

SPECIFIC BIOLOGICAL EFFECTS OF URBAN DUST SAMPLES-SEASONAL VARIATION

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Abstract: *Air particulate matter and dust has been associated mainly with lung and hearth diseases until recently. Recent studies indicate that pollutants present within PM could act also as endocrine disruptors (ED). Assessment of this type of effects cannot be based only on data from chemical analyses. Thus, it is important to include specific bioassays into evaluation part of the air pollution monitoring programs. In this work, we show usage of set of in vitro biotests in assessment of specific biological effects of samples of urban dust (particles smaller than 400 µm) from Brno and Ostrava at summer and wintertime. The sample extracts were assessed for PAHs and specific effects using in vitro bioassays with reporter genes linked with aryl hydrocarbon receptor (AhR) and estrogen receptor, the receptors that are traditionally linked with ED.*

Keywords: urban dust, dioxin-like activity, estrogenicity

INTRODUCTION

Dust particles in urban and industrial areas often contain complex mixtures of environmental pollutants. Contamination of dust by organic compounds is mostly evaluated by analysis of indicator classes of contaminants such as polycyclic aromatic hydrocarbons (PAHs). However, most of environmental samples contain very complex mixtures of chemicals, some of which could be even unidentified yet. These mixtures could potentially produce toxic effects that would not be expected, based on the available analytical data. Specific effects of the pollutant mixtures 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 (Novak et al. 2009).

In this study, bioassays were used to assess potential of extracts of urban

dust samples to interact with intracellular estrogen receptor and aryl hydrocarbon receptor (AhR) mediating dioxin-like activity. These receptors are known to be involved in mediating some endocrine disruptive effects of xenobiotics. AhR-mediated effects are considered a valuable marker of contamination by dioxin-like compounds that can negatively affect liver functions as well as immunity, endocrine and nervous system (Mukerjee 1998).

MATERIAL AND METHODS

The urban dust samples were collected using broom and dust extractor in Brno at locality Kotlářská and in Ostrava-Bartovice at locality Nad obcí. The sampling was performed twice at each locality. First sampling was done in January 2009 and the second in June 2009. The samples were analyzed gravimetrically and then extracted with dichloromethane. Part of the extracts was used for analyses of PAHs and the other part was transferred to DMSO and assessed for specific toxic effects by *in vitro* bioassays.

H4IIE-*luc*, rat hepato-carcinoma cells stably transfected with the luciferase gene under control of the AhR were used for analysis of dioxin-like activity of the samples. This bioassay is a well-established model for evaluation of AhR-mediated activities of pure substances as well as environmental samples. MVLN, human breast carcinoma cells transfected with a luciferase gene under control of estrogen receptor activation (Demirpence et al. 1993), was used for assessment of estrogenicity. More detailed description of the bioassay procedure can be found in Novak et al. (2009).

Results of the bioassays were reported as bioTEQ or bioEEQ expressed as [ng] of TCDD or estradiol, respectively, per [g] of urban dust based on EC25 or EC50 values from calibration curve of the respective model compound (Villeneuve et al. 2000). All samples in the bioassays were assessed in triplicates and the data comes from at least three independent experiments.

RESULTS AND DISCUSSION

The data obtained with H4IIE-*luc* model assessing AhR-mediated toxicity indicates that there is not significant difference between bioTEQ values of dust samples from Brno and Ostrava collected in the same season (fig. 1). This is in contrast with data from chemical analyses that shows that the samples from Brno contained almost three times greater PAHs levels than the samples from Ostrava. This fact indicates that the assessed PAH representatives were not the most important dioxin-like compounds in the analyzed samples. There seems to be greater dioxin-like activity produced by winter samples compared to summer dust extracts. This increase in winter is probably caused by pollutants from local heating and by greater condensation of volatile organic compounds due to lower temperatures. To our knowledge, there is no other

work assessing AhR-mediated toxicity of urban dust so far. However, more information exists on dioxin-like properties of ambient air particulate matter extracts. There have been several studies describing AhR-mediated effects of the PM₁₀ particulate phase fraction of ambient air (Clemons et al. 1998). This type of effect is a useful marker of contamination by dioxin-like compounds, which have been shown to be involved in numerous health effects such as impairment of immunity and nervous system or reproduction (Mukerjee 1998).

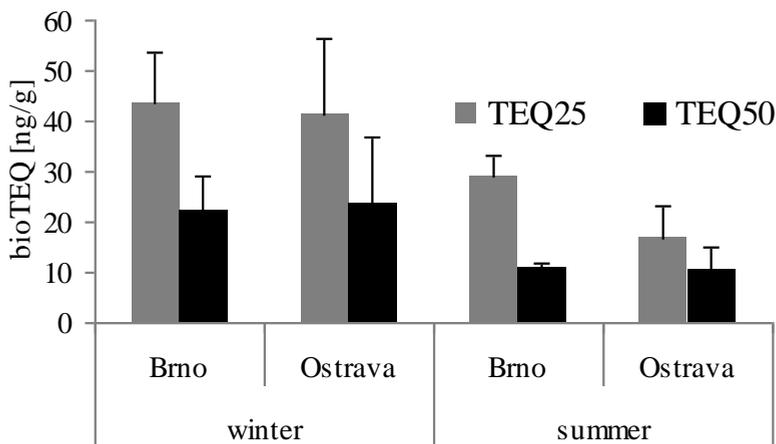


Fig. 1: TCDD-Equivalents (ng bioTEQ/g; mean + SEM) of the urban dust samples

While both samples from Ostrava and Brno winter samples did not produce estrogenic effects, the Brno summer sample extract was significantly estrogenic. Although the assessed activity did not reach EC50 of estradiol, it was sufficient to calculate EEQ based on EC25 value (tab. 1). Interactions of air pollutants with estrogen receptors has been reported previously. Estrogenic activity was found in extracts of the particulate matter of air from several urban localities (Clemons et al. 1998; Matsumoto et al. 2005). Because some PAHs have been shown to be estrogenic it has been proposed that the estrogenic activity of extracts was due to the presence of these compounds (Clemons et al. 1998). Alternatively, another report suggested that the estrogenicity of the particulate phase of air could be caused by bisphenol A, which was relatively abundant in the air on the studied urban locality (Matsumoto et al. 2005). However, based on the available data, it cannot be assumed which of these theories could explain the observed estrogenicity of Brno summer dust sample. Our data indicates that urban dust contains significant levels of dioxin-like compounds that could play a role in mediating of adverse health effects of exposed individuals; moreover, we have described presence of estrogenic compounds in one of the dust samples. This might indicate that urban dust could be a source of endocrine disruptive chemicals. This research was

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Tab. 1: Chemical biological analyses of the dust samples; PAHs- polycyclic aromatic hydrocarbons; bioTEQ25 (50)- assessed toxic equivalent of TCDD calculated from EC25 (EC50) of TCDD; EEQ- assessed estrogenic equivalent of estradiol calculated from EC25 of estradiol

Locality	Brno		Ostrava	
Season	winter	summer	winter	summer
\sum 19 PAHs [μ g/g]	16.42	13.08	5.97	3.35
bioTEQ25 [ng/g]	43.76	29.08	41.30	16.76
bioTEQ50 [ng/g]	22.07	11.11	23.54	10.33
bioEEQ25 [ng/g]	n.a.	31.26	n.a.	n.a.

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