Environment International 139 (2020) 105634

Contents lists available at ScienceDirect

Environment International

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

Toxic potentials of particulate and gaseous air pollutant mixtures and the role of PAHs and their derivatives

Zuzana Nováková[a], Jiří Novák[a], Zoran Kitanovski[b], Petr Kukučka[a], Marie Smutná[a], Marco Wietzoreck[b], Gerhard Lammel[a,b], Klára Hilscherová[a,*]

[a] RECETOX, Faculty of Science, Masaryk University, Kamenice 753/5, 625 00 Brno, Czech Republic
[b] Max Planck Institute for Chemistry, Multiphase Chemistry Department, Hahn-Meitner-Weg 1, 55128 Mainz, Germany

ARTICLE INFO

Handling Editor: Xavier Querol

Keywords:
Air pollutant mixture
Toxic potential
Endocrine disruption
Bioassay
PAHs, OPAHs, NPAHs
Human health

ABSTRACT

Background: Air pollution, which represents a major environmental risk to human health, comprises a complex mixture of compounds where only little is known about its specific toxicities.
Objectives: This study examined the specific toxicities associated with ambient air pollutant mixtures with respect to gas/particle partitioning, particulate matter (PM) size, pollutant polarity and bioaccessibility from PM, and evaluated the contribution of PAHs and their oxygenated and nitrated derivatives (OPAHs, NPAHs).
Methods: Air samples (gas phase, PM_{10} and size-segregated PM), were collected at urban (in winter and summer) and background (winter) sites in the Czech Republic. The total and bioaccessible concentrations were addressed using organic solvent extraction and simulated lung fluid extraction, respectively. Organic extracts were also further fractionated according to polarity. Aryl hydrocarbon receptor (AhR)-mediated activity, anti-/estrogenicity, anti-/androgenicity, thyroid receptor (TR)-mediated activity and cytotoxicity for bronchial cells were determined by human cell-based *in vitro* bioassays. The contribution of studied compounds to observed effects was assessed by both modelling and reconstructing the mixtures.
Results: Significant effects were detected in the sub-micrometre size fraction of PM (estrogenicity, androgenicity, TR- and AhR-mediated activities) and in the gas phase (TR-mediated activity, antiandrogenicity). Compounds interacting with TR showed high bioaccessibility to simulated lung fluid. Relatively lower bioaccessibility was observed for estrogenicity and AhR-mediated activity. However, the toxicity testing of reconstructed mixtures revealed that the targeted pollutants are not the main contributors, except for urban PM air pollution in winter, where they accounted for 5–88% of several effects detected in the original complex environmental samples.
Discussion: Studied toxicities were mostly driven by polar compounds largely attributed to the easily inhalable PM_1, which is of high relevance for human health risk assessment. Except of parent PAHs in some cases, the targeted compounds contributed to the detected effects mostly to a relatively low extent implying huge data gaps in terms of endocrine disruptive potencies of targeted substances and the significance of other polar compounds present in ambient air.

1. Introduction

Exposure to ambient fine particulate matter ($PM_{2.5}$) is a major human health concern. In 2016, 91% of the world population was living in areas which failed to meet air quality criteria (WHO, 2018). Not only has poor air quality been linked to a higher incidence of pulmonary and cardiovascular diseases (Kim et al., 2015), ambient air pollution has also been classified as carcinogenic by the International Agency for Research on Cancer (IARC, 2016). The risk related to various $PM_{2.5}$-attributable causes of death is estimated to account for 7.5–10.3 million deaths worldwide in 2015 (95% confidence interval; Burnett et al., 2018). Gaseous air pollutants contribute to the health risk, although their relationships to mortality and morbidity endpoints are less well established (WHO, 2018).

Air pollution comprises complex mixtures of chemicals. While some compounds are subject to regulatory monitoring due to their known toxic properties, other constituents may be omitted. Thus, in regulatory air pollution monitoring, the complexity of air contaminant mixtures as well as the interactions among mixture constituents are not covered.

One group of the most abundant and widespread pollutants are

* Corresponding author.
E-mail address: klara.hilscherova@recetox.muni.cz (K. Hilscherová).

https://doi.org/10.1016/j.envint.2020.105634
Received 4 September 2019; Received in revised form 13 February 2020; Accepted 2 March 2020
Available online 20 May 2020

polycyclic aromatic hydrocarbons (PAHs). Their abundance increases due to anthropogenic emissions and intense carbon-based fossil fuels use. PAHs can affect human health *via* several mechanisms like aryl hydrocarbon receptor-mediated toxicity, genotoxicity or estrogenicity (Bandowe and Meusel, 2017). Moreover, several types of cancer have been associated with PAHs exposure (Rengarajan et al., 2015) making them compounds of toxicological concern and of interest in this study. Even though selected PAHs have been used as air quality indicators and are studied intensively in terms of their environmental levels and toxic potentials, little is known about the other constituents of ambient air mixtures. Besides parent PAHs (containing only carbon and hydrogen), there are many PAHs derivatives, like oxygenated PAHs (OPAHs) or nitrated PAHs (NPAHs). However, their concentrations and toxic potentials are frequently ignored, even though they have also been associated with various adverse effects manifested in exposed populations. Similarly to their precursors, OPAHs and NPAHs have been recognized as direct-acting mutagens and carcinogens (Howard et al., 1990; IARC, 2014; Misaki et al., 2016; Purohit and Basu, 2000; Sen and Field, 2013). Moreover, other toxic effects, e. g. cytotoxicity for respiratory cells or inflammatory potential, were reported (Bolton et al., 2000; Koike et al., 2014). The mechanisms underlying some of these toxicological effects of PAHs derivatives include DNA damage, DNA adduct formation, aryl hydrocarbon receptor activation, changes in gene and protein expression, cell cycle alternations, elevated levels of reactive oxygen species and pro-inflammation (Bandowe and Meusel, 2017). PAHs and their metabolites can also interfere with the endocrine system, primarily through their estrogenic activity, and have been associated with endocrine-related cancers (Annamalai and Namasivayam, 2015; Idowu et al., 2019).

To study the bioactive potential of complex mixtures, *in vitro* bioassays represent a sensitive and very effective tool for toxicological profiling. Not only do they cover the combined effect of the mixture constituents, like concentration-addition or synergism, but, together with chemical analyses, they also enable to prioritize toxicity drivers. Health effects associated with poor air quality may be linked to various molecular mechanisms. Although air toxicity assessment has mostly been focused on genotoxicity or oxidative stress, recent studies have pointed out that air pollution may also be linked to endocrine disruption *via* various molecular mechanisms (reviewed in Darbre, 2018).

Sensitive mediators of endocrine disruptive effect are intracellular receptors such as the estrogen receptor (ER), androgen receptor (AR), aryl hydrocarbon receptor (AhR), glucocorticoid receptor (GR) or thyroid receptor (TR). They play a crucial role in physiological processes like growth, development, reproduction, energy homeostasis or behaviour as well as in receptor-mediated pathways involved in detoxification and cell proliferation (Janošek et al., 2006). The interference of environmental pollutants with signalling of these receptors is often connected with adverse health effects such as carcinogenesis, immunosuppression, altered neurodevelopment or adverse effects on the reproduction system (De Coster and van Larebeke, 2012). Even though the data on toxic potentials of air pollutant mixtures are quite scarce, there is growing evidence that air pollutant mixtures can have toxic potentials to interfere with intracellular receptors. Several studies have already reported airborne estrogenic activity (Klein et al., 2006; Oziol et al., 2017; Wenger et al., 2009). The antagonistic effect of extracts from both gas and particulate phases of outdoor air on the AR has been found in studies of Novák et al. (2008) and Oziol et al. (2017). AhR activation has been related to the particulate phase of outdoor air in the study of Klein et al. (2006) and Misaki et al. (2008) and further investigated towards PM size sub-fractions in the paper of Novák et al. (2014). While most previous studies focused on the thyroid-disrupting compounds indoors, a recent study by Oziol et al. (2017) detected thyroid receptor-mediated activity also in outdoor air.

To better understand the mechanisms underlying potential toxic effects of air pollutant mixtures and to identify the key substances contributing to observed effects, further investigation is needed. Although both chemical composition and bioaccessibility of PM-associated pollutants strongly vary with particle size, to our knowledge only a few studies so far focused on the distribution of the toxic potentials across PM size fractions (Cho et al., 2009; Gilmour et al., 2007; Mirowsky et al., 2013; Novák et al., 2014). Obviously, the bioaccessibility is strongly dependent on gas-particle partitioning of pollutants, too. While the bioaccessibility of toxic transition metals associated with inhaled PM has been addressed (Kastury et al., 2017), almost nothing is known about the bioaccessibility of organic pollutant mixtures with toxic potential.

This study aims to explore the potential of air pollutant mixtures to act through several important molecular mechanisms of endocrine disruption and to cause cytotoxicity to bronchial cells and the role of PAHs and their derivatives in the mixture effects. Moreover, we aim to describe the specific distribution of both toxic potentials and pollutant levels with respect to gas-particle partitioning, PM size, pollutant polarity, sampling site and season. Additionally, the bioaccessibility of the air pollutant mixtures associated with PM is studied based on extraction using simulated lung fluids as solvents.

For the purpose of this study, two sampling sites in the Czech Republic were selected; one heavily polluted urban site affected by industry, traffic and domestic heating, and a regional background site for Central Europe, part of the European Monitoring and Evaluation Programme (EMEP) monitoring network. The urban site was located in Ostrava city, which is situated in the north-eastern Czech Republic and, due to its numerous industrial and traffic sources, is considered one of the most polluted areas not only in the Czech Republic but also in Europe (Šrám et al., 2013). The worst air quality occurs in winter when these pollution sources are accompanied by coal-based domestic heating. Such environmental conditions can be associated with significantly impaired human health (Šrám et al., 2013). At this urban site, samples were collected in summer and winter to study also seasonal variability. Furthermore, the gas phase, particulate matter (PM_{10}), and six PM_{10} size sub-fractions were sampled to assess the phase- and particle size-specific distributions of studied toxic potentials and pollutants. In this study, human cell-based *in vitro* bioassays were employed to examine endocrine-disruptive potentials, AhR-mediated toxicity and cytotoxicity for the human respiratory tract. Toxicological results are discussed together with the results of the chemical analysis which focused on PAHs and their derivatives, OPAHs and NPAHs, to examine their contribution to observed effects.

2. Material and methods

2.1. Sampling and sample processing

Samples were collected during 12 consecutive days at an urban and a background site in the Czech Republic (Fig. S1 in Supplementary material 1). Three sampling campaigns were carried out. The urban site (Ostrava city) was sampled in both winter and summer periods (February and September 2016), whereas the background site (Košetice monitoring station, part of EMEP) was sampled only in winter (February 2017). Both the gas and particulate phases were collected by four active air samplers employed side-by-side to increased sampled air volume: two high volume Digitel DH77 samplers (Digitel, Switzerland) and two high-volume 6-stage cascade impactors Baghirra HV-100P (Baghirra, Czech Republic). The Digitel samplers were equipped with PM_{10} sampling cartridge containing quartz fibre filter (QFF, Whatman GE, UK) and, downstream, two polyurethane foam plugs (PUF; Molitan, Czech Republic, density 0.030 g/cm^3, placed in a glass cartridge) in series. The Baghirra samplers were equipped with a multi-stage cascade impactor (Andersen Instruments Inc., Fultonville, New York, USA, series 230, model 235) with five impactor stages, corresponding to 7.2–10, 3–7.2, 1.5–3, 0.95–1.5, 0.49–0.95 μm of aerodynamic particle size, and backup filter collecting particles <0.49 μm. In the impactor, PM was collected on slotted quartz fibre filters (QFFs, TE-230-QZ, Tisch

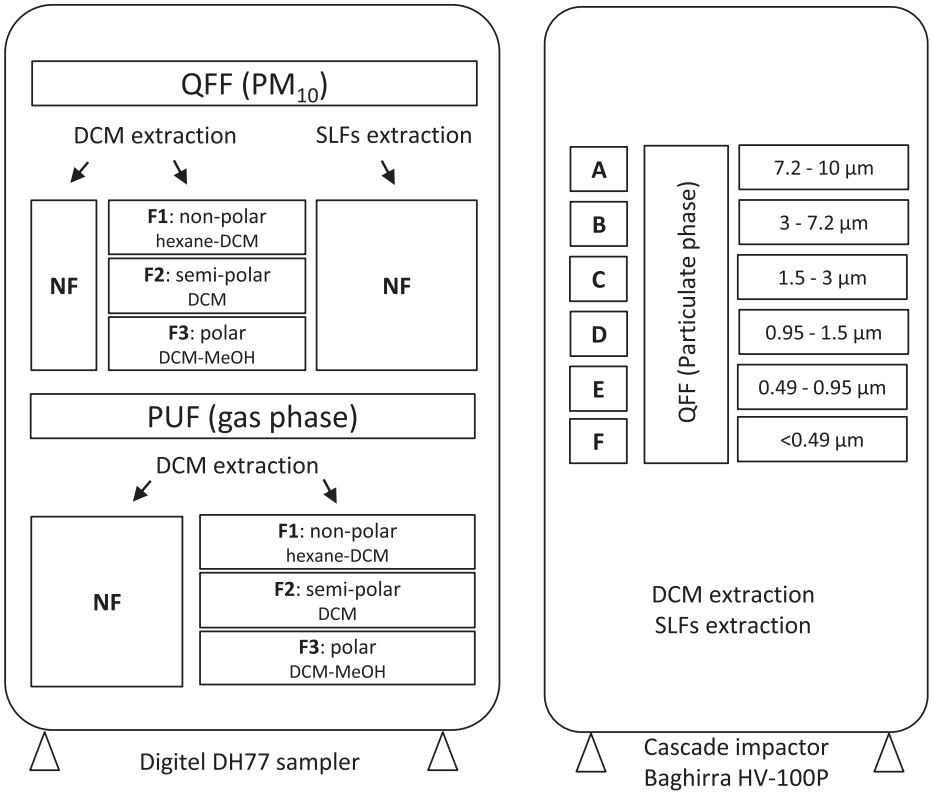

Fig. 1. Samplers and sample processing scheme. Air pollutants were sampled by 2 types of high volume air samplers. The Digitel sampler collected particulate phase (PM_{10}) and gas phase using quartz fibre filter (QFF) and polyurethane foam plug (PUF), respectively. These sampling media were extracted by dichloromethane (DCM) and a portion of each extract was further fractionated according to polarity resulting in fractions F1, F2 and F3. The parent non-fractionated extract is marked as NF. Secondly, a cascade impactor, Baghirra, was employed to collect 6 size sub-fractions of PM_{10} using QFF. The stages of the impactor are marked as A – F. All filters from cascade impactor were extracted by DCM. A portion of QFFs from both samplers was extracted also by simulated lung fluids (SLFs).

Environmental Inc., Cleves, USA, 14.3 × 13.7 cm) and the backup filter was another QFF (Whatman GE, UK). PUFs were pre-cleaned (8 h-extraction in acetone and 8 h in dichloromethane), wrapped in two layers of aluminium foil, placed into zip-lock polyethylene bags and kept in a freezer prior to deployment. The samplers were operated at constant flow rates of ≈30 (Digitel, 24 h sampling) and ≈68 m^3/h (Baghirra, 96 h sampling).

High volume air sampler (Digitel) provided enough material for fractionation according to pollutant polarity (Fig. 1). The cascade impactor, deployed in parallel to Digitel sampler, was used to address the distribution of pollutants among six PM fractions according to particle size. One filter set from each sampler type (PUF plug and QFF from the Digitel sampler and 6 QFFs from the Baghirra sampler) was used for organic extraction with dichloromethane (DCM), while another filter set (only QFFs from both sampler types) was used for the bioaccessibility assessment using simulated lung fluid extraction (Section 2.2). The organic extraction was performed for both PUFs and QFFs using automated Soxhlet extraction (40 min warm Soxhlet followed by 20 min of solvent rinsing) with DCM in a B-811 extraction unit (Büchi, Switzerland). After extraction, the time series sample extracts were pooled to obtain a 12-day composite sample for each sampler and filter type. Blanks (i.e. solvent control and extracts of field blank filters) were prepared and analysed using the same procedures as collected samples.

For a portion of the gas phase and PM_{10} sample extracts, a fractionation was performed on a deactivated silica column (as above, 10% deactivated). The first fraction was eluted by 15 mL *n*-hexane followed by 20 mL DCM:*n*-hexane (1:1), the second fraction by 10 mL DCM and the third and final fraction by 15 mL DCM:methanol (1:1).

All extracts were split for chemical and toxicological analysis (Sections 2.3 and 2.4, respectively).

2.2. Bioaccessibility assessment using simulated lung fluids

To assess the bioaccessibility of studied PAHs and derivatives and potential other bioactive compounds associated with the PM of different sizes, a portion of QFFs from the side-by-side PM_{10} and PM_{10}-subfraction samplers were extracted by simulated lung fluids (SLFs). Two types of SLFs were used. An artificial lysosomal fluid (ALF) was prepared following Marques et al. (2011). This ALF mimics the lung fluid which inhaled particles come in contact with after phagocytosis by alveolar and interstitial macrophages. This solution is similar to the traditionally used Gamblés solution but with more acidic pH (4.5) and higher organic content by the addition of, for example, citric acid (to mimic natural proteins while avoiding solution foaming), glycine and sodium lactate. The second SLF was simulated epithelial lung fluid (SELF). In addition to electrolytes, it also contains antioxidants i.e. glutathione, uric and ascorbic acid, proteins (albumin, mucin), and a surfactant. Due to the latter components, the SELF composition is more similar to the real human lung fluid than e.g. the Gamble's solution and it can better mimic the airways' fluid surfactant properties and lipophilicity (Boisa et al., 2014). The exact composition of both SLFs and leaching procedure are described in detail in Supplementary material 1.

After leaching for 24 h, the leachate was filtered through a cellulose acetate membrane filter and further transferred into a 50 mL amber vial with a PTFE lined cap. The vials were stored at 4 °C until liquid–liquid extraction (extracted three times by 5 mL DCM:*n*-hexane 1:3). Blanks (i.e. leachates with no filter and leachates with field blank filters) were prepared using the same procedure.

2.3. Chemical analyses

The chemical analyses focused on PAHs and their derivatives, OPAHs and NPAHs (Table 1). After splitting the extracts for chemical and toxicological analysis, the extract portions for chemical analysis were spiked with deuterated PAHs and NPAHs (listed in Supplementary material 1).

The extracts were cleaned-up using a silica column (5 g of silica, 0.063–0.200 mm, activated at 150 °C for 12 h, 10% deactivated with ultrapure water) and 1 g Na_2SO_4. Then they were loaded and eluted with 5 mL *n*-hexane followed by 50 mL DCM. The eluate volume was

Table 1
List of targeted compounds.

			PAHs		
ACE	Acenaphthene	BGF	Benzo[ghi]fluoranthene	FLT	Fluoranthene
ACY	Acenaphthylene	BJF	Benzo[j]fluoranthene	CHR	Chrysene
ANT	Anthracene	BKF	Benzo[k]fluoranthene	INP	Indeno[123-cd]pyrene
ATT	Anthanthrene	BPE	Benzo[ghi]perylene	PER	Perylene
BAA	Benzo[a]anthracene	COR	Coronene	PHE	Phenantrene
BAP	Benzo[a]pyrene	CPP	Cyclopenta-[cd]-pyrene	PYR	Pyrene
BBF	Benzo[b]fluoranthene	DBA	Dibenzo[ah]anthracene	TPH	Triphenylene
BBN	Benzo[b]fluorene	DCA	Dibenzo[ac]anthracene		
BEP	Benzo[e]pyrene	FLN	Fluorene		
			OPAHs		
O_2NAP	1,4-Naphthoquinone	(1,4)O_2ANT	1,4-Anthraquinone	BAN	Benzanthrone
1(CHO)NAP	Naphthalene-1-aldehyde	(9,10)O_2PHE	9,10-Phenanthroquinone	(7,12)O_2BAA	Benz[a]anthracene-7,12-dione
9OFLN	9-Fluorenone	11OBaFLN	Benzo[a]fluoren-11-one	(5,12)O_2TET	5,12-Naphthacenequinone
(9,10)O_2ANT	9,10-Anthraquinone	11OBbFLN	Benzo[b]fluoren-11-one		
			NPAHs		
1NNAP	1-Nitronaphthalene	9NPHE	9-Nitrophenanthrene	7NBAA	7-Nitrobenzo[a]anthracene
2NNAP	2-Nitronaphthalene	3NPHE	3-Nitrophenanthrene	6NCHR	6-Nitrochrysene
3NACE	3-Nitroacenaphthene	2NFLT	2-Nitrofluoranthene	(1,3)N_2PYR	1,3-Dinitropyrene
5NACE	5-Nitroacenaphthene	3NFLT	3-Nitrofluoranthene	(1,6)N_2PYR	1,6-Dinitropyrene
2NFLN	2-Nitrofluorene	1NPYR	1-Nitropyrene	(1,8)N_2PYR	1,8-Dinitropyrene
9NANT	9-Nitroanthracene	2NPYR	2-Nitropyrene	6NBAP	6-Nitrobenzo[a]pyrene

then reduced by nitrogen stream in TurboVap II concentrator unit (Caliper LifeSciences, USA) and transferred into a GC vial. The *p*-terphenyl and PCB 121 were added as syringe standards, the final volume was 200 µL.

GC–MS analysis of PAHs was performed on a 6890 GC (Agilent, USA) equipped with a 60 m × 0.25 mm × 0.25 µm Rxi-5Sil MS column (Restek, USA) coupled to a Quattro Micro GC (Waters, UK). The injection was 1 µL splitless at 280 °C, with helium as carrier gas at a constant flow of 1.5 mL/min. The GC programme was 80 °C (1 min hold), then 15 °C/min to 180 °C, followed by 5 °C/min to 310 °C (20 min hold). The MS was operated in EI + mode with multiple reaction monitoring mode (MRM).

OPAHs and NPAHs were analysed by gas chromatography-atmospheric pressure chemical ionization-tandem mass spectrometry (GC-APCI-MS/MS) on a Waters Xevo TQ-S MS coupled to Agilent 7890 GC. The MS was operated under dry source conditions in MRM mode. The GC was fitted with a 30 m × 0.25 mm × 0.25 µm Rxi-5Sil MS column (Restek, USA). The injection 1 µL was splitless at 270 °C. Helium was used as carrier gas at a constant flow of 1.5 mL/min. The oven temperature programme was 90 °C (1 min hold), then 40 °C/min to 180 °C, followed by 5 °C/min to 320 °C (6 min hold). As for the extract stability, repeated analyses confirmed that the targeted compounds (PAHs, OPAHs, NPAHs) are stable in the extracts. All results are blank-corrected using data from a set of field and solvent blanks. The method was validated using PUFs for air and simulated lung fluids spiked with native compounds. Recovery of native analytes can be found in the Supplementary material 1 (Table S2). Limits of quantification (LOQs) for parent PAHs were derived from the calibration curve based on the signal-to-noise ratio (S/N) of ten. For OPAHs and NPAHs, the S/N of ten in sample chromatogram was used for the LOQ estimation. LOQ values can be found in the Supplementary material 2.

2.4. Toxicological analyses

Five cell lines (Table 2) were employed to assess the toxic potentials of air sample extracts while covering interactions among mixture constituents. To increase the relevance of the study towards human health, only human-based cell lines were used. Studied endpoints included AhR-mediated toxicity, anti-/estrogenicity, anti-/androgenicity, TR-mediated activity and cytotoxicity for bronchial cells characterised by 3 different reagents assays. A detailed description of used cell lines and testing procedures is provided in Supplementary material 1.

Based on data from chemical analyses, mixtures of detected chemicals were reconstructed from standard chemicals according to the composition of non-fractionated PM_{10} and gas phase organic extracts (i.e. Digitel samples) from all 3 campaigns. For each of these extracts, four variants of the mixture were prepared. A complex mixture containing all three analysed groups of chemicals (PAHs, NPAHs and OPAHs) and three mixtures representing each of the groups separately. The quality of the mixture preparation was confirmed by chemical analyses and the mixture toxicity was assessed by the reporter-gene bioassay battery.

2.5. Data analysis

The data from bioassays were evaluated using GraphPad Prism 6.0 software, using a log-logistic dose-response model to estimate the effective concentration, EC_x or IC_x values (preferably EC_{20} or IC_{20}). In cases where the observed effect did not reach the level of EC_{20} or IC_{20}, EC_{10} or IC_{10} was estimated. These effective concentrations were calculated from the statistically significant responses compared to the solvent controls obtained from independent experiments (one-way-ANOVA, Dunnett's test, $p < 0.05$).

The obtained EC and IC (inhibition concentration) values for reference compounds and extracts were converted into bioanalytical equivalents (BEQ_{bio}) using Eq. (1), with the EC_{20}/EC_{10} or IC_{20}/IC_{10} value of the reference compound (rc) and the corresponding EC_x or IC_x level of the air sample extract. When the response did not reach the level of EC_{10}, but was statistically significant compared to the solvent control, point-estimate was used. Limits of quantification (LOQs) for the samples with no statistically significant effects were based on the fact that even the greatest non-cytotoxic concentration of the sample did not cause effect that would correspond to the effect of the EC_{10} value of the reference chemical. Thus, LOQs were defined as the EC_{10} of the reference chemical per the highest tested air concentration with no cytotoxic effect (viability not significantly different from the solvent control). LOQs can be found in the Supplementary material 2. Cytotoxicity was expressed as the index of cytotoxicity ($1/IC_{20}$).

Table 2
List of employed bioassays.

Endpoint	Endpoint abbreviation	Bioassay	Ref. compound	Ref. compound concentration range	Seeding density (cells/well)	EC_X derived
AhR-mediated activity	AhR	AZ-AHR	TCDD	0.016–50 nM	24,000	EC_{20}
Estrogenicity	ER	HeLa9903	Estradiol	1.2–500 pM	12,000	EC_{10}
Antiestrogenicity	AntiER	HeLa9903	Fulvestrant	0.008–4 nM	12,000	IC_{20}
Androgenicity	AR	MDA-kb2	DHT	0.01–100 pM	40,000	EC_{10}
Antiandrogenicity	AntiAR	MDA-kb2	Flutamide	0.008–1 μM	40,000	IC_{20}
Thyroid receptor-mediated activity	TR	PZ-TR	T3	0.2–150 nM	24,000	EC_{10}
Cytotoxicity	Cyto	BEAS-2B	–	–	12,000	IC_{20}

$$BEQ_{bio} = \frac{EC_X(rc)}{EC_X(extract)} \; or \; \frac{IC_X(rc)}{IC_X(extract)} \quad (1)$$

Further, relative effect potency (REP_i) of the detected chemicals was calculated from earlier in-house measurements, complemented by information from literature or calculated based on data from the CompTox database (US EPA, 2015). The list of REPs with references can be found in Supplementary material 2_5. REP_i was calculated using Eq. (2), with EC_x of the reference compound (rc) and the corresponding EC_x value of detected chemical *i*.

$$REP_i = \frac{EC_X(rc)}{EC_X(i)} \; or \; \frac{IC_X(rc)}{IC_X(i)} \quad (2)$$

Moreover, BEQ_{chem} (analytically determined equivalent) based on the concentration of each chemical quantified in the air sample extract by targeted chemical analysis ($c_{a,\,i}$) and corresponding REP_i value was modelled using the concentration-addition approach described by Eq. (3), which reflects the current knowledge of mixture toxicity (Kortenkamp et al., 2009). Even though the BEQ_{chem} value was calculated in molar units, by introducing the coefficient MW_{RC}:MW_i, (which covers the molecular weight difference between studied and reference compound), final BEQ_{chem} is expressed in mass units.

$$BEQ_{chem} = \sum_{i=1}^{n} REP_i \times C_{a,i} \times \frac{MW_{RC}}{MW_i} \quad (3)$$

3. Results

3.1. Toxicological analyses of organic extract samples

The analyses characterized the mean exposure situation over twelve-day sampling periods at the sites. The *in vitro* testing detected significant effects of air sample extracts for most studied endpoints: estrogenicity, anti-/androgenicity, AhR-mediated activity, thyroid receptor-mediated activity and cytotoxicity for respiratory cells. The specific toxic potentials expressed as the bioanalytical equivalents (BEQ_{bio}) i.e. the concentration of the corresponding reference compounds are shown in Fig. 2A–E. A polarity-dependent distribution of toxic potential was found for AhR-, ER-, AR- and TR-mediated activities. In most cases, the parent sample (non-fractionated, NF) and the most polar fraction (F3) showed the strongest effects. Furthermore, the AhR-, ER- and AR-mediated activities and cytotoxicity for lung cells were associated mostly with fine/ultrafine PM fractions. For results see Fig. 2, for detailed information Supplementary material 2_1. Except for field blank relevant for the urban winter PM cascade impactor samples extracted with DCM, which elicited cytotoxicity in AlamarBlue assay, all blanks were negative in all employed bioassays. In this exceptional case, the data were blank-corrected. There was no blank correction needed for any other effect data.

AhR-mediated activity was associated only with the PM phase, with urban winter samples eliciting the strongest effects. Relatively high activity was found at the background site in winter and the lowest activity at the urban site in summer. Moreover, the cascade impactor data showed clear PM size-dependent distribution of AhR-mediated activity, with the sub-micrometre PM fractions being the most potent. Even though this activity was detected also in the non-polar fraction, it was found predominantly in the polar fraction (Fig. 2A).

Estrogenic activity was also associated only with the PM phase and significant effects were observed in both winter and summer urban campaigns. The estrogenic effect was elicited mostly by the fine PM fractions ($PM_{<1.5}$ in winter and $PM_{<0.95}$ in summer). Again, this activity was mostly elicited by polar pollutants, even though some activity was observed also in the non-polar fraction of urban winter samples (F1; Fig. 2B). Antiestrogenicity was not detected in any sample (< LOQ of 43 pg/m^3).

Androgenicity was greatest in urban winter samples. It was associated only with fine/ultrafine particles and predominantly the polar (F3) fractions with a smaller contribution from non-polar compounds (F1) similarly to estrogenicity (Fig. 2C). Even though the employed cell line MDA-kb2 can detect also GR-mediated activity, the co-exposure to samples and AR antagonist flutamide abolished the effect of the active samples (data not shown), confirming that the observed effects are mediated by AR only. Interestingly, while androgenic potential was detected specifically for particulate phase, gas phase was associated with antiandrogenic activity (Fig. 2C and D). The fractionation of gas phase samples showed that this activity can be elicited by compounds from both polar and non-polar fractions.

The thyroid receptor-mediated activity was the most frequently quantified in the urban summer samples. It was associated with polar compounds in both gas and particulate phases. As for the PM size distribution, the activity was associated namely with the particles sized 0.49–0.95 μm (stage E). Interestingly, this activity was also detected in the gas phase samples from the background site in winter (Fig. 2E).

Cytotoxicity to respiratory cells assessed by 3 different assays (Fig. 2F–H) was strongly associated with the PM phase and strongly dependent on the particle size. It was detected in all three campaigns. Also, cytotoxic effects were often more pronounced in the polar fraction (F3). Cytotoxicity of the gas phase showed a similar pattern but these results were detected only by the most sensitive assay (neutral red uptake, NRU).

3.2. Chemical analyses of organic extract samples

A total of 54 compounds including PAHs (25), OPAHs (11) and NPAHs (18) were analysed (Table 1). Three compounds (1,6N_2PYR; 1,8N_2PYR; 6NBAP) were not detected in any sample and, therefore, are not shown in any graphs. In general, the total concentration (particulate and gas phases) of detected compounds followed two trends. First: urban winter > urban summer > background winter, and second: PAHs > OPAHs ≫ NPAHs (Figs. 3 and 4). At the urban site, the concentration of PM-associated PAHs and NPAHs was approximately 5 times higher in winter than in summer, whereas for OPAHs this ratio was ≈10. Moreover, a PM size-dependent distribution was found for all 3 compound groups with the highest concentration associated with the smallest particles. Detailed results of organic extract chemical analyses can be found in the Supplementary material 2_3.

The total concentration of the targeted parent PAHs (≈140 ng/m^3) at the urban site was higher in winter than in summer (Fig. 4A). In winter, similar total concentrations were detected in both studied phases, but in summer the total concentration of parent PAHs was higher in the gas phase. The PAH mass fraction associated with the

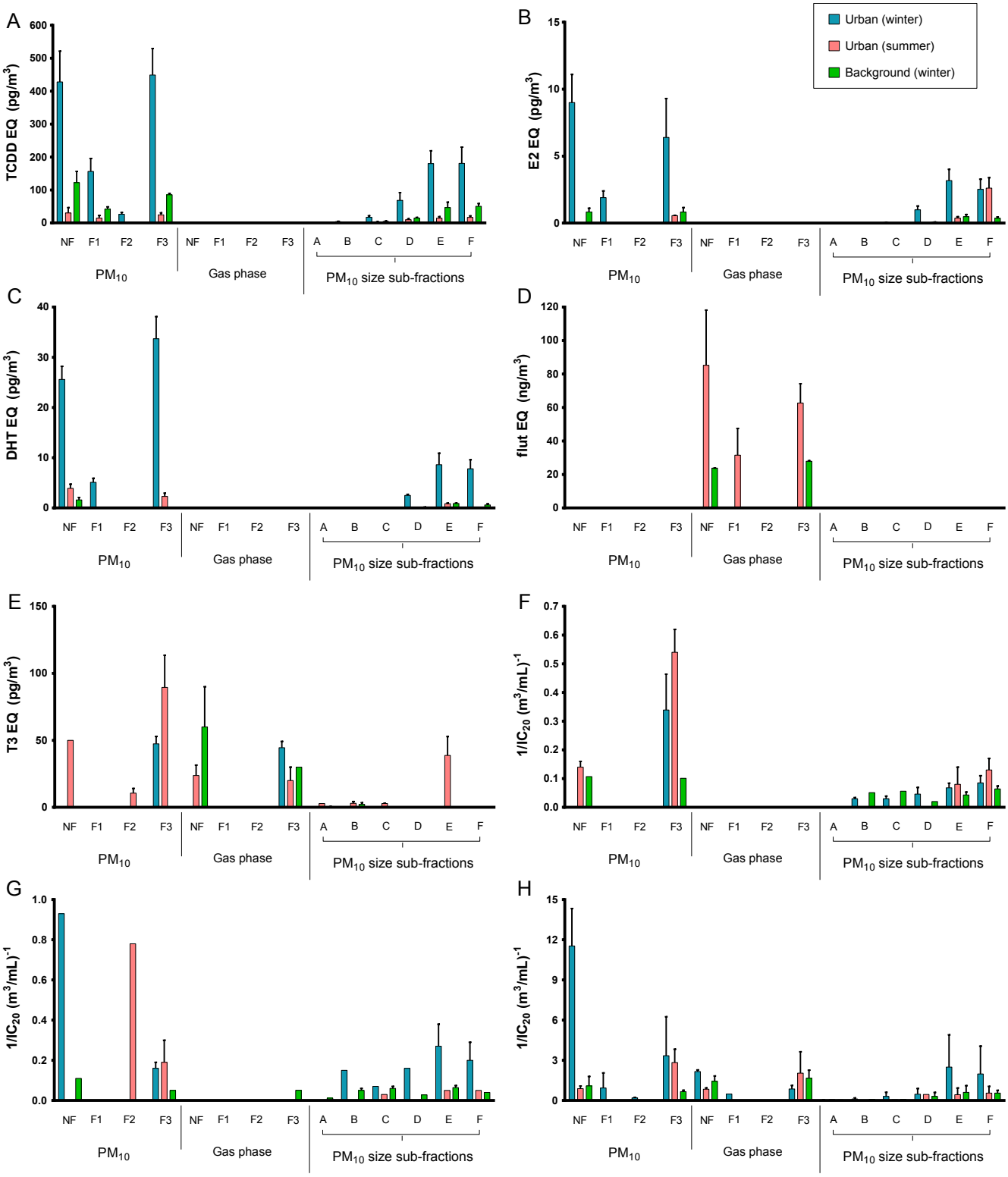

Fig. 2. Toxicological profiling of air samples organic extracts. Toxic potentials assessed *in vitro* using reporter-gene bioassays (A = AhR-mediated activity, B = estrogenicity, C = androgenicity, D = antiandrogenicity, E = thyroid receptor-mediated activity) and cytotoxicity for lung cells BEAS-2B measured by 3 reagents (F = Calcein-AM, G = Alamar Blue, H = neutral red). Results from reporter-gene assays are expressed as equivalent of corresponding reference compound (Table 2), cytotoxicity is expressed as index of cytotoxicity ($1/IC_{20}$). All graphs show the distribution of toxic potentials between gas and particulate phases and also the distribution according to pollutant polarity (NF = non-fractionated sample, F1 = non-polar, F2 = semi-polar, F3 = polar fraction) and PM_{10} size sub-fractions (A = 7.2–10 μm, B = 3–7.2 μm, C = 1.5–3 μm, D = 0.95–1.5 μm, E = 0.49–0.95 μm, F < 0.49 μm). All graphs cover seasonal variability (winter vs summer) at urban sampling site compared to the background site in winter. All graphs show mean + SD. Missing bars represent activity < LOQ. LOQ values are provided in Supplementary material 2. The same data plotted in logarithmic scale can be found in the Fig. S2 in Supplementary material 1.

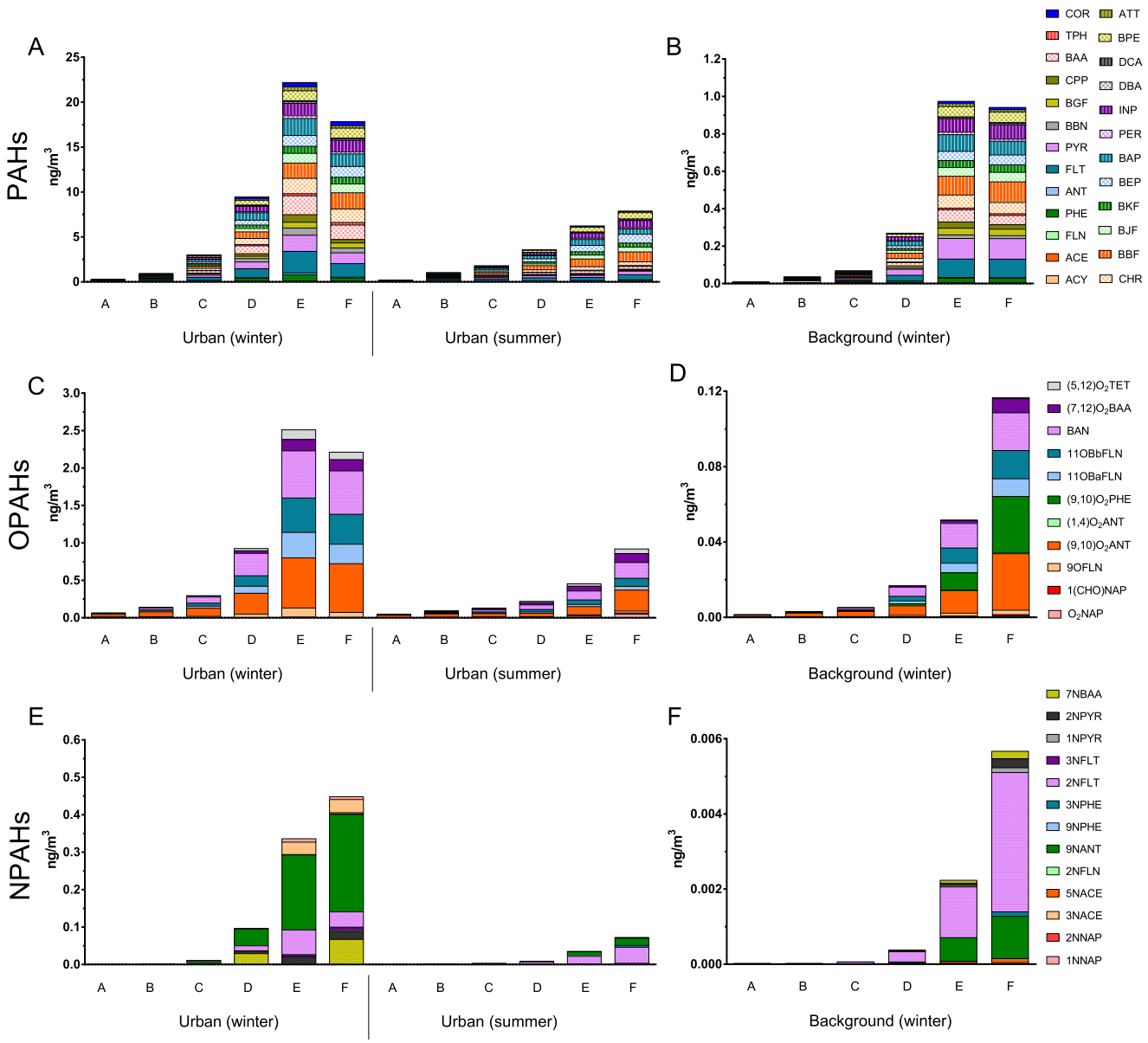

Fig. 3. The distribution of parent PAHs and their oxygenated and nitrated derivatives among PM_{10} sub-fractions: A = 7.2–10 μm, B = 3–7.2 μm, C = 1.5–3 μm, D = 0.95–1.5 μm, E = 0.49–0.95 μm, F < 0.49 μm of aerodynamic particle size at the polluted urban site in winter and summer (graph A, C and E) and the background site in winter (graph B, D and F). Analysed compounds are listed in Table 1.

particulate phase showed a pronounced size dependency, with the highest concentrations associated with the sub-micrometre fractions (Fig. 3A and B). As for pollutant polarity, PAHs were detected mainly in the non-polar fraction (F1) with phenanthrene, fluoranthene, fluorene and pyrene being most abundant among detected parent PAHs in the gas phase and pyrene, fluoranthene, chrysene and benzo[b]fluoranthene in the particulate phase (Fig. 4A and B).

Contrary to the quite abundant parent PAHs, the total concentration of targeted OPAHs in both phases was below 12 ng/m^3 at the urban site during both sampling seasons and below 1 ng/m^3 at the background site (Fig. 4C and D). The concentrations were higher in winter when OPAHs were associated more with the particulate matter, while in summer, OPAHs were less abundant and more associated with the gas phase. The most abundant substances were benzanthrone and 9,10-anthraquinone in winter and 1,4-naphthoquinone and 9,10-phenanthroquinone in summer. OPAHs were detected mainly in the polar fraction (F3) – in both particulate and gas phases (Fig. 4C and D). The particle size-dependent distribution of OPAHs was similar to the parent PAHs distribution with greatest levels in the smallest particle size fractions (Fig. 3C and D).

Compared to parent PAHs and OPAHs, the nitro derivatives were the least abundant. NPAHs were detected in both particulate and gas phases, with a total concentration in both phases below 1.2 ng/m^3, regardless the site or the season (Fig. 4E and F). The concentrations of all individual NPAHs were always less than 1 ng/m^3. In winter, they were associated more with the particulate phase and mainly with the sub-micrometre fractions (most abundant 9-nitroanthracene, Fig. 3E and F), but in summer they were more abundant in the gas phase with 1-nitronaphthalene being the most abundant. NPAHs did not show any clear distribution pattern among the polarity fractions (Fig. 4E and F).

3.3. Toxicological and chemical analyses of simulated lung fluid (SLF) extract samples

The toxicological assessment revealed AhR-mediated activity, estrogenicity and TR-mediated activity in SLF extracts (Figs. 5 and S3). In

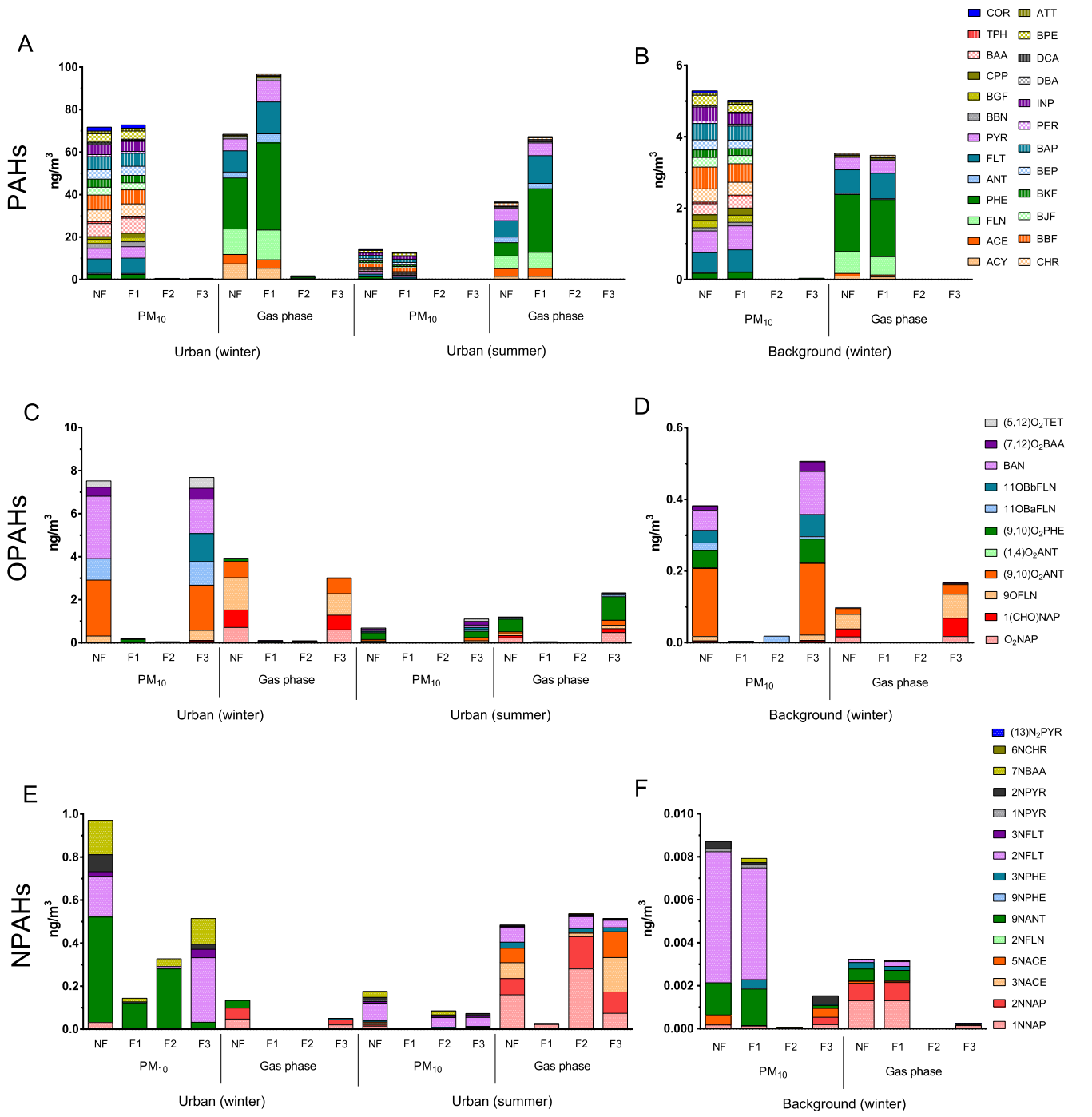

Fig. 4. The distribution of parent PAHs and their oxygenated and nitrated derivatives among polarity fractions (NF = non-fractionated sample, F1 = non-polar, F2 = semi-polar, F3 = polar fraction) in the particulate and gas phases at the urban site in winter and summer (graph A, C and E) and the background site in winter (graph B, D and F). Analysed compounds are listed in Table 1. Detailed numeric results can be found in the Supplementary material 2_3.

ALF extracts, AhR-mediated activity and estrogenicity were detected in both urban and background winter samples. In SELF extracts, these two activities were detected also in urban summer samples. The TR-mediated activity was found in SLF extracts of urban samples from both sampling seasons. All studied effects showed pronounced particle size-dependent pattern (Fig. 5). Androgenicity was not detected in any SLF extract (LOQs in Supplementary material 2_2). Comparison of the bioactivity of organic and lung fluid extracts showed that, except for the TR-mediated activity, SLF extractable compounds mostly reached only several per cents of the effects of organic extracts. More specifically, it was by average 18.7% (3.1–148%) and 2.6% (0.1–11.3%) for estrogenicity and AhR-mediated toxicity, respectively. On the other hand, TR-mediated activity was often relatively higher in SLF extracts with a mean of >208% (8.7–>918%) of the effects caused by organic extracts. In several cases, namely for fractions C, D, E and F of urban winter samples, the TR-mediated activity detected in SLFs was up to 9 times higher than the LOQ value derived for the organic extract (Supplementary material 2_2).

Parent PAHs were extracted more effectively using SELF than ALF (Fig. 6A–C). Total concentrations ranged from several pg/m^3 to 284 pg/m^3 and were about 2–3 orders of magnitude lower compared to extraction by organic solvents. As for seasonal variability, concentrations

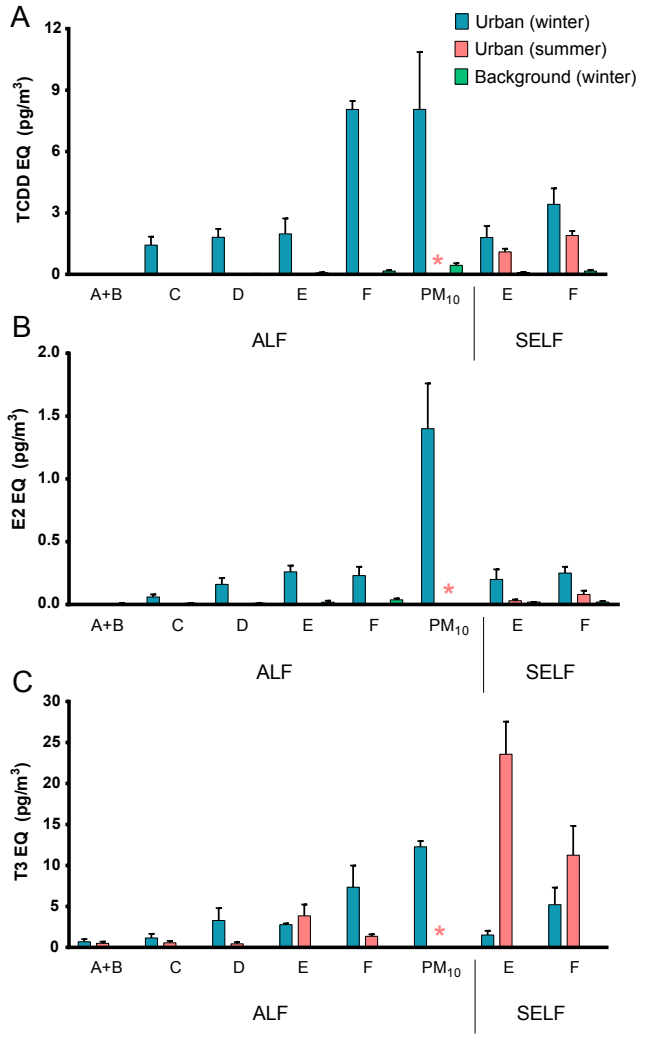

Fig. 5. Toxicological profiling of air samples lung fluid extracts. The distribution of detected toxic potentials in either total PM_{10} sample or PM_{10} size sub-fraction samples extracted by artificial lysosomal fluid (ALF) or by simulated epithelial lung fluid (SELF). Graphs represent results for AhR-mediated activity (A), estrogenicity (B) and TR-mediated activity (C). All results are expressed as mean + SD. PM_{10} sub-fractions according to aerodynamic particle size were: A + B = 3–10 μm, C = 1.5–3 μm, D = 0.95–1.5 μm, E = 0.49–0.95 μm, F < 0.49 μm of aerodynamic particle size. Missing bars represent activity < LOQ. LOQ values are provided in Supplementary material 2. Asterisk indicates that the urban summer PM_{10} sample was not tested. The same data plotted in logarithmic scale can be found in the Fig. S3 in Supplementary material 1.

in SLFs were approximately ten times higher in winter compared to summer (Fig. 6A vs B). The most abundant compound among parent PAHs was phenanthrene.

Oxygenated PAHs were detected in similar concentrations as parent PAHs (from several pg to 446 pg/m^3) with 9,10-anthraquinone and 9,10-phenanthroquinone being the most abundant. Again, significantly higher concentrations were detected in winter (Fig. 6D and E). Compared to organic extract, the total OPAHs concentrations detected in SLFs were about 1–2 orders of magnitude lower. The concentrations of all studied NPAHs were below the limit of detection in all SLF extracts. Detailed results of SLF extract chemical analyses can be found in the Supplementary material 2_4.

3.4. Contribution of the detected compounds to observed bioactivities

To assess the contribution of the detected chemicals to the observed biological effects, BEQ_{chem} was calculated using the concentration-addition approach (Eq. (3)). Data on REPs (relative effect potency) were collected from peer-reviewed literature, CompTox database or based on our own measurements. Unfortunately, the available data on REPs are quite scarce. Most of the relevant REP values were found for parent PAHs and only a few for oxy- and nitro- derivatives (Supplementary material 2_5). From these values, preferentially REPs derived from human-based cell models, which focused on the studied receptors, were included in the calculation. For each studied endpoint, all REP values for the individual chemical effect data are adjusted/related to the same reference compound from the same assay. Among the observed effects, AhR-mediated toxicity and estrogenicity were the most studied ones (REPs for 15 and 12 substances, respectively), while the information on the other toxicological endpoints is often not available. A summary of the obtained data, calculated BEQs and the ratio BEQ_{chem}/BEQ_{bio} (a fraction of the observed effect explicable by the targeted substances) for both organic and SLF extracts can be found in Supplementary material 2_1 and 2_2.

As for AhR-mediated toxicity, up to 42% of the observed effect was explained in urban winter samples, and up to 98% in summer samples by modelling the effect of REP-covered PAHs. Both maximum values were found for the non-polar fraction of the PM_{10} sample. Even though the concentration of REP-covered compounds in this fraction was approximately 5 times higher in winter than in summer, the detected AhR-mediated toxicity was approximately 10 times higher. Thus the ratio of BEQ_{chem}/BEQ_{bio} (the explicable fraction) in this fraction was lower in winter implying other non-polar AhR-activators play a more significant role in winter air pollutant mixture. The highest BEQ_{chem}/BEQ_{bio} was associated with particle-bound parent PAHs with known REPs, which were found in quite high concentrations compared to their derivatives. Moreover, parent PAHs are also characterised by higher REP values than their derivatives in case of AhR-mediated toxicity. Therefore, in our study, they contributed the most to the observed effect among studied compound groups. Similarly to AhR-mediated activity, the detected estrogenicity could be partly explained (in average 4%) by detected compounds with available REP values. The highest explicability was found for the C fraction of PM phase in the urban winter sample (23%).

REPs for androgenicity were found only for 3 compounds (benzo[a]anthracene, indeno[123-cd]pyrene and dibenzo[ah]anthracene) and explained at maximum only 0.85% of the detected effect. As for the antiandrogenicity, REP values for 15 compounds were found. The calculated BEQ_{chem} explained in average less than 2% of the observed effect.

Contrary to organic extracts, where REP-covered compounds explained the detected activities at least partly, in case of SLF extracts, the REP-based modelling did not bring any more light into toxicity drivers identification. At maximum, the detected compounds with known REPs were able to explain 3.5% of the detected effect (AhR mediated activity, background site in winter, stage C extracted by ALF) but in average the explicability in SLFs was below 1% for both AhR-mediated activity and estrogenicity. Androgenicity was not detected and antiandrogenicity not tested in SLF extracts (Supplementary material 2_2).

Regardless the extract solvent, other effects could not be explained due to the lack of relevant REP data. Given these huge data gaps, the potential role of PAHs and their derivatives in these effects could not be evaluated by modelling. To assess their contribution, REPs for these compounds and employed assays are needed. Both complex chemical and effect-driven analyses are required for the identification of the effect-drivers.

In the testing of reconstructed chemical mixtures, quantifiable effects were elicited mostly by the mixture based on PM_{10} filter sample from the urban winter campaign and its PAHs fraction. Detected effects of these reconstructed mixtures included AhR-mediated activity, estrogenicity and androgenicity (Supplementary material 2_6). Additionally, AhR-mediated activity was quantifiable for the mixture

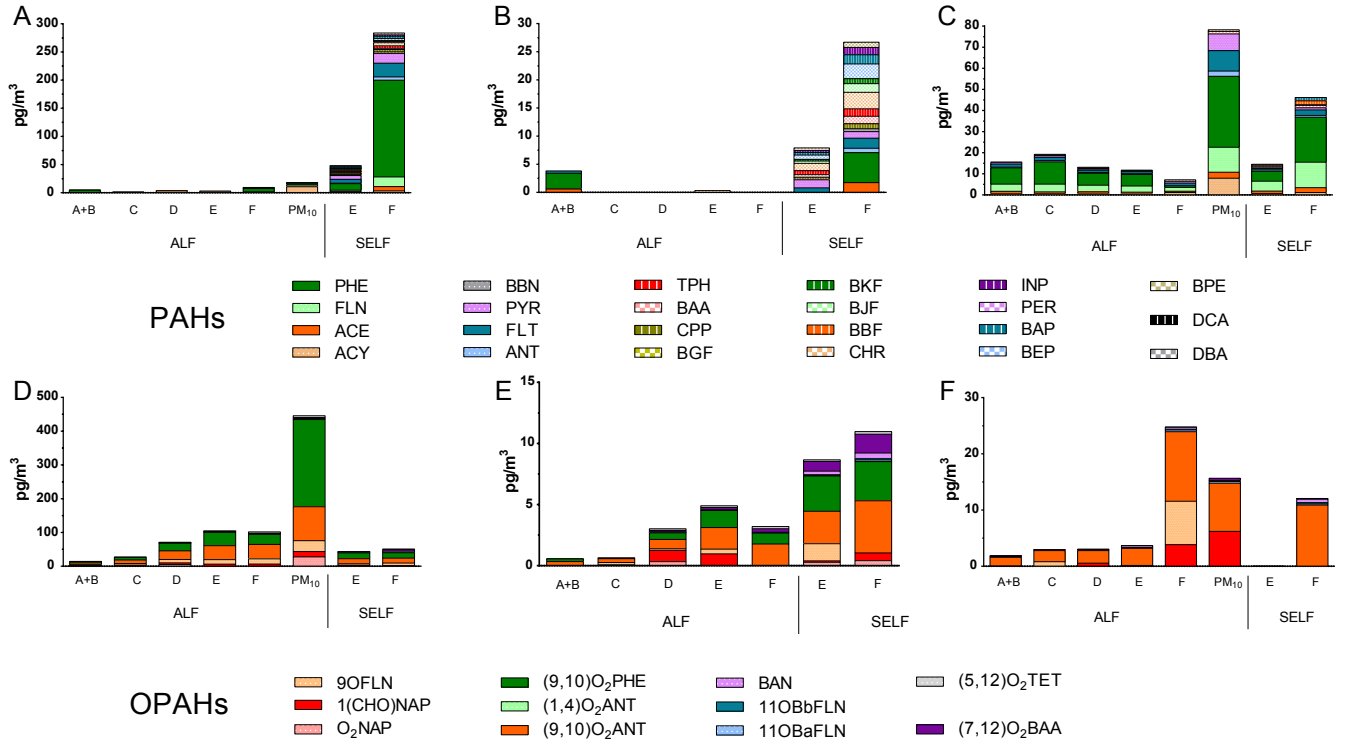

Fig. 6. Concentrations of parent PAHs and their oxygenated derivatives extracted into simulated lung fluids. The distribution of quantified compounds in either total PM_{10} sample or PM_{10} size sub-fraction samples extracted by artificial lysosomal fluid (ALF) or by simulated epithelial lung fluid (SELF). Levels of PAHs at urban site in winter, in summer and at background site in winter are shown in graph A, B and C, respectively; levels of OPAHs at graphs D, E and F. PM_{10} sub-fractions: A + B = 3–10 μm, C = 1.5–3 μm, D = 0.95–1.5 μm, E = 0.49–0.95 μm, F < 0.49 μm of aerodynamic particle size. Analysed compounds are listed in Table 1. Detailed numeric results can be found in the Supplementary material 2_4.

based on PM_{10} filter sample collected at the background site and anti-androgenicity was found in the reconstructed gas phase mixture from the urban winter campaign. Effects observed in the reconstituted mixtures of parent PAHs and OPAHs were compared to those detected for the non-polar (F1) and polar (F3) fraction, respectively, since most of the parent PAHs was detected in F1 and OPAHs in F3.

The AhR-mediated activity of the reconstructed mixture ($recBEQ_{bio}$) reached 24% and 4% of the original effect (BEQ_{bio}) in urban and background winter PM_{10} samples, respectively. While the calculated effect (BEQ_{chem}) of these 2 samples represented 16 and 4% of the activity detected in the original samples, it represented 68 and 81% of the activity detected in the reconstructed mixtures ($recBEQ_{bio}$) of urban and background winter samples, respectively. Nevertheless, some of the compounds in reconstructed mixtures could not be taken into account in the calculated effect (BEQ_{chem}) due to the lack of their REP values.

The AhR-mediated activity of the non-polar fraction (F1) of the original urban and background winter samples was explained by reconstructed mixtures of parent PAHs by 69 and 17%, respectively. Whereas in the original F1 samples, the effect calculated from known REPs (BEQ_{chem}) represented 42 and 8% of the AhR-mediated activity detected *in vitro* (BEQ_{bio}), it represented 61 and 51% of the activity ($recBEQ_{bio}$) in reconstructed parent PAH mixtures of urban and background winter samples, respectively.

Quantifiable estrogenicity was only detected in two reconstructed mixtures. The reconstructed mixture based on the composition of PM_{10} urban winter sample reached 5% of the complex sample activity (BEQ_{bio}) whereas the mixture reconstructed from parent PAHs reached 37% of the activity detected in the non-polar fraction (F1). While the estrogenicity modelled from known REPs (BEQ_{chem}) explained 3 and 13% of the original activity (BEQ_{bio}), it explained 58 and 35% of the activity detected in reconstructed mixtures ($recBEQ_{bio}$) of PM_{10} urban winter sample and its F1 fraction containing parent PAHs, respectively.

Similarly, also androgenicity was detected only in these two reconstructed mixtures of urban winter PM samples: in the non-fractionated sample and in the parent PAHs mixture (corresponding to the F1 fraction). These two reconstructed mixtures elicited 88 and 252% of the original activity (BEQ_{bio}), respectively. Since only 3 androgenicity REPs were available, the modelled level of BEQ_{chem} explained only 0.2 and 0.3% of the reconstructed mixture activities ($recBEQ_{bio}$), respectively.

The antiandrogenicity of the reconstructed mixture was detected only for the gas phase of urban winter sample, and the detected recBEQ was below the LOQ for the activity of the original sample. Nevertheless, the comparison of this LOQ with the $recBEQ_{bio}$ indicates that the reconstructed mixture would elicit at least 45% of the original sample activity. The calculated BEQ_{chem} explained 13% of the effect observed in the reconstructed mixture. The TR-mediated activity was not quantifiable for any reconstructed mixture.

4. Discussion

Ostrava city located in the northeast of the Czech Republic is considered one of the most polluted areas in Europe due to its industry and intense traffic (Pokorná et al., 2015). The pollution has been associated with significantly impaired human health (Šrám et al., 2013). Apart from the identified and regulated air pollutants, there are other compounds, which upon inhalation can affect human health. The diversity of air pollutants results in the exposure to complex mixtures, the specific toxic potentials of which were assessed by the *in vitro* bioassays. The collected air sample extracts were pooled across 12-day sampling periods to increase the relevance of obtained results towards the longer-term exposure situation. The detected levels of chemicals (54 PAHs and their derivatives) were used for modelling of their effects (BEQ_{chem}) and their contribution to the air samples effects detected by *in vitro* assays (BEQ_{bio}). However, the modelling approach was impaired by rather low

availability of relative potency values (REPs) of the chemicals for studied endpoints. To bypass the problem with non-available REPs for many analysed pollutants, detected chemical mixtures were reconstructed and assessed with bioassays.

4.1. Toxicological analyses of organic extracts of air samples

4.1.1. AhR-mediated activity

The activation of the aryl-hydrocarbon receptor can trigger the induction of enzymes involved for instance in hormone signalling, detoxification and cell proliferation and its activation has been studied in both toxicological and pharmaceutical studies since it can be linked to carcinogenesis or immunotoxicity (Safe and Wormke, 2003). In our study, the AhR-mediated activity was associated with the particulate phase only, strongly PM size-dependent, and elicited mostly by compounds in polar fraction. The AhR-mediated activity at the background site in winter was higher than at the urban site in summer. This implies that the dispersion cannot compensate for the pollution of the background atmosphere in Central Europe in winter. The highest activity detected in winter urban air is in agreement with the data published previously by Klein et al. (2006) who reported this activity to be associated with PM regardless of site or season in Canada. Similarly, our previous study (Novák et al., 2014) showed that the potency to activate AhR is inversely proportional to the particle size. The AhR-mediated activity elicited by compounds in the polar fraction had also been found by Misaki et al. (2008) who related their findings to diesel exhaust particles in Japan. Furthermore, diesel exhaust particles from an urban site in Japan were found to be AhR active in transfected human prostate carcinoma cells (Okamura et al., 2004).

Out of the 25 targeted parent PAHs, REP values were available only for 10 (in general the most abundant ones), which accounted for 80% of the mass of the targeted parent PAHs (urban winter samples, PM_{10}). On the other hand, these most abundant PAHs only explained 16% of the observed effect. Additionally, the rest of the targeted compounds (OPAHs and NPAHs) with known REP values explained 2×10^{-3}% (REP values found for 5 out of 29 OPAHs and NPAHs). However, data from reconstructed mixtures show that all targeted PAHs together were able to elicit 24% of the effect of the complex sample and even 69% of parent PAHs-containing fraction. This implies that the PAHs without available REP played a big role in the effect and it might be also possible that the chemicals in the mixture act synergistically, even though in complex mixtures it is not as common as concentration-addition mechanism. Anyway, there is 76% of the effect unexplained, obviously elicited by non-targeted compounds.

The AhR-mediated activity in the non-polar fraction was partly explained by REP-based modelling (42% in winter and 98% in summer), and only parent PAHs contributed to the explanation. This corresponds with the finding that the usual AhR-activity suspects (parent PAHs) were detected mostly in the non-polar fraction. On the other hand, the AhR-mediated activity detected *in vitro* dominated in the polar fraction and we were unable to explain it by the contribution of the identified substances or by the reconstructed mixture. This implies that the observed effect was caused by other polar PAH derivatives and/or by other polar substances associated with PM not addressed in this study.

4.1.2. Anti-/estrogenicity

The estrogen receptor is a crucial player in physiological processes like growth, development and reproduction and even though estrogenic compounds are mostly studied in aquatic environment, airborne estrogenic activity has been reported in previous studies. Oziol et al. (2017) detected significant estrogenic activity in an urban environment in both gas and PM phases during both winter and autumn. Even though the activity was more attributed to the gas phase, it was higher in winter. Similarly, Klein et al. (2006) found that the estrogenicity elicited by compounds in the gas phase is often similar or even higher than PM-related estrogenicity in both urban and rural environments. Contrary to their findings, in our study, the estrogenic activity was exclusively particle-borne and driven mostly by the polar compounds associated in all 3 campaigns with the fine particles <0.95 μm. The estrogenic activity of the gas phase samples was always below LOQ. The differences may originate from different pollution sources or from different sorbents used (i. e. highly absorbent resin XAD vs PUF). The difference can be also affected by different sensitivities of some assays. Despite the detected estrogenicity in F3 of the urban summer PM organic extract, no estrogenicity was detected in the corresponding NF sample. However, the detected estrogenicity in the fractionated sample was comparable to LOQ of NF sample. The effects in fractions could be detected even below or close to the LOQ for the nonfractionated samples, that could be only tested at lower concentrations due to their greater cytotoxicity. Another factor that could play a role is the potential presence of ER antagonists in NF sample and their removal in fractionation process.

As for the level of estrogenic activity, our study showed higher levels of E2 equivalents in pg/m^3 compared to the study in Paris (Oziol et al., 2017). However, a direct comparison is not possible, since different cell lines were employed. As for the compounds responsible for detected estrogenicity, some PAHs were found to have estrogenic potential and corresponding REP values were derived. In our study, the calculated BEQ_{chem} could be based only on 7, 3 and 2 out of 25 PAHs, 11 OPAHs and 18 NPAHs, respectively (non-availability of REP values). In most cases REP-covered PAHs or OPAHs represented approximately 50% of the total detected mass of the targeted groups, but they explained in average 3.9% of the estrogenic effect observed for organic extract samples. The only exception was the organic extract of C size fraction of urban winter PM_{10} sample, where the REP-covered PAHs explained 23% of the observed effect. This finding again implies that the PAHs and their derivatives without known REP values, PAHs not addressed in this study, and/or other substance classes may play an important role for the ambient aerosols' estrogenic potential.

Contrary to our findings, antiestrogenicity associated with both fine PM and gaseous phase was detected in the study of Novák et al. (2014) covering two locality types in the Czech Republic. Also, it was detected in diesel exhaust particle extracts from Korea by Oh et al. (2008), who associated this activity with both crude and pH neutral extracts. Both studies used human breast cancer cell lines, MVLN and MCF7, respectively, but tested the antiestrogenicity in different exposure times.

On the other hand, another research paper (Oziol et al., 2017) addressing also the antiestrogenicity by human breast cancer cell line (MELN), did not detect any antiestrogenic effect in air samples from Paris. Similarly, in our study, where HeLa9903 cells were employed, no antiestrogenicity was detected. The different outcomes could be affected by different cell line sensitivity. Also, various pollution sources and sampling techniques may contribute to inter-studies variability. Last but not least, different extraction solvents were often used. The availability of LOQs would also improve the comparison, however, these values are often not reported.

4.1.3. Anti-/androgenicity

The androgen receptor is involved in signalling pathways important for example for the development of male sexual characteristics, reproductive functions, growth and behaviour (Janošek et al., 2006). Its function can be influenced in both the agonistic and antagonistic way. In the present study, significant androgenicity was completely associated with the PM phase, whereas antiandrogenicity was detected only in the gas phase. This finding is in a good agreement with findings in the same region in summer (bioluminescent yeast assay; Novák et al., 2008) and in Paris in both autumn and winter season (Oziol et al., 2017), the latter using the same *in vitro* model as our study. No further evaluation of androgenicity BEQ_{chem} was possible due to the lack of REP data (only 3 values found that explained less than 1% of the detected effect). Out of 54 targeted compounds, for 15 of them

antiandrogenicity REPs were available. These compounds explained less than 2% of the detected antiandrogenicity even though they contribute in average by 45% to the detected mixture composition mass. However, the reconstructed mixture was able to explain 88% of urban winter PM_{10} organic extract androgenicity and in a non-polar fraction of this sample the explicability exceeded 100%. The exceedance was within the variability of the bioanalysis. In winter the antiandrogenicity was detected in the gas phase organic extract from the background site, but not in the urban site, which could be associated with high LOQ value in NF urban sample caused by its greater cytotoxicity. This LOQ was actually higher than the BEQ detected for the background site. Several other factors could play a role in this observation, like, unspecified local sources in the background site or presence of the androgenic compounds decreasing the antagonistic effect at the urban site in winter.

4.1.4. Thyroid receptor-mediated activity

Thyroid receptor signalling contributes to the regulation of numerous physiological processes like growth, neurodevelopment and energy homeostasis. However, this endocrine signalling network can be disrupted by various compounds like polybrominated diphenyl ethers or polychlorinated biphenyls (Calsolaro et al., 2017). Moreover, some pesticides have been indicated as potential TR agonists, while others as antagonists (Xiang et al., 2017). Although compounds interfering with thyroid signalling were found in both indoor and outdoor environments, there is very limited information regarding thyroid receptor-mediated activity of ambient air gas phase or PM. To our present knowledge, only one study dealing with thyroid receptor-mediated activity in outdoor air was published so far (Oziol et al., 2017). They reported significant thyroid receptor-mediated activity associated with urban PM phase in Paris with higher activity observed in winter than in autumn (2.8 vs 0.6 equiv. T3 EQ pg/m^3). We detected thyroid activity associated with both particulate and gas phases and being more prominent in summer. The more frequent detection of TR-mediated activity can result from higher mobility of indoor TR-activating pollutants to outdoor air due to both higher temperature and increased ventilation rate. Also, the pesticide application or evaporation during summer could contribute to detected effects. This is supported by the fact that several pesticides were found to be able to bind the human TR (Xiang et al., 2017). As for site comparison, the NF gas phase organic extract from winter elicited higher activity at background site than at urban site (62 vs $<$24 T3 EQ pg/m^3). Local sources at the background site, such intense local heating in winter, might play role in this observation. Furthermore, also some compounds with antagonistic activity towards TR could be present at the urban site in winter lowering the overall TR activity in NF sample, which is supported by the fact that TR activity was detected in F3, but not in the NF sample. However, more research is needed for the identification of TR-mediated activity drivers. Our reporter-gene assay often detected greater TR-mediated activity than in the above-mentioned study (up to 90 equiv. T3 pg/m^3, PM_{10}-F3 urban-summer sample). Moreover, our model is based on human cell line (Oziol et al., 2017 applied a model derived from rat adrenal cancer), hence should be more relevant for human health risk assessment. While most previous studies focused on the thyroid-disrupting compounds indoors, our study confirms that the thyroid receptor-mediated activity can be significant also in ambient air and might, therefore, pose a hazard for the exposed population.

4.1.5. Cytotoxicity

Significant cytotoxicity detected by all 3 applied assays i.e. Calcein-AM, Alamar Blue and neutral red uptake (NRU), was mostly particle-related and dominated in polar fraction. The NRU assay was proven to be the most sensitive due to the highly energy-demanding storage of neutral red in lysosomes. Thus, this assay can reflect the energy imbalance at sub-cytotoxic levels. In this assay, significant cytotoxicity was also detected in the gas phase. These results demonstrate the potential of the particle-associated air pollutants to adversely affect cells of the respiratory system *via* more general mechanisms of toxicity. Even though the cytotoxicity assessment does not address any specific mode of action, as the other employed receptor-based bioassays do, it comprises a highly relevant tool for addressing the non-specific toxicity mechanisms related to the inhalation exposure.

Our toxicological analysis showed that the studied effects are often strongly PM size-dependent with greatest effects associated with submicrometre particles. This finding shows as extremely important for human exposure, since these particles penetrate into the deep lung (alveolar-interstitial region) and reside there longer (clearance), while the super-micrometre particles are preferentially deposited further up (Kappos, 2010; Pokorná et al., 2015). Also, this study documents that the studied bioactivities are largely attributable to the polar fraction that contains the OPAHs, but also other polar compounds. The polar fraction of particulate organic matter is the least characterized (Claeys et al., 2012; Graber and Rudich, 2006; Saxena and Hildemann, 1996), which, despite recent progress in analytical chemistry, leaves us with thousands of unidentified chemicals (e.g. Sippula et al., 2014).

4.2. Bioaccessibility assessment using SLFs

The overall level of SLF extractable PAHs and OPAHs was in general much lower compared to the amount obtained by the total (organic solvent) extraction, implying that only a small portion of studied particle-associated pollutants can leach into lung fluid. When comparing the extractability of targeted compound groups, it can be concluded that OPAHs are more extractable into SLFs than PAHs, due to the double bonded oxygen in the structure, which increases their polarity. Also, since SLF is a mix containing, among others, carbohydrates and lipoproteins, these may increase extraction efficiency for OPAHs, either as freely dissolved or by forming structures similar to micelles. The concentration of SLF extractable NPAHs was always below the limit of detection, which corresponds to the much lower levels of NPAHs in organic extracts. As for the bioactivities detected in SLF extracts, significant estrogenicity and AhR-mediated toxicity were quantified, nevertheless mostly in much lesser extent compared to the bioactivities elicited by the corresponding organic extracts. As for the comparison of the bioactivities detected in both ALF and SELF extracts of E and F fractions, we can conclude, that the winter samples elicited higher effects in ALF extracts, whereas effects of summer samples were more pronounced in SELF extracts. Interestingly, TR-mediated activity was detected in SLF extracts from both winter and summer urban PM samples and it was quantifiable more frequently in SLFs compared to organic extracts. Moreover, in contrast to other activities detected in SLF, the detected TR-mediated activity was in similar order of magnitude as the T3 EQ levels found in organic extracts. In case of fine PM fractions, where TR-mediated activity was quantifiable only in SLF extracts, the activity in SLF even exceeded the LOQ values of the organic extracts with no detected effect (Supplementary material 2_2). This implies the presence of not targeted, more easily bioaccessible substances contributing to the TR-mediated activity mainly in the fine PM fractions. These findings could be of importance since thyroid signalling pathways play role in regulation of growth, development and metabolism and are involved for example in neurodevelopment, but further research is definitely needed to examine the potential link between long-term exposure to airborne TR-activating compounds and its possible health impact. No such study to directly compare with has been performed, to the best of our knowledge. Vuong et al. (2017) determined the toxicity of water-soluble and water-insoluble fractions of urban air PM_{10} from Ottawa for lung epithelial cells A549 and found that the insoluble fraction is responsible for the detected toxicity and that soluble and insoluble fractions can differently alter the expression of proteins linked to cell death, proliferation and inflammation. BEQ_{chem} modelled in SLFs explained significantly less activity compared to organic extract-based BEQ_{chem}, proving that REP-covered

targeted compounds are not the toxicity drivers in SLFs and that the original mixture is much more complex.

4.3. Concentrations of PAHs and their derivatives in outdoor air samples

This study focuses on the role of PAHs and their derivatives in the studied bioactivities, therefore following paragraphs just briefly summarize the levels of targeted pollutants to provide the reader with general context. Detailed results can be found in the Supplementary material 2_3. Detailed discussion of both concentration and distribution of targeted compounds will be provided in an accompanying paper (Lammel et al., 2020).

The total concentration of studied parent PAHs was higher at the urban site compared to the rural one. This trend, as well as the PAHs levels, are in a good agreement with findings of Morville et al. (2011). On the other hand, parent PAHs can reach tens of ng/m^3, especially during impaired weather conditions in winter (Landlová et al., 2014). PM size-dependent distribution was observed to be the same as in previous studies (Kawanaka et al., 2009; Lammel et al., 2010; Landlová et al., 2014) confirming the observation that particles of the smallest size fractions often carry most of the studied toxic compound mass.

OPAHs were detected at similar concentrations as reported previously in Europe and Canada. They are mostly found at levels below 10 ng/m^3 regardless the place or sampling season (Albinet et al., 2007; Ringuet et al., 2012; Tomaz et al., 2017; Wnorowski and Charland, 2017). As for their distribution, OPAHs dominated in PM in winter with the highest concentration associated with small particles, whereas in summer higher concentrations were found in the gas phase.

The chemical analysis of selected NPAHs confirmed that they are the least abundant among the studied substance classes. Compared to PAHs and OPAHs, NPAHs were found at 2 and 1 order of magnitude lower concentration, which corresponds to previous studies (Albinet et al., 2007; Lammel et al., 2017). In this study, the highest total NPAHs concentration was 1.11 ng/m^3, which is in a good agreement with other studies from polluted environments (Albinet et al., 2007; Liu et al., 2017; Ringuet et al., 2012). However, the knowledge on their toxicity is very limited and further investigation is needed to estimate their contribution to the observed effects.

The chemical analysis confirmed that the parent PAHs are fractionated into the least polar solvent, while the more polar OPAHs dominated in the polar fraction. The distribution of NPAHs according to polarity varies with both sampling season and site.

4.4. Effects of reconstructed mixtures

For reconstructed mixtures based on analytical data from gas phase samples across sites and urban summer PM_{10} samples, there were no quantifiable effects detected. Thus, the targeted chemicals were probably not the main effect-drivers in the gas phase. Similarly, the detected effects of environmental pollutant mixtures associated with PM_{10} in summer were elicited mainly by chemicals non-analysed in this study. In case of winter PM_{10} samples, the analysed chemicals were more significant for the studied toxicity endpoints. This was the case mainly for the winter sample from the urban region, since these reconstructed mixtures elicited also quantifiable estrogenic and androgenic activities compared to reconstructed mixtures of background winter samples, which elicited only AhR-mediated activity. The mixture data show that among the analysed chemicals parent PAHs were the most effective. The effects detected in the reconstructed mixtures were elicited mainly by parent PAHs since the mixture containing all chemicals together produced a similar effect as the mixture consisting of parent PAHs only. Moreover, OPAHs and NPAHs mixtures were without quantifiable effects when tested separately by chemical groups. However, the most potent fraction of the original samples was the polar one (F3). Thus, the targeted OPAHs were not the effect-drivers for the assessed endpoints. Therefore, there is a need to identify those other polar chemicals which contributed significantly to the detected effects.

5. Conclusions

This study represents an efficient assessment of outdoor air pollution linking both chemical and toxicological perspectives. The results confirm that air pollutants possess several toxic potentials, that can interfere with human health *via* various molecular mechanisms like estrogenicity, AhR-mediated activity or thyroid receptor-mediated activity as well as toxicity for bronchial cells. Furthermore, our results emphasize the seasonal variability and distribution of the studied toxic potentials among the particulate and gas phases of the ambient air and across size fractions of inhalable particles. It documents that the bioactivities are largely attributed to PM_1 and the polar fractions of both gas and particulate phases.

The effects of the targeted PAHs and their derivatives represent only a part of the mixture toxicity humans are exposed to in both urban and background environments. The calculated contribution of the targeted PAHs and OPAHs together accounted on average for 21 (0.004–98) % of the total AhR-mediated activity and on average for 4 (0.06–23) % of the total estrogenicity. However, the toxicity testing of the mixtures reconstructed according to chemical analyses revealed that, apart from an important role of parent PAHs in several effects associated with urban PM pollution in winter, the targeted pollutants are not the main contributors to the effects detected for the studied complex environmental samples.

This study is the first one focusing on the more specific distribution of the thyroid receptor-mediated activity, which is highly relevant for the health of the exposed population. Especially if we take into account that compounds causing TR-mediated activity were the most bioaccessible compared to the other effects detected in the simulated lung fluids. While for some effects specific toxicity carriers have been at least partly identified, effect-directed analysis should be conducted to identify further pollutants contributing to the effects, namely for the thyroid receptor-mediated activity in outdoor air, where the drivers are unknown.

This study confirms the suitability of specific *in vitro* bioassays for assessing the mixture toxicity. As for most effects the particulate phase sample extraction by simulated lung fluids mobilised only a portion of the biological effects attributable to the total extract, the results emphasise the significance of addressing the bioaccessible mass fraction for the assessment of PM toxicity. Obviously, there is an urgent need to close the data gaps with regard to REP values of aromatic air pollutants. The availability of further REPs would definitely help in assessing the effects of environmentally relevant air pollutant mixtures.

CRediT authorship contribution statement

Zuzana Nováková: Investigation, Writing - original draft, Formal analysis, Visualization. **Jiří Novák:** Investigation, Formal analysis, Writing - review & editing. **Zoran Kitanovski:** Methodology. **Petr Kukučka:** Investigation. **Marie Smutná:** Investigation. **Marco Wietzoreck:** Investigation. **Gerhard Lammel:** Conceptualization, Project administration, Writing - review & editing. **Klára Hilscherová:** Conceptualization, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Roman Prokeš, Ondřej Sáňka (MU), and Libor

Černikovský (Czech Hydrometeorological Institute) for sampling and on-site support and Anetta Martináková for performing the analysis of reconstructed mixtures. This research was supported by the Czech Science Foundation (P503 16-11537S), by CETOCOEN EXCELLENCE Teaming 2 project supported by Horizon2020 (857560), and by the Czech Ministry of Education, Youth and Sports (LM201812, 102.1.01/0.0/0.0/18_046/0015975) and by the Max Planck Society.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2020.105634.

References

Albinet, A., Leoz-Garziandia, E., Budzinski, H., Viilenave, E., 2007. Polycyclic aromatic hydrocarbons (PAHs), nitrated PAHs and oxygenated PAHs in ambient air of the Marseilles area (South of France): concentrations and sources. Sci. Total Environ. 384, 280–292. https://doi.org/10.1016/j.scitotenv.2007.04.028.

Annamalai, J., Namasivayam, V., 2015. Endocrine disrupting chemicals in the atmosphere: their effects on humans and wildlife. Environ. Int. 76, 78–97. https://doi.org/10.1016/J.ENVINT.2014.12.006.

Bandowe, B.A.M., Meusel, H., 2017. Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment – a review. Sci. Total Environ. 581–582, 237–257. https://doi.org/10.1016/J.SCITOTENV.2016.12.115.

Boisa, N., Elom, N., Dean, J.R., Deary, M.E., Bird, G., Entwistle, J.A., 2014. Development and application of an inhalation bioaccessibility method (IBM) for lead in the PM10size fraction of soil. Environ. Int. 70, 132–142. https://doi.org/10.1016/j.envint.2014.05.021.

Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., 2000. Role of quinones in toxicology. Chem. Res. Toxicol. 13, 135–160. https://doi.org/10.1021/TX9902082.

Burnett, R., Chen, H., Szyszkowicz, M., Fann, N., Hubbell, B., Pope, C.A., Apte, J.S., Brauer, M., Cohen, A., Weichenthal, S., Coggins, J., Di, Q., Brunekreef, B., Frostad, J., Lim, S.S., Kan, H., Walker, K.D., Thurston, G.D., Hayes, R.B., Lim, C.C., Turner, M.C., Jerrett, M., Krewski, D., Gapstur, S.M., Diver, W.R., Ostro, B., Goldberg, D., Crouse, D.L., Martin, R.V., Peters, P., Pinault, L., Tjepkema, M., van Donkelaar, A., Villeneuve, P.J., Miller, A.B., Yin, P., Zhou, M., Wang, L., Janssen, N.A.H., Marra, M., Atkinson, R.W., Tsang, H., Quoc Thach, T., Cannon, J.B., Allen, R.T., Hart, J.E., Laden, F., Cesaroni, G., Forastiere, F., Weinmayr, G., Jaensch, A., Nagel, G., Concin, H., Spadaro, J.V., 2018. Global estimates of mortality associated with long-term exposure to outdoor fine particulate matter. Proc. Natl. Acad. Sci. USA 115, 9592–9597. https://doi.org/10.1073/pnas.1803222115.

Calsolaro, V., Pasqualetti, G., Niccolai, F., Caraccio, N., Monzani, F., 2017. Thyroid disrupting chemicals. Int. J. Mol. Sci. 18. https://doi.org/10.3390/ijms18122583.

Cho, S.-H., Tong, H., McGee, J.K., Baldauf, R.W., Krantz, Q.T., Gilmour, M.I., 2009. Comparative toxicity of size-fractionated airborne particulate matter collected at different distances from an urban highway. Environ. Health Perspect. 117, 1682–1689. https://doi.org/10.1289/ehp.0900730.

Claeys, M., Vermeylen, R., Yasmeen, F., Gómez-González, Y., Chi, X., Maenhaut, W., Mészáros, T., Salma, I., 2012. Chemical characterisation of humic-like substances from urban, rural and tropical biomass burning environments using liquid chromatography with UV/vis photodiode array detection and electrospray ionisation mass spectrometry. Environ. Chem. 9, 273–284. https://doi.org/10.1071/EN11163.

Darbre, P., 2018. Overview of air pollution and endocrine disorders. Int. J. Gen. Med. 11, 191–207. https://doi.org/10.2147/IJGM.S102230.

De Coster, S., van Larebeke, N., 2012. Endocrine-disrupting chemicals: associated disorders and mechanisms of action. J. Environ. Public Health 2012, 1–52. https://doi.org/10.1155/2012/713696.

Gilmour, M.I., McGee, J., Duvall, R.M., Dailey, L., Daniels, M., Boykin, E., Cho, S.-H., Doerfler, D., Gordon, T., Devlin, R.B., 2007. Comparative toxicity of size-fractionated airborne particulate matter obtained from different cities in the United States. Inhal. Toxicol. 19, 7–16. https://doi.org/10.1080/08958370701490379.

Graber, E.R., Rudich, Y., 2006. Atmospheric HULIS: How humic-like are they? A comprehensive and critical review. Atmos. Chem. Phys. 6, 729–753. https://doi.org/10.5194/acp-6-729-2006.

Howard, P.C., Hecht, S.S., Beland, F.A. (Eds.), 1990. Nitroarenes: Their Detection, Mutagenicity and Occurrence in the Environment, first ed., Nitroarenes. Springer US, Boston, MA. https://doi.org/10.1007/978-1-4615-3800-4_5.

IARC, 2016. Outdoor Air Pollution. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.

IARC, 2014. Diesel and gasoline engine exhausts and some nitroarenes. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.

Idowu, O., Semple, K.T., Ramadass, K., O'Connor, W., Hansbro, P., Thavamani, P., 2019. Beyond the obvious: environmental health implications of polar polycyclic aromatic hydrocarbons. Environ. Int. 123, 543–557. https://doi.org/10.1016/j.envint.2018.12.051.

Janošek, J., Hilscherová, K., Bláha, L., Holoubek, I., 2006. Environmental xenobiotics and nuclear receptors—Interactions, effects and in vitro assessment. Toxicol. Vitr. 20, 18–37. https://doi.org/10.1016/J.TIV.2005.06.001.

Kappos, A.D., 2010. Health risks of urban airborne particles. In: Zereini, Fathi, Wiseman, C.L.S. (Eds.), Urban Airborne Particulate Matter Origin, Chemistry, Fate and Health Impacts, pp. 527–551. https://doi.org/10.1007/978-3-642-12278-1_27.

Kastury, F., Smith, E., Juhasz, A.L., 2017. A critical review of approaches and limitations of inhalation bioavailability and bioaccessibility of metal(loid)s from ambient particulate matter or dust. Sci. Total Environ. 574, 1054–1074. https://doi.org/10.1016/J.SCITOTENV.2016.09.056.

Kawanaka, Y., Tsuchiya, Y., Yun, S.-J., Sakamoto, K., 2009. Size distributions of polycyclic aromatic hydrocarbons in the atmosphere and estimation of the contribution of ultrafine particles to their lung deposition. Environ. Sci. Technol. 43, 6851–6856. https://doi.org/10.1021/es900033u.

Kim, K.-H., Kabir, E., Kabir, S., 2015. A review on the human health impact of airborne particulate matter. Environ. Int. 74, 136–143. https://doi.org/10.1016/J.ENVINT.2014.10.005.

Klein, G.P., Hodge, E.M., Diamond, M.L., Yip, A., Dann, T., Stern, G., Denison, M.S., Harper, P.A., 2006. Gas-phase ambient air contaminants exhibit significant dioxin-like and estrogen-like activity in vitro. Environ. Health Perspect. 114, 697–703. https://doi.org/10.1289/EHP.8496.

Koike, E., Yanagisawa, R., Takano, H., 2014. Toxicological effects of polycyclic aromatic hydrocarbons and their derivatives on respiratory cells. Atmos. Environ. 97, 529–536. https://doi.org/10.1016/j.atmosenv.2014.04.003.

Kortenkamp, A., Backhaus, T., Faust, M., 2009. State of the art report on mixture toxicity. Final Report to the European Commission under Contract Number 070307/2007/485103/ETU/D.1.

Lammel, G., Klánová, J., Ilić, P., Kohoutek, J., Gasić, B., Kovacić, I., Škrdlíková, L., 2010. Polycyclic aromatic hydrocarbons in air on small spatial and temporal scales – II. Mass size distributions and gas-particle partitioning. Atmos. Environ. 44, 5022–5027. https://doi.org/10.1016/J.ATMOSENV.2010.08.001.

Lammel, G., Mulder, M.D., Shahpoury, P., Kukuaka, P., Lišková, H., Přibylová, P., Prokeš, R., Wotawa, G., 2017. Nitro-polycyclic aromatic hydrocarbons - gas-particle partitioning, mass size distribution, and formation along transport in marine and continental background air. Atmos. Chem. Phys. 17, 6257–6270. https://doi.org/10.5194/acp-17-6257-2017.

Lammel, G., Kitanovski, Z., Kukučka, P., Novák, J., Arangio, A.M., Codling, G.P., Filippi, A., Hovorka, J., Kuta, J., Leoni, C., Příbylová, P., Prokeš, R., Sáňka, O., Shahpoury, P., Tong, H., Wietzoreck, M., 2020. Oxygenated and nitrated polycyclic aromatic hydrocarbons in ambient air - levels, phase partitioning, mass size distributions, and inhalation bioaccessibility. Environ. Sci. Technol., American Chemical Society 54 (5), 2615–2625. https://doi.org/10.1021/acs.est.9b06820.

Landlová, L., Čupr, P., Franců, J., Klánová, J., Lammel, G., 2014. Composition and effects of inhalable size fractions of atmospheric aerosols in the polluted atmosphere: Part I. PAHs, PCBs and OCPs and the matrix chemical composition. Environ. Sci. Pollut. Res. 21, 6188–6204. https://doi.org/10.1007/s11356-014-2571-y.

Liu, D., Lin, T., Syed, J.H., Cheng, Z., Xu, Y., Li, K., Zhang, G., Li, J., 2017. Concentration, source identification, and exposure risk assessment of PM2.5-bound parent PAHs and nitro-PAHs in atmosphere from typical Chinese cities. Sci. Rep. 7, 10398. https://doi.org/10.1038/s41598-017-10623-4.

Marques, M.R.C., Loebenberg, R., Almukainzi, M., 2011. Simulated biological fluids with possible application in dissolution testing. Dissolution Technol. https://doi.org/10.14227/DT180311P15.

Mirowsky, J., Hickey, C., Horton, L., Blaustein, M., Galdanes, K., Peltier, R.E., Chillrud, S., Chen, L.C., Ross, J., Nadas, A., Lippmann, M., Gordon, T., 2013. The effect of particle size, location and season on the toxicity of urban and rural particulate matter. Inhal. Toxicol. 25, 747–757. https://doi.org/10.3109/08958378.2013.846443.

Misaki, K., Suzuki, M., Nakamura, M., Handa, H., Iida, M., Kato, T., Matsui, S., Matsuda, T., 2008. Aryl hydrocarbon receptor and estrogen receptor ligand activity of organic extracts from road dust and diesel exhaust particulates. Arch. Environ. Contam. Toxicol. 55, 199–209. https://doi.org/10.1007/s00244-007-9110-5.

Misaki, K., Takamura-Enya, T., Ogawa, H., Takamori, K., Yanagida, M., 2016. Tumour-promoting activity of polycyclic aromatic hydrocarbons and their oxygenated or nitrated derivatives. Mutagenesis 31, 205–213. https://doi.org/10.1093/mutage/gev076.

Morville, S., Delhomme, O., Millet, M., 2011. Seasonal and diurnal variations of atmospheric PAH concentrations between rural, suburban and urban areas. Atmos. Pollut. Res. 2, 366–373. https://doi.org/10.5094/APR.2011.041.

Novák, J., Hilscherová, K., Landlová, L., Čupr, P., Kohút, L., Giesy, J.P., Klánová, J., 2014. Composition and effects of inhalable size fractions of atmospheric aerosols in the polluted atmosphere. Part II. In vitro biological potencies. Environ. Int. 63, 64–70. https://doi.org/10.1016/j.envint.2013.10.013.

Novák, J., Jálová, V., Giesy, J.P., Hilscherová, K., 2008. Pollutants in particulate and gaseous fractions of ambient air interfere with multiple signaling pathways in vitro. Environ. Int. 35, 43–49. https://doi.org/10.1016/j.envint.2008.06.006.

Oh, S., Ryu, B., Chung, K., 2008. Identification of estrogenic and antiestrogenic activities of respirable diesel exhaust particles by bioassay-directed fractionation. Arch. Pharm. Res. 31 (1), 75–82. https://doi.org/10.1007/s12272-008-1123-8.

Okamura, K., Kizu, R., Hayakawa, K., Toriba, A., Mizokami, A., Burnstein, K.L., Klinge, C.M., Kato, S., 2004. Variation in the antiandrogenic activity of diesel exhaust particulates emitted under different engine loads. Polycycl. Aromat. Compd. 24, 743–757. https://doi.org/10.1080/10406630490472518.

Oziol, L., Alliot, F., Botton, J., Bimbot, M., Huteau, V., Levi, Y., Chevreuil, M., 2017. First characterization of the endocrine-disrupting potential of indoor gaseous and particulate contamination: comparison with urban outdoor air (France). Environ. Sci. Pollut. Res. 24, 3142–3152. https://doi.org/10.1007/s11356-016-8045-7.

Pokorná, P., Hovorka, J., Klán, M., Hopke, P.K., 2015. Source apportionment of size resolved particulate matter at a European air pollution hot spot. Sci. Total Environ. 502, 172–183. https://doi.org/10.1016/j.scitotenv.2014.09.021.

Purohit, V., Basu, A.K., 2000. Mutagenicity of Nitroaromatic Compounds. Chem. Res.

Toxicol. 13, 673–692. https://doi.org/10.1021/TX000002X.
Rengarajan, T., Rajendran, Peramaiyan, Nandakumar, N., Lokeshkumar, B., Rajendran, Palaniswami, Nishigaki, I., 2015. Exposure to polycyclic aromatic hydrocarbons with special focus on cancer. Asian Pac. J. Trop. Biomed. 5, 182–189. https://doi.org/10.1016/S2221-1691(15)30003-4.
Ringuet, J., Albinet, A., Leoz-Garziandia, E., Budzinski, H., Villenave, E., 2012. Diurnal/nocturnal concentrations and sources of particulate-bound PAHs, OPAHs and NPAHs at traffic and suburban sites in the region of Paris (France). Sci. Total Environ. 437, 297–305. https://doi.org/10.1016/J.SCITOTENV.2012.07.072.
Safe, S., Wormke, M., 2003. Inhibitory aryl hydrocarbon receptor − estrogen receptor α cross-talk and mechanisms of action. Chem. Res. Toxicol. 16 (7), 807–816. https://doi.org/10.1021/tx034036r.
Saxena, P., Hildemann, L.M., 1996. Water-soluble organics in atmospheric particles: a critical review of the literature and application of thermodynamics to identify candidate compounds. J. Atmos. Chem. 24, 57–109. https://doi.org/10.1007/BF00053823.
Sen, S., Field, J.M., 2013. Genotoxicity of polycyclic aromatic hydrocarbon metabolites. In: Fishbein, James C., Heilman, J.M. (Eds.), Advances in Molecular Toxicology. Elsevier, pp. 83–127. https://doi.org/10.1016/B978-0-444-62645-5.00003-1.
Sippula, O., Stengel, B., Sklorz, M., Streibel, T., Rabe, R., Orasche, J., Lintelmann, J., Michalke, B., Abbaszade, G., Radischat, C., Gröger, T., Schnelle-Kreis, J., Harndorf, H., Zimmermann, R., 2014. Particle emissions from a marine engine: chemical composition and aromatic emission profiles under various operating conditions. Environ. Sci. Technol. 48, 11721–11729. https://doi.org/10.1021/es502484z.
Šrám, R.J., Dostál, M., Líbalová, H., Rössner, P., Rössnerová, A., Švecová, V., Topinka, J., Bartoňová, A., 2013. The European Hot Spot of B[a]P and PM 2.5 Exposure—The Ostrava Region, Czech Republic: Health Research Results. ISRN Public Health 2013, pp. 1–12. https://doi.org/10.1155/2013/416701.
Tomaz, S., Jaffrezo, J.-L., Favez, O., Perraudin, E., Villenave, E., Albinet, A., 2017. Sources and atmospheric chemistry of oxy- and nitro-PAHs in the ambient air of Grenoble (France). Atmos. Environ. 161, 144–154. https://doi.org/10.1016/J.ATMOSENV.2017.04.042.
US EPA, 2015. ToxCast; Tox21 Summary Files from invitrodb_v3. [WWW Document]. URL https://www.epa.gov/chemical-research/toxicity-forecaster-toxcasttm-data (accessed 4.17.18).
Vuong, N.Q., Breznan, D., Goegan, P., O'Brien, J.S., Williams, A., Karthikeyan, S., Kumarathasan, P., Vincent, R., 2017. In vitro toxicoproteomic analysis of A549 human lung epithelial cells exposed to urban air particulate matter and its water-soluble and insoluble fractions. Part. Fibre Toxicol. 14, 39. https://doi.org/10.1186/s12989-017-0220-6.
Wenger, D., Gerecke, A.C., Heeb, N.V., Schmid, P., Hueglin, C., Naegeli, H., Zenobi, R., 2009. *In vitro* estrogenicity of ambient particulate matter: contribution of hydroxylated polycyclic aromatic hydrocarbons. J. Appl. Toxicol. 29, 223–232. https://doi.org/10.1002/jat.1400.
WHO, 2018. Ambient (outdoor) air quality and health [WWW Document]. URL http://www.who.int/en/news-room/fact-sheets/detail/ambient-(outdoor)-air-quality-and-health (accessed 6.18.18).
Wnorowski, A., Charland, J.-P., 2017. Profiling quinones in ambient air samples collected from the Athabasca region (Canada). Chemosphere 189, 55–66. https://doi.org/10.1016/J.CHEMOSPHERE.2017.09.003.
Xiang, D., Han, J., Yao, T., Wang, Q., Zhou, B., Mohamed, A.D., Zhu, G., 2017. Structure-based investigation on the binding and activation of typical pesticides with thyroid receptor. Toxicol. Sci. 160, 205–216. https://doi.org/10.1093/toxsci/kfx177.