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RESEARCH ARTICLE

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Abstract Chemicals in air were characterized for potential interference with signaling of estrogen, androgen, and arylhydrocarbon (AhR) receptors, which are known to play an important role in endocrine-disruptive changes in vivo. Previously, effects of this type have been studied mainly in particulate matter in the ambient air from various localities. In this study, both volatile and particulate fractions of air from three sites in Banja Luka region (Bosnia and Herzegovina) were investigated to describe the distribution of endocrine-disrupting contaminants on a small spatial scale. Circadian variability of air pollution was investigated by collecting samples during both day and night. Air samples collected from urban localities at night were more potent in producing the AhR-mediated effects than those collected during daytime. This trend was not observed at the reference rural location. None of the samples showed significant estrogenic or androgenic activity. On the other hand, anti-androgenicity was detected in both particulate and vapor phases, while anti-estrogenicity was detected only in the particulate fraction of air from all localities. The AhR-mediated potencies of samples were associated primarily with non-persistent compounds. Based on the concentrations of 28 individual compounds, PAHs accounted for approximately 30 % of the AhR-mediated potency determined by the bioassay. The results show that there can be a significant difference between levels of bioactive compounds in air between daytime and nighttime.

Keywords Ambient air · Particulate matter · Gas phase · AhR-mediated toxicity · Estrogenicity · Androgenicity

Introduction

Pollution of ambient air has been shown to play a role in mediating a range of adverse effects on respiratory, cardiovascular, immune, hematological, nervous, and reproductive systems (Curtis et al. 2006). There is an effort to improve guidelines for ambient air quality to protect human health. However, these guidelines are available for relatively few pollutants. Many of them are inorganic constituents of ambient air such as sulfur oxides and nitrogen oxides. Evaluation of the potential risks due to organic contaminants is mostly limited to characterization of individual chemicals in air by use of instrumental analyses. Of the organic compounds of concern, several representatives of indicator classes such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), or organochlorine pesticides (OCPs) are monitored (Holoubek et al. 2007; Klanova et al. 2009). However, air can contain mixtures of numerous pollutants, some of which probably have not been identified yet. These mixtures could potentially produce adverse effects that cannot be predicted only from concentrations of the individual chemicals because the overall toxic outcome could be affected by interactions among the effects of the pollutants in the mixtures

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(Jager et al. 2010). Thus, it seems advisable to employ bioassays for the detection of toxicity because they, unlike chemical analyses, integrate the effects of all chemicals in the complex mixture and their potential interactions.

Besides systemic responses, air pollutants have been shown to elicit specific biological effects such as modulation of endocrine system. Several studies have described the presence of known endocrine disruptors in ambient and indoor air (Garcia-Jares et al. 2009; Hwang et al. 2008; Rudel et al. 2003; Rudel and Perovich 2009). Moreover, endocrine disruptive potential of air pollutants was shown in vivo (Jeng and Yu 2008; Mukerjee 1998; Ropstad et al. 2006) and in vitro (Clemons et al. 1998; Kennedy et al. 2009; Matsumoto et al. 2005; Novak et al. 2009; Wenger et al. 2009b). Another potential mode of action that is widely used in the toxicity evaluation of complex samples is associated with aryl hydrocarbon receptor (AhR) and is referred to as AhR-mediated or dioxin-like toxicity. It is produced mainly by planar organic compounds, and it has been associated with many adverse health consequences such as neurotoxicity, embryotoxicity, immunotoxicity, or carcinogenicity (Janosek et al. 2006). There are several reports that have described this type of effect in ambient air (Ciganek et al. 2004; Clemons et al. 1998; Mason 1994; Novak et al. 2009; Wenger et al. 2009a). Until relatively recently, the specific effects of complex mixtures of pollutants in air were studied mainly in particulate matter (PM), but it has been shown that considerable part of the active compounds is present also in gas phase fraction (GP; Klein et al. 2006; Novak et al. 2009).

The present study focuses on the potential interference of pollutants associated with PM as well as GP fractions of ambient air with the signaling of AhR, estrogen, and androgen receptor (ER and AR, respectively), which are traditionally linked with endocrine disruption. These effects were assessed using three mammalian cell line models that were transfected with luciferase reporter gene whose expression is triggered by activation of the respective receptor. To describe circadian differences in specific biological potencies of pollutants in the air, samples of air were collected at three relatively close localities separately during daytime and nighttime.

Material and methods

Air sampling

Samples were collected at three sites in Banja Luka region (Bosnia and Herzegovina). Banja Luka, a city of 225,000 inhabitants, is located in a valley surrounded by hills approximately 160 km from the Adriatic coast. The first sampling location, which is referred to as an urban site, was located on a roof of the Banja Luka institute of the Hydrometeorological Service of Bosnia and Herzegovina, which is located in a mixed residential, commercial, and agricultural area, 2.7 km

NNE of the city center. The main pollution source at this site was probably traffic. The second location, referred to as an industrial site was situated on the grounds of a former paper factory, which is 2.9 km SE from the city center. The site was affected by remaining on-going industrial activities and also by sporadic open waste burning in the vicinity as well as inside the factory grounds. The third sampling site, referred to as rural location was on the NW slope of a hill on a rural/forest site at Banj Brdo. This location, situated at a seismological station of Banja Luka, is 5.6 km SSW of the urban sites. The site is located in afforested area without any significant local sources of pollution. For more information on sampling sites and weather conditions, see previously published papers (Gasic et al. 2010; Lammel et al. 2010a, b, 2011).

Air pollutants were collected using a high-volume sampler ($F \approx 12$ m^3/h; GMW, Graseby, Village of Cleves, USA) which was equipped with PM$_{10}$ size-selective inlet with a quartz filter (Whatman, Germany) and a precleaned polyurethane foam filter (Gumotex, Czech Republic) in tandem. This allowed for separate collection of inhalable particulate matter-associated fraction PM$_{10}$ (referred to as PM) and pollutants in the gas phase fraction (GP), which were associated with quartz and polyurethane foam filters, respectively. The duration of sampling was 72 h, starting at 2100 hours of July 11, 2008. Six 4-h subsamples were collected during three nights and nine subsamples collected during three daytime periods, i.e., two (2050–0050 and 0120–0520 hours) and three subsamples (0650–1050, 1120–1520, and 1550–1950 hours), respectively, each 24 h. Chemical constituents were extracted from filters by use of dichloromethane in an automatic extractor (Büchi B-811). The volume of the extract was reduced under a gentle stream of nitrogen at ambient temperature. Both PM and GP samples were analyzed using a gas chromatograph (Agilent 6890) equipped with a J&W Scientific fused silica column DB-5MS coupled to a mass spectrometer (Agilent HP 5973) for 28 polycyclic aromatic hydrocarbons, 7 indicator PCBs, and OCPs (4 HCHs, 6 DDTs, PeCB, HCB) (Gasic et al. 2010; Lammel et al. 2010a, 2011). Terphenyl and PCB 121 were used as internal standards for PAH and PCB analyses, respectively. A portion of the extracts was used for the assessment of biological parameters with in vitro bioassays. To provide sufficient concentration for the assessment of biological potencies, extracts of subsamples taken during the daytime were combined together. The same thing has been done with nighttime samples. The combined samples were transferred to DMSO by evaporation under a gentle stream of nitrogen using DMSO as a keeper. The final volume of samples was adjusted by addition of DMSO to contain pollutants from the same volume of collected air. Prior to the transfer to DMSO, a subsample of each composite sample was treated with sulfuric acid to eliminate the non-persistent compounds.

Bioassay procedures

Three different bioassays were used to assess biological potencies of air samples. The assays were performed in microplate format in triplicates for each sample concentration, and each air sample was assessed in at least three independent experiments for each bioassay. The cells were exposed for 24 h to solvent control (0.5 % DMSO), dilution series of respective reference compound, and dilution series of the air extracts.

The H4IIE-*luc*, rat hepatocarcinoma cells stably transfected with luciferase gene under control of the AhR were used to quantify the dioxin-like activity of the samples (Sanderson et al. 1996). This bioassay is a well-established model for evaluation of AhR-mediated activities of pure substances as well as environmental samples (Janosek et al. 2006). The cells were grown and exposed in Dulbecco's modified Eagle medium (DMEM) medium (PAA, Austria) supplemented with a 10 % fetal bovine serum (PAA, Austria) without antibiotics at 37 °C, 5 % v/v CO_2, and high relative humidity. Tetrachlorodibenzo-*p*-dioxin (TCDD) was used as reference AhR-activating compound. A portion of the AhR-mediated activity elicited by persistent organic pollutants was determined by testing the extracts after treatment with sulfuric acid. Estrogenic and anti-estrogenic effects were examined by use of the MVLN cell line, human breast carcinoma cells transfected with a luciferase gene under control of estrogen receptor (Demirpence et al. 1993; Freyberger and Schmuck 2005; Villeneuve et al. 2002). Cells were grown in DMEM without phenol red (PAA, Austria) supplemented with 10 % fetal bovine serum (PAA, Austria). Cells were exposed in 96-well plate in DMEM without phenol red (PAA, Austria) supplemented with 5 % dialyzed fetal bovine serum (PAA, Austria) that was additionally treated with dextran-coated charcoal suspension (Sigma-Aldrich, Czech Republic) to further decrease the level of estrogens in the medium. 17β-Estradiol (E2) served as a reference estrogenic compound. Effects of air sample extracts on MVLN cells were assessed in the presence or absence of competing endogenous ligand. Anti-estrogenicity was assessed by simultaneous exposure of the extract and E2 (11pM). Androgenicity and anti-androgenicity were assessed using MDA-kb2 cell line stably transfected with luciferase reporter gene triggered by AR activation (Wilson et al. 2002). The cells were grown in Leibovitz's L-15 medium (PAA, Austria) supplemented with 10 % fetal bovine serum (PAA, Austria) at 37 °C and normal air with high relative humidity. Experiment was performed in microplates in L-15 medium supplemented with 10 % dialyzed fetal bovine serum (PAA, Austria) that was additionally treated with dextran-coated charcoal suspension (Sigma-Aldrich, Czech Republic). Androgen dihydrotestosterone (DHT) was used as a reference compound. To assess the effect in interaction with physiological ligand of the AR, cells were exposed to extracts alone or in combination with DHT (0.5 nM).

Luminescence elicited by luciferase, which was produced by the reporter gene in H4IIE-*luc* and MVLN cells, was detected after 24-h exposure using Steady-Glo® luciferase kit (Promega, Madison, WI, USA) following manufacturer's instructions. For MDA-kb2 cells, lysis buffer (Promega, Germany) was added to the cells. Plates were shaken for 20 min. After shaking, 100 μL of substrate for luciferase described previously (Wilson et al. 2002) was added and the luminescence was measured with luminometer Synergy 4 (BioTek, Winooski, VT, USA).

Data analysis

AhR-mediated potencies determined in the H4IIE-*luc* assay were reported as toxic equivalents (bioTEQ) expressed in femtograms of TCDD per cubic meter of air. The calculation was based on EC_{50} values as described previously (Villeneuve et al. 2000). The calculated TEQ (chemTEQ) were derived from analytical data on 28 PAHs with relative potency values and toxic equivalency factor approach that were described previously (Machala et al. 2001; Safe 1998).

IC_{25} and IC_{50} values (cubic meters of air per milliliter of exposure medium) for anti-estrogenicity and anti-androgenicity were calculated from dose–response curves compared to signal of competitive concentration of added natural ligand 11pM E2 and 0.5nM DHT, respectively, which were considered to be the maximal response (100 %). Thus, anti-estrogenicity and anti-androgenicity were determined from the decrease of signal given by specified amount of competing E2 or DHT, in the medium. The values in graphs and statistical analyses are expressed as an index of anti-estrogenicity (iAE) or anti-androgenicity (iAA), which correspond to reciprocal value of IC_{50} and IC_{25}, respectively.

Because there were insufficient data to confirm normal distribution, correlations were calculated by use of nonparametric Kendall tau procedure. The Kendall tau procedure was chosen because it is more robust than Spearman correlation based on ranks. The significance of differences between observed responses was evaluated using nonparametric Wald–Wolfowitz Run test and Kruskal–Wallis ANOVA. Statistical significance of the tests was defined with type I (p) errors of less than 0.05. Principal component analysis (PCA) was performed with standardized data using STATISTICA for Windows 10.0 (StatSoft, Inc., USA).

Results and discussion

Most previously published studies describe biological activities of air samples collected over relatively long-term sampling periods. The variation of biological potencies of ambient air pollutants during the circadian cycle has not been studied so far. To our knowledge, this is the first study to describe differences in toxic properties of air during the day and night. The

region of Banja Luka was chosen because of weather stability during the summer season to eliminate confounding factor of meteorological variation. However, at the end of the sampling campaign, there were significant precipitations and temperature decrease because of an approaching cold front. The meteorological conditions during the sampling has been described in detail previously together with the levels and relative concentration profile of PAHs (Lammel et al. 2010a, b) as well as data on PCBs (Gasic et al. 2010) and chlorinated pesticides (Lammel et al. 2011). The chemical analysis of these samples revealed highest levels of PAHs at night (Table 1) with the concentrations being inversely proportional to oxidants such as O_3 in the atmosphere (Lammel et al. 2010a). There was a significant difference in PAH profiles among the three localities and a gradient of PAHs levels between both sites in the city and the rural site namely due to volatile PAHs. Authors have shown that 24-h sampling could significantly mask differences in concentration of pollutants among sites that would be obvious with higher temporal resolution. Gasic et al. (2010) have described that PCB emissions per capita in Banja Luka are an order of magnitude lower than in Zurich or Chicago. That indicates lower usage of PCBs at Banja Luka region in the past than in Western countries.

AhR-mediated activity

Aryl hydrocarbon receptor (AhR) activation is a well-described mode of toxic action of a wide range of environmental pollutants. These compounds have been shown to be involved in adverse health effects such as impairment of reproduction and immune and nervous systems (White and Birnbaum 2009). This mode of action can lead to indirect activation of ER that could play a role in endocrine disruptive potential of dioxin-like compounds (Safe and Wormke 2003). Several studies have shown that AhR-mediated toxicity could be one of potential modes of action of ambient air pollutants (Brown et al. 2005; Ciganek et al. 2004; Clemons et al. 1998; Novak et al. 2009; Wenger et al. 2009a). In our study, this type of effect was produced by both air fractions from all localities (Fig. 1) and it was elicited almost exclusively by non-persistent compounds. Persistent fractions of samples did not contain concentrations of AhR agonists such as polychlorinated dibenzo-*p*-dioxins and furans sufficient enough to produce quantifiable AhR-dependent response (data not shown). This is consistent with data from chemical analyses where concentrations of PCBs, the only analyzed persistent compounds with significant dioxin-like toxic potential, were relatively low compared to situation in other cities (Gasic et al. 2010). Levels of AhR-mediated activity expressed as concentrations of bioTEQ were comparable to those measured in similar studies for samples collected in summer (Kennedy et al. 2010; Novak et al. 2009) and about one order of magnitude lower compared to those from winter (Brown et al. 2005; Ciganek et al. 2004; Wenger et

al. 2009a). This is likely due to the fact that the activity of combustion sources is more pronounced and degradation of pollutants is slower during winter. The most significant part of the effect was produced by non-volatile compounds because PM fraction extracts were more potent than the ones from the GP. The greatest AhR-mediated activities were elicited by PM fractions collected at night. There was significantly greater AhR-mediated potency in the PM fraction collected at night than in the one collected during the day at urban and industrial locations. However, this trend was not observed for the GP. At the rural location, there was a significantly more bioTEQ in the GP during the day than at night. A similar trend was observed for the concentrations of PAHs (Table 1). The trend was shown also with PAHs data from higher time-resolution sampling (Lammel et al. 2010a). This, together with lesser concentrations of persistent AhR-active compounds, could indicate that AhR-mediated effects were elicited by chemicals with physical–chemical properties similar to PAHs.

However, concentrations of TEQs calculated from concentrations of 28 priority PAHs (chemTEQ), which significantly correlated with bioTEQ levels ($\tau=0.81$) in PM fraction but not in GP-one, indicate that these compounds were responsible for 8–27 % of bioTEQ in PM fraction (Table 1). In the GP fraction, these compounds represented less than 4 % of the concentration of bioTEQ. The major part of the effect was therefore elicited by non-persistent compounds that are not routinely assessed. These could be other PAHs or their substituted (e.g., alkylated) derivatives that have been detected in the atmosphere in substantial levels previously (Lehndorff and Schwark 2009) or compounds such as polycyclic aromatic ketones and quinones (Misaki et al. 2007) because these compounds have been described to activate AhR in vitro (Vondracek et al. 2007; Misaki et al. 2007). To minimize the differences in assessed bioTEQ and chemTEQ, the latter have been calculated using relative potencies of PAHs that have been acquired with the same bioassay as in our study (Machala et al. 2001). However, TEQ concept (Safe 1998) might not predict bioTEQ precisely because there could be other interactions in the mixtures than additivity. Anyway, the difference between chemTEQ contribution in PM and GP fractions indicates that there are probably other volatile AhR-active compounds than PAHs.

The greater AhR-mediated activity in PM observed at night was probably caused by municipal pollution sources such as hot water generation by burning of fossil fuels at the urban and industrial locality because this trend was not observed at the rural location. Because some AhR-active PAHs are known to be susceptible to photodegradation and oxidation by ozone (Liu et al. 2006), this phenomenon was probably a function of lesser photolysis and lesser concentrations of reactive molecules during nighttime as it has been shown with levels of ozone although statistically significant negative correlation was not observed (Lammel et al. 2010a). Greater PAHs levels during the

Table 1 Concentrations of contaminants and calculated percentage of contribution of PAHs (chemTEQ) to assessed TCDD equivalent (bioTEQ) in extracts of daytime and nighttime air samples (nanograms per cubic meter)
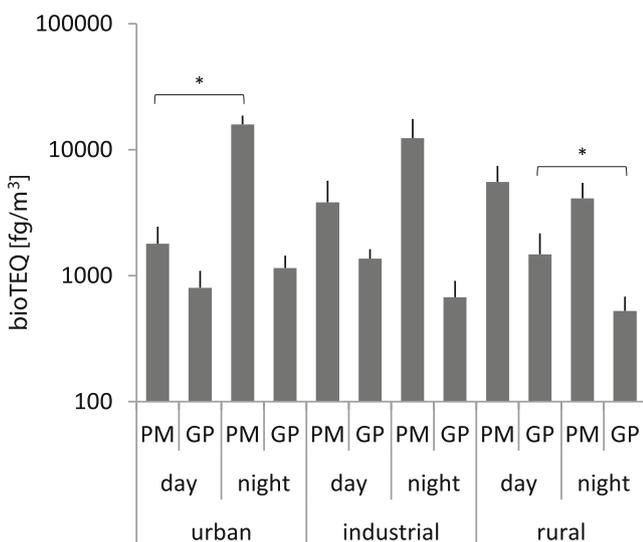
Site	Time	Phase	∑PAHs	∑PCBs	∑OCPs	chemTEQ [%]
Urban	Day	PM	5.46	0.0018	0.0202	25.2
		GP	39.71	0.1544	0.3495	4.0
	Night	PM	32.13	0.0052	0.0299	23.4
		GP	94.26	0.3224	0.2834	5.0
Industrial	Day	PM	4.48	0.0065	0.0091	8.8
		GP	39.87	0.1717	0.3199	2.0
	Night	PM	15.06	0.0152	0.0167	11.8
		GP	98.52	0.1179	0.4967	3.9
Rural	Day	PM	8.15	0.0030	0.0108	13.3
		GP	47.67	0.1433	0.4499	2.2
	Night	PM	11.13	0.0045	0.0118	26.2
		GP	27.38	0.0653	0.2537	4.2

∑PAHs sum of 28 polycyclic aromatic hydrocarbons, *∑PCBs* sum of seven polychlorinated biphenyls, *∑OCPs* sum of organochlorinated pesticides, *PM* particulate phase, *GP* gas phase
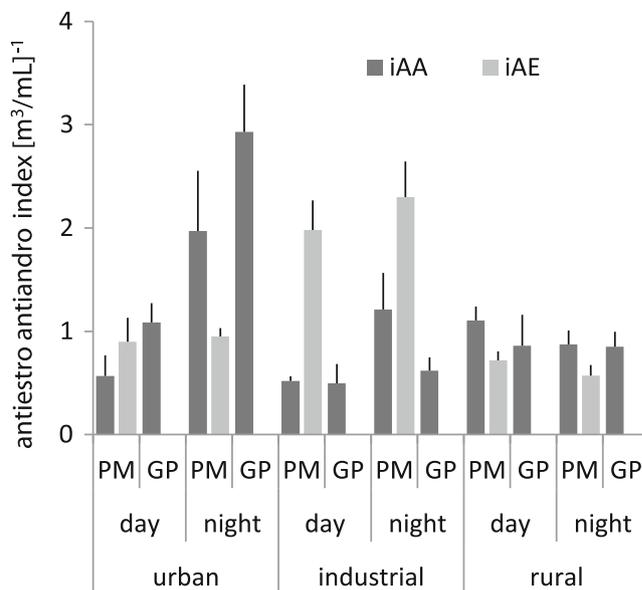
night were observed also in region of Paris (Ringuet et al. 2012) and during the cold season also in Germany (Sklorz et al. 2007).

Interactions with estrogen receptor

Contaminants in air have been previously reported to interact with ER. Most of the studies describe estrogenic effects of chemicals associated with PM (Clemons et al. 1998; Kennedy et al. 2009; Wang et al. 2004; Wenger et al. 2009b), but estrogenic activity has also been detected in the GP of air (Klein et al. 2006). In the present study, no significant estrogenic response was observed in either fraction, but anti-estrogenic effects were observed in both of them (Fig. 2).

Anti-estrogenic properties have been ascribed to some PAHs (Arcaro et al. 1999; Santodonato 1997), but other authors describe rather their estrogenic potential (Santodonato 1997; Villeneuve et al. 2002). Anti-estrogenicity was reported to be produced by compounds from combustion engine exhausts, which should at least partly represent traffic-derived air particle contamination. This has been shown for diesel (Okamura et al. 2002) and motorcycle engine emissions both in vitro and in vivo (Ueng et al. 2004). It has been proposed that the mechanism of action could be connected with AhR activation because known inhibitor of AhR and cytochrome P450 α-

Fig. 1 TCDD equivalents (femtograms bioTEQ per cubic meter; mean + SEM) of the particulate (*PM*) and gas (*GP*) phases of daytime and nighttime air samples

Fig. 2 Index of anti-estrogenicity (*iAE*) and anti-androgenicity (*iAA*; reciprocal value of IC_{50} and IC_{25}, respectively [per cubic meter per milliliter] mean + SEM) of the particulate (*PM*) and gas (*GP*) phases of daytime and nighttime air samples

naphthoflavone suppressed the effect (Ueng et al. 2004). The mechanism of cross-talk between AhR and ER signaling pathway has been thoroughly reviewed previously (Safe and Wormke 2003). This mechanism, however, is not unequivocal because it has been shown that activated AhR activates signaling of unliganded ER while liganded ER activity is inhibited (Ohtake et al. 2003). The most probable explanation of the discrepancy between our results and some previous studies are the differences in the composition of the complex pollutant mixtures in different regions. The sensitivity of our detection system was comparable or greater than that of the bioassays employed in the previous studies (Clemons et al. 1998; Matsumoto et al. 2005; Novak et al. 2009). Some differences in sensitivity can occur namely between the mammalian cells and yeast-based models. Cross-talk of AhR and ER signaling does not occur in the yeast models. Moreover, mammalian cell lines cannot be cultivated without serum that, despite dialysis or dextran-charcoal stripping, always contains at least a trace amount of estrogens. Thus, samples that would be estrogenic in media without steroids might be anti-estrogenic in media with certain estrogen levels. It is possible that the exposure medium, although it contained reduced concentration of dialyzed/charcoal-dextran-treated serum, contained greater concentrations of estrogens than in the abovementioned studies and it might lead to the differences in the observed effect. However, conditions within organisms exposed to air pollutants are hardly ever completely estrogen free, so it is not clear what kind of in vitro exposure conditions are more relevant to the in vivo situation.

The results of the study reported here are consistent with previous results from a sampling campaign in the Czech Republic where no estrogenic potency was detected, while significant anti-estrogenic effects in both PM and GP fractions were observed using the same detection system as in this study (Novak et al. 2009). However, data from Banja Luka show that anti-estrogenic activity was produced by all samples of PM from all localities, but samples of GP were devoid of quantifiable effect (data not shown). There was significant difference among the studied sites and the greatest effects were elicited by extracts of samples from industrial sites where night samples were significantly more anti-estrogenic than samples from the other localities. Anti-estrogenic potential did not differed between day and night samples, and it was significantly correlated only with concentrations of bioTEQ. This result is consistent with the theory of AhR and ER signaling cross-talk. Nevertheless, the correlation was not too high ($\tau = 0.38$) and it was statistically significant only for bioTEQ and iAE$_{25}$, and thus, the evidence is not very strong.

Interactions with androgen receptor

Unlike anti-estrogenicity, anti-androgenic effects were produced by both PM and GP from all three localities without

statistically significant differences among samples from different locations, fractions or daytimes (Fig. 2). The only significant difference was observed between day and night PM samples from industrial locality. This is generally in agreement with the results of a previous study of ambient air in the Czech Republic where PM and GP from one of the studied region displayed similar effects, but the magnitude of response was greater. This was probably caused by different pollutants in Central Europe and/or, more likely, by the fact that the studies differ in the bioassay employed for the detection of anti/androgenicity (Novak et al. 2009). In the current study, the anti-androgenic index iAA$_{25}$ in PM was correlated with bioTEQ ($\tau = 0.35$). In GP, iAA$_{25}$ was correlated with concentrations of PCBs ($\tau = 0.33$). It has been described that some PCB congeners have anti-androgenic potency (Hamers et al. 2011). iAA$_{25}$ was also associated with the concentrations of OCPs ($\tau = 0.35$); anti-androgenic properties of some OCPs have been described previously (Kojima et al. 2004). Samples from urban and industrial site seem to show opposite trends. While anti-androgenicity dominates over anti-estrogenicity at urban site during the night, this trend is the opposite for industrial site at both sampling periods (Fig. 2). This could indicate differences in composition of the pollutant mixtures at the sites and document that specific toxic potencies of air could be quite different even at relatively close sites that vary in main pollutant sources. The relative concentration profile of PAHs also differed significantly between these two sites (Lammel et al. 2010a).
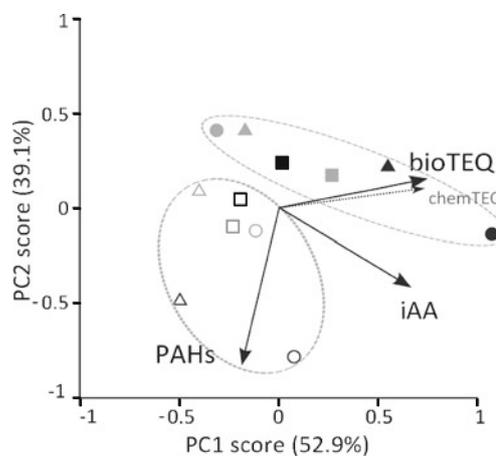
Fig. 3 Presentation of air phase-specific pattern of pollution based on principal component analysis of data on TCDD equivalent (*bioTEQ*), index of anti-androgenicity (*iAA*), and PAHs levels, with TCDD equivalent calculated from PAH levels (*chemTEQ*) as a supplementary variable. The samples from different sites and fractions are distinguished by shape and color of the points: *circle* (urban), *triangle* (industrial), *square* (rural). Daytime samples are *gray*, nighttime are *black*. The air phases are distinguished by the point pattern: *empty* (gas phase), *filled* (particulate phase)

Principal component analysis

To show the combination of several assessed parameters together, PCA has been performed. Because this study was based on a limited number of cases, it was necessary to reduce the number of parameters used in the analysis. Anti-estrogenicity was excluded because it was not detected in GP fraction. PCA of data on bioTEQ, iAA$_{25}$, and the sum of 28 PAHs clearly separated the cases according to PM and GP fractions (Fig. 3). Unlike the rural location, samples collected during the day and night from urban and industrial sites were relatively well separated on the plot. This might be due to local pollution sources at night at these two localities because the night samples were shifted along vectors describing the air pollution parameters in the opposite direction than the rural location. This might indicate a decreased rate of pollutant transport from the city during nighttime. While the level of PAHs was an important factor in separating GP samples from PM-ones, PM fraction separation was driven mainly by bioTEQ levels. This is caused by the fact that AhR-active PAHs are less volatile, and thus, they are associated with PM fraction. On the other hand, great portion of the assessed PAHs in this study was volatile and without AhR-mediated toxic properties, and so they were associated with the gas phase of the air samples. This supports the fact, which has been already mentioned, that bioTEQ of gas phase fraction is mostly elicited by other compounds than the assessed PAHs. The fact that iAA vector is associated neither with the vector for bioTEQ nor for PAHs indicates that anti-androgenic effects are probably elicited by other compounds which are not playing also a crucial role in the AhR-mediated potency.

Conclusions

The results of this study confirmed that pollutants in particulate as well as gas phase of ambient air can elicit dioxin-like, anti-estrogenic, and anti-androgenic effects in vitro. Concentrations of active compounds were greater during the night at sites with local sources. The results show that routinely assessed 28 priority PAHs can explain only relatively small portion of detected AhR-mediated activity. This was especially significant in gas phase fraction of the air samples.

References

Arcaro KF, O'Keefe PW, Yang Y, Clayton W, Gierthy JF (1999) Antiestrogenicity of environmental polycyclic aromatic hydrocarbons in human breast cancer cells. Toxicology 133:115–127

Brown LE, Trought KR, Bailey CI, Clemons JH (2005) 2,3,7,8-TCDD equivalence and mutagenic activity associated with PM10 from three urban locations in New Zealand. Sci Total Environ 349:161–174

Ciganek M, Neca J, Adamec V, Janosek J, Machala M (2004) A combined chemical and bioassay analysis of traffic-emitted polycyclic aromatic hydrocarbons. Sci Total Environ 334–35:141–148

Clemons JH, Allan LM, Marvin CH, Wu Z, McCarry BE, Bryant DW, Zacharewski TR (1998) Evidence of estrogen- and TCDD-like activities in crude and fractionated extracts of PM10 air particulate material using in vitro gene expression assays. Environ Sci Technol 32:1853–1860

Curtis L, Rea W, Smith-Willis P, Fenyves E, Pan Y (2006) Adverse health effects of outdoor air pollutants. Environ Int 32:815–830

Demirpence E, Duchesne MJ, Badia E, Gagne D, Pons M (1993) MVLN cells—a bioluminescent Mcf-7-derived cell-line to study the modulation of estrogenic activity. J Steroid Biochem Mol Biol 46:355–364

Freyberger A, Schmuck G (2005) Screening for estrogenicity and anti-estrogenicity: a critical evaluation of an MVLN cell-based transactivation assay. Toxicol Lett 155:1–13

Garcia-Jares C, Regueiro J, Barro R, Dagnac T, Llompart M (2009) Analysis of industrial contaminants in indoor air. Part 2. Emergent contaminants and pesticides. J Chromatogr A 1216:567–597

Gasic B, MacLeod M, Klanova J, Scheringer M, Ilic P, Lammel G, Pajovic A, Breivik K, Holoubek I, Hungerbuhler K (2010) Quantification of sources of PCBs to the atmosphere in urban areas: a comparison of cities in North America, Western Europe and former Yugoslavia. Environ Pollut 158:3230–3235

Hamers T, Kamstra JH, Cenijn PH, Pencikova K, Palkova L, Simeckova P, Vondracek J, Andersson PL, Stenberg M, Machala M (2011) In vitro toxicity profiling of ultrapure non-dioxin-like polychlorinated biphenyl congeners and their relative toxic contribution to PCB mixtures in humans. Toxicol Sci 121:88–100

Holoubek I, Klanova J, Jarkovsky J, Kohoutek J (2007) Trends in background levels of persistent organic pollutants at Kosetice observatory, Czech Republic. Part I. Ambient air and wet deposition 1996–2005. J Environ Monit 9:557–563

Hwang HM, Park EK, Young TM, Hammock BD (2008) Occurrence of endocrine-disrupting chemicals in indoor dust. Sci Total Environ 404:26–35

Jager T, Vandenbrouck T, Baas J, De Coen WM, Kooijman S (2010) A biology-based approach for mixture toxicity of multiple endpoints over the life cycle. Ecotoxicology 19:351–361

Janosek J, Hilscherova K, Blaha L, Holoubek I (2006) Environmental xenobiotics and nuclear receptors–Interactions, effects and in vitro assessment. Toxicol in Vitro 20:18–37

Jeng HA, Yu L (2008) Alteration of sperm quality and hormone levels by polycyclic aromatic hydrocarbons on airborne particulate particles. J Environ Sci Health Part A Toxic/Hazard Subst Environ Eng 43:675–681

Kennedy K, Macova M, Leusch F, Bartkow ME, Hawker DW, Zhao B, Denison MS, Mueller JF (2009) Assessing indoor air exposures using passive sampling with bioanalytical methods for estrogenicity and aryl hydrocarbon receptor activity. Anal Bioanal Chem 394:1413–1421

Kennedy K, Macova M, Bartkow ME, Hawker DW, Zhao B, Denison MS, Mueller JF (2010) Effect based monitoring of seasonal ambient air exposures in Australia sampled by PUF passive air samplers. Atmos Pollut Res 1:50–58

Klanova J, Cupr P, Holoubek I, Boruvkova J, Pribylova P, Kares R, Tomsej T, Ocelka T (2009) Monitoring of persistent organic pollutants in Africa. Part 1: Passive air sampling across the continent in 2008. J Environ Monit 11:1952–1963

Klein GP, Hodge EM, Diamond ML, Yip A, Dann T, Stem G, Denison MS, Harper PA (2006) Gas-phase ambient air contaminants exhibit significant dioxin-like and estrogen-like activity in vitro. Environ Health Perspect 114:697–703

Kojima H, Katsura E, Takeuchi S, Niiyama K, Kobayashi K (2004) Screening for estrogen and androgen receptor activities in 200 pesticides by in vitro reporter gene assays using Chinese hamster ovary cells. Environ Health Perspect 112:524–531

Lammel G, Klanova J, Ilic P, Kohoutek J, Gasic B, Kovacic I, Lakic N, Radic R (2010a) Polycyclic aromatic hydrocarbons in air on small spatial and temporal scales—I. Levels and variabilities. Atmos Environ 44:5015–5021

Lammel G, Klanova J, Ilic P, Kohoutek J, Gasic B, Kovacic I, Skrdlikova L (2010b) Polycyclic aromatic hydrocarbons in air on small spatial and temporal scales—II. Mass size distributions and gas-particle partitioning. Atmos Environ 44:5022–5027

Lammel G, Klanova J, Eric L, Ilic P, Kohoutek J, Kovacic I (2011) Sources of organochlorine pesticides in air in an urban Mediterranean environment: volatilisation from soil. J Environ Monit 13:3358–3364

Lehndorff E, Schwark L (2009) Biomonitoring airborne parent and alkylated three-ring PAHs in the Greater Cologne Conurbation I: temporal accumulation patterns. Environ Pollut 157:1323–1331

Liu Y, Sklorz M, Schnelle-Kreis J, Orasche J, Ferge T, Kettrup A, Zimmermann R (2006) Oxidant denuder sampling for analysis of polycyclic aromatic hydrocarbons and their oxygenated derivates in ambient aerosol: evaluation of sampling artefact. Chemosphere 62:1889–1898

Machala M, Vondracek J, Blaha L, Ciganek M, Neca J (2001) Aryl hydrocarbon receptor-mediated activity of mutagenic polycyclic aromatic hydrocarbons determined using in vitro reporter gene assay. Mutat Res Genet Toxicol Environ Mutagen 497:49–62

Mason GGF (1994) Dioxin-receptor ligands in urban air and vehicle exhaust. Environ Health Perspect 102:111–116

Matsumoto H, Adachi S, Suzuki Y (2005) Bisphenol A in ambient air particulates responsible for the proliferation of MCF-7 human breast cancer cells and its concentration changes over 6 months. Arch Environ Contam Toxicol 48:459–466

Misaki K, Kawami H, Tanaka T, Handa Y, Nakamura M, Matsui S, Matsuda T (2007) Aryl hydrocarbon receptor ligand activity of polycyclic aromatic ketones and polycyclic aromatic quinones. Environ Toxicol Chem 26:1370–1379

Mukerjee D (1998) Health impact of polychlorinated dibenzo-p-dioxins: a critical review. J Air Waste Manage 48:157–165

Novak J, Jalova V, Giesy JP, Hilscherova K (2009) Pollutants in particulate and gaseous fractions of ambient air interfere with multiple signaling pathways in vitro. Environ Int 35:43–49

Ohtake F, Takeyama K, Matsumoto T, Kitagawa H, Yamamoto Y, Nohara K, Tohyama C, Krust A, Mimura J, Chambon P, Yanagisawa J, Fujii-Kuriyama Y, Kato S (2003) Modulation of oestrogen receptor signalling by association with the activated dioxin receptor. Nature 423:545–550

Okamura K, Kizu R, Toriba A, Klinge CM, Hayakawa K (2002) Antiestrogenic activity of extracts of diesel exhaust particulate matter in MCF-7 human breast carcinoma cells. Polycyclic Aromat Compd 22:747–759

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 Tot Environ 437:297–305

Ropstad E, Oskam IC, Lyche JL, Larsen HJ, Lie E, Haave M, Dahl E, Wiger R, Skaare JU (2006) Endocrine disruption induced by organochlorines (OCs): field studies and experimental models. J Toxicol Environ Health A 69:53–76

Rudel RA, Perovich LJ (2009) Endocrine disrupting chemicals in indoor and outdoor air. Atmos Environ 43:170–181

Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG (2003) Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environ Sci Technol 37:4543–4553

Safe SH (1998) Hazard and risk assessment of chemical mixtures using the toxic equivalency factor approach. Environ Health Perspect 106:1051–1058

Safe S, Wormke M (2003) Inhibitory aryl hydrocarbon receptor-estrogen receptor a cross-talk and mechanisms of action. Chem Res Toxicol 16:807–816

Sanderson JT, Aarts J, Brouwer A, Froese KL, Denison MS, Giesy JP (1996) Comparison of Ah receptor-mediated luciferase and ethoxyresorufin-O-deethylase induction in H4IIE cells: implications for their use as bioanalytical tools for the detection of polyhalogenated aromatic hydrocarbons. Toxicol Appl Pharmacol 137:316–325

Santodonato J (1997) Review of the estrogenic and antiestrogenic activity of polycyclic aromatic hydrocarbons: Relationship to carcinogenicity. Chemosphere 34:835–848

Sklorz M, Schnelle-Kreis J, Liu YB, Orasche J, Zimmermann R (2007) Daytime resolved analysis of polycyclic aromatic hydrocarbons in urban aerosol samples—impact of sources and meteorological conditions. Chemosphere 67:934–943

Ueng TH, Wang HW, Huang YP, Hung CC (2004) Antiestrogenic effects of motorcycle exhaust particulate in MCF-7 human breast cancer cells and immature female rats. Arch EnvironContam and Toxicol 46:454–462

Villeneuve DL, Blankenship AL, Giesy JP (2000) Derivation and application of relative potency estimates based on in vitro bioassay results. Environ Toxicol Chem 19:2835–2843

Villeneuve DL, Khim JS, Kannan K, Giesy JP (2002) Relative potencies of individual polycyclic aromatic hydrocarbons to induce dioxinlike and estrogenic responses in three cell lines. EnvironToxicol 17:128–137

Vondracek J, Svihalkova-Sindlerova L, Pencikova K, Marvanova S, Krcmar P, Ciganek M, Neca J, Trosko JE, Upham B, Kozubik A, Machala M (2007) Concentrations of methylated naphthalenes, anthracenes, and phenanthrenes occurring in Czech river sediments and their effects on toxic events associated with carcinogenesis in rat liver cell lines. Environ Toxicol Chem 26:2308–2316

Wang JX, Xie P, Xu Y, Kettrup A, Schramm KW (2004) Differing estrogen activities in the organic phase of air particulate matter collected during sunny and foggy weather in a Chinese city detected by a recombinant yeast bioassay. Atmos Environ 38:6157–6166

Wenger D, Gerecke AC, Heeb NV, Hueglin C, Seiler C, Haag R, Naegeli H, Zenobi R (2009a) Aryl hydrocarbon receptor-mediated activity of atmospheric particulate matter from an urban and a rural site in Switzerland. Atmos Environ 43:3556–3562

Wenger D, Gerecke AC, Heeb NV, Schmid P, Hueglin C, Naegeli H, Zenobi R (2009b) In vitro estrogenicity of ambient particulate matter: contribution of hydroxylated polycyclic aromatic hydrocarbons. J Appl Toxicol 29:223–232

White SS, Birnbaum LS (2009) An overview of the effects of dioxins and dioxin-like compounds on vertebrates, as documented in human and ecological epidemiology. J Environ Sci Heal C 27:197–211

Wilson VS, Bobseine K, Lambright CR, Gray LE (2002) A novel cell line, MDA-kb2, that stably expresses an androgen- and glucocorticoid-responsive reporter for the detection of hormone receptor agonists and antagonists. Toxicol Sci 66:69–81