"Exploring the full-chain approach for PAHs using 2 case studies in Flanders and the Czech Republic"

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Aim of the study:
- Comparing PAH exposure levels in Flanders vs Czech Republic.
- Linking external PAH exposure to internal biomarker measurements.
- Getting insight into which biomarkers are useful to evaluate environmental exposure to PAHs and to interpreat health impact assessment.
- Improving exposure-effect functions for: (i) linking environmental exposure with internal dose and (ii) linking internal dose with biological effective dose and/or health impact.

Material and Methods

Study population

Flanders
48 non-smokers (25 males, 23 females) between 32 and 45y old, living in 25 residences were recruited in 2 seasons in 2010: Winter season between February 18 and March 2, and Summer season: between June 1 and June 25, and another 2 people on August 17.

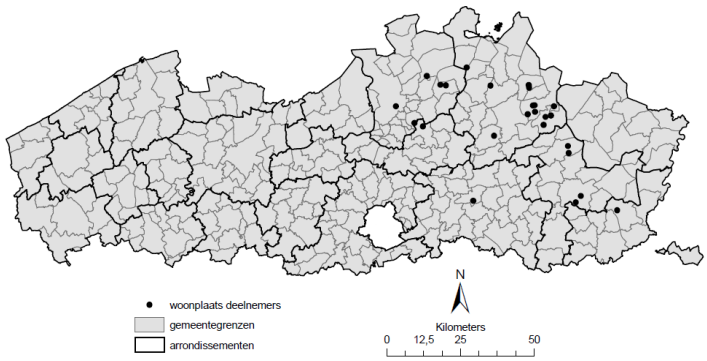

Figure 1: Location of residences included in the Flemish study: participants houses are indicated with dots.

Czech Republic
214 male non-smokers living in two locations in the Czech Republic with different levels of air pollution: Prague (66 subjects), a control location, and the polluted Ostrava region (including 31 subjects from Karvina, 12 subjects from Havirov, 28 subjects from Radvanice-Bartovice and 78 subjects from the city of Ostrava). The subjects were followed in Winter 2010, exposure to c-PAHs and VOC was assessed and biomarkers were analyzed.

External exposure data

Flanders

24-hours average outdoor air concentrations of 16 EPA PAHs were obtained every 4th day from one stationary monitor of the Flemish Environmental Agency. The monitor was located in Borgerhout (in the urban Antwerp area). The sampling device consisted of an active sampler using a pump with flow of ca. 6L/h and cartridges filled with both polydimethyl siloxane and Tenax TA, to collect particles (with size of ca. PM_{10}) and the gas phase respectively (Wauters *et al.*, 2008). The PAHs were analysed using GC-MS. For the indoor air monitoring, a similar cartridge was used and 24h-sampling took place in the 25 residences (in the living area of the house) using a stand of ca. 1.5m high on which the cartridge was attached.

Sedimented house dust was collected by the inhabitants of the residences during 3 weeks using a vacuum cleaner. The fine dust (ca. < 100µg) in between the two paper layers of the vacuum cleaner dust bag was extracted using soxhlet extraction, gel permeation chromatography, Si-column fractionation and analysed with GC-MS.

Czech Republic

The subjects' exposure to c-PAHs was monitored by personal samplers worn during two consecutive days (48 hours). The samplers were equipped with filters collecting particles of aerometric diameter < 2.5 µm ($PM_{2.5}$). After extraction of the filters with dichlormethane, quantitative chemical analysis of c-PAHs, (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene) was performed by HPLC with fluorescence detection according to the EPA method in the certified laboratory ALS Czech Republic, Prague.

Ambient air quality during the sampling periods was monitored using stationary HiVol samplers in Prague. The samplers continuously measured the levels of c-PAHs and $PM_{2.5}$. The study subjects lived and worked within the range of the samplers.

Table 1: External PAH (and some other) exposure measurements in both study populations

Flanders	**Czech Republic**
Outdoor stationary monitoring	
cPAHs from Flemish Environmental Agency (VMM) *Interpollated concentrations of NO_2, PM_{10} and ozone (on 4x4km grid) by VMM*	cPAHs + $PM_{2.5}$ +VOC
Personal monitoring	
NO_2 passive dosimetry (IVL sampler)	c-PAHS active dosimetry + VOC passive dosimetry
Indoor stationary monitoring	
PAH gas + particulates *PAH in house dust (vacuüm cleaner)*	none

Biomarker measurements

Table 2: Biomarker measurements included in the Flemish and Czech project

Flanders	**Czech Republic**
Internal PAH exposure markers	
1 hydroxypyrene - urine *1-OH and 2-OH naphtalene - urine* *B[a]P tetrol - urine* *BPDE-DNA adducts - blood*	1 hydroxypyrene - urine 1-OH and 2-OH naphtalene - urine B[a]P tetrol - urine
Oxidative stress markers	
8-oxodeoxyguanosine - urine *15-F2T-isoprostane - plasma* *Protein carbonyls - plasma* *Comet assay - whole blood and isolated mononuclear cells*	8-oxodeoxyguanosine - urine 15-F2T-isoprostane - plasma Protein carbonyls - plasma

Table 2: Biomarker measurements included in the Flemish and Czech project

Flanders	**Czech Republic**
Exhaled NO, temp exhaled air	
Genotox markers	
Micronucleus - blood	Chromosomal aberrations, FISH, micronucleus - blood
Markers of susceptibility	
Triglycerides, LDL,HDL, cholesterol *Vit E - plasma* *Genotyping - blood in biobank*	Triglycerides, LDL,HDL, cholesterol Vit A,C,E, folic acid - plasma Genotyping - blood
Others	
Gene expression - blood *Cotinin - urine*	Gene expression - blood Cotinin - urine

Results and Discussion

PAHs measured outdoor, indoor and via personal monitoring

PAH exposure via air is still an important issue. Outdoor and indoor B[a]P concentrations measured are currently at or above the guideline value of 1 ng/m^3, set out by WHO for 2012, both in Flanders and Czech Republic. Winter outdoor B[a]P levels in the Czech Republic were a factor 5 (for Prague) to 50 (for polluted Ostrava region) higher compared to the outdoor levels measured in the urban Antwerp region in Flanders (Table 3). Levels assessed via personal sampling were in the range of outdoor concentrations (only done in Czech study).

Table 3: Outdoor air concentrations (median (min-max)) of B[a]P measured in Flanders and Czech Republic in the period of the study (2009-2010)

FLANDERS		**CZECH REPUBLIC**		
	Outdoor air B[a]P (ng/m³)		**Outdoor air B[a]P (ng/m³)**	**Personal exposure B[a]P winter '10**
Anwerp, Winter '10	**0.59 (0.27-3.81)[2]**	**Prague**	**2.65 (Winter '10)**	**2.43 (0.28-11.50)**
Antwerp, Summer '10	**0.16 (0.09-0.27)**	**Karvina**	**24.59 (Winter '09)**	**12.75 (5.66-36.20)**
		Ostrava		
		Bartovice	**29.01 (Winter '10)**	**8.28 (2.19-74.20)**
		Poruba	**4.73 (Spring '10)**	
		Havirov	**-**	**12.50 (5.93-18.80)**
		Radvanice/Bartovice	**-**	**8.17 (4.70-29.20)**

[2] *significant difference Winter vs. Summer (p<0.05, Mann-Whitney U-test).*

In the Flemish study repeated measurements in both seasons in 25 residences as well as outdoors, showed, that Summer (indoor and outdoor) air levels of B[a]P were 2-3 times lower than Winter values (p<0.05, Mann Whitney-U test). Also house dust B[a]P levels decreased in Summer. Heating with a stove or open fire caused significant higher indoor air and house dust concentrations in the winter time (p<0.05, Mann Whitney-U test) (Table 4).
Furthermore, outdoor concentrations were correlated with indoor levels. B[a]P levels in indoor air and sedimented house dust were correlated mainly in winter time (r=0.38, p=0.08, Spearman rank correlations) (Figure 2).

Table 4: Indoor B[a]P concentrations (median (min-max)) measured in 25 residences in Flanders

Season	**Outdoor air B[a]P (Antwerp) (ng/m³)**	**Indoor air B[a]P (ng/m³)**		**Indoor vacuum cleaner B[a]P (ng/g dust)**	
		Heating stove/open fire used in Winter?			
		Yes (N=9)	**No (N=16)**	**Yes (N=9)**	**No (N=16)**
Winter '10	**0.59 (0.27-3.81)[2]**	**0.88 (0.09-9.20)[1,2]**	**0.56 (0.15-2.70)[3]**	**170 (76-742)[1]**	**133 (37-545)**
Summer '10	**0.16 (0.09-0.27)**	**0.28 (0.21-0.90)**	**0.24 (0.04-0.69)**	**148 (64-331)**	**114 (50-567)**

[1] *p< 0.05 stove vs. to no stove,* [2] *p< 0.05 Winter vs. Summer,* [3] *p=0.07 Winter vs. Summer (Mann-Whitney U-test)*

Table 5: Spearman rank correlations between B[a]P concentrations in outdoor and indoor air and between indoor air and indoor dust

	B[a]P outdoor vs. indoor air			B[a]P indoor air vs. indoor dust		
	N	*r*	*p-level*	*N*	*r*	*p-level*
All seasons	39	**0,45**	**0,004**	45	**0,36**	**0,02**
Winter '10	21	0,33	0,15	22	**0,38**	**0,08**
Summer '10	18	0,37	0,13	21	0,15	0,53

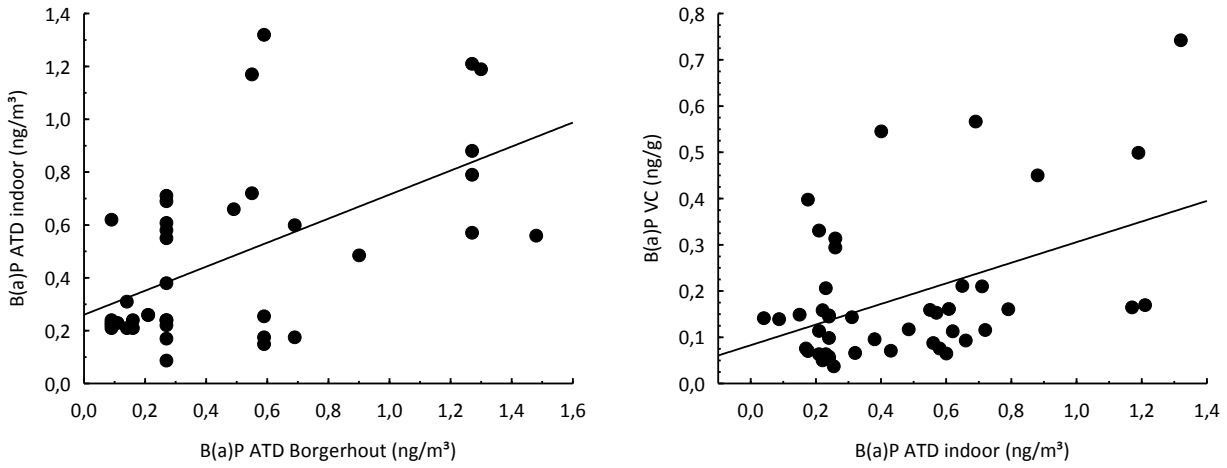

Figure 2: Scatter plot of B[a]P: outdoor air vs. indoor air measurements (outdoor measurements were done in the week before the indoor sampling) (left); and indoor air vs. indoor sedimented dust concentrations (right).

PAH metabolites in urine

B[a]P tetrols are the hydrolysis products of the dihydrodiolepoxide of B[a]P (benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide) in the metabolism of B[a]P. They are potentially better markers for carcinogenic risk than phenolic compounds, but detection in urine is more difficult. Indeed, in both studies, the levels of B[a]P tetrols in urine were low. In respectively 24 and 20% of the Flemisch and Czech urine samples, the most apparent steriochemic variant benzo[a]pyrene,r-7,t-8,t-9,**t-10**-tetrahydrotetrol (B[a]P tetrol 2), was below the limit of detection of 0.49 pg/mL (or 0.38 pg/mg CRT). There was a difference between sexes, with females having higher values compared to males (also if not corrected for creatinine, data not shown). Comparing the males in both countries, showed similar levels for Flanders and Prague, and somewhat higher urinary values in the more PAH contaminated areas of the Czech study (Table 5).

Table 5: Levels (median (min-max))of B[a]P tetrols and 1-OH pyrene measured in urine in both study populations

	FLANDERS		CZECH REPUBLIC	
	Men (n=25)	Women (N=24)		Men
	total B[a]P tetrols (pg/mg CRT)[1]		total B[a]P tetrols (pg/mg CRT)[1]	
Winter'10	1,75 (0,17-24.15)	4,89 (0.30-175.31)	**Prague**	1.83 (0.16-37.04)
Summer'10	1,60 (0.14-23.23)	3,30 (0.14-189.76)	**Karviná**	2.37 (0.16-18.29)
			Havířov	3.74 (1.85-6.90)
			Radvanice/Bartovice	2.88 (0.39-17.89)
			Ostrava	2.65 (0.11-88.01)
	B[a]P tetrol 2 (pg/mg CRT)[2]		B[a]P tetrol 2 (pg/mg CRT)[2]	
Winter'10	1.43 (0.05-21.69)	1.91 (0.28-46.34)	**Prague**	1.38 (0.05-27.35)
Summer'10	1.14 (0.03-22.24)	1.39 (0.10-30.20)	**Karviná**	1.47 (0.11-18.01)
			Havířov	3.56 (1.83-6.41)
			Radvanice/ Bartovice	1.32 (0.11-12.00)
			Ostrava	1.34 (0.02-41.74)
	1-OH pyrene (ng/g CRT)		1-OH pyrene (ng/g CRT)	
Winter'10	0.15 (0.04-1.81)	0.16 (0.09-0.82)	**Prague**	0.15 (0.003-0.83)
Summer'10	0.15 (0.04-2.38)	0.12 (0.01-0.84)	**Karviná**	0.24 (0.002-1.93)

Havířov	0.14 (0.01-0.24)
Radvanice/ Bartovice	0.19 (0.02-1.02)
Ostrava	0.19 (0.004-2.61)

*[1]Total B[a]P tetrol = Benzo[a]pyrene,r-7,t-8,t-9,**c-10**-tetrahydrotetrol + Benzo[a]pyrene,r-7,t-8,t-9,**t-10**-tetrahydrotetrol, [2]B[a]P tetrol 2 = Benzo[a]pyrene,r-7,t-8,t-9,**t-10**-tetrahydrotetrol. All measurements were done in the Flemish lab. CRT: creatinine*

Biological effective dose and effect markers to assess the impact of PAH exposure

For the biomarkers, there was a clear influence of the sampling season: in Flanders higher levels of micronuclei and DNA breaks in blood, urinary 8-oxodG levels and oxidative damage to DNA (FPG sites in comet assay) and plasma lipids (15-F2t-isoprostane) in Summer compared to Winter. The differences in median biomarker levels between both countries, were less pronounced.

Table 6: Levels (median (min-max) of biological effective dose and effect markers in both study populations

	FLANDERS		CZECH REPUBLIC	
	Men (N=25)	**Women (N=24)**		**Men**
Genotox				
Micronucleus test (# micronuclei/1000 BN cells)				
Winter '10	2,8 (1.0-5.7)	4,1 (1.9-20.0)	**Prague Winter '10 (N=65)**	6.7 (3.0-14.0)
Summer '10	4,7 (2.1-12.6)	6,8 (0.0-22.1)	**Karviná Winter '10 (N=31)**	6.0 (2.3-15.0)
			Havířov Winter '10 (N=12)	6.7 (3.7-9.0)
			Radvanice/Bartovice Winter '10 (N=28)	6.2 (3.3-15.0)
			Ostrava Winter '10 (N=78)	6.5 (2.7-13.7)
Comet assay on MWBC (% DNA in tail)				
Winter '10	3,5 (1.5-6.0)	2,9 (1.4-5.6)	**Prague Winter '10 (N=65)**	1.50 (0.72-3.87)
Summer '10	6,2 (2.0-8.2)	5,3 (2.4-17.4)	**Karviná Winter '10 (N=31)**	1.14 (0.83-6.04)
			Havířov Winter '10 (N=12)	2.43 (1.02-5.90)
			Radvanice/Bartovice Winter '10 (N=28)	1.14 (0.46-2.66)
			Ostrava Winter '10 (N=78)	1.56 (0.59-12.67)
Oxidative stress				
8-oxodG (nmol/mmol CRT)				
Winter '10	5.1 (0.02-17.1)	4.7 (0.3-9.4)	**Prague Winter '10 (N=65)**	4.9 (0.5-9.9)
Summer '10	3.9 (2.6-9.6)	4.1 (2.7-10.9)	**Ostrava Winter '10 (N=149)**	4.8 (0.2-10.4)
Comet assay FPG sites in WBC (% DNA in tail)				
Winter '10	15,4 (9.7-22.0)	15,3 (4.4-28.0)	**Prague Winter '10 (N=65)**	1.54 (0.28-7.43)
Summer '10	22,7 (12.3-26.1)	22,4 (14.5-35.8)	**Karviná Winter '10 (N=31)**	2.46 (0.37-4.41)
			Havířov Winter '10 (N=12)	5.31 (0.14-16.50)
			Radvanice/Bartovice Winter '10 (N=28)	2.41 (0.08-10.25)
			Ostrava Winter '10 (N=78)	1.32 (0.05-11.17)
15-F2t-Isoprostane (pg/mL plasma)				
Winter '10	71,8 (26.5-135.9)	58,5 (22.8-173.7)	**Prague Winter '10 (N=65)**	236.9 (119.6-647.8)
Summer '10	82,9 (23.4-289.6)	78,5 (16.5-160.8)	**Ostrava Winter '10 (N=149)**	252.3 (90.6-814.6)
protein carbonyls (nmol/mL plasma)				
Winter '10	38,7 (27.4-50.2)	40,4 (30.4-58.8)	**Prague Winter '10 (N=65)**	22.8 (10.0-39.1)
Summer '10	29,0 (19.2-47.4)	30,1 (18.1-42.3)	**Ostrava Winter '10 (N=149)**	21.3 (11.3-43.3)

MWBC = mononuclear white blood cells. The micronucleus assay and 8-oxodG analysis were done in different labs for both countries. 15-F2t-isoprostane and protein carbonyl samples were all measured in the Czech lab.

In the Flemish study following relations were observed for the winter time period: The indoor air measurements of 16 EPA PAHs and naphtalene were correlated with urinary 1-OH pyrene levels in 41 individuals. Breaks in DNA of mononuclear white blood cells (MWBC) measured via the comet assay, were significantly associated with nearly all measured PAH air levels indoors. Furthermore 1-OH pyrene was significantly associated with breaks in DNA of white blood cells from whole blood.

Table 7: Relationship between external and internal exposure and biological effective dose markers in the Flemish study in 49 individuals, living in 25 resisdences. Results of multiple regression statistics with residence

as random factor for the winter season.

Indoor measurements and biomarkers		ß	SE	P>|t|	Covariates in the model
[a] Naphtalene in indoor air	1-OH pyrene in urine	0,21	0,10	0,04	Age, sex, consumption of grilld fish/meat, cotinine (ng/mg CRT), vitamin E
16 EPA PAHs in indoor air	1-OH pyrene in urine	0,23	0,11	0,04	
[a] Benzo[a]pyrene in indoor air	Comet assay breaks in MWBC	0,16	0,05	0,003	
[a] Naphtalene in indoor air	Comet assay breaks in MWBC	0,19	0,04	0,0001	
[a] Pyrene in indoor air	Comet assay breaks in MWBC	0,12	0,06	0,06	
[a] volatile PAHs	Comet assay breaks in MWBC	0,20	0,04	0,0001	
[a] c-PAHs in indoor air	Comet assay breaks in MWBC	0,19	0,06	0,002	
[a] 16 EPA PAHs in indoor air	Comet assay breaks in MWBC	0,21	0,05	0,0001	
1-OH pyrene in urine	Comet assay breaks in white blood cells of whole blood	0,31	0,10	0,002	Age, sex, consumption of grilld fish/meat, cotinine (ng/mg CRT), vitamin E, LDL en HDL

[a] *log10 of the variable*