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Pollutants in particulate and gaseous fractions of ambient air interfere with multiple signaling pathways *in vitro*

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ABSTRACT

Traditionally, contamination of air has been evaluated primarily by chemical analyses of indicator contaminants and these studies have focused mainly on compounds associated with particulates. Some reports have shown that air contaminants can produce specific biological effects such as toxicity mediated by the aryl hydrocarbon receptor (AhR) or modulation of the endocrine system. This study assessed the dioxin-like toxicity, anti-/estrogenicity, anti-/androgenicity and anti-/retinoic activity of both the particulate and gas phase fractions of air in two regions with different types of pollution sources and a background locality situated in an agricultural area of Central Europe. The first region (A) is known to be significantly contaminated by organochlorine pesticides and chemical industry. The other region (B) has been polluted by historical releases of PCBs, but the major current sources of contamination are probably combustion sources from local traffic and heating. Samples of both particle and gas fractions produced dioxin-like (AhR-mediated) activity, anti-estrogenic and antiandrogenic effects, but none had any effect on retinoid signaling. AhR-mediated activities were observed in all samples and the TEQ values were comparable in both fractions in region A, but significantly greater in the particulate fraction in region B. The greater AhR-mediated activity corresponded to a greater coincident antiestrogenicity of both phases in region B. Our study is the first report of antiestrogenicity and antiandrogenicity in ambient air. Anti-androgenicity was observed in the gas phase of all regions, while in the particulate phase only in one region due to the specific type of pollution in that area. Even though based on concentrations of individual compounds, except for the OCPs, the level of contamination of the two regions was similar, there were strong differences in responses in the bioassays between the two regions. Moreover, AhR-mediated activity and antiestrogenic potencies were greater in region B, where the pollution level according to the chemical analysis was similar or less than in the other region, which indicates the presence of other atmospheric pollutants with specific effects. The results document the advantage and utility of the simultaneous use of bioassays and chemical analysis in risk assessment of complex environmental samples.

1. Introduction

Ambient air in urban and industrial areas often contains complex mixtures of environmental contaminants that are present in the particulate and/or gas phases of air (Englert, 2004). Traditionally, air pollution monitoring has focused on assessment of concentrations of particles, inorganic oxides, and ozone. Pollution of air by organic compounds is mostly evaluated by analysis of indicator classes of contaminants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs). However, air contamination consists of very complex mixtures of chemicals, some of which are unidentified, and these mixtures could potentially produce toxic effects that would not be expected, based on the available analytical data. Specific effects of air pollutants can be assessed by bioassays, which unlike chemical analyses can integrate the effects of all chemicals in the complex mixture, also taking in account interactions such as additivity, antagonism, or synergism.

Air pollution has been linked with adverse health consequences in exposed animals (Bernstein et al., 2004; Kotwal et al., 2005) and humans (for review see Curtis et al., 2006; de Kok et al., 2006). Polluted air has been associated mainly with lung and heart diseases. However, recent studies indicate that air pollutants can possibly impair reproduction. Inhalation of diesel exhaust has been reported to cause reproductive effects in rodents (Li et al., 2006a; Watanabe and Kurita, 2001; Yoshida et al., 1999) and birds (Li et al., 2006b). The effects of air pollution on reproduction have been validated in

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experiments with mice raised either in filtered or non-filtered city air (Lichtenfels et al., 2007; Mohallem et al., 2005). A statistically significant negative correlation between concentrations of particulate matter in air and male to female ratio was observed in newborn children in Sao Paulo, Brazil (Lichtenfels et al., 2007). These authors also observed significantly poorer semen quality in men occupationally exposed to traffic (De Rosa et al., 2003). Some of these effects could possibly be related to endocrine disruptive properties of air pollutants. This hypothesis is supported by the fact that pollutants which are known as potential endocrine disruptors have been detected in indoor air and dust (Rudel et al., 2003) and that the contaminants in extracts of ambient air have been shown to interact with receptors for steroid hormones *in vitro*. Some authors have observed *in vitro* estrogenic (Clemons et al., 1998; Klein et al., 2006; Ueng et al., 2004) and antiprogesteronic (Wang et al., 2005b) effects of air extracts or antiestrogenic and antiandrogenic *in vitro* and *in vivo* effects of traffic exhaust particles (Kizu et al., 2003; Okamura et al., 2004). Another potential mode of action that is widely used in the toxicity evaluation of complex samples is the dioxin-like toxicity and there are several works describing this type of effect in ambient air samples (Arrieta et al., 2003; Ciganek et al., 2004; Clemons et al., 1998; Mason, 1994).

Previous studies dealing with effects of ambient air pollution at the molecular level have focused almost exclusively on pollutants associated with air particles. Nevertheless, a large number of air contaminants is present at least partly in the gas phase (Fernandez et al., 2002; Mandalakis et al., 2002). Moreover, Klein et al. (2006) have shown that a portion of compounds with dioxin-like or estrogenic activity is present in the gas phase of ambient air. This finding, together with the fact that the gas fraction of the air contaminants is readily bioavailable, document that the gas phase of ambient air can be a potentially important source of biologically active contaminants.

In this study, bioassays were used to examine interactions of extracts of both the gaseous and particulate phases of air with intracellular receptors for estrogens (estrogen receptor; ER), androgens (androgen receptor; AR), compounds with dioxin-like activity (AhR-mediated), and retinoids (retinoic acid receptor; RAR). These receptors are known to be involved in mediating some endocrine disruptive effects of xenobiotics. While ER and AR are considered potential targets for endocrine disruptors, AhR- and RAR-mediated effects are usually neglected in the study of this phenomenon (Janosek et al., 2006). AhR-mediated effects are considered a valuable marker of contamination by dioxin-like compounds (Whyte et al., 2004) that can negatively affect liver functions as well as immunity, endocrine and nervous system (Mukerjee, 1998). Although there is no known endogenous physiological ligand for the AhR (Hahn, 2002) and thus it is not considered a part of hormonal signaling pathways, it has been described to cross-talk with estrogen receptor signaling (Ohtake et al., 2003; Safe and Wormke, 2003). Moreover, there is evidence for cross-talk between AhR-dependent signaling pathways and other hormone pathways (Puga et al., 2005; Safe and McDougal, 2002; Widerak et al., 2006).

The RAR is a part of a signaling pathway of retinoids that controls processes such as morphogenesis, development, reproduction, or apoptosis. Although retinoids are not strictly endogenous since they are derived from dietary sources of vitamin A or its precursors, the levels of their active forms are regulated in a hormone-like way such that they are sometimes referred to as 'dietary' hormones (Ross and Zolfaghari, 2004; Simms and Ross, 2000). Moreover, the involvement of retinoid signaling as a target of endocrine disruptive environmental pollutants has been proposed in several studies (Nishikawa et al., 2004; Novak et al., 2007; Smith et al., 2003).

This study evaluated the potential of air pollutants to act through several modes of action. The hypothesis that pollutants from different regions with different profiles of contaminants could modulate endocrine function was tested by collecting air samples in two regions with different sources of pollution and a background locality. Bioassays were used in conjunction with instrumental identification and quantification of residues to assess the specific activities of pollutants in both particulate and gas phases of air.

2. Materials and methods

2.1. Collection and preparation of samples

Air samples were collected in July and August 2005 from three regions (Fig. 1). The first region (A) was represented by 6 discrete sampling locations in a region that is known to be contaminated by organochlorine pesticides and chemical industry. The second region (B), represented by 8 sampling stations, has been polluted by historical releases of PCBs, but the current major sources of contamination are probably combustion sources from local traffic and heating. The third region was a reference area where a composite sample (REF) was prepared from four one-day samples from a background locality at the Košetice observatory, Czech Republic. The reference location is a part of long-term monitoring project EMEP (UN-ECE's European Monitoring and Evaluation Programme) and the level of contamination at that location has been measured continuously since 1988 (Holoubek et al., 2007). The reference location represents a rural region devoid of larger settlements or major sources of contamination. Another set of 6 composite samples, each composed of five 24-hour sub-samples,

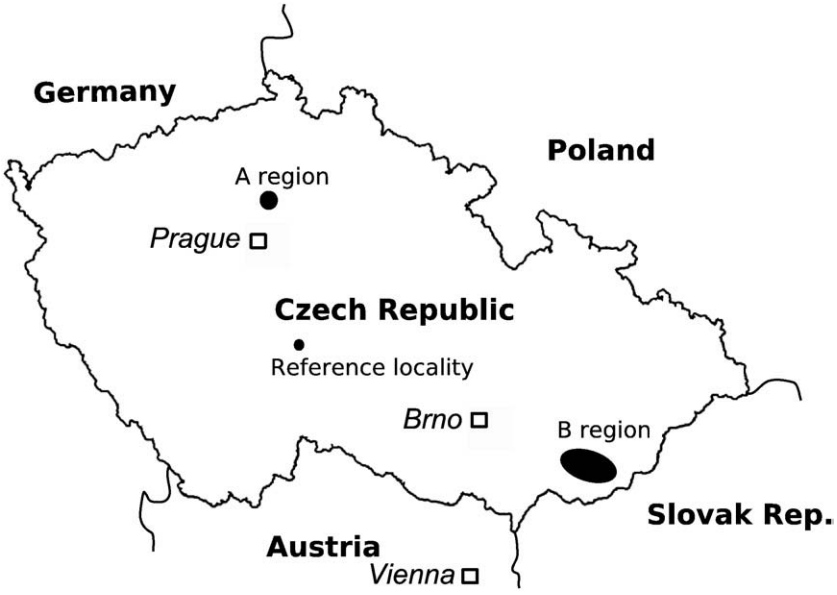

Fig. 1. Locations of studied regions within the Czech Republic.