

A COMBINED CHEMICAL AND BIOASSAY ANALYSIS OF TRAFFIC EMITTED PAHs

Ciganek^{a*}, Neca^a, Adamec^b, Janosek^a, Machala^a

^aVeterinary Research Institute, Hudcova 70, 62132 Brno, Czech Republic

^bTransport Research Centre, Krizikova 70, 63600 Brno, Czech Republic

* Corresponding author. Tel.: +420-5-41321241; fax: +420-5-412112269.

E-mail address: ciganek@vri.cz

Abstract

The objective of this study was to determine concentrations of a large series of polycyclic aromatic hydrocarbons (PAHs) and nitrated PAHs in outdoor air samples collected in the low-contaminated urban areas affected mainly by traffic emissions, and to estimate in vitro mutagenic and dioxin-like toxicity of extracts from these samples. Data on concentrations of PAHs and toxic in vitro potencies were compared in extracts obtained by different sampling methods. PAHs and their derivatives were analysed by high performance liquid chromatography with diode array and fluorescence detection and gas chromatography with mass spectrometry. The total sum of 39 PAHs under study ranged from 6.7 to 62.7; of this, sum of 16 US EPA Priority PAHs in urban air samples ranged from 3.2 to 6.2 ng.m⁻³. Phenanthrene was the prevalent PAH in all air samples tested, with concentrations up to 17.6 ng.m⁻³, followed by fluorene, fluoranthene and pyrene present mostly in the gaseous phase. Also other low-molecular-weight PAHs (with MW up to 228) were distributed mostly in gaseous phase. The particulate phase contained mostly carcinogenic PAHs, among which benzo[a]pyrene, indeno[1,2,3-cd]pyrene and benzofluoranthenes were predominant compounds (with benzo[a]pyrene reaching levels up to 1.57 ng.m⁻³). Traffic emissions were confirmed as the major source of PAHs in the airborne samples due to the presence of elevated concentrations of benzo[ghi]perylene and coronene. The most abundant nitrated PAH derivatives were nitronaphthalenes present exclusively in vapor phase; 9-nitroanthracene, 9-nitrophenantrene and 3-nitrofluoranthene were associated mostly with a PM₁₀. Bioassays for detection of the Ah receptor-mediated activity and mutagenicity in vitro were used as screen of potential adverse effects of air pollutants emitted from traffic. The major part of mutagenic and AhR-mediated activities were found to be present in the PM₁₀ fraction. Although the PM₁₀ sampling technique was found to be a suitable method regarding the subsequent determination of mutagenic and AhR-mediated activities in vitro, relative toxic potencies, associated with low-molecular-weight PAHs (such as tumor promotion and other adverse effects), could be underestimated.

Keywords: urban air, polycyclic aromatic compounds, dioxin-like toxicity, mutagenicity

1. Introduction

Traffic emissions are considered to be one of the major contributors to high concentrations of polycyclic aromatic hydrocarbons (PAHs) in urban air (Wild and Jones, 1995). Human and ecotoxicological risks associated with these airborne contaminants are related to carcinogenic and mutagenic character many of these substances and their environmental mixtures (Harada et al., 1984; Møller et al., 1985; Tokiwa, 1986; Hannigan et al., 1994; Nielsen et al., 1996; WHO, 1998). Durant et al. (1996, 1999) tested human cell mutagenicity of a large series of unsubstituted, oxygenated, and nitrated PAHs; these results provided further evidence of the

human cell mutagenicity of airborne PAHs. Also significant nongenotoxic effects of PAHs and derivatives have been reported, especially aryl hydrocarbon receptor (AhR)-mediated toxicity in vitro (Clemons et al., 1998; Machala et al., 2001).

A number of both environmental and epidemiological studies suggested that the chemical analysis alone may not provide a sufficient information about toxic potencies of a wide variety of air pollutants. Thus, biomonitoring of in vitro of toxic potencies based on specific modes of action could provide an essential supplement to chemical analysis. Urban air particulate material is a complex mixture of non-volatile chemicals released both from transportation and industrial sources. Of particular concern to human health are particles in the inhalable and respirable size ranges, referred to as PM₁₀. The basic goal of this study was to determine, whether the toxic and mutagenic compounds are associated with particulate matter. Concentrations of both unsubstituted and nitrated PAHs were determined, and the in vitro mutagenic and dioxin-like toxicity were estimated in outdoor air samples collected in relatively moderately polluted city of Brno, Czech Republic, in the areas known to be affected mainly by traffic emissions. Concentrations of PAHs and the mutagenicity and AhR-mediated activity in vitro were determined in two different types of samples reflecting either contribution of airborne particulate matter (PM₁₀) or total amount of airborne contaminants in gaseous phases.

2. Materials and Methods

2.1 Chemicals

Naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fla), pyrene (Py), benzo[a]anthracene (BaA), chrysene (Chry), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DBahA), benzo[ghi]perylene (BPE), indeno[1,2,3-cd]pyrene (IPY), biphenyl (Bip), cyclopenta[def]phenanthrene (CPPhe), benzo[c]phenanthrene (BcPhe), benzo[a]fluorene (BaFlu), benzo[b]fluorene (BbFlu), triphenylene (Tri), 1-methylpyrene (1-MePy), cyclopenta[cd]pyrene (CPP), 5-methylchrysene (5-MeChry), benzo[j]fluoranthene (BjF), benzo[e]pyrene (BeP), dibenz[a,c]anthracene (DBacA), dibenz[a,j]anthracene (DBajA), dibenz[a,l]pyrene (DBalP), 1-methylbenzo[a]pyrene (1-MeBaP), dibenz[a,e]pyrene (DBaeP), dibenz[a,i]pyrene (DBaiP), dibenz[a,h]pyrene (DBahP), were obtained from Ehrenstorfer (Augsburg, Germany). Picene (Pic), coronene (Cor), naphtho[1,2,3,4-ghi]perylene (NPer) and benz[a]coronene (BaCor) were purchased from Promochem (Wesel, Germany). 1-Methylbenzo[a]pyrene (1-MeBaP) was supplied by Midwest Research Institute (Kansas City, Missouri, USA). The other chemicals used were of the highest purity available.

Acetonitrile and methanol, both gradient grade for liquid chromatography, and tetrahydrofuran for liquid chromatography were purchased from Merck (Merck, Darmstadt, Germany). The other organic solvents used were for organic trace analysis. Ultrapure water was obtained from a Milli-Q UF Plus water system (Millipore, Molsheim, France).

2.2 Air Sampling

The study covered measurements at two sampling sites in the city of Brno, Czech Republic. Site A was at busy Kotlarska street, close to a frequented crossroad in the centre of the city. Site B was located at Kroftova street, with only a moderate traffic. Air samples were collected using two types of sampling protocols. First, particles with a diameter < 10 µm (PM₁₀) were collected by the High Volume Sampler PM₁₀ (Graseby-Andersen, Bedford, MA, USA) on a

Teflon coated glass filters Pallflex T60 20A. About 1600 m³ of air was collected during 24 hours in the sampling period of 4 consequent days. This sampling method allowed analyses of substances just adsorbed on PM₁₀ particles. Substances in other physical state were not collected. Second, the total suspended particles were captured in a series of glass fibre filter (GF) and gaseous components were adsorbed in polyurethane foam filter (PUF) (PS-1 Graseby-Andersen, Bedford, MA, USA). The following sampling materials were used: glass microfibre filters Z-5 (Filpap, Steti, Czech Republic) with a diameter of 102 mm and thickness of 350 µm and polyurethane foam filters of the polyether type N 2227 (Gumotex, Breclav, Czech Republic) with a density of 0.022 g . cm⁻³. About 400 m³ of air was sucked at a defined volume velocity through the filters placed in a large-volume pump during 24 h. Polyurethane foam filters (PUF) were pre-treated in a Soxhlet apparatus with acetone for 9 h and with dichloromethane for 8 h before use. The cleaned filters, wrapped in aluminium foil and sealed in polyethylene bags, were stored at -22 °C. Glass fibre filters (GF) were pre-treated with dichloromethane for 8 h and packed and stored as given above. After combining the extracts of both GF and PUF (samples denoted GF/PUF), this sampling method is appointed to collecting both total suspended air particles and compounds presented in vapour phase. All the samples were collected during four days (from Monday 11:00 a.m. till Friday 11:00 a.m.) in October 2001.

2.3 Extraction, Clean-up and Fractionation

The 24 h air samples (PM₁₀ or GF/PUF) from one location and sampling technique were combined and extracted with dichloromethane in a Soxhlet apparatus for 8 hours. Extracts were concentrated by rotary evaporator. Aliquots of crude extracts were used for the HPLC analysis and in vitro bioassays, while the second part of extract was cleaned-up and fractionated to four fractions (aliphatic, aromatic, slightly polar and polar fractions) using a low-pressure silica gel column. Aliquot of sample in dichloromethane was evaporated just to dryness; the residue was redissolved in 0.5 ml of hexane and applied to the top of open silica gel column. Silica gel (Silica gel 60, particle size 0.063-0.2 mm, 60 A, Brockmann and Schodder activity 2-3, Merck, Darmstadt, Germany) was activated 1 hour at 200 °C prior to their use. Columns (250x10 mm) were slurry-packed with 5 g of silica gel. Two grams of dry natrium sulphate was placed on the top of the bed. Column was washed with 10 ml of hexane prior to application of extract. Crude extracts were eluted with 10 ml of hexane (aliphatic fraction 1) followed by 21 ml of hexane:dichloromethane 1:1 (aromatic fraction 2), 20 ml of dichloromethane (semipolar fraction 3), and 30 ml of methanol (polar fraction 4). All the operations with samples were done within a shortest time possible, and lighting was reduced to a minimum. Aliquots of fractions were redissolved in acetonitrile or isooctane for HPLC/DAD and GC/MS analysis, respectively. The confirmation of HPLC data on analytes with molecular weight from 128 (naphthalene) to 278 (dibenz[a,h]anthracene) was achieved by GC/MS.

2.4 HPLC

The HPLC system consisted of a 717plus autosampler, 600E multisolvent delivery system, 996 photodiode array detector, and fluorescence detector (Waters, Milford, MA, USA). A Supelcosil LC-PAH column with particle diameter 5 µm (150x3 mm) (Supelco, Bellefonte, PA, USA) was used. A gradient with water, methanol, acetonitrile and tetrahydrofuran was applied for separation of the analytes: 0-55 min. 40-0% water, 30% acetonitrile and 30-70% methanol, 55-72 min. 30-100% acetonitrile and 70-0% methanol, 72-100 min. 100-72%

acetonitrile and 0-28% tetrahydrofuran. The flow rate of the mobile phase was 0.6 ml/min; the column temperature was set at 35 °C.

2.5 GC/MS

The GC/MS separation of PAHs was performed in a DB 5 ms fused silica capillary column (25 x 0.25 mm I.D., 0.25 µm, J. & W. Scientific, Folsom, USA). Helium at a column head pressure of 70 kPa was used as the carrier gas. The separation was achieved by programming the GC oven temperature from a 1 min hold at 70 °C to 150 °C (20 °C/min), to 260 °C (5 °C/min), and to the final temperature of 280 °C (3 °C/min) with a 2.5 min hold. The splitless injection volume was 2 µl sample in isoctane with 0.5 µl of solvent wash at the injector temperature of 250 °C. An ion trap mass spectrometer (Saturn 2100T, Varian, Palo Alto, CA, USA) was used for the detection and identification of the analytes. The transfer line and MS were kept at 250 °C and 220 °C, respectively. The mass spectrometer was operated in EI mode at electron ionisation energy of 70 eV. A mass range of m/z 80-310 was scanned at a rate of 1 scan/s (4 µScans). Solvent delay was 100 seconds.

2.6 Biological analysis

2.6.1 Mutagenicity assay

Mutagenic activity of crude extracts was determined using the Ames plate incorporation assay (Ames et al., 1975) with the Salmonella typhimurium test strains TA98 and YG1041; the latter one possessing high nitroreductase and O-acetyltransferase activities (Hagiwara et al., 1993). The mutagenicity of the crude extracts was assayed in five doses, ranging from 3 to 300 µg EOM per plate for TA98, and from 0.3 to 300 µg EOM/plate for YG1041. All assays were carried out in triplicates, either with or without S9 mix. The data presented are mean values from three independent experiments.

2.6.2 DR-CALUX assay

The AhR-inducing potencies of the DCM extracts were determined in the DR-CALUX assay as described previously (Vondracek et al., 2001). Briefly, the rat hepatoma H4IIE cell line, stable transfected with a luciferase reporter gene under the control of dioxin-responsive enhancers, was used to screen for compounds which elicit AhR-mediated activity (Murk et al., 1997). The cells were grown in minimal essential medium (α -MEM, Sigma) supplemented with 10% heat-inactivated fetal bovine serum. All assays were performed in 96-well cell culture plates (Costar, Cambridge, MA, USA); 24 h after seeding (90–100% confluence), the cells were dosed with serial dilution of the samples or the reference toxicant TCDD dissolved in dimethylsulfoxide (DMSO). After 24 h of exposure, the medium was removed, the cells were washed with 0.5× phosphate-buffered saline, luciferase was extracted with a low salt lysis buffer (10mM Tris, 2mM DTT, 2mM 1,2-diamin cyclic hexane-N,N,N',N'-tetraacetic acid, pH 7.8) and the plates were frozen at -80°C. The luciferase activity was then measured on a luminometer Luminoscan using the Luciferase Monitoring Kit (Labsystems, Turku, Finland). The toxic equivalents related to 2,3,7,8-TCDD were calculated as pg TCDD/micrograms of EOM from EC₅₀ dose-response curves (i.e. picograms of EOM causing the same induction of luciferase activity a EC₅₀ of TCDD) and the statistical analysis was carried out using the MS Excel software (Microsoft, USA) and SlideWrite (Advanced Graphics Software, Carlsbad, CA, USA).

3. Results and Discussion

3.1. Concentrations of polycyclic aromatic hydrocarbons (PAHs)

In the GF/PUF and PM₁₀ samples collected at two urban stations, concentrations of 43 unsubstituted PAHs were determined by HPLC (Tab. 1-2). The data on concentrations of lower-molecular-weight PAHs (up to molecular weight 272) were confirmed by the GC/MS analysis. Nine nitrated PAH derivatives were determined after fractionation of airborne samples on a silica gel column and results are shown in Table 3. Several high-molecular-weight PAHs, benzo[a]perylene, dibenzo[a,k]fluoranthene, dibenzo[a,e]fluoranthene, and naphtho[2,3-a]pyrene, were not detected in the samples (in the detection limit 0.01 ng . m⁻³). The composition of remaining 39 PAHs led to the following conclusions:

1. PAHs concentrations in all airborne samples were low and only slightly above the background levels reported elsewhere. The average concentration of 16 PAHs in the city air samples range from a few ng.m⁻³ up to 100 ng.m⁻³ (Lin et al., 1999; Schnelle-Krei et al., 2001; Yassaa et al., 2001; Park et al., 2001). In the Central European regional background monitoring, the average observed concentrations of 16 US EPA priority PAHs 36. 3 ng.m⁻³ have been reported in the year 1997 (Holoubek et al., 2001). In our study, concentrations of 39 PAHs were determined by HPLC/DAD/FLD and GC/MS. Total average concentrations of 16 US EPA priority PAHs and other PAHs in GF/PUF and PM₁₀ samples ranged from 3.2 to 46.2 and 3.5 to 16.5 ng.m⁻³, respectively. The samples were collected from city areas with low industrial and domestic heating emissions. This fact and elevated concentrations of benzo[ghi]perylene and coronene in airborne samples demonstrated a high contribution of traffic emissions to PAHs concentration (Nielsen, 1996). Phenanthrene was the prevalent PAH in all the air samples, with concentrations up to 23.3 ng.m⁻³ and average concentration up to 17. 6 ng.m⁻³. Particulate phase contained mainly carcinogenic and mutagenic PAHs, among which benzo[a]pyrene and indeno[1,2,3-cd]pyrene were predominated with concentration up to 1.57 and 1.42 ng.m⁻³, respectively.
2. Compared with the high volume sampling of the PM₁₀ fraction, the sampling by GF/PUF method gave several times higher amount of low-molecular-weight PAHs yielded several times higher amount of low-molecular-weight US EPA priority PAHs (Nos. 1-8 in Table 1) and other low-molecular-weight PAHs (Nos. 17-23, see Table 2). The carcinogenic US EPA priority PAHs (Nos. 9-16 in Table 1) and additional 15 high-molecular-weight PAHs detected in the samples were found in the PM₁₀ samples in concentrations approaching those found in the GF/PUF samples. Thus, selection of the high-volume PM₁₀ sampling protocol can significantly affect results of chemical analysis of PAHs and other contaminants. This fact may lead to underestimation of overall in vitro toxicity of air samples, determined in bioassays.
3. With exception of nitronaphthalenes, nitrated PAHs were found at significant concentrations in the PM₁₀ samples (Table 3). Higher-molecular-weight nitrated PAH derivatives under study (6-nitrochrysene and 6-nitrobenzo[a]pyrene) were not detected. The concentration of nitro-PAHs in urban air have been previously reported in the range from 0.004 to 0.431 ng.m⁻³ (Dimashki et al., 2000; Marino, et al., 2000; Feilberg et al., 2001). In this study, the total concentrations of nitrated PAHs surpassed 1 ng.m⁻³ at the most contaminated site. Thus, their relatively high concentrations form a relatively important part of air contaminants produced by transportation.

3.2 In vitro bioassays of mutagenic and AhR-mediated activities

The genotoxic potencies of air sample extracts are often monitored by the use of the Salmonella typhimurium/mammalian microsome assay (Ames test). Ambient air contains a complex mixture of organic chemicals such as aliphatic hydrocarbons, PAHs, polar substituted PAHs and other polar organic substances (Bodzek et al., 1993; Yassaa et al., 2001; Lee et al., 2001). Some PAHs are potentially responsible for the mutagenic activity detected in the presence of the hepatic mammalian S9 fraction (+S9), because they usually require metabolic activation to form biologically reactive metabolites. Direct-acting mutagenicity (without metabolic activation by S9 fraction) has been attributed to nitro-PAHs and other, mostly chemically not characterised compounds (Møler et al., 1985; Hannigan et al., 1994; Cerna et al., 2000). The DR-CALUX assay detects one of most important nongenotoxic effects of PAHs, AhR-mediated toxicity, which presents a different mode(s) of toxic action (Machala et al., Mutation Res., 2001). Therefore, a combination of the two bioassays is suitable to screen toxic potencies of contaminants present in the environmental samples.

In vitro mutagenic and AhR-inducing potencies of the GF/PUF and PM10 crude extracts were determined in the Ames and DR-CALUX bioassays, respectively (Table 4-5). Persistent aromatic contaminants possessing the AhR-mediated activity (polychlorinated biphenyls, dibenzo-p-dioxins and dibenzofurans) did not affect this type of activity in our samples as shown by results of DR-CALUX bioassay after treatment of samples on H₂SO₄/silica gel column (Machala, personal communication). Toxic in vitro potencies of PAHs slightly lower in PM10 samples, however, most of the toxic potencies was associated with particulate matter. This is in agreement with the reported mutagenic and AhR-inducing potencies of individual PAHs under study (ref. Table 1-2). Also the presence of majority of direct mutagens in PM10 samples was confirmed by Ames test without metabolic activation(-S9), although the chemical identification of these mutagens was not performed. Therefore, the study clearly showed that sampling of airborne PM10 fraction is suitable for in vitro assays of mutagenicity and AhR-mediated activity.

Acknowledgements Authors wish to thank to Dr. J. Totusek (Faculty of Medicine, Masaryk University, Brno) for his skilful performance of the Ames assay. This work was supported by the grant No. VaV 801/210/109 by the Czech Ministry of Transport and Communications and supported in part by the Czech Ministry of Agriculture, grant No. MZE-M03-99-01, respectively.

References

- Ames BN, McCann J, Yamasaki E. Methods for carcinogens and mutagens with *Salmonella* mammalian microsome mutagenicity test. *Mutat Res* 1975; 31: 347-364.
- Bodzek D, Tyrpien K, Warzecha L. Identification of oxygen derivatives of polycyclic aromatic hydrocarbons in airborne particulate matter of Upper Silesia (Poland). *Intern J Environ Anal Chem* 1993; 52: 75-85.
- Cerna M, Pochmanova D, Pastorkova A, Benes I, Lenicek J, Topinka J, Binkova B. Genotoxicity of urban air pollutants in the Czech Republic Part I. Bacterial mutagenic potencies of organic compounds adsorbed on PM10 particles. *Mutat Res* 2000; 469: 71-82.
- Clemons JH, Allan LM, Marvin CH, Wu Z, McCarry BE, Bryant DW, Zacharewski TR. Evidence of Estrogen- and TCDD-like activities in crude and fractionated extracts of PM₁₀ air particulate material using in vitro gene expression assay. *Environ Sci Technol* 1998; 32: 1853-1860.
- Dimashki M, Harrad S, Harrison RM. Measurement of nitro-PAH in the atmospheres of two cities. *Atmos Environ* 2000; 34: 2459-2469.

- Durant JL, Busby WF Jr, Lafleur AL, Penman BW, Crespi CL. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. *Mutat Res* 1996; 371: 123-157.
- Durant JL, Lafleur AL, Busby WF Jr, Donhoffner LL, Penman BW, Crespi CL. Mutagenicity of C₂₄H₁₄ PAH in human cells expressing CYP1A1. *Mutat Res* 1999; 446: 1-14.
- Feilberg A, Poulsen MWB, Nielsen T, Skov H. Occurrence and sources of particulate nitro-polycyclic aromatic hydrocarbons in ambient air in Denmark. *Atmos Environ* 2001; 35: 353-366.
- Hagiwara Y, Watanabe M, Oda Y, Sofuni T, Nohmi T. Specificity and sensitivity of *Salmonella typhimurium* YG1041 and YG1042 strains possessing elevated levels of both nitroreductase and acetyltransferase activity. *Mutat Res* 1993; 291: 171-180.
- Hannigan MP, Cass GR, Lafleur AL, Longwell JP, Thilly WG. Bacterial mutagenicity of urban organic aerosol sources in comparison to atmospheric samples. *Environ Sci Technol* 1994; 28: 2014-2024.
- Harada T, Yamauchi T, Sawai K, Yamamura T, Koseki Y, Ishii T. In situ emission levels of carcinogenic and mutagenic compounds from diesel and gasoline engine vehicles on an expressway. *Environ Sci Technol* 1984; 18: 895-902.
- Holoubek I, Ansorgová A, Shatalov V, Dutchak S, Kohoutek J. Regional background monitoring of PBT compounds. *Environ Sci Pollut Res* 2001; 8: 1-11.
- Lee SC, Ho KF, Chan LY, Zielinska B, Chow JC. Polycyclic aromatic hydrocarbons (PAHs) and carbonyl compounds in urban atmosphere of Hong Kong. *Atmos Environ* 2001; 35: 5949-5960.
- Lin LH, Harrison RM, Harrad S. The contribution of traffic to atmospheric concentrations of polycyclic aromatic hydrocarbons. *Environ Sci Technol* 1999; 33 3538-3542.
- Machala M, Vondracek J, Blaha L, Ciganek M, Neca J. Aryl hydrocarbon receptor-mediated activity of mutagenic aromatic hydrocarbons determined using in vitro reporter gene assay. *Mutat Res* 2001; 497: 49-62.
- Marino F, Cecinato A, Siskos PA. Nitro-PAH in ambient particulate matter in the atmosphere of Athens. *Chemosphere* 2000; 40: 533-537.
- Møller M, Hagen I, Ramdahl. Mutagenicity of polycyclic aromatic compounds (PAC) identified in source emissions and ambient air. *Mutat Res* 1985; 157: 149-156.
- Murk AJ, Leonards PEG, Bulder AS, Jonas AS, Rozemeijer MJC, Denison MS, Koeman JH, Brouwer A. The CALUX (chemical-activated luciferase expression) assay adopted and validated for measuring TCDD equivalents in blood plasma. *Environ Toxicol Chem* 1997; 16: 1583-1589.
- Nielsen T. Traffic contribution of polycyclic aromatic hydrocarbons in the center of a large city. *Atmos Environ* 1996; 20: 3481-3490.
- Park JS, Wade L, Sweet S. Atmospheric distribution of polycyclic aromatic hydrocarbons and deposition to Galveston Bay, Texas, USA. *Atmos Environ* 2001; 35: 3241-3249.
- Schnelle-Kreis J, Gebefügi I, Welzl G, Jaensch T, Kettrup A. Occurrence of particle-associated polycyclic aromatic compounds in ambient air of the city of Munich. *Atmos Environ Suppl No. 1* 2001; 35: S71-S81.
- Tokiwa H, Ohnishi Y. Mutagenicity and carcinogenicity of nitroarenes and their sources in the environment. *CRC Critical Reviews in Toxicology* 1986; 17: 23-60
- Vondracek J, Machala M, Minksova K, Blaha L, Murk AJ, Kozubik A, Hofmanova J, Hilscherova K, Ulrich R, Ciganek M, Neca J, Svrckova D, Holoubek I. Monitoring river sediments contaminated predominantly with polycyclic aromatic hydrocarbons by chemical and in vitro bioassay techniques. *Environ Toxicol Chem* 2001; 20: 1499-1506.
- Yassaa N, Meklati BY, Cecinato A, Marino F. Particulate n-alkanes, a-alkanoic acids and polycyclic aromatic hydrocarbons in the atmosphere of Algiers city area. *Atmos Environ* 2001; 35: 1843-1851.
- WHO. Selected non-heterocyclic polycyclic aromatic hydrocarbons, *Environmental Health*

Criteria 202, World Health Organization, Geneva, 1998, pp 883.

WHO. Guidelines for air quality. World Health Organisation, Geneva, 2000, pp 185;
<http://who.int/peh>.

Wild SR, Jones KC. Polynuclear aromatic hydrocarbons in the United Kingdom environment: A preliminary source inventory and budget. Environ Polut 1995; 88: 91-108.

Table 1: Average concentration of unsubstituted PAHs in airborne samples

Analytes	MW	Mutagenic activity ¹	AhR-mediated activity ²	Concentration [ng.m ⁻³]			
				Site A		Site B	
				GF/PUF	PM ₁₀	GF/PUF	PM ₁₀
low molecular weight US EPA priority PAHs							
1 Nap	128	n.d.	-	0,12	0,01	0,04	0,01
2 Acy	152	+	-	3,62	0,01	1,27	0,00
3 Ace	154	n.d.	-	1,85	0,01	0,47	0,03
4 Flu	166	-	-	5,34	0,01	2,14	0,01
5 Phe	178	-	-	17,6	0,20	8,71	0,10
6 Ant	178	-	-	2,13	0,02	0,68	0,01
7 Fla	202	-	(+)	5,24	0,55	2,60	0,30
8 Py	202	-	(+)	5,39	0,57	1,95	0,27
"carcinogenic" US EPA priority PAHs							
9 BaA	228	+	+	0,71	0,43	0,38	0,26
10 Chry	228	+	++	0,76	0,53	0,50	0,35
11 BbF	252	+	++	0,63	0,54	0,45	0,45
12 BkF	252	+	+++	0,33	0,27	0,22	0,22
13 BaP	252	++	++	0,64	0,52	0,38	0,38
14 DBahA	278	+	+++	0,02	0,02	0,02	0,02
15 BPE	276	+	-	1,17	0,95	0,48	0,41
16 IPY	276	+	+++	0,69	0,57	0,40	0,37
Sum of PAHs (Nos. 1-16)				46,2	5,2	20,7	3,2
Sum of carcinogenic PAHs (Nos. 9-16)				3,8	2,9	2,4	2,1

Abbreviations in description of analytes, see Materials and Methods

Toxic potencies are expressed semiquantitatively [(+) < + < ++ < +++]

MW – molecular weight

¹ (Durant et al., 1996)

² (Machala et. al., 2001)

n.d. – not determined

Table 2: Average concentrations of other unsubstituted PAHs in airborne samples

Analytes	MW	Mutagenic activity ¹	AhR-mediated activity ²	Concentration [ng.m ⁻³]			
				Site A		Site B	
				GF/PUF	PM ₁₀	GF/PUF	PM ₁₀
17 Bip	154	n.d.	n.d.	1,76	0,07	0,53	0,04
18 CPPhe	190	n.d.	(-) ³	5,08	0,05	1,91	0,03
19 BcPhe	228	n.d.	+	0,32	0,12	0,18	0,07
20 BaFlu	216	-	+	0,80	0,20	0,63	0,14
21 BbFlu	216	-	+	0,76	0,15	0,47	0,10
22 Tri	228	+	(+) ³	0,53	0,18	0,28	0,13
23 1-MePy	216	n.d.	+	0,40	0,10	0,19	0,05
24 CPP	226	+++	+	2,01	1,27	0,47	0,31
25 5-MeChry	242	++	++	0,32	0,27	0,08	0,07
26 BjF	252	+	+++	0,91	0,79	0,67	0,66
27 BeP	252	+	+	0,98	0,81	0,59	0,58
28 Per	252	-	(-) ³	0,61	0,48	0,32	0,32
29 DBacA	278	n.d.	++	0,03	0,03	0,02	0,02
30 DBajA	278	+	+++	0,02	0,02	0,02	0,02
31 DBalP	302	+++	+	0,07	0,07	0,04	0,04
32 1-MeBaP	266	+	(++) ³	0,01	0,01	0,01	0,01
33 DBaeP	302	+	++	0,04	0,02	0,04	0,02
34 Pic	278	-	++	0,62	0,53	0,61	0,61
35 Cor	300	-	(-) ³	0,99	0,73	0,32	0,23
36 DBaiP	302	++	++	0,05	0,04	0,03	0,03
37 NPer	326	n.d.	(+) ³	0,06	0,04	0,02	0,01
38 DBahP	302	+	++	0,01	0,00	0,01	0,00
39 BaCor	350	n.d.	n.d.	0,10	0,07	0,05	0,03
Sum of other PAHs (Nos. 17-39)				16,5	6,0	7,5	3,5

Abbreviations in description of analytes, see Materials and Methods

Toxic potencies are expressed semiquantitatively [(+) < + < ++ < +++]

MW – molecular weight

¹ (Durant et al., 1996, 1999)

² (Machala et. al., 2001)

³ Machala, personal communication

n.d. – not determined

Table 3: Average concentrations of nitroderivatives of PAHs in airborne samples

Analytes	Concentration [ng.m ⁻³]			
	Site A		Site B	
	GF/PUF	PM ₁₀	GF/PUF	PM ₁₀
1 1-Nitronaphthalene	0,22	< 0.005	0,08	< 0.005
2 2-Nitronaphthalene	0,26	< 0.005	0,12	< 0.005
3 3-Nitrofluorene	< 0.005	< 0.005	< 0.005	< 0.005
4 9-Nitroanthracene	0,14	0,05	0,09	0,03
5 9-Nitrophenanthrene	0,12	0,13	0,16	0,05
6 3-Nitrofluoranthene	0,19	0,17	0,10	0,08
7 1-Nitropyrene	0,08	0,03	0,04	< 0.02
8 6-Nitrochrysene	< 0.02	< 0.02	< 0.02	< 0.02
9 6-Nitrobenzo[a]pyrene	< 0.02	< 0.02	< 0.02	< 0.02
Sum of Nitro-PAHs	1,01	0,38	0,59	0,16

Table 4: Mutagenicity of crude extracts of airborne samples in the Ames test

	Site A [revertants/m ³]				Site B [revertants/m ³]			
	GF/PUF		PM10		GF/PUF		PM10	
	- S9	+ S9	- S9	+ S9	- S9	+ S9	- S9	+ S9
S. typhimurium TA 98	7,7	16,3	7,2	9,6	7,6	9,7	6,5	8,1
S. typhimurium YG 1041	475	268	255	198	335	198	220	227

Comparison of two sampling techniques (TSP, PM₁₀, see Materials and Methods)

Table 5: Toxic equivalents related to 2,3,7,8-TCDD in crude extracts of airborne samples determined by the DR-CALUX assay

	Site A [pg TCDD/m ³]		Site B [pg TCDD/m ³]	
	GF/PUF	PM ₁₀	GF/PUF	PM ₁₀
crude extract	130 ± 20	100 ± 20	69 ± 9	64 ± 06

Comparison of two sampling techniques (TSP, PM₁₀, see Materials and Methods)