	0	0	1	0	0	3670.0	1507.1	963.0	25.0	77.0	78.0	5169.0	2122.6	1356.3	35.2	108.5	109.9	71
06/02/2017 23:00									**2**	0	0	0	0	0	3520.0	1631.1	922.0	163.0	80.0	90.0	3555.6	1647.6	931.3	164.6	80.8	90.9	99
07/02/2017 07:00	1.33	0.40	0.92	4.62	2.72	0.51	2.21	5.78	0	0	0	0	0	0	2750.0	1567.1	1281.0	248.0	72.0	74.0	2644.2	1506.9	1231.7	238.5	69.2	71.2	104
07/02/2017 18:00	2.80	0.53	2.27	6.05	1.77	0.52	1.25	5.95	**6**	0	0	0	0	0	5050.0	1148.7	1404.0	25.0	71.0	64.0	5872.1	1335.7	1632.6	29.1	82.6	74.4	86
07/02/2017 21:00	5.12	0.73	4.39	8.33	1.92	0.52	1.39	5.96	**4**	0	0	0	0	0	2850.0			219.0	66.0	67.0	5000.0			384.2	115.8	117.5	57
08/02/2017 07:00	0.93	0.25	0.68	2.86	1.73	0.43	1.30	4.90	0	0	0	0	0	0	1950.0	341.4	997.0	25.0	39.0	43.0	3823.5	669.3	1954.9	49.0	76.5	84.3	51

	Indoor air (µg/ m^3)				outdoor air (µg/ m^3)				not at home (h)	home outside (h)	WB indoor	com-plaint score	food	ex-haust	urine (per volume)						urine (per creatinine)						CRT
Time point	BC_in	BC_WB	BC_FF	PM_WB	BC_out	BC_WB	BC_FF	PM_WB							LEV (µg/ L)	unid. OH-PAH (ng/ L)	2OH-NAP (ng/ L)	2+3OH-FLU (ng/ L)	2+3OH-PHE (ng/ L)	1OH-PYR (ng/ L)	LEV (µg/ g CRT)	unid. OH-PAH (ng/ g CRT)	2OH-NAP (ng/ g CRT)	2+3OH-FLU (ng/ g CRT)	2+3OH-PHE (ng/ CRT)	1OH-PYR (ng/ g CRT)	(mg/ dL)
HV-05																											
08/ 02/ 2017 16:00					3.51	0.80	2.71	9.12	0	0	1	0	0	0	4830.0	828.8	3616.0	214.0	143.0	63.0	4514.0	774.6	3379.4	200.0	133.6	58.9	107
08/ 02/ 2017 20:00					5.55	1.71	3.84	19.54	0	0	3	0	0	0	3460.0	469.8	3094.0	164.0	100.0	31.0	3295.2	447.5	2946.7	156.2	95.2	29.5	105
08/ 02/ 2017 22:00					6.11	1.61	4.50	18.40	0	0	3	0	0	0	2970.0	102.1	2846.0	173.0	70.0	32.0	2700.0	92.9	2587.3	157.3	63.6	29.1	110
09/ 02/ 2017 07:00					3.16	0.61	2.55	6.97	0	0	0	0	1	0	2300.0	173.1	2493.0	25.0	64.0	30.0	2346.9	176.6	2543.9	25.5	65.3	30.6	98
09/ 02/ 2017 17:00					5.18	1.48	3.70	16.88	**4**	0	1	14	2	0	3290.0	389.0	3518.0	208.0	110.0	48.0	3427.1	405.2	3664.6	216.7	114.6	50.0	96
09/ 02/ 2017 23:00					4.72	1.13	3.59	12.91	**4**	0	5	0	1	0	4010.0	311.0	3505.0	180.0	98.0	49.0	4177.1	324.0	3651.0	187.5	102.1	51.0	96
10/ 02/ 2017 08:00					3.24	0.46	2.78	5.19	0	0	0	0	2	0	2800.0	232.6	3349.0	25.0	93.0	44.0	3181.8	264.4	3805.7	28.4	105.7	50.0	88
10/ 02/ 2017 16:00					5.46	1.41	4.05	16.05	**1**	0	0	0	1	0	3950.0	840.2	3873.0	220.0	125.0	54.0	3038.5	646.3	2979.2	169.2	96.2	41.5	130
10/ 02/ 2017 20:00					5.92	1.78	4.14	20.27	0	0	4	0	1	0	3670.0	635.9	3144.0	158.0	96.0	33.0	2823.1	489.1	2418.5	121.5	73.8	25.4	130
10/ 02/ 2017 22:00					6.62	1.91	4.71	21.73	0	0	6	0	1	0	3630.0	322.4	3185.0	25.0	109.0	33.0	2904.0	257.9	2548.0	20.0	87.2	26.4	125
11/ 02/ 2017 07:00					5.66	1.96	3.70	22.32	0	0	0	0	1	0	1940.0	176.2	2561.0	158.0	76.0	29.0	2179.8	197.9	2877.5	177.5	85.4	32.6	89
11/ 02/ 2017 19:00					8.03	2.10	5.93	23.90	**4**	0	3	0	2	0	9110.0	621.2	4463.0	25.0	104.0	34.0	8133.9	554.7	3984.8	22.3	92.9	30.4	112
11/ 02/ 2017 22:00					8.55	2.26	6.29	25.82	**3**	0	6	0	1	0	10440.0	705.1	4498.0	25.0	126.0	43.0	9405.4	635.3	4052.3	22.5	113.5	38.7	111
12/ 02/ 2017 09:00					4.50	1.25	3.25	14.26	0	0	0	0	1	0	9030.0	447.0	4395.0	25.0	126.0	51.0	6738.8	333.6	3279.9	18.7	94.0	38.1	134
12/ 02/ 2017 21:00					3.39	1.19	2.20	13.58	**6**	0	4	0	1	0	5600.0	25.0	4937.0	274.0	150.0	50.0	3943.7	17.6	3476.8	193.0	105.6	35.2	142
13/ 02/ 2017 08:00					3.27	0.48	2.80	5.43	0	0	0	0	1	0	6520.0	443.1	3349.0	25.0	141.0	34.0	4527.8	307.7	2325.7	17.4	97.9	23.6	144
13/ 02/ 2017 17:00					2.05	0.74	1.31	8.39	**1**	0	1	0	1	0	5650.0	25.0	6931.0	507.0	207.0	79.0	3284.9	14.5	4029.7	294.8	120.3	45.9	172
13/ 02/ 2017 20:00					2.78	1.20	1.58	13.74	0	0	4	0	1	0	2490.0	469.3	2130.0	25.0	101.0	26.0	2465.3	464.7	2108.9	24.8	100.0	25.7	101
13/ 02/ 2017 22:00					3.38	1.36	2.02	15.55	0	0	6	0	1	0	2060.0	294.7	2709.0	184.0	107.0	28.0	1839.3	263.2	2418.8	164.3	95.5	25.0	112
14/ 02/ 2017 07:00					2.76	0.58	2.18	6.63	0	0	0	0	1	0	1730.0	170.0	2946.0	216.0	94.0	25.0	1517.5	149.1	2584.2	189.5	82.5	21.9	114
14/ 02/ 2017 18:00					2.08	1.08	1.01	12.28	**5**	0	1	0	1	0	6500.0	1183.4	4436.0	25.0	159.0	61.0	4545.5	827.5	3102.1	17.5	111.2	42.7	143
14/ 02/ 2017 22:00					3.66	1.60	2.06	18.24	**1**	0	5	0	0	0	5060.0	25.0	2902.0	25.0	130.0	54.0	4642.2	22.9	2662.4	22.9	119.3	49.5	109
15/ 02/ 2017 08:00					3.98	0.74	3.24	8.42	0	0	0	0	0	0	3420.0	25.0	2763.0	25.0	112.0	54.0	3257.1	23.8	2631.4	23.8	106.7	51.4	105
HV-06																											
08/ 02/ 2017 23:00					3.04	0.80	2.24	9.13	0	0	2	4	0	0	1810.0						1160.3						156
09/ 02/ 2017 06:00					2.38	0.30	2.08	3.39	0	0	0	0	0	0	630.0	25.0	3197.0	203.0	51.0	10.0	1050.0	41.7	5328.3	338.3	85.0	16.7	60
09/ 02/ 2017 15:00					2.23	0.35	1.88	3.98	**6**	0	0	0	2	0	1350.0	25.0	2585.0	25.0	53.0	10.0	4218.8	78.1	8078.1	78.1	165.6	31.3	32
09/ 02/ 2017 20:00					2.46	0.33	2.14	3.49	**4**	0	2	2	2	2	3750.0	641.8	5879.0	25.0	174.0	41.0	4746.8	812.4	7441.8	31.6	220.3	51.9	79
10/ 02/ 2017 01:00					2.21	0.43	1.78	4.91	**4**	0	5	0	1	0	2610.0	751.7	7829.0	25.0	142.0	34.0	3262.5	939.6	9786.3	31.3	177.5	42.5	80
10/ 02/ 2017 06:00					2.24	0.63	1.61	7.17	0	0	0	0	0	0	1220.0	25.0	3515.0	25.0	83.0	10.0	3297.3	67.6	9500.0	67.6	224.3	27.0	37
10/ 02/ 2017 17:00					3.01	0.73	2.28	8.30	**5**	0	1	2	2	1	37040.0	25.0	7153.0	462.0	150.0	44.0	39828.0	26.9	7691.4	496.8	161.3	47.3	93
11/ 02/ 2017 01:00					4.57	1.08	3.49	12.22	**1**	0	5	10	0	1	27400.0	1282.2	6850.0	25.0	167.0	62.0	25607.5	1198.3	6401.9	23.4	156.1	57.9	107
11/ 02/ 2017 07:00					4.88	1.31	3.57	14.91	0	0	0	0	0	0	12690.0	2234.9	5285.0	25.0	112.0	41.0	19523.1	3438.4	8130.8	38.5	172.3	63.1	65
11/ 02/ 2017 19:00					5.53	0.93	4.60	10.62	**6**	0	0	0	1	2	7070.0	2809.6	4546.0	25.0	127.0	44.0	10100.0	4013.7	6494.3	35.7	181.4	62.9	70
12/ 02/ 2017 01:00					7.07	1.65	5.42	18.83	**4**	0	5	0	1	1	12510.0				286.0	98.0	7189.7				164.4	56.3	174
12/ 02/ 2017 10:00					3.59	0.82	2.77	9.35	0	0	0	0	0	0	4010.0	25.0	5480.0	344.0	135.0	35.0	4556.8	28.4	6227.3	390.9	153.4	39.8	88
12/ 02/ 2017 13:00					2.62	0.58	2.04	6.60	**2**	0	0	0	1	1	2480.0	1797.5	4620.0	25.0	122.0	34.0	2883.7	2090.1	5372.1	29.1	141.9	39.5	86
12/ 02/ 2017 21:00					2.34	0.50	1.84	5.69	**3**	**3**	1	0	2	3	17610.0	7931.8	8943.0	25.0	251.0	77.0	12760.9	5747.7	6480.4	18.1	181.9	55.8	138
13/ 02/ 2017 00:00					2.84	0.68	2.15	7.73	0	**3**	4	0	1	3	9480.0	5624.9	8056.0	25.0	179.0	60.0	7523.8	4464.2	6393.7	19.8	142.1	47.6	126
13/ 02/ 2017 06:00					2.86	0.70	2.16	7.95	0	0	0	0	0	0	1630.0	349.5	2564.0	245.0	51.0	10.0	3975.6	852.5	6253.7	597.6	124.4	24.4	41
13/ 02/ 2017 17:00					1.72	0.27	1.45	3.05	**5**	0	0	0	3	1	1260.0	825.3	2214.0	25.0	42.0	10.0	4344.8	2846.0	7634.5	86.2	144.8	34.5	29
13/ 02/ 2017 19:00					1.73	0.32	1.42	3.56	**3**	0	0	0	3	1	3060.0	2023.9	5884.0	249.0	150.0	47.0	3517.2	2326.4	6763.2	286.2	172.4	54.0	87
13/ 02/ 2017 20:00									**2**	0	1	0	4	1	2980.0	1685.3	6651.0	347.0	169.0	66.0	2684.7	1518.3	5991.9	312.6	152.3	59.5	111
14/ 02/ 2017 00:00					2.25	0.57	1.67	6.53	**3**	0	5	0	1	0	2160.0	1624.3	6271.0	161.0	140.0	42.0	2427.0	1825.0	7046.1	180.9	157.3	47.2	89
14/ 02/ 2017 06:00					2.01	0.38	1.63	4.37	0	0	0	0	0	0	1850.0	666.9	2848.0	177.0	86.0	10.0	5285.7	1905.3	8137.1	505.7	245.7	28.6	35
14/ 02/ 2017 17:00					2.25	0.60	1.65	6.85	**5**	0	0	0	2	1	44980.0	2247.8	7182.0	429.0	244.0	76.0	39113.0	1954.6	6245.2	373.0	212.2	66.1	115
14/ 02/ 2017 19:00					2.43	0.72	1.71	8.22	**4**	0	1	0	2	2	29180.0	2107.1	7785.0	372.0	319.0	91.0	21776.1	1572.5	5809.7	277.6	238.1	67.9	134
14/ 02/ 2017 22:00					3.58	1.10	2.49	12.50	**1**	0	4	0	3	2	14580.0	1284.2	5832.0	351.0	201.0	49.0	16022.0	1411.3	6408.8	385.7	220.9	53.8	91
15/ 02/ 2017 00:00					4.55	1.37	3.18	15.58	**1**	0	6	0	2	1	4900.0	477.9	3276.0	201.0	90.0	10.0	10652.2	1038.9	7121.7	437.0	195.7	21.7	46
15/ 02/ 2017 06:00					3.53	1.20	2.32	13.71	0	0	0	0	0	0	2860.0	400.6	2857.0	223.0	74.0	10.0	6975.6	977.0	6968.3	543.9	180.5	24.4	41

CHAPTER 6 CONCLUSIONS AND RESULTS INTERPRETATION – POLICY RECOMMENDATIONS

Since the current project considered evaluation of methodologies for determination of personal exposure to chemicals originating from wood burning, conclusions and recommendations are formulated together, as the main question was: which biomarker and sampling approach to be used in general population studies. Furthermore some ideas are formulated on how to interpret the (bio)markers in terms of health impact.

6.1. BIOMARKER FOR WOOD SMOKE, ARE WE THERE?

The urinary markers (OH-PAHs and levoglucosan) that were measured in the current study were all indicators of wood smoke exposure. In the feasibility pilot study 2 with repeated analysis in six individuals it was on some days possible to pinpoint rather precisely wood smoke exposure in a timeframe of 6-12h after exposure.
However, the analysed markers were **not exclusively indicators for wood smoke**. Since these markers are potentially present in urine in case of dietary sources, smoking/traffic/other air contaminants exposure, they are not that straight forward to use for detection of daily-life (low-medium) personal exposure levels.
This was shown by the analysis of biobanked samples of the first pilot feasability study. Although on some days the winter levels of levoglucosan in the outdoor environment of the five inhabitants peaked to 500-700 ng/m^3, it was not possible to systematically discover this exposure in the inhabitants, and there was no similarity in biomarker levels among couples of the same household. Furthermore, PAH and levoglucosan sources, other than wood smoke, probably disturbed the urinary exposure patterns. Detailed information on daily activities, would have been needed to interpret the biomarker values, such as time spent at home, or in traffic, complaints/nuisance reporting due to burning, time spent indoors/outdoor. It should also be remarked that the individuals of that study were exposed to woodsmoke only via outdoor sources, and the time frame for which the accumulated exposure to outdoor woodsmoke was measured was broad, namely 48 hours, compared to the half-life of the biomarkers (range of 4-12 hours). **Indeed, the pilot 2 feasibility study clearly showed that BC levels in the (6-12) hours before collection of urine** samples are needed to allow precise assessment of inhalation exposure to wood smoke PAHs in relation to the measured urinay biomarkers.

6.1.1. INTERPRETATION OF BIOMARKERS IN COMBINATION WITH AIR SAMPLING

Different biomarkers and samplers were tested. Quantifying a suite of compounds as a screen for exposure to woodsmoke, overcomes the difficulties of interpretation for individual compounds (Dills et al., 2001). Furthermore it was also stipulated that **assessing biomarkers in combination with**

ambient air quality measurements, could allow characterization of background wood smoke exposure (Dills, Zhu, & Kalman, 2001). Air quality concentrations in combination with human biomarkers are furthermore effective methods for education and stimulation of reduction of exposure to wood combustion products.

→ Air sampling of wood buring compounds

BC, a major wood burning compound can be sampled using an aethalometer (generating levels on minute base). Using multiwavelength aethalometers, as was used in the current study, the BC fraction from wood burning (BC_WB) can be derived, allowing even better estimation of the wood smoke exposure. In the current study, we did not always have BC levels indoor as well as outdoor. Even if the levels of BC_WB were mostly lower indoors compared to outdoors, assessing indoor concentrations is very relevant, as people, especially in winter, spend more hours inside than outside. Shin et al. (2013) reported that the major exposure route of NAP, FLU, PHE and PYR is likely indoor inhalation. This includes all indoor environments, also other than home. Indeed, it was observed in the current study, that spending several hours indoors at other locations with possible wood smoke exposure, had its impact on LEV and OH-PAH levels in the evening-next morning urine samples (e.g. HV-01 visiting other wood stove residence). Therefore, the most optimal way to assess the complete wood smoke exposure would be by personal sampling e.g. via new available personal multi-wavelength microaethalometer samplers (such as MA200, https://aethlabs.com/microaeth).

Assessing personal exposure was in the current study mainly tested by applying the methodology of PAH and/or levoglucosan analysis in silicone wristbands or hair, passively collecting these compounds:

- Analysing levoglucosan in hair was possible, but in some chromatograms, high background levels were present. It did not appear to be a straightforward matrix for this analysis.
- Levoglucosan collected on the silicone wristband, could not anymore being extracted from it, in other words the wristband was not a suitable sampler for levoglucosan.
- The wristband was however suitable for PAH analysis (as published by (O'Connell et al., 2014). A precleaning and LC-MS analysis methodology was tested and worked out. In the wood smoke experiment, it could be seen that mainly the low molecular weight PAHs (NAP, ACY, ACE, FLU, PHE, ANT, FLA) were dectectable on the wristband after exposure to the wood fire, and a gradient of decreasing concentrations away from the fire was observed. These lower molecular weigh compouds are known quantitatively the most apparent tracers for wood smoke exposure. In the feasibility pilot 2, the six participants were wearing a wristband during four weeks. A considerable amount of dermal compounds were accumulated on the wristband, and visible in the chromatogram. Within the frame of the current study no clean-up of the extract was done yet, but the crude extract allowed to compare individuals considering extend of (mainly lower molecular weight) PAH exposure. Mainly the two suspected higher wood smoke exposed individuals HV-02 (from neighborhood wood stove) and HV-06 (own wood stove), had levels of both FLU and PHE that were higher than the other paricipants. FLU and PHE may be interesting potential wood smoke biomarkers for comparative passive sampling of wood smoke exposure assessment via the wristband or possibly an alternative in shape of silicone 'patch'.

→ Interpretation of internal exposure markers for wood burning compounds

The urinary markers (OH-PAHs and levoglucosan) that were measured in the current study were all indicators of wood smoke exposure. In the feasibility pilot study 2 with repeated analysis in six individuals it was on some days possible to pinpoint rather precisely wood smoke exposure in a timeframe of 6-12h after exposure.
However, the analysed markers were not exclusively indicators for wood smoke. Since these markers are potentially present in urine in case of dietary sources, smoking/traffic/other air contaminants exposure, they are not that straight forward to use for detection of daily-life (low-medium) personal exposure levels. This was shown by the analysis of biobanked samples of the first pilot feasability study. Although on some days the winter levels of levoglucosan in the outdoor environment of the five inhabitants peaked to 500-700 ng/m^3, it was not possible to systematically discover this exposure in the inhabitants, and there was no similarity in biomarker levels among couples of the same household. Furthermore, PAH and levoglucosan sources, other than wood smoke, probably disturbed the urinary exposure patterns. Detailed information on daily activities, would have been needed to interpret the biomarker values, such as time spent at home, or in traffic, complaints/nuisance reporting due to burning, time spent indoors/outdoor. For both wood smoke and traffic exposure time-activity patterns were ineed indicated as critical determinants of exposure (Laumbach & Kipen, 2012). One could argue that quesstionnaires may be sufficient for assessment of wood smoke exposure. To some extend, this may be through. In case of limited budget, one could do the query combined with collection of urine. Based on the questionnaire data one could select the individuals which are complaining/mentioning wood smoke expsore, and analyse those samples in comparison to a pool of the other non-exposed individuals.
It should also be remarked that the individuals of the current pilot1 study were exposed to woodsmoke only via outdoor sources, and the time frame for which the accumulated exposure to outdoor woodsmoke was measured was broad, namely 48 hours, compared to the half-life of the biomarkers (range of 4-12 hours). Indeed, the pilot 2 feasibility study clearly showed that BC levels in the (6-12) hours before collection of urine samples are needed to allow precise assessment of inhalation exposure to wood smoke PAHs in relation to the measured urinay biomarkers. In all other sampling shemes, there is still uncertainty on the urinary markers, to which extend they originate from wood smoke exposure. The markers should therefore be interpreated as indicators that need to be checked by air quality measurements (prefereably by personal samplers). Since the latter measurements are more expensive, urinary markers can be used to do a prescreening.

Urinary Levoglucosan appeared to be elevated in case of wood smoke exposure, but not in a consistant way. The inhabitant HV-02 had (mostly) the highest levels at the home environment, but had only temporarly higher levels of urinary levoglucosan. This compound tends to be mainly visible in case the air concentration of wood smoke compounds is clearly higher and if urine is sampled quickly after exposure (within the first hours). The increase in urinary levels of LEV, and also other markers in the controlled wood smoke exposure experiment was maximal a factor 2. Bergauff et al. (2010) reported a non-consistent response to a controlled campfire exposure (majority of participants between minor and 2-5 times increase, sometimes showing multiple peaks post exposure). It may be speculated that a urinary LEV increase of more than 5 to 10 times the background value of a person, may be due to a peak from food exposure. The main dietary source of urinary LEV is food intake of caramelized products (as was clearly seen in current study with 100-fold increase of urinary LEV after consumption of pancakes with candy sugar by one of the participants).

The pattern of all the assessed OH PAHs, was usefull for assessing the exposure source. In case there is consistency in the increased levels of the parameters (possibly in combination with increased urinary LEV), it is more plausible that wood smoke exposure appeared. Although, 1OH PYR is not a marker for wood smoke, as the levels were lower in case the individuals were exposed to wood smoke (HV-01, HV-02, HV-05 and HV-06). Also the unidentified OH-PAH metabolite close to OH-NAP in the chromatogram, was relatively low in participants (occasionally) exposed to BC_WB (HV-01, HV-05). 2+3OH fluorene in combination with the unidentified OH-PAH metabolite were higher in individuals reporting daily exposure to traffic exhausts (HV-04 and HV-06). Both 2+3OH FLU and 1OH PYR may therefore be helpful in understanding that there were other than wood burning sources of PAHs.

In summary: Based on the limited amount of results from the current study, it can be speculated that indoor wood smoke exposure may be one of the major sources, causing an increase of the urinary OH-PAHs, although locally outdoor levels may be of more importance. Overall it can be advised to screen OH PAHs in urine, minimal in combination with detailed information on daily activities (see above), or if possible in combination with a personal BC sampler, or passive silicone sampler type, although the latter still needs to be worked out.

6.2. SAMPLING METHODOLOGY AND HUMAN BIOMONITORING – RECOMMENDATIONS

Overall, since the LEV and OH PAH biomarkers, may be to a variable larger or lesser extend influenced, by other than woodsmoke sources, such as e.g. diet, traffic and/or indoor sources, applying different biomarkers and/or personal samplers combined with a good sampling strategy to bypass confounding.

Considering improving the biomarker sampling strategy, some recommendations can be done based on the current study:

- Season: Wood smoke exposure studies only need to be set up in the winter, assuring that a considerable proportion of the urinary OH PAHs originates from wood smoke. In case the aim is to study the relationship between wood smoke exposure and measured or recorded health effect markers, a sampling in both seasons is favorable;
- Since wood burning for domestic heating mainly occurs in the evenings, urine samples should be collected in the evening and/or morning;
- Sampling urine in the evening as well as at the first void in the morning, allows to assess the consistency of the exposure. Pooling of those samples could be a good option (not tested in the current study);
- Different individuals tend to have different levoglucosan background levels (most probably because of different basic diet intakes). Ideally it would be good to have this level assessed e.g. in the second void urine of the individual;
- Pooling: Collection of urine in several individuals of a same close geographical area, and pooling them to assess small-regional levels of woodsmoke exposure, and at the same time randomizing the influence of peak exposure and/or dietary influences was suggested by Wallner et al. (2013b). This concept is interesting and should be further explored. However,

we speculate that this allows mainly identification of high exposure. Pooling of the samples should according to the research question, e.g.:

- Comparing individuals with wood burning indoors vs. no indoor wood burning;
- Individuals experiencing nuisance and/or health complaints from wood burning vs. those without complaints;
- Individuals (without wood buring indoors), using continuous mechanic ventilation (type C and D) having more exposure to outdoor wood burning compounds vs. those individuals living in homes with non-mechanical ventilation?
- Are individuals in lower-populated (rural/suburban) geographical areas, more exposed to woodsmoke, than people living in urban areas.

- Food intake and other confounders:
  - There are some main confounders which are known to influence the urinary levels of the tested wood smoke biomarkers in the current study (Table 25~~Table 25~~).
  - In the pilot 2 feasibility study (of the current study) the participants were asked to fill out a diary table, which appeared very useful for interpretation of the data (See annex 2). Hourly reporting on time spent at home, or in traffic, complaints/nuisance due to burning, time spent indoors/outdoors, food intake, and cooking activity by the participant are useful as information.
  - Considering food, for levoglucosan it is most feasible to ask participants to fill out a (diary) table indicating if they were eating one of those food items in the past 6-12 hours. There are a lot of food items, that can contribute to the intake. For OH-PAHs, Li et al. (2010) recommended that participants should be asked not to eat grilled, smoked and barbecued food 12 hours before urine collection, to avoid confounding (Li et al., 2010).

Table 25: Potential confounders for the tested wood smoke biomarkers

Parameters of influence	OH-PAHs	Methoxyphenols (guaiacol, syringol)	Levoglucosan
Sigarette smoking	Active and passive smoking	Possible influence	Possible influence
Other air pollution	Traffic, industry	-	-
Diet	grilled, smoked and barbecued food (Li et al., 2010)	Smoked foods, Smoke flavoring (Dills et al., 2001) Smoke flavoring: mainly cresols, to lesser extend guaiacols and syringols (Russell L Dills et al., 2006)	Caramelized sugar containing food (Li et al., 2015a). List of possible food items (non-exhaustive) containing caramel (colors): baking ingredients, dark beers, cooking/table sauces, bouillon, energy/sport drinks, coca cola, gums/jellies, hard candies, ice cream, bread/cakes/biscuits, chocolate sweets, ready meals, soups (Sprong et al. 2014)

Medicines	1-OH NAP metabolite of beta-blocker propranolol (Li et al., 2015a)	Coughing medicines (guafenesin = glyceryl guaiacolate) possibly for guaiacol (Dills et al., 2006)	-
Pesticides	1-OHNAP main metabolite of carbamate insecticide carbaryl, and the herbicide napropamide (Li et al., 2015a)	-	-
Indoor	Incense burning, mothballs, candles, cooking aerosols	-	-

- Collection conditions: There is no special requestment to sampling materials. Urine is collected in polypropylene tubes. After collection, urine can be left for 2 days at room temperature (Li et al., 2015a) and afterwards it is stored in the freezer at -20°C.
- Correction for urine density: and instead of correction of urine density for creatinine, the excretion rate of the compound can be calculated. The calculation of the excretion rate is based on the amount of the compound excreted, during the period between the time point of the previous and current void. Therefore information on these time points, as well as volume are needed to be collected when sampling urine. This is not affected by diuresis and the hydration state of the individual.
- Applicacability of markers/samplers relevant to assess wood smoke exposure in human biomonitoring on short or longer term:

Table 26: Markers/samplers relevant for human wood smoke exposure and their applicability on short or longer term

Biomarker or sampler	Phase of applicability	Indicative price (EUR)
URINE		
OH-PAHs	Methodology ready for use (2OH NAP, 2+3 FLU, 2+3 PHE, 1OH PYR). NB: still unidentified NAP, which might be interesting	70
levoglucosan	Methodology ready for use. NB: other food caramel compounds that could be measured in urine for confounder correct, still in test phase	85
Urinary guaiacol, syringol	Methodology ready for use	140
Levoglucosan	Methodology ready for use	60
PASSIVE + ACTIVE SAMPLERS		
Silicon wristband: PAHs	Methodology with potential. Precleaning essential. Easy storage at room temperature in airtight PTFE bags with closure. After use storage in same bag at room temperature. Improvements needed and/or to be tested within short term (weeks): - clean-up of liquid extract - sampling period - storage conditions after wearing of wristband	150 (wristband + analysis)

Biomarker or sampler	Phase of applicability	Indicative price (EUR)
Silicon patch: PAHs	Similar methodology as for silicon wristband (see above). Improvements needed and/or to be tested within short term (weeks): - sampling period - storage conditions after wearing of patch	150? (patch + analysis)
Passive sampler type radiello: PAHs and guaiacol, syringol	Potentially useful. Uptake rate of PAHs need to be determined. Needs some weeks to be determined (+ associated working hours)	<100 (sampler and analysis)
Personal aethalometer (multiwavelenght)	Ready to use, once available on the market. Measures on different wavelengths. Calculation of BC from wood smoke possible. (e.g. MA200, https://aethlabs.com/microaeth)	10 000 (per sampler)+costs for data analysis
Personal filter + pump on batteries	To be constructed, analysis of levoglucosan and PAHs on the filter for personal use. Needs several months to be developed	Target price: <150 (?)

6.3. HEALTH INTERPRETATION

Woodsmoke is considered to contribute to the toxicity of PM outdoor as well as indoor. 'Indoor emissions from combustion of biomass fuel (primarily wood)' has been classified by the International Agency for Research on Cancer as a Group 2A carcinogen i.e. probably carcinogenic to humans (http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf). It has been reported that PM resulting from burning of plants (wood) has a higher oxidant capacity compared to particles from diesel exhaust (EPA, 2010, cited by Wallner et al., 2013b). Furthermore, recently, Manoli et al. (2016) reported that the wintertime carcinogenic and mutagenic potencies of particles and the PAH-induced inhalation cancer risk were almost equal at an urban and background site in Thessaloniki (northern Greece). Although traffic was the dominant contributor, also wood smoke contributed considerably namely 17 and 28 % to total ambient BaP at the traffic and at the urban background site, respectively. Its importance is also linked to the fact that sources of biomass (as well as traffic emissions) are close to habitation. Biomass emissions often occur primarily indoors, and further distribution outdoors via residential chimneys.

There are several studies on wood smoke exposure and the link with effect biomarkers or health. Health effects examined are respiratory health, cardiovascular outcomes and genotoxicity/cancer risk (Laumbach & Kipen, 2012). Health effects of wood smoke exposure can be examined via **long function test, fractional analysis of exhaled nitric oxide, asthma symptoms, examination of inflammation and oxidative stress markers in nasal mucus or exhaled breath, systemic inflammation, coagulation** (e.g. fibrinogen, platelet counts), **hearth rate variability parameters, lipid peroxidation markers** (F_2-isoprostane, malondealdehyde), **genotoxicity testing** (comet assay, micronucleus). Some studies show (limited) effects after exposure to wood smoke (Barregard et al., 2008; Stockfelt et al., 2013; Bønløkke et al., 2014)(Ghio et al., 2012), whereas others report on increased risk (Stockfelt et al., 2013; Murgia et al., 2016).

A direct health interpretation of the in the current study used biomarkers is limited because of the lack of health guidance values for the OH PAH metabolites in urine. A possible approach for health interpretation, is to do a simplified calculation of the intake of PAHs via the airways. In earlier studies, and from the current wood smoke project, 2OH NAP, OH fluorenes and OH anthracenes appeared to be the more specific biomarkers for wood smoke exposure.

Small PAHs with two or three rings, such as NAP, FLU and PHE, exist mainly in the gaseous phase, whereas those with four or more rings (e.g. PYR or BaP) occur in the particle phase (Li et al., 2015a). The major exposure route of naphthalene, fluorene, phenanthrene and pyrene was estimated to be inhalation of indoor air, calculated based on the indoor concentrations and urinary levels of the OH-metabolites measured in the NHANES human biomonitoring campaign (Shin et al., 2013). For these PAHs with a low vapor pressure (below 0.01 Pa for NAP, FLU, PHE), 97% of the intake was due to indoor sources, and for less volatile/more persistent PAHs, such as benzo[a]pyrene, more than 95% of the intake was from food consumption (Shin et al., 2013). Based on mass balance calculation done in the current study, during the controlled wood smoke exposure study, it could be seen that the amount of those compounds inhaled and excreted in urine, is in the same magnitude of order (Table 17~~Table 17~~).

For estimation of the health impact one could follow a rough calculation (could be checked using PBPK modeling such as IndusChemFate model). Urinary OH PAH levels can be back calculated to the levels in air, assuming complete conversion of the PAH to the OH PAH metabolite and complete absorption. OH-PAHs are a result of initial metabolism of the compound by cytochrome P_{450} enzymes. Further metabolism products are not measured when analysing the OH-PAHs. This is a source of uncertainty that might lead to an underestimation of cumulative PAH intake (Shin et al., 2013). For the calculation one can assume a urine production of 1500 mL per day, and inhalation of 16-20 m^3 of air per day. This means that the urine concentration is then back-calculated to an air concentration as follows: ng/m3 air = (ng/L urine * 1.5 L/day)/(16 m^3/day)

As urinary NAP, PHE, FLU metabolites also originate from other than wood burning sources, one needs to multiply calculated exposure with the fraction that is really wood burning related. Since there are no non-occupational inhalation exposure values for the targeted PAH compounds, the toxicity needs to be estimated based on the comparison with the unit cancer risk of BaP being being 0.01 ng/m^3 air for a cancer risk of $1/10^6$. For calculation of the PAH relative to BaP, a toxicological equivalence factor can be used, which is only applicable for relative estimation of the PAH toxicity with respect to cancer outcome.

CHAPTER 7 EXPERT CONSULTATION

7.1. EXPERT CONSULTATION ON STUDY APPROACH AND WOOD SMOKE BIOMARKERS

Some experts were consulted on their ideas about how to tackle the assessment of wood smoke exposure in the general population using biomarkers and/or external markers.

Zheng Li

Lead Environmental Health Scientist - Environmental Epidemiology Branch (EEB), Division of Toxicology and Human Health Sciences (DTHHS). Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, USA. She has extensively worked on urinary PAH markers and wood smoke exposure (Li et al., 2016b; Li et al., 2015b;Riojas-Rodriguez et al., 2011;Jung et al., 2014;Li et al., 2011):

"Based on your description, I think it'll be challenging to tease out woodsmoke exposure from the general environmental exposure in such a study. However, it should be achievable by measuring ***multiple woodsmoke biomarkers*** *(in urine) and* ***markers*** *(in external samples such as dermal wipe, hair sample, personal air sample).*

Measuring all three groups of proposed urinary woodsmoke biomarkers (levoglucosan, methoxyphenols, PAH metabolites) should serve as a ***"triple" confirmation****, although the low sensitivity of urinary levoglucosan means it may not be able to capture low woodsmoke exposure (see discussion on these biomarkers in* (Li et al., 2015a)*. I myself have not worked on measuring levoglucosan and methoxyphenols in urine, so I cannot speak for the analytical method performance on these two analytes/groups.*

Using external samples, e.g., dermal wipes, hair, and personal air samples, should be very helpful, with good analytical methods and sufficient QA/QC measures to account for potential external contaminations/background "noise". Retene, an alkylated phenanthrene (1-methyl-7-isopropylphenanthrene), has been proposed as a woodsmoke marker in air and I previously had found that it was very responsive to wood smoke source in air (see attached paper:(Li et al., 2009)).

Therefore, I ***highly recommend measuring retene, in addition to levoglucosan, methyoxyphenol and PAH, if you decide to collect external samples****." (e-mailing in period of November2016-January 2017)*

Rémy Slama

Inserm, Team of Environmental Epidemiology Applied to Reproduction and Respiratory Health, U1209 (IAB), Grenoble, France, and Univ. Grenoble-Alpes, Team of Environmental Epidemiology applied to Reproduction and Respiratory Health, IAB, Grenoble, FranceInserm- Grenoble, France

Dr. Slama indicated that in Grenoble air measurements of levoglucosan are performed as they are cheaper (centrally measured). In case one choses for urine biomarkers (with which they do not have experience themselves), he suggested, there might be a specificity issue. "*In case a few people eat food containing caramel or if these can be identified by questionnaire, then in the case of a short half-life, which is not specific to air-born levoglucosan, the solutions would be:*

- to ***collect repeated samples within subject*** *(see e.g. our recent paper on this within-subject pooling approach, attached) (Perrier, Giorgis-Allemand, Slama, & Philippat, 2016);*

- to collect ***one sample per subject, but at random times****, which would give a valid answer of the average population exposure - this is assuming you just want to describe exposures, not relate them to health." (e-mailing: November 2016).*

Spyros Karakitsios

Aristotle University of Thessaloniki, Department of Chemical Engineering, Environmental Engineering Laboratory, 54124 Thessaloniki, Greece, and Centre for Research and Technology Hellas, Chemical Process and Energy Resources Institute, Natural and Renewable Resource Exploitation Laboratory, 57001 Thessaloniki, Greece.

Personal contact during meeting (November 2016): Dr. Karakitsios indicated that biomarkers for assessment of wood smoke exposure are a difficult issue, as there is no specific biomarker available. He suggested a combination of internal expsosure measurement of e.g. OH PAHs and external assessment of specific wood smoke compounds, would be possibly the only feasible way.

Laura Campo

Department of Occupational and Environmental Health, University of Milan, Italy. Personal contact during meeting (December 2016)

Dr. Campo had no experience with measuring specific wood smoke compounds in human. They are specialized in analyzing parent PAH compounds in urine. It might be an option to assess unmetabolized PAHs in urine, followed by calculation of diagnostic ratios for source apportionment, such as FLA/(FLA+PYR), and IcdP/(IcdP+BghiP). In case these ratios are above 0.5, there is an indication of grass, wood, coal combustion origine of the PAHs. However this approach is approximative, as also differences in diet and food preparation and changes in the relative appearance of the compounds in urine (due to different metabolisation in the human body) compared to the composition in the air, may occur (Campo et al., 2007; De Craemer et al., 2016).

Gerard Hoek

Institute for Risk Assessment Sciences (IRAS), Environmental Epidemiology, Utrecht University, The Netherlands:

Dr. Hoek indicated that he had no experience in wood smoke biomarkers. There had been some contacts with Dr. Greg Evans of the University of Toronto (USA) on using wristbands for personal monitoring of semi-volatiles (others than PAHs). The problem with PAH monitoring is their unspecificity for wood smoke. In air, levoglucosan is used, but in urine it might be too much confounded.

7.2. EXPERT CONSULTATION ON HEALTH INTERPRETATION METHODOLOGY

Considering the health interpretation of the data, four experts were consulted.

Spyros Karakitsios

Aristotle University of Thessaloniki, Department of Chemical Engineering, Environmental Engineering Laboratory, 54124 Thessaloniki, Greece, and Centre for Research and Technology Hellas, Chemical Process and Energy Resources Institute, Natural and Renewable Resource Exploitation Laboratory, 57001 Thessaloniki, Greece.

Dr. Krakitsios is experienced in estimation of health risk associated with particulate matter-bound PAHs originating from biomass combustion. In the recent publications on the effects of biomass combustion, (Sarigiannis et al., 2015; Sarigiannis et al., 2014).

Annette Rohr

Principal Project Manager, Air Quality and Health, Electric Power Research Institute, 16541 Redmond Way #176C, Redmond, WA 98052, U.S.A.

Dr. Rohr made a review paper on health impacts of organic aerosols, with a focus on specific sources of organic material. In the review it was stated that the literature suggests clear health impacts from emissions containing carbon-containing PM, but that the difficulty remains in apportioning responsed to certain groups of carbonaceous materials, such as organic and elemental carbon, condensed and gas phases, and primary and secondary material (Rohr & McDonald, 2016). Dr. Rohr was contacted via mail, and communicated that she did not have experience with organic biomarkers of wood smoke exposure, and their health impact estimation.

Kathleen de Brouwere and Lieve Geerts

VITO, Environmental Risk and Health, Flemish Institute for Technological Research, Mol, Belgium.

The approach of the approximate calculation of exposure to PAHs was discussed with them.

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ANNEX A CHEMICAL COMPOSITION OF WOOD SMOKE

After: Larson & Koenig (1994)

Table 1 Chemical composition of wood smoke

Specie[1]	g/kg wood[2]	Physical state[3]	Reference
Carbon monoxide	80–370	V	25, 74
Methane	14–25	V	74
VOCs (C_2–C_7)	7–27	V	74
Aldehydes	0.6–5.4	V	25, 62
Formaldehyde	0.1–0.7	V	25, 62
Acrolein	0.02–0.1	V	62
Propionaldehyde	0.1–0.3	V	25, 62
Butryaldehyde	0.01–1.7	V	25, 62
Acetaldehyde	0.03–0.6	V	25, 62
Furfural	0.2–1.6	V	30, 34
Substituted furans	0.15–1.7	V	30, 34
Benzene	0.6–4.0	V	74
Alkyl benzenes	1–6	V	89
Toluene	0.15–1.0	V	89
Acetic acid	1.8–2.4	V	30
Formic acid	0.06–0.08	V	30
Nitrogen oxides (NO,NO_2)	0.2–0.9	V	25, 74
Sulfur dioxide	0.16–0.24	V	25
Methyl chloride	0.01–0.04		48
Napthalene	0.24–1.6	V	89
Substituted napthalenes	0.3–2.1	V/P	89
Oxygenated monoaromatics	1–7	V/P	89
Guaiacol (and derivatives)	0.4–1.6	V/P	35
Phenol (and derivatives)	0.2–0.8	V/P	35
Syringol (and derivatives)	0.7–2.7	V/P	35
Catechol (and derivatives)	0.2–0.8	V/P	35
Total particle mass	7–30	P	74
Particulate organic carbon	2–20	P	21
Oxygenated PAHs	0.15–1	V/P	89
PAHs			
Fluorene	4×10^{-5}–1.7×10^{-2}	V/P	1, 19, 21, 46, 92, 99
Phenanthrene	2×10^{-5}–3.4×10^{-2}	V/P	1, 19, 21, 46, 92, 99
Anthracene	5×10^{-5}–2.1×10^{-2}	V/P	1, 19, 21, 46, 92, 99
Methylanthracenes	7×10^{-5}–8×10^{-3}	V/P	1, 19, 21, 92, 99
Fluoranthene	7×10^{-4}–4.2×10^{-2}	V/P	1, 19, 21, 92, 99
Pyrene	8×10^{-4}–3.1×10^{-2}	V/P	1, 19, 21, 92, 99
Benzo(a)anthracene	4×10^{-4}–2×10^{-3}	V/P	1, 19, 21, 92, 99
Chrysene	5×10^{-4}–1×10^{-2}	V/P	1, 19, 21, 92, 99
Benzofluoranthenes	6×10^{-4}–5×10^{-3}	V/P	1, 19, 21, 92, 99
Benzo(e)pyrene	2×10^{-4}–4×10^{-3}	V/P	1, 19, 21, 92, 99
Benzo(a)pyrene	3×10^{-4}–5×10^{-3}	V/P	1, 19, 21, 92, 99
Perylene	5×10^{-5}–$3 - 10^{-3}$	V/P	1, 19, 21, 92, 99
Ideno(1,2,3-cd)pyrene	2×10^{-4}–1.3×10^{-2}	V/P	1, 19, 21, 92, 99
Benz(ghi)perylene	3×10^{-5}–1.1×10^{-2}	V/P	1, 19, 21, 92, 99
Coronene	8×10^{-4}–3×10^{-3}	V/P	1, 19, 21, 92, 99

Table 1 (*Continued*)

Specie[1]	g/kg wood[2]	Physical state[3]	Reference
Dibenzo(a,h)pyrene	$3 \times 10^{-4} - 1 \times 10^{-3}$	V/P	1, 19, 21, 92, 99
Retene	$7 \times 10^{-3} - 3 \times 10^{-2}$	V/P	21, 46
Dibenz(a,h)anthracene	$2 \times 10^{-5} - 2 \times 10^{-3}$	V/P	19, 21, 46, 92, 99
Trace elements			
Na	$3 \times 10^{-3} - 1.8 \times 10^{-2}$	P	21, 46, 96
Mg	$2 \times 10^{-4} - 3 \times 10^{-3}$	P	21, 46, 96
Al	$1 \times 10^{-4} - 2.4 \times 10^{-2}$	P	21, 46, 96
Si	$3 \times 10^{-4} - 3.1 \times 10^{-2}$	P	21, 46, 96
S	$1 \times 10^{-3} - 2.9 \times 10^{-2}$	P	21, 46, 96
Cl	$7 \times 10^{-4} - 2.1 \times 10^{-1}$	P	21, 46, 96
K	$3 \times 10^{-3} - 8.6 \times 10^{-2}$	P	21, 46, 96
Ca	$9 \times 10^{-4} - 1.8 \times 10^{-2}$	P	21, 46, 96
Ti	$4 \times 10^{-5} - 3 \times 10^{-3}$	P	21, 46, 96
V	$2 \times 10^{-5} - 4 \times 10^{-3}$	P	21, 46, 96
Cr	$2 \times 10^{-5} - 3 \times 10^{-3}$	P	21, 46, 96
Mn	$7 \times 10^{-5} - 4 \times 10^{-3}$	P	21, 46, 96
Fe	$3 \times 10^{-4} - 5 \times 10^{-3}$	P	21, 46, 96
Ni	$1 \times 10^{-6} - 1 \times 10^{-3}$	P	21, 46, 96
Cu	$2 \times 10^{-4} - 9 \times 10^{-4}$	P	21, 46, 96
Zn	$7 \times 10^{-4} - 8 \times 10^{-3}$	P	21, 46, 96
Br	$7 \times 10^{-5} - 9 \times 10^{-4}$	P	21, 46, 96
Pb	$1 \times 10^{-4} - 3 \times 10^{-3}$	P	21, 46, 96
Particulate elemental carbon	0.3–5	P	21, 78
Normal alkanes ($C_{24}-C_{30}$)	$1 \times 10^{-3} - 6 \times 10^{-3}$	P	21
Cyclic di- and triterpenoids			
Dehydroabietic acid	0.01–0.05	P	87
Isopimaric acid	0.02–0.10	P	87
Lupenone	$2 \times 10^{-3} - 8 \times 10^{-3}$	P	87
Friedelin	$4 \times 10^{-6} - 2 \times 10^{-5}$	P	87
Chlorinated dioxins	$1 \times 10^{-5} - 4 \times 10^{-5}$	P	72
Particulate acidity	$7 \times 10^{-3} - 7 \times 10^{-2}$	P	72

[1]Some species are grouped into general classes as indicated by italics.
[2]To estimate the weight percentage in the exhaust, divide the g/kg value by 80. This assumes that there are 7.3 kg combustion air per kg of wood. Major species not listed here include carbon dioxide and water vapor (about 12 and 7 weight percent, respectively, under the assumed conditions).
[3]At ambient conditions; V = vapor, P = particulate, and V/P = vapor and/or particulate (i.e. semivolatile).

ANNEX2: INSTRUCTIONS FOR THE PARTICIPANTS TO PILOT STUDY 2

Pilootstudie houtverbrandings-biomerkers

In deze pilootstudie testen we of stoffen die ontstaan bij houtverbranding – verbrandingsmerkers, o.a. levoglucosan, polycyclische aromatische koolwaterstoffen (PAK's) en methoxyfenolen – meetbaar zijn in urine, op haren en op silicone-armbandjes die u persoonlijk draagt. De studie wordt door VITO uitgevoerd in opdracht van de Vlaamse Overheid - Leefmilieu Natuur en Energie (LNE), dienst Milieu en Gezondheid.

OVERZICHT STUDIE

In de week van de sampling , moet u het volgende doen:

- Luchtsampling black carbon in het oog houden: de luchtsampler wordt opgesteld en weer afgehaald door een veldwerker van VITO. Dit toestel meet continue de black carbon in de omgeving. Op regelmatige tijdstippen moet u de goede werking van het toestel nakijken. Dit is zichtbaar op het apparaat. Indien stroompanne of het toestel afslaat, kan je het volgende doen:
  1. aanzetten : zie instructiefoto's AE33 1 t/m 4 in bijlage 1:
     - schakel de power switch op de achterzijde van het toestel naar "ON (I)"
     - schakel de power switch op de binnenzijde van het toestel (achter de deur) naar "ON (I)"
     - het toestel doorloopt een self-test (screen "welcome to aethalometer start")
     - het toestel voert een " auto start " uit en na een 5-tal minuten verschijnt "instrument status 0" (= measuring)
  2. uitschakelen : vanuit het "home" screen ; alleen op vraag van VITO : zie instructiefoto's AE33 5 t/m 9 in bijlage 1:
     - ga naar het screen "operation"
     - druk op de toets "stop", "shutdown", "yes"
     - wacht tot de shutdown procedure beëindigd is
     - schakel de power switch op de binnenzijde van het toestel (achter de deur) naar "OFF (0)"
     - schakel de power switch op de achterzijde van het toestel naar "OFF (0)"

- Silicone armbandjes dragen: op de dag van start van de luchtsampling begin je met het dragen van 4 silicone armbandjes. Deze armbandjes zullen de verbrandingsmerkers in je omgeving opnemen. Later kunnen die in het laboratorium worden gemeten. Haal ze uit de verpakking en houd deze verpakking zorgvuldig bij. Op de laatste dag, steek je 2 armbandjes terug in de originele verpakking, sluit je deze en bewaar je ze in de koelkast. Draag de armbandjes zoveel mogelijk boven je kledij (boven mouw van bv. trui of jas, als het kan). Twee armbandjes draag je nog 3 weken langer. Daarna steek je ook die armbandjes terug in de originele verpakking, sluiten en bewaren in de koelkast.

- Elke dag van de staalname week urine verzamelen: bij het opstaan (ochtendurine), 's avonds bij het thuiskomen of ca. 17-18u, in ieder geval voor het avondeten indien mogelijk, en verder alle urine tot voor het slapengaan.
  Vul op de lijst onderaan de nummer in van het recipiënt dat je hebt gebruikt en het tijdstip. De recipiënten mogen worden ingevroren. Gekoeld bewaren mag ook.

Haar laten samplen: de laatste dag van de week wordt door een veldwerker 100mg haar gecollecteerd met een schaar. Dit is gelijk aan 1 plukje haar. De staalname is van die aard dat je er achteraf niets van merkt.

- Logboekje + vragenlijst invullen: per dag aanduiden wanneer thuis op de woonplaats, wanneer urine gecollecteerd, wanneer houtkachel/open haard aangestoken en wanneer last van houtrook.

Samenvatting van de taken die worden uitgevoerd in de week van de staalname:

Lucht staalname Black Carbon gedurende 1 week	2 silicone armbandjes 1 week dragen; 2 bandjes 4 weken	Dagelijkse staalname urine	Staalname haar op einde van de week	Dagelijks logboek + vragenlijst invullen
Apparatuur wordt aangezet bij begin week en uitgezet op het einde van de week	Haal uit verpakking en draag 1 week (2 bandjes) en 4 weken (2 bandjes) Daarna, terug in verpakking steken	- ochtendurine - ca. 17-18u of voor het avondeten - bij slapen gaan	Plukje haar laten nemen op eind van de week	Vul aan voor elke dag van de week

JE GEGEVENS

Graag hieronder je gegevens invullen. We zullen alle gegevens gecodeerd verwerken.

Naam: _____
Adres: _____
Telefoon nummer.: _____
E-mail adres (eventueel): _____

Datum bij invullen van dit deel van de vragenlijst:...../...../.....

Info over uzelf

1. Geboortedatum: ...../...../.....
2. Geslacht: O man O vrouw
3. Gewicht:.....kg
4. Lengte:.....cm

Werk/beroep

5. Werkt u momenteel? o neen
   o ja

6. Belangrijkste activiteit op het werk.....

7. Postcode en naam van de gemeente/stad waar u werkt.....

INSTRUCTIES + INVULTABEL tubes urinecollectie

Elke dag verzamelt u urine op volgende tijdstippen: (1) ochtendurine, (2) 's avonds bij het thuiskomen bv. ca. 17-18u, of in ieder geval voor het avondeten en (3) voor u gaat slapen (kunnen meerdere monsters zijn).
De urinerecipiënten zijn genummerd. Haal het recipiënt uit het plastic zakje en noteer het codenummer in de tabel hier onderaan.

- Open de schroefdop en in plas rechtstreeks in het recipiënt met urine. Niet volledig vol maken. Indien dit het geval zou zijn, kan je een beetje uitgieten in het toilet.
- Schroefdop terug op het recipiënt plaatsten en in het geheel in het plastic zakje steken.
- Bewaar dit recipiënt in de diepvriezer en vul de bijhorende vragenlijst in.

Invultabel voor inventarisatie van de gevulde urinerecipiënten

Staal	Identificatienummer urinerecipiënt	datum	uur	Opmerkingen (bv. niet in diepvriezer bewaard,...)
1				
2				
3				
4				
5				
6				
7				
8				

Staal	Identificatienummer urinerecipiënt	datum	uur	Opmerkingen (bv. niet in diepvriezer bewaard,...)
9				
10				
11				
12				
13				
14				
15				
16				
17				
18				
19				
20				
21				
22				
23				
24				
25				
26				
27				
28				

Staal	Identificatienummer urinerecipiënt	datum	uur	Opmerkingen (bv. niet in diepvriezer bewaard,...)
29				
30				
31				
32				
33				
34				
35				
36				
37				
38				
39				
40				

LOGBOEK: staalname en overlast van houtkachelrook

In dit logboek kan je aangeven hoeveel last je had van rook van houtkachels binnen of buiten (hinder en/of gezondheidsklachten). Het gaat om de overlast gedurende de week dat er bij uw huis gemeten wordt.

VOORBEELD

Thuis binnen/buiten: welke uren van de dag was je thuis binnen of buiten? -> Duidt aan met kruisje Indien je niet thuis was, hoef je niets te noteren.
Kachel/haard: Welke uren van de dag was in huis de houtkachel/open haard aan? -> Duid aan met kruisje
Overlast: Welke uren had je overlast door houtrook van eigen houtkachel of uit de buurt? -> Met een cijfer tussen 1 en 10 geef je aan hoeveel overlast je had, d.w.z. hinder en/of gezondheidsklachten. Als je geen overlast had, dan vul je niets in. Als je heel erge overlast had, dan vul je een '10' in. Als je overlast had maar niet in belangrijke mate, dan vul je een cijfer van 1 tot 9 in: hoe meer overlast hoe hoger het cijfer.
Maaltijd Gegeten: Om welk uur heb je een maaltijd gegeten? -> Duid aan met een kruisje
Snack gegeten: tijdstip waarop je een snack of snoep hebt gegeten, denk vooral aan chocolade, koekjes, snoep en suikerwaren
Uitlaatgassen: contact met uitlaatgassen in verkeer, via motorcross, in garage-> Duid aan met een kruisje
Sigarettenrook: in gezelschap van sigaretten of sigaren roker(s) -> Duid aan met een kruisje
Venster open: venster minimum 30 minuten open gehad, bv. ook slapen met open venster-> Duid aan met een kruisje
Buiten sporten: gesport in open lucht -> Duid aan met een kruisje

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen	x	x	x	x	x	x	x	x									x	x	x	x		x	x	x
Thuis buiten																								
Kachel/haard																			x	x	x	x		
Overlast(1-10)																	3			8	8			
Maaltijd							x					x							x					
Snack																								
Uitlaatgassen									x	x							x							
Sigarettenrook																								
Venster open							x																	
Buiten sporten																				x				

Voorbeeld: Thuis tot 8u 's ochtends. Ontbijt om 7u. Venster voor minstens 30 minuten open gezet om 7u. Tussen 9 en 10u in verkeer. Tijdens ochtend geen overlast van houtrook. Thuis vanaf 17u, voordien in verkeer gezeten voor 15 minuten. Houtkachel aangestoken tussen 19 en 22u. Gegeten om 19u. Urine verzameld om 19, 22, en 24u

Geef per dag aan - door het plaatsen van een kruisje - welke uren je thuis binnen of buiten was, wanneer je de houtkachel/open haard in huis hebt aangemaakt. Duid aan hoeveel last van houtrook je had, door eigen kachel of door houtkachels in de buurt (cijfer tussen 1-10). Geeft met een kruisje ook aan wanneer je gegeten hebt of snack hebt gebruikt, of je in contact kwam met uitlaatgassen, sigaretten/sigarenrook, of het venster minimaal 30 minuten open was thuis en of je buiten gesport hebt.

DAG 1: datum: .....

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								
Thuis buiten																								
Kachel/haard																								
Overlast(1-10)																								
Maaltijd																								
Snack																								
Uitlaatgassen																								
Sigarettenrook																								
Venster open																								
Buiten sporten																								

DAG 2: datum: .....

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								
Thuis buiten																								
Kachel/haard																								
Overlast(1-10)																								
Maaltijd																								
Snack																								
Uitlaatgassen																								
Sigarettenrook																								
Venster open																								
Buiten sporten																								

DAG 3: datum: .....

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								
Thuis buiten																								
Kachel/haard																								
Overlast(1-10)																								

Maaltijd																							
Snack																							
Uitlaatgassen																							
Sigarettenrook																							
Venster open																							
Buiten sporten																							

DAG 4: datum: .....

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								
Thuis buiten																								
Kachel/haard																								
Overlast(1-10)																								
Maaltijd																								
Snack																								
Uitlaatgassen																								
Sigarettenrook																								
Venster open																								
Buiten sporten																								

DAG 5: datum: .....

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								

Thuis buiten																							
Kachel/haard																							
Overlast(1-10)																							
Maaltijd																							
Snack																							
Uitlaatgassen																							
Sigarettenrook																							
Venster open																							
Buiten sporten																							

DAG 6: datum: .....

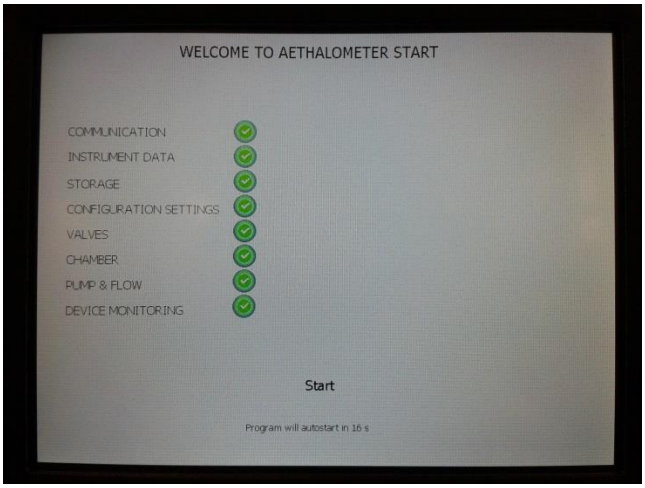
Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								
Thuis buiten																								
Kachel/haard																								
Overlast(1-10)																								
Maaltijd																								
Snack																								
Uitlaatgassen																								
Sigarettenrook																								
Venster open																								
Buiten sporten																								

DAG 7: datum: .....

Tijdstip(u)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Thuis binnen																								
Thuis buiten																								
Kachel/haard																								
Overlast(1-10)																								
Maaltijd																								
Snack																								
Uitlaatgassen																								
Sigarettenrook																								
Venster open																								
Buiten sporten																								

BIJLAGE 1 : instructiefoto's AE33 :

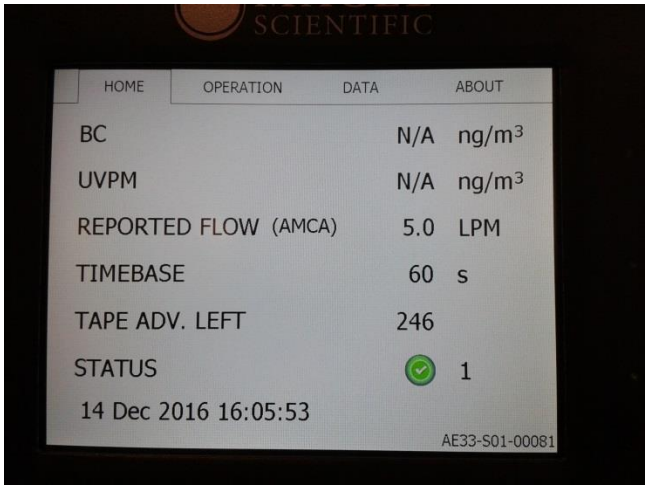
1



2

foto 1 : self test

foto 2 : auto start

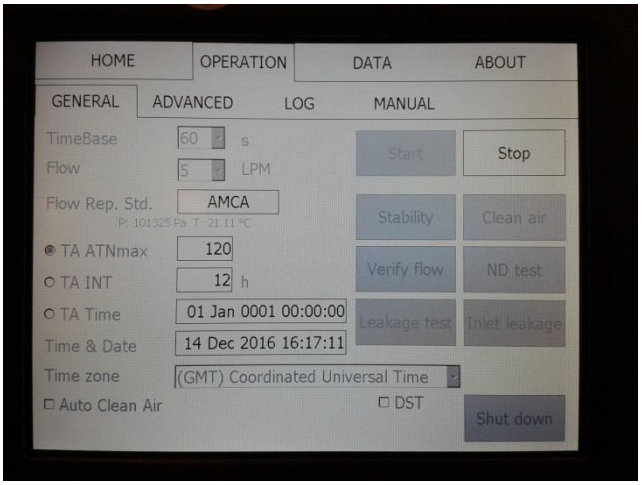


3

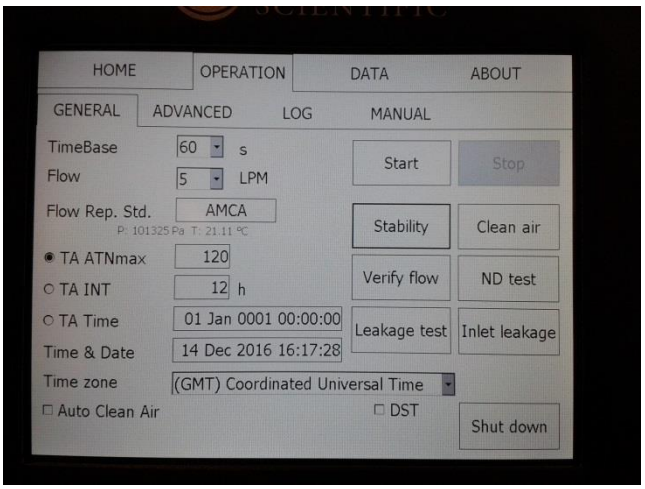
4

foto 3 : status 1

foto 4 : status 0 = measuring



5

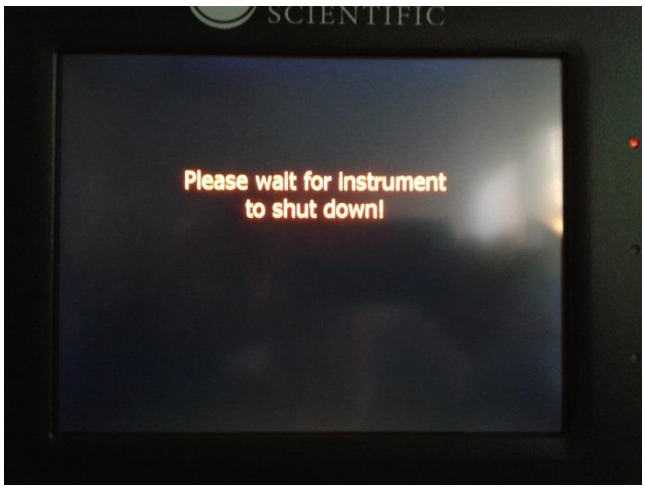


6

foto 5 : stop - key

foto 6 : shut down - key

7



8

foto 7 : sure ? => yes - key

foto 8 : wait

9

foto 9 : safe to turn off

ANNEX 3: INFORMED CONSENT PILOT STUDY 2

Pilootstudie houtverbrandings-biomerkers

Studie uitgevoerd door de Vlaamse Instelling voor Technologisch Onderzoek te Mol, gefinancierd door het departement Leefmilieu, Natuur en Energie (LNE) van de Vlaamse Overheid.

Beste,
In deze pilootstudie testen we of stoffen die ontstaan bij houtverbranding, meetbaar zijn in urine, haren en armbandjes persoonlijk gedragen door individuen die niet beroepshalve blootgesteld zijn aan houtverbranding. De pilootstudie kadert in een studie van LNE naar het uittesten van gepaste houtverbrandings(bio)merkers.

Wie kan deelnemen?
Wij zoeken 6 personen die bereid zijn om deel te nemen aan deze studie. De metingen zullen lopen in januari-februari 2017. De volgende selectiecriteria gelden:

- Niet roken en geen rokers in huis
- Geen huidige beroepsblootstelling aan PAK's (bv. werkplaatsen waar petroleum-producten worden verbrand, cokes productie, gasproductie, metaal- of staal-productie, roofing teer en asfaltverwerking of plaatsing, afvalverbrandingsinstallatie, aluminium smelters)
- Bereid zijn om meetapparatuur te plaatsen aan de woning.

Hoe zal het onderzoek praktisch verlopen?
Black carbon (zwarte koolstof) luchtmetingen zullen gedurende 1 week uitgevoerd worden in de achtertuin van je woning. Een veldwerker van VITO komt het meettoestel plaatsen en ophalen. De metingen lopen automatisch gedurende 1 week.
Als deelnemer verzamel je elke dag van de staalnameweek minstens 3 urinestalen: (1) ochtendurine, (2) 's avonds bij het thuiskomen bv. ca. 17-18u, of in ieder geval voor het avondeten en (3) alle monsters tot u gaat slapen.
Tijdens de staalnameweek draag je 4 silicone armbandjes: op de dag van start van de luchtsampling begin je met het dragen van deze armbandjes. Twee armbandjes zal langer dragen, nl. in totaal 4 weken. Verder zal op het einde van de staalnameweek, een veldwerker 100mg haar collecteren met een schaar. Dit is gelijk aan de hoeveelheid van een plukje haar.

De urinestalen, de armbandjes en het haar worden geanalyseerd op de aanwezigheid van levoglucosan (verbrandingsproduct van cellulose in hout), polycyclische aromatische koolwaterstoffen (PAK's = verbrandingsproducten) en methoxyfenolen (verbrandingsproduct van lignine in hout). De resten van de stalen worden bewaard als reserve en voor stockage voor mogelijk latere analyse.

Tijdens de staalnameweek vul je elke dag een logboekje in over wanneer je thuis was op de woonplaats, wanneer de houtkachel/open haard werd aangestoken, wanneer en in welke mate je last had van houtrook van eigen stookactiviteiten of van de buren, wanneer je hebt gegeten, of je in contact kwam met sigarettenrook of uitlaatgassen en wanneer je buitenshuis was.

Kosten
Er zijn voor u geen kosten verbonden aan dit onderzoek.

Risico's en ongemakken
Er is geen enkel risico verbonden aan deze studie.

Voordelen
U krijgt uw persoonlijke resultaten en het eindrapport van deze studie.

Vertrouwelijkheid
Alle informatie die u ons geeft, wordt vertrouwelijk behandeld overeenkomstig de wet op de privacy. Indien de resultaten van deze studie gepubliceerd worden in een rapport of wetenschappelijk tijdschrift zal uw naam niet genoemd worden.

Vrijwillige deelname / intrekking van deelname uit de studie
U neemt vrijwillig deel aan de studie. U kunt op eender welk moment beslissen uw deelname aan de studie stop te zetten om gelijk welke reden, ook al ondertekende u dit toestemmingsformulier. Wij vragen u wel onze diensten hiervan op de hoogte te stellen (adres en telefoonnummers staan verder in deze brief).

Hoe gaat het nu verder?
Als u wilt deelnemen, vult u bijgevoegd toestemmingsformulier in. U kan dit aan ons terugsturen via mail, of meegeven met de veldwerker.

Meer informatie nodig?
Voor vragen rond deze studie:

Gudrun Koppen
VITO
Tel: 014/33 51 65 of mail naar gudrun.koppen@vito.be

Dit voorstel werd voorgelegd aan de Adviescommissie Medische Ethiek van de Universiteit Antwerpen, een comité dat er moet voor zorgen dat deelnemers aan een wetenschappelijk onderzoek geen nadeel ondervinden.

TOESTEMMINGSFORMULIER
VOOR DEELNAME AAN DE STUDIE

Pilootonderzoek naar houtverbrandings biomerkers

Gelieve dit document terug te bezorgen aan VITO in bijgevoegde omslag

Ik heb de informatie over deze studie gelezen en begrepen. Ik neem vrijwillig deel aan deze studie.

Wenst u een persoonlijk resultaat te ontvangen van de metingen? (1 bolletje kleuren)

O ja, ik wens persoonlijke resultaten te ontvangen.

O neen, ik wens geen persoonlijke resultaten te ontvangen

Naam: .....

Adres: .....

Telefoonnummer/GSM: .....

e-mail adres: .....

geslacht: O **man** O **vrouw**

geboortedatum: …./……/19….

…….//……..
Handtekening **Datum**