Effect-based assessment of passive air samples from four countries in Eastern Europe

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Abstract Although passive sampling has been previously used for the monitoring of volatile and semi-volatile contaminants in air, there are limited data on the use of this technique coupled with bioassays based on specific biological responses. Biological responses including those mediated by the aryl hydrocarbon (AhR) receptor as well as (anti-)estrogenicity and (anti-)androgenicity of samples from four Eastern European countries (Lithuania, Slovakia, Romania, and Serbia) were determined. To address the potential differences of specific toxic potencies of pollutant mixtures in ambient air in Eastern Europe, each country was characterized by a single more remote location that served to determine regional background conditions and one location in more urbanized and industrialized locations, which were defined as "impacted" areas. Besides samples from Lithuania, a significant gradient in concentrations of AhR-mediated potency from background and impacted localities was observed. Greatest potencies were measured in samples from impacted locations in Romania and Slovakia. Concentrations of polycyclic aromatic hydrocarbons (PAHs) that were quantified accounted for 3–33 % of the 2,3,7,8-tetrachlorodibenzo-p-dioxin equivalents determined by use of the bioassay. No significant estrogenic potency was detected but anti-estrogenic effects were produced by air from two background locations (Lithuania, Slovakia) and three impacted locations (Lithuania, Romania, and Serbia). Anti-androgenic potency was observed in all samples. The greatest anti-estrogenic potency was observed at the background location in Slovakia. Anti-estrogenic and anti-androgenic potencies of studied air samples were probably associated with compounds that are not routinely monitored. The study documents suitability of passive air sampling for the assessment of specific toxic potencies of ambient air pollutants.

Keywords Ambient air · Passive sampling · AhR-mediated toxicity · Estrogenicity · Androgenicity

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Introduction

Organic pollutants present in ambient air have been associated with several adverse effects on respiratory, cardiovascular, immune, and reproductive systems

(Curtis et al. 2006). Monitoring of organic pollutants in ambient air has focused mainly on relatively few indicator classes of compounds such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), or organochlorine pesticides (OCPs; Holoubek et al. 2007; Klánová et al. 2009). Despite the fact that knowledge of concentrations of these classes of contaminants is important for estimation of exposure of humans as well as for determining sources, these data are difficult to link directly with the observed adverse effects because mixtures of pollutants in the environment can contain thousands of individual compounds and their transformation products, some of which are probably not yet characterized (Lewtas 2007). There is a need for methods that could integrate the effects of chemicals in the mixture with their possible interactions with biota, such as receptor-mediated responses as well as accounting for additivity, antagonism, or synergism. This can be addressed by bioassays, including transactivation assays based on in vitro responses of genetically modified cells (Janošek et al. 2006). Bioassays can also be used in directed fractionation and identification schemes (Hecker and Giesy 2011) and potency balance studies to identify novel chemicals in the environment (Hilscherová et al. 2002).

Although some of the relatively less specific toxic effects of polluted air such as production of oxidative stress, inflammation, and genotoxicity are often being connected with inorganic components of particulate matter (Jeng 2010; Wessels et al. 2010), other more specific effects, such as endocrine disruption, seem to be associated with organic pollutants in the atmosphere (Rudel and Perovich 2009). At least part of the specific toxic effects is mediated by intracellular receptors such as estrogen receptor (ER), androgen receptor (AR), or by aryl hydrocarbon receptor (AhR). These receptors function as transcription factors and their dysregulation by xenobiotics has been shown to be connected with adverse in vivo effects such as carcinogenesis, immunosuppression, or reproductive disorders (Janošek et al. 2006). These receptor-related modes of action were intensively studied and described in the literature. However, the toxic potencies and effects of environmental mixtures of pollutants such as the atmospheric pollutants remain to be elucidated (Hotchkiss et al. 2008). While ER and AR signaling is clearly connected with hormone signaling, AhR affect estrogenic signaling indirectly (Safe and Wormke 2003). However, AhR is activated by many non-polar compounds, many of

which are routinely assessed environmental pollutants such as some PAHs, PCBs or polychlorinated dioxins and dibenzofurans (PCDD/Fs). Because it is possible to calculate a toxic equivalent from chemical as well as effect-directed analyses, the data from both types of analyses can be relatively easy to compare and synthesize, which increases the scientific relevance of dioxin-like toxicity mediated by AhR (Behnisch et al. 2001; Van den Berg et al. 2006).

To describe long-term toxic potentials of organic pollutants in air at multiple localities, effect-based monitoring needs a suitable tool for collection of the organic chemicals from air. Contaminants in air have mostly been studied by use of active sampling devices that are not very suitable for large-scale or long-term monitoring. Active collection of samples could be particularly complicated at remote locations because of its demands on technical equipment, and lack of availability of electricity and full-time, on-site operators during sampling. Relatively recent methods for passive sampling of air have been introduced that eliminate many of the limitations previously associated with sampling of contaminants in air (Harner et al. 2004; Shoeib and Harner 2002). While not sensitive to short-term fluctuations in concentrations, passive air samplers are suitable for measurement of long-term, average concentrations of contaminants without requirements for electrical power or daily maintenance. This makes them suitable for the monitoring of pollutants from sources with rather stable releases, such as traffic or long-range transport. On the other hand, most types of passive samplers collect mainly volatile compounds, thus omitting a portion of pollutants that are less volatile, and so they are associated with particulate matter (Klánová et al. 2008). Moreover, the volume of sampled air cannot be measured precisely so it is difficult to normalize concentrations of pollutants on volumetric basis (Harner et al. 2004; Shoeib and Harner 2002). However, the advantages make this approach convenient for long-term monitoring (Klánová et al. 2006; Peng et al. 2013) even at localities without expert technical support, such as Africa, where active sampling would be more difficult (Klánová et al. 2009).

Although there are relatively many studies that have measured concentrations of some classes of pollutants by use of passively sampled air, few of them have focused on the potential of the mixture to cause effects on specific pathways. The presence of compounds with genotoxic potential (Bonetta et al. 2009; Čupr et al. 2006; Isidori et al. 2003; Kennedy et al. 2010;

Slapšytė et al. 2006), dioxin-like toxicity modulated via the AhR (Kennedy et al. 2009, 2010) and estrogenicity (Kennedy et al. 2009) have been detected in passive samples of ambient air. To demonstrate the utility of passive air sampling in effect-based monitoring, a set of biological potentials of air samples was measured. Extracts of passive sampler filters were assessed for potential to modulate signaling pathways of ER, AR, and AhR. The results were compared to data from chemical analyses and used in a mass potency assessment by comparing the total potential as measured in the bioassays to concentrations of relatively routinely analyzed pollutants, by use of relative potency factors. Unlike most previous studies, passive air samplers were exposed at diverse localities including background, urban, and industrial locations in four countries to bring important information on possible variability of composition of pollutant mixtures in ambient air of Eastern Europe. Samples came from the passive air monitoring network (MONET) that was established in this region for the purpose of the Global Monitoring Plan (Přibylová et al. 2012).

Materials and methods

Collection of samples

Samples of ambient air were collected at all studied locations simultaneously for 5 months between March and August of 2006 by use of passive samplers with precleaned polyurethane foam (PUF) disks (15-cm diameter, 1.5-cm thick, density 0.033 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) inside two stainless steel protective domes. Each PUF disk was used for 28-day sampling and thus five disks were deployed sequentially at each location. For more detail on the sampling procedure, see Přibylová et al. (2012). A theory of passive air sampling with similar devices has been described elsewhere (Harner et al. 2004, 2006; Shoeib and Harner 2002).

Contaminants in air were sampled in four countries from Eastern Europe—Slovakia, Lithuania, Romania, and Serbia. Each country was represented by a background and an impacted location (Fig. 1; Table 1). In Lithuania, the background location was located in Preila (LT-1) at a background environmental research station of the Lithuanian Institute of Physics that is located on a sandy coast of the Baltic Sea on a 70-km long and 2–3-
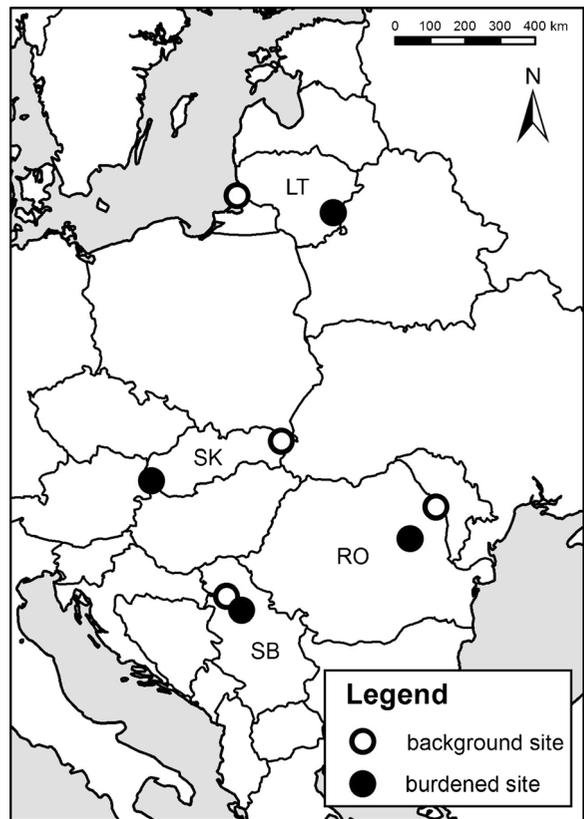
Fig. 1 Map of studied localities in Europe. *LT* Lithuania, *SK* Slovakia, *RO* Romania, *SB* Serbia

km wide Curonian Spit separating the Curonian Lagoon from the Baltic Sea. The other sampling location in Lithuania (LT-2) was located in the center of the city of Vilnius at a street with intensive traffic. In Slovakia, background level of contamination was represented by Starina dam location (SK-1), which is a background monitoring station of the European Monitoring and Evaluation Programme (EMEP) and is located in a national park in the eastern part of Slovakia. The second location was Trnavské mýto (SK-2), an important crossroad in the capital Bratislava with intensive traffic. In Romania, the location in Raducaneni (RO-1) was a rural locality near borders with Moldova. Onesti-Borzesti (RO-2) in central Romania was a location with high density of chemical industry. The Chimcomplex Borzesti is one of the most important chemical manufacturers in Romania and produce chlorine and caustic soda, organic solvents, inorganic chlorides, and pesticides. There is also production of synthetic rubber (CAROM Onesti) and oil refinery (RAFO Onesti). Serbia was characterized by a background location at

Table 1 Sampling sites characteristics

Code	LT-1	LT-2	SK-1	SK-2	RO-1	RO-2	SB-1	SB-2
Country	Lithuania	Lithuania	Slovakia	Slovakia	Romania	Romania	Serbia	Serbia
Sampling location	Preila, research institute	Vilnius, center	Starina, dam, EMEP station	Bratislava, Trnavské mýto	Raducaneni, garden	Onesti-Borzesti	Fruška Gora	Beograd
Characteristics, pollution sources	Background location, seashore, local heating	Industrial location, intensive traffic	Background location	Urban location, traffic pollution	Rural area, agriculture	Industrial location, chemical industry and oil refinery	Background location	Urban location, traffic pollution
Northern lat.	55°21′49.39″	54°42′42.09″	49° 2′32.34″	48° 9′31.73″	46°55′59.00″	46°14′17.00″	45°09′33.00″	44°47′27.58″
Eastern long.	21°3′10.35″	25°2041.77″	22°15′39.89″	17°744.33″	27°55′59.00″	26°48′33.97″	19°51′46.10″	20°22′36.62″
Altitude (m)	9	195	362	145	195	239	511	76
Sampling period	25.3–13.8	21.3–13.8	2.3–9.8	21.3–8.8	24.3–13.7	26.3–11.7	30.3–17.8	27.3–6.9
Average temperature (°C)[a]	13	12.4	13.7	17.6	15.9	16.3	18.1	17.5
Average month temperatures (°C)[b]	3.6	1.1	5.5	9.7	8.8	9.7	12.4	11.3
	10.8	10.6	12.1	14.9	13.0	11.2	16.0	14.2
	12.4	11.3	12.2	15.6	16.0	15.7	16.5	16.2
	19.3	19.8	19.1	23.3	20.0	24.0	22.8	22.7
	18.8	19.0	19.8	24.4	21.5	21.0	22.6	23.0

[a] Average temperature during whole sampling period

[b] Average temperature during each 5 months of sampling

Fruška Gora (SB-1) in mountain region near Novi Sad and in the city of Beograd (SB-2) as an urban locality.

Preparation of samples and identification and quantification of chemicals

Exposed PUF disks were extracted with dichloromethane in a Büchi System B-811 automatic extractor (Büchi, Switzerland) as described earlier (Klánová et al. 2009; Přibylová et al. 2012). One laboratory blank and one reference material sample were analyzed with each set of ten samples. A sulfuric acid-modified silica gel column was used for the removal of less persistent chemicals in a portion of samples that were used for the assessment of PCBs/OCPs as well as for the determination of the contribution of persistent pollutants to the observed toxic effects. Standards for PAHs and OCPs were obtained from Absolute Standards (USA) and PCBs from Sigma-Aldrich (Czech Republic). Surrogate recovery standards were used for PAHs analysis ($d8$-naphthalene, $d10$-phenanthrene, $d12$-perylene) and PCBs analysis (PCB 30 and PCB 185). Terphenyl and PCB 121 served as internal standards for PAH and PCB/OCP analyses, respectively. Extracts were split into two parts and one of them was treated with sulfuric acid-modified silica gel column to remove the non-persistent chemicals. For bioassays, the sample volume was reduced after extraction under a gentle stream of nitrogen at ambient temperature with dimethyl sulfoxide as a keeper. Samples were analyzed by use of GC-MS (HP 6890–HP 5975; Agilent Technologies, Czech Republic) with a J&W Scientific fused silica column DB-5MS (5 % Ph; Agilent Technologies) in electron impact ionization mode for PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180), and OCPs (α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH, δ-HCH, 1,1-dichloro-2,2-bis (p-chlorphenyl) ethylene (p,p'-DDE), 1,1-dichloro-2,2-bis (p-chlorphenyl) ethan (p,p'-DDD), 1,1,1-trichloro-2,2-bis (p-chlorphenyl) ethan (p,p'-DDT),o,p'-DDE, o,p'-DDD, o,p'-DDE, hexachlorobenzene (HCB), and pentachlorobenzene (PeCB)). 16 US EPA polycyclic aromatic hydrocarbons were determined by use of GC-MS (HP 6890–HP 5972) using a J&W Scientific fused silica column DB-5MS.

Bioassays

Total receptor-mediated potencies of mixtures in extracts were determined by use of three bioassays. H4IIE-*luc* rat hepatoma cell line stably transfected with a luciferase gene under control of AhR was used for analysis of dioxin-like potency of extracts of PUF disks (Sanderson et al. 1996). The H4IIE-*luc* cell line was grown and exposed in DMEM medium (PAA, Austria) containing 10 % fetal bovine serum Mycoplex (PAA). Human breast carcinoma cells MVLN, which are transfected with the estrogen receptor-triggered luciferase gene (Demirpence et al. 1993; Freyberger and Schmuck 2005; Villeneuve et al. 2000), were cultivated in DMEM/F12 medium without phenol red (Sigma-Aldrich) supplemented with 10 % fetal bovine serum Mycoplex (PAA). Exposure was performed in the same medium supplemented with 5 % dialyzed fetal bovine serum (PAA) that was additionally treated with dextran-coated charcoal suspension (Sigma-Aldrich) to further decrease concentrations of interfering steroids. A human MDA-kb2 breast adenocarcinoma cells, which were stably transfected with luciferase reporter gene construct under control of the AR, were cultivated in L-15 medium (PAA) supplemented with 10 % fetal bovine serum Mycoplex (PAA) and exposed in the same medium supplemented with 10 % dialyzed fetal bovine serum (PAA) treated with dextran-charcoal suspension (Sigma-Aldrich).

Exposure of cells was performed in 96-well plates (Greiner Bio-One, Belgium). For the experiment, cells were seeded at densities 15,000; 20,000; and 50,000 cells per well for H4IIE-*luc*, MVLN and MDA-kb2 cells, respectively. After 24-h plating, cells were exposed to a dilution series of the samples in triplicates together with solvent control and calibration series of model compound, i.e., 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD; Ultra Scientific, USA), 17β estradiol (E2; Sigma-Aldrich), or 5α-dihydrotestosteron (DHT; Sigma-Aldrich) in case of H4IIE-*luc*, MVLN, and MDA-kb2, respectively. Anti-estrogenic and anti-androgenic effects were assessed by simultaneous exposure of cells to the sample extracts together with competing agonist that was 11-pM 17β-estradiol and 0.5-nM DHT in case of MVLN or MDA-kb2, respectively. At least three independent analyses were conducted for each sample within all test systems. After 24 h of exposure, intensity of bioluminescence corresponding to the respective receptor activation was measured by use of

Steady-Glo Kit (Promega, USA) with H4IIE-*luc* and MVLN models and luciferin kit described previously when used with MDA-kb2 (Wilson et al. 2002). Cytotoxicity of tested dilutions of the samples was assessed using neutral red uptake assay (Freyberger and Schmuck 2005) and data from cytotoxic sample dilutions were excluded from the calculations.

Data analysis

Concentrations of contaminants and responses of cells in vitro were expressed per cubic meter of sampled air assuming that the passive sampler had collected air pollutants from 100 m^3 of air during 28-day sampling based on data from calibration studies that have been performed previously (Chaemfa et al. 2008, 2009; Klánová et al. 2006, 2008; Shoeib and Harner 2002). Dioxin-like potency assessed with H4IIE-*luc* assay was reported as toxic equivalents (bioTEQ) of TCDD per cubic meter. The calculation was based on EC$_{50}$ values of TCDD as described previously (Villeneuve et al. 2000). A portion of bioTEQ elicited by persistent organic pollutants (popTEQ) was assessed with H4IIE-*luc* cells by use of the part of samples treated with sulfuric acid-modified silica gel column that had removed less persistent compounds such as PAHs. Concentrations of TEQ (chemTEQ) levels were calculated as the sum of the products of concentrations of individual chemicals multiplied by their relative potencies. The individual PAHs considered included pyrene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[*1,2,3-c,d*]pyrene, dibenz[*a,h*]anthracene, cyclopenta[*c,d*]pyrene, benzo[*j*]fluoranthene, benzo[*e*]pyrene, and dibenzo[*a,c*]anthracene. Relative potencies were determined for H4IIE-*luc* cells (Machala et al. 2001) based on toxic equivalency factor approach that was described previously (Safe 1998).

Anti-estrogenicity and anti-androgenicity potencies were expressed based on IC$_{50}$, i.e., concentration of the sample that produced 50 % decrease of response of added natural ligand of the respective receptor (11-pM estradiol and 0.5-nM DHT, respectively). Values in graphs and statistical analyses are expressed as an index of anti-estrogenicity (iAE) or anti-androgenicity (iAA), respectively, which correspond to the reciprocal value of IC$_{50}$.

Correlations were calculated by use of the nonparametric Kendall tau test ($P < 0.05$) because there was insufficient data to confirm normal distribution, which is one of the assumptions for parametric testing. Kendall tau was chosen because it is more robust than Spearman's rank correlation coefficient. Differences between pollutant levels in air samples were calculated using Kruskal–Wallis or Mann–Whitney test ($P < 0.05$).

Results and discussion

Passive sampling is beginning to be a well-established method for long-term, integrated monitoring of pollutants in air. It provides data on average concentrations of pollutants during relatively long sampling periods. Passive samplers are reported to collect representatively mainly semi-volatile compounds (Klánová et al. 2008); volatile compounds (with low K_{OA} values) could reach concentration equilibrium between atmosphere and the filter sooner than the 28-day sampling period. With non-volatile particle-bound chemicals such as higher molecular weight PAHs, the situation is less clear. While Harner et al. (2013) did not observe significant differences in sampling rates for volatile and particle-bound PAHs during cold season in Canada, Klánová et al. (2008) have described that particle-bound chemicals are collected with approximately 10 lower effectiveness than the semi-volatile compounds. Authors of the latter study argue that it is because the passive sampler collects mainly fine and ultra-fine particles, which behave in similar way as gas particles, but coarse particles do not enter the sampling chamber. However, the sampling rate of less volatile chemicals such as many PAHs is closely related to temperature because at warmer temperatures, these compounds partition more to the gas phase and this, together with factor of wind that also affects sampling rate significantly, might play a role in the discrepancy of these studies. Sampling rate of less volatile chemicals is probably lower than for the semi-volatile compounds.

Because it is difficult to quantify the volume of air that has passed through the sampler, the pollutant levels are often expressed in amount of the contaminants per sample and sampling period. Previous studies in which passive samplers have been calibrated have shown that the type of sampling device used in our studies typically collects chemicals associated with 3.5–7 m^3 of air per day, i.e., 100–200 m^3 during the 28-day sampling period (Chaemfa et al. 2008, 2009; Klánová et al. 2006, 2008; Shoeib and Harner 2002). Rates of accumulation of

chemicals are influenced mostly by physicochemical properties of the individual chemicals (Halse et al. 2010), especially their particle/gas partitioning coefficients that are closely connected to temperature. An estimation of volumetric data is provided to be able to compare our results to those of similar studies that have been performed by use of active collection. The studied samples were chosen from a larger set of the MONET passive sampling network (Přibylová et al. 2012) to include several countries that are located in warm summer continental climate (Peel et al. 2007). Each country was described by a background and an impacted locality (Table 1). The latter localities of interest were mostly urban with intense traffic. However, the main source of pollution at the impacted location from Romania was the chemical industry. Moreover, this locality contained substantial amounts of organochlorine compounds and other pollutants that were probably a legacy of the former production (Přibylová et al. 2012). Background sites were chosen to be minimally affected by local sources of pollution to show levels of the studied potentials that would describe effects of disperse sources of pollutants, such as agriculture, or effect of long-range transport. This is probably the case especially with LT-1 site that is located on the Curonian spit and so the long-range transport from shore and/or from shipping probably plays an important role there.

Chemical analysis

Concentrations of assessed pollutants were generally significantly lower at the background localities compared to the respective impacted locations (Table 2). The greatest concentrations of PAHs were observed at the impacted location from Romania (RO-2) but differences from those at other locations were mostly within an order of magnitude and in similar range as in previous studies. Concentrations of PAHs at rural localities have been reported to be 3.5–11.5 ng/m^3 in Canada (Motelay-Massei et al. 2005) or 33–157 ng/m^3 in the Czech Republic (Klánová et al. 2006), while those from impacted locations were reported to be 7.4–71 and 40–538 ng/m^3 in Canada and the Czech Republic, respectively. The greatest concentrations of PCBs were observed at localities RO-2, SB-2, and SK-2. At location RO-2, the greater concentrations of PCBs probably originated from historical uses in the chemical industry, assuming that current chemical technologies do not release substantial amounts of chlorinated compounds into the environment. In Serbia, relatively large concentrations of PCBs were reported to be connected with damage caused during the Balkan war (Klánová et al. 2007). Slovakia, when a part of the former Czechoslovakia, was one of the greatest producers of PCBs in Europe and these compounds were extensively used in many areas so the large concentrations were probably a legacy of the former use (Holoubek et al.

Table 2 Concentrations of contaminants and receptor-mediated equivalents expressed per cubic meter of air (sampling rate estimated to 100 m^3/28 days)

Samples	ΣPAH [ng/m^3]	ΣPCB [pg/m^3]	ΣHCH [pg/m^3]	ΣDDT [pg/m^3]	ΣOCP [pg/m^3]	chemTEQ [pg/m^3; %]	popTEQ [pg/m^3; %]	iAE [m^3/ml]$^{-1}$	iAA [m^3/ml]$^{-1}$
LT-1	33.4	91.4	170.7	38.6	335.9	0.08 (7.7)	<0.08 (n.q.)	0.913	0.349
LT-2	33.9	117.6	205.3	192.7	485.8	0.07 (6.4)	0.13 (11.5)	6.80	0.406
SK-1	11.8	49.6	182.9	50.4	316.5	0.11 (21.0)	<0.08 (n.q.)	3.41	2.55
SK-2	98.8	366.9	1079.6	224.7	1438.6	0.34 (32.4)	0.27 (3.0)	<0.12	1.46
RO-1	54.7	63.4	904.4	329.9	1303.9	0.29 (26.2)	<0.08 (n.q.)	<0.12	0.541
RO-2	139.6	337.1	11065.2	448.2	11687.5	0.37 (9.0)	0.37 (8.3)	2.41	0.326
SB-1	13.6	184.1	321.5	91.0	1695.5	0.07 (10.8)	<0.08 (n.q.)	<0.12	0.494
SB-2	104.4	346.1	1631.9	985.4	1164.2	0.44 (3.3)	<0.08 (n.q.)	4.92	1.40

ΣPAH sum of 16 polycyclic aromatic hydrocarbons; ΣPCB sum of 7 polychlorinated biphenyls; ΣHCH sum of α-, β- and γ-hexachlorocyclohexane; ΣDDT sum of DDT and its degradation-products; ΣOCP sum of HCHs, DDTs, HCB, and PeCB; chemTEQ TCDD-equivalent calculated from PAHs levels (percentage contribution to bioassay-derived TCDD-equivalent (bioTEQ)); popTEQ bioassay-derived TCDD-equivalents of persistent sample fraction (percentage contribution to bioTEQ); iAE index of anti-estrogenicity (reciprocal value of IC$_{50}$); iAA index of anti-androgenicity (reciprocal value of IC$_{50}$); n.q. not quantified; LT Lithuania; SK Slovakia; RO Romania; SB Serbia; 1 background location; 2 impacted location

2007). The detected background concentrations were of a similar magnitude to those that have been previously reported (Klánová et al. 2006, 2007). Concentrations of OCP were greatest at localities RO-2 and SB-2 and were due to historical releases from the chemical industry and former uses, respectively. This was particularly the case for concentrations of HCHs at RO-2 that were at least 10-fold greater than that in samples from most of other locations.

AhR-mediated potency

AhR-mediated potency has been suggested to cause several kinds of adverse effects including carcinogenicity and endocrine disruption (Safe and Wormke 2003; White and Birnbaum 2009). The employed H4IIE-*luc* bioassay is widely used for rapid screening of environmental samples for AhR-active PAHs and related compounds, such as nitrogen heterocyclics and sulfur-, oxygen-, nitro-, amino-, and alkyl-substituted PAHs, as well as persistent PCDD/Fs, some PCBs and naphthalenes, as well as the brominated analogs of these compounds (Behnisch et al. 2003). Besides samples from Lithuania, samples from impacted locations contained significantly greater concentrations of AhR-activating compounds in air than were observed at background locations (Fig. 2). Both the greatest and lowest concentrations of bioTEQ were observed in samples from
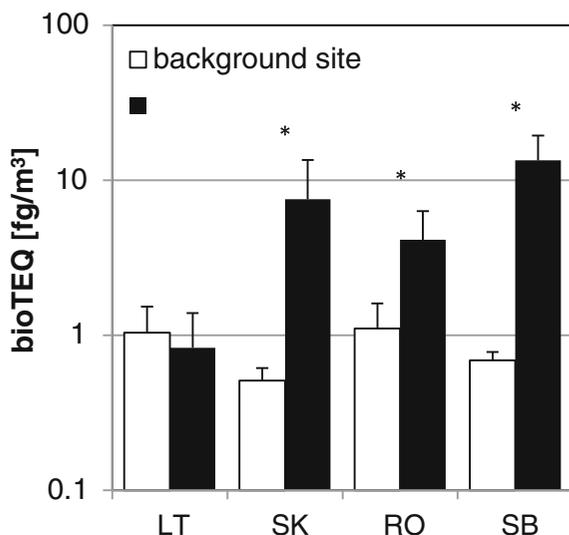
Fig. 2 Bioteq: TCDD-equivalents per cubic meter of air (fg bioTEQ/m^3; mean+SD; sampling rate estimated to 100 m^3/ 28 days); *LT* Lithuania, *SK* Slovakia, *RO* Romania, *SB* Serbia

Slovakia and Serbia, at background and burdened sites, respectively. In Lithuania, AhR-activation potencies of samples from background and impacted location were not different. Similar trends were seen for concentrations of PAH (Table 2), which were correlated with concentrations of bioTEQ ($\tau=0.79$). This indicates the contribution PAHs to AhR-mediated potency. It has been shown previously that in the gas phase, as well as in particulate phase of ambient air, this potency was caused primarily by nonpersistent compounds, of which PAHs are most likely responsible (Andrysík et al. 2011; Kennedy et al. 2010; Novák et al. 2009). This finding is consistent with the results observed in the study that are reported here. Persistent fractions of extracts (popTEQ) were quantifiable only for three samples and it was not responsible for more than 12 % of bioTEQ (Table 2). AhR-mediated potencies calculated from concentrations of PAHs (chemTEQ) have shown that PAHs were responsible for 7.7–26 % of observed bioTEQ at background locations and 6.4–32 % at impacted locations (Table 2) and were correlated with bioTEQ levels ($\tau=0.64$). This contribution was greater than that in passive samples collected in Australia where PAHs elicited 0.55–1.4 % of bioTEQ assessed by CAFLUX bioassay (Kennedy et al. 2010). This could be caused by differences in pattern of PAHs in the air in Australia and Eastern Europe, different sensitivities of employed bioassays, or by using different set of relative potencies of PAHs for calculation of chemTEQ. In the present study, there were used previously published potencies obtained with the same bioassay (Machala et al. 2001). Another set of relative potencies has been derived specifically for the CAFLUX bioassay (Kennedy et al. 2010). However, the difference is probably due to the diverse sources of pollutants and meteorological situation that were different in Australia and Europe. This would result in mixtures of pollutants with different characteristics. The greatest contribution to the overall chemTEQ was attributed to benzo[*k*]fluoranthene, benzo[*j*]fluoranthene, and chrysene that were, on average, responsible for 52, 15, and 11 % of chemTEQ, respectively (Fig. 3), but this profile was not uniform. For example, dibenz[*a,h*]anthracene contributed 34 % to the chemTEQ at location RO-2 while its contribution at the rest of locations was less than 3 %. In samples from Australia, most of the potency measured in the bioassays was contributed by chrysene and indeno[*1,2,3-c,d*]pyrene (Kennedy et al. 2010). In extracts of PM from Switzerland, most of the potency was due to benzo[*k*]fluoranthene and indeno[*1,2,3-c,d*]pyrene
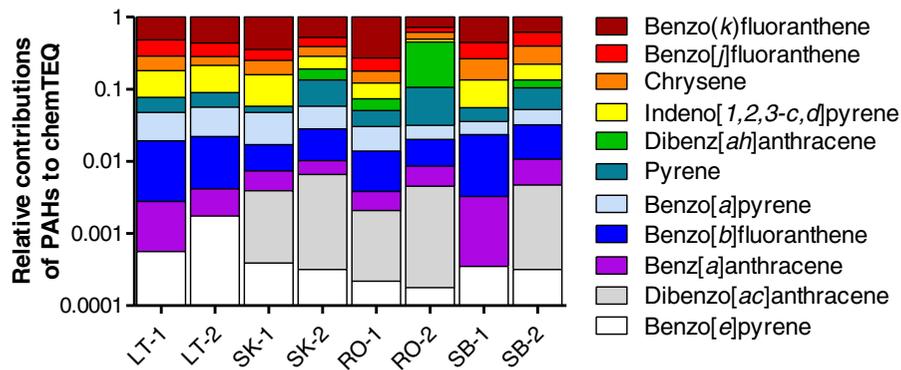
Fig. 3 Relative contributions of PAHs to chemTEQ, PAHs are sorted according to their average contribution; *LT* Lithuania, *SK* Slovakia, *RO* Romania, *SB* Serbia; *1* background location, *2* impacted location

(Wenger et al. 2009), but this fraction contains less volatile PAHs, not efficiently collected by passive samples. Profiles of PAHs were relatively location-specific and closely associated with characteristics of the present pollution sources. These results indicate that concentrations of 28 indicator PAHs do not sufficiently describe the overall potency of non-persistent AhR-active compounds in air because there are other non-persistent AhR activators besides PAHs such as their methylated and oxygenated derivatives or polycyclic aromatic ketones and quinones as it has been shown previously (Bekki et al. 2009; Misaki et al. 2007).

Interactions with estrogenic and androgenic receptors

In previous studies of ambient air investigated by use of passive sampling, the air was determined to have estrogenic potency (Kennedy et al. 2009; Klein et al. 2006). In the present study, quantifiable estrogenic potency was observed with neither sample (data not shown). However, when exposed simultaneously with E2, extracts of passive samplers caused anti-estrogenic responses (Table 2). The anti-estrogenic effects could be quantified in only five samples and two of them were from background locations in Lithuania and Slovakia. The relatively great anti-estrogenic potency of SK-1 was confirmed by six repeated independent assessments and it is probably caused by pollutants from local sources such as agriculture. Besides samples collected directly from sources of pollution, such as emissions from engines (Ueng et al. 2004), the anti-estrogenicity has been show in actively collected samples of ambient air both in particulate as well as gas phase previously and the potential had a clear location-specific pattern (Novák

et al. 2009). The difference in the sampling techniques prevent the direct comparison of the results with our study, even though the gas phase fraction from active sampling could possess similar characteristics as passively collected air samples. However, in the present study, no significant associations of anti-estrogenicity and other assessed parameters could be derived because of the limited set of samples with the quantifiable effect.

Anti-androgenic effect has been ascribed to pollutants emitted during combustion of wood (Owens et al. 2006), particles in the exhaust of diesel engines (Okamura et al. 2004), or pesticides (Kojima et al. 2004). Anti-androgenic effects have been shown to be associated with mixtures of pollutants in the gas phase of ambient air (Novák et al. 2009). In the present study, similar results were obtained. Samples did not produce any androgenic responses and the only significant effect was anti-androgenicity in co-exposure with standard androgen DHT (Table 2). The greatest anti-androgenic potential was associated with samples from the background location in Slovakia and urban locations in Slovakia and Serbia. Thus, anti-androgenic potential did not show clear linkages with concentrations of pollutants, but there were significant differences in anti-androgenic potentials among countries that could be connected with country-specific composition of mixtures that could affect the anti-androgenic potency. The only study showing anti-androgenic effects of extracts of ambient air has used actively collected air samples and the anti-androgenicity was assessed using a yeast bioassay so the data are not directly comparable (Novák et al. 2009). The relatively greater anti-androgenic and anti-estrogenic potential of SK-1 sample could be connected with compounds whose levels are not associated

with levels of assessed types of pollutants and AhR-active compounds.

Summary

The results of this study show applicability of passive sampling for effect-based long-term assessment of pollutants in ambient air. There was greater AhR-mediated potential at the urban/industrial localities compared to those at background locations in Slovakia, Romania, and Serbia. The potential was elicited mostly by non-persistent compounds such as PAHs. While AhR-mediated effects clearly correlated with PAHs levels, the analyzed PAHs were responsible for relatively small portion of the dioxin-like toxicity. This indicates that chemical analyses assessing only a limited set of compounds with the toxic potential could significantly underestimate the toxic effect of the air samples. The studied samples elicited anti-estrogenic and anti-androgenic effects that could not be clearly linked to the chemicals detected in the samples. It seems that these effects are associated with chemicals that are not routinely monitored.

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