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VLIV ZNEČIŠTĚNÉHO OVZDUŠÍ NA OXIDAČNÍ POŠKOZENÍ DNA

IMPACTS OF AIR POLLUTION ON OXIDATIVE DNA DAMAGE

Dizertační práce

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Prohlášení:

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Poděkování

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ABSTRAKT

Práce pojednává o vlivu znečištěného ovzduší na lidský organismus. Studovány byly biomarkery biologicky efektivní dávky - biomarkery oxidačního poškození DNA, lipidů a proteinů. Studium vlivu znečištěného ovzduší bylo zaměřeno na vyhodnocení významnosti jednotlivých složek ovzduší, měření personální expozice a analýzu ukazatelů oxidačního poškození makromolekul.

Jako biologicky nejvýznamnější byla vyhodnocena frakce aerosolových částic menších než 2,5μm (PM2.5) a na ni vázané karcinogenní polycyklické aromatické uhlovodíky (k-PAU). Sledovány byly také těkavé organické látky (benzen, toluen, ethylbenzen, m,p,o-xylen; BTEX). Byla změřena personální expozice k-PAU vázaných na PM2.5 spolu s koncentracemi ve venkovním ovzduší a personální expozice BTEX. Koncentrace polutantů byly korelovány s hladinami biomarkerů v různých obdobích a v různých lokalitách. Modelovými populacemi byli řidiči autobusů v Praze, 6-10 leté děti z Teplic a Prachatic, městští strážníci a úředníci z Ostravska.

Oxidační poškození DNA bylo studováno pomocí 8-oxodeoxyguanosinu (8-oxodG), oxidační poškození lipidů pomocí 15-F2t-isoprostanu (15-F2t-IsoP) a oxidace proteinů pomocí karbonylových skupin. Oxidační poškození DNA korelovalo s expozicí PM10 a PM2.5. Jako nejlepší biomarker odrážející vliv znečištěného ovzduší se jevil 15-F2t-IsoP v krevní plazmě, který byl významně pozitivně ovlivňován personální expozicí k-PAU, B[a]P i některými VOC (benzen, toluen, m,p-xyleny). Vyšší hladiny vitamínu C a E měly protektivní vliv proti oxidačnímu poškození DNA. Exprese genu XRCC1 byla spojena s vyššími hladinami vitamínu C, může zvyšovat bázovou excizní reparaci, což by mohlo vést k rychlejšímu odstranění 8-oxodG z DNA.

Pomocí podrobného dotazníkového šetření během personálního monitoringu byly vyhodnoceny faktory významně ovlivňující personální expozici k-PAU i BTEX. Jako nejvýznamnější faktory ovlivňující expozice k-PAU byly určeny venkovní ovzduší, doprava, ETS (tabákový kouř v prostředí), domácí topení uhlím, dřevem a plynem, frekvence využívání digestoře, vaření a cestování autem. Pro BTEX byly určující vnitřní prostředí, ETS, vaření, domácí topení v krbu či plynem, cestování autem a čas trávený v restauraci. Venkovní koncentrace benzenu byly významné pro ostravskou populaci.

ABSTRACT

This thesis deal with impacts of air pollution on human health. The biomarkers of biologicaly effective dose, biomarkers of oxidative damage to DNA, lipids and proteins, were studied. We aimed at importance of individual pollutants, measured the personal exposure to these pollutants and analysed the biomarkers of oxidative damage to macromolecules.

c-PAHs (carcinogenic polycyclic aromatic hydrocarbons) bound to airborne PM2.5 (particulate matter ≤ 2.5 μm) and volatile organic compounds (benzene, toluene, ethylbenzene and m,p,o-xylenes, BTEX) were studied as ones of the biologicaly most important pollutants. Personal and outdoor concentration of c-PAHs together with personal exposure to BTEX were measured. The concentrations of pollutants were correlated with biomarker levels in different seasons and localities. Bus drivers in Prague, 6-10 years old children from Teplice and Prachatice and policemen with office workers from Ostrava region were the model populations.

Oxidative damage to DNA were measured by 8-oxodeoxyguanosine (8-oxodG), 15-F2t-isoprostanes (15-F2t-IsoP) were used to measure oxidative damage to lipids and carbonyl groups for protein oxidation. Oxidative damage to DNA correlated with PM10 and PM2.5 exposure. As the best biomarker to see the impacts of air pollution seemed to be 15-F2t-IsoP in blood plasma, was significantly influenced by personal exposure to c-PAHs, benzo[a]pyrene, benzene, toluene and m,p-xylene. The protective effect against oxidative damage to DNA had vitamins C and E. Expression of XRCC1 were connected with higher levels of vitamin C, could support base excision repair and result in better removal efficiency.

Detailed time acitivity and life style questionnaires during personal monitoring were used to assess factors significantly affecting personal exposure to c-PAHs and BTEX. As main factors affecting personal exposure to c-PAHs were evaluated outdoor air pollution, traffic, ETS (environmental tobacco smoke), home heating fuel of coal, wood or gas, frequency of exhaust fan use, cooking and commuting by car. The main determinants of BTEX personal exposure were indoor environment, ETS, cooking, home heating fireplace or gas stove, automobile use and being in a restaurant. Ostrava`s outdoor benzene pollution was a significant factor for Ostrava study participants.

OBSAH

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Příloha 9

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SEZNAM ZKRATEK

%AB.B.	procento aberantních buněk	percentage of aberrant cells (%AB.C.)
15-F2t-IsoP	15-F2t-isoprostan	15-F2t-isoprostane
8-oxodG	8-oxo-7,8-dihydro-2‘-deoxyguanosin	8-oxo-7,8-dihydro-2‘-deoxyguanosine
APEX1	apurinová/apirimidinová endonukleasa	apurinic/apyrimidinic endonuclease
B[a]P	benzo[a]pyren	benzo[a]pyrene
BER	bázová excizní reparace	base excision repair
BPDE	benzo[a]pyren diolepoxid	benzo[a]pyrene diolepoxide
BTEX	benzen, toluen, ethylbenzen, m,p,o-xylen	benzene, toluene, ethylbenzene, m,p,o-xylene
CYP1A1	cytochrom P4501A1	cytochrome P4501A1
CYP2E1	cytochrom P4502E1	cytochrome P4502E1
DNA	deoxyribonukleová kyselina	deoxyribonucleic acid
DNP	dinitrofenylhydrazon	dinitrophenylhydrazon
ELISA	imunologická metoda sloužící k detekci protilátek	Enzyme-Linked ImmunoSorbent Assay
EPHX1	epoxidhydrolasa 1	epoxide hydrolase 1
ETS	tabákový kouř v prostředí	environmental tobacco smoke
FG/100	genomická frekvence translokací na 100 buněk	genomic frequency of translocation per 100 cells
GSTM1	glutathion S-transferasa M1	glutathione S-transferase M1
GSTP1	glutathion S-transferasa P1	glutathione S-transferase P1
GSTT1	glutathion S-transferasa T1	glutathione S-transferase T1
HDL	vysokodenzitní lipoprotein	high-density lipoprotein
HiVol	velkoobjemový vzorkovač ovzduší	High Volume Air Sampler
HPLC	vysokoúčinná kapalinová chromatografie	High Performance Liquid Chromatography
IARC	Mezinárodní agentura pro výzkum rakoviny	International Agency for Research on Cancer
k-PAU	karcinogenní polycyklické aromatické uhlovodíky	carcinogenic polycyclic aromatic hydrocarbons (c-PAHs)

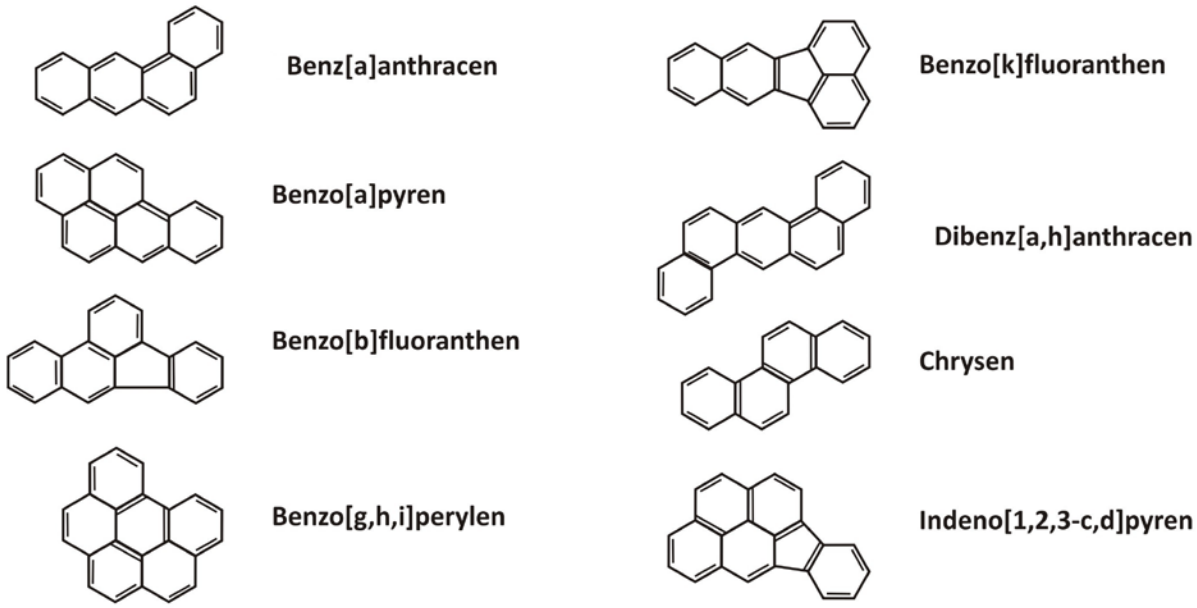
LDL	nízkodenzitní lipoprotein	low-density lipoprotein
LIG4	ligasa 4	ligase 4
MDA	malondialdehyd	malondialdehyde
MHD	městská hromadná doprava	public transport
NHEJ	nehomologní rekombinace	non-homologous end-joining
NOx	souhrnné označení pro oxidy dusíku	a term for the nitrogen oxide air pollutants NO and NO_2
OGG1	8-oxoguanin DNA glykosylasa	8-oxoguanine DNA glycosylase
PAU	polycyklické aromatické uhlovodíky	polycyclic aromatic hydrocarbons
PM	aerosolové částice	particulate matter
PM2.5	aerosolové částice o aerodynamickém průměru ≤ 2,5 μm	particulate matter of aerometric diameter ≤ 2.5 μm
PM10	aerosolové částice o aerodynamickém průměru ≤ 10 μm	particulate matter of aerometric diameter ≤ 10 μm
PV1.7	personální vzorkovač o průtoku 1,7 l/min.	personal sampler with flow rate 1.7 l/min.
ROS	reaktivní formy kyslíku	reactive oxygen species
VOC	těkavé organické látky	volatile organic compounds
WHO	Světová zdravotnická organizace	World Health Organization
XRCC (1, 4, 5, 6)	DNA reparační proteiny	DNA repair proteins

1. ÚVOD

Znečištěné ovzduší je dnes jedním z hlavních environmentálních problémů. Globální změny klimatu spolu s vlivy znečištěného ovzduší na živé organismy, člověka nevyjímaje, jsou nejvíce řešenými problémy v této oblasti zájmu. Ovzduší, které je směsí plynů s vodními kapičkami či ledovými krystalky, obsahuje i různé další znečišťující příměsi původu přírodního, ale i antropogenního. Znečišťující látky mohou být neškodné, ale také mohou ovlivňovat chování celé planety (vliv CO_2 na globální oteplování), či mohou mít vliv na živé organismy (toxicita určitých látek do ovzduší vypouštěných). S průmyslovou činností člověka přibylo látek do ovzduší uvolňovaných a s přibývajícími dopady na zdraví žijících organismů přibyly i studie věnující se této problematice.

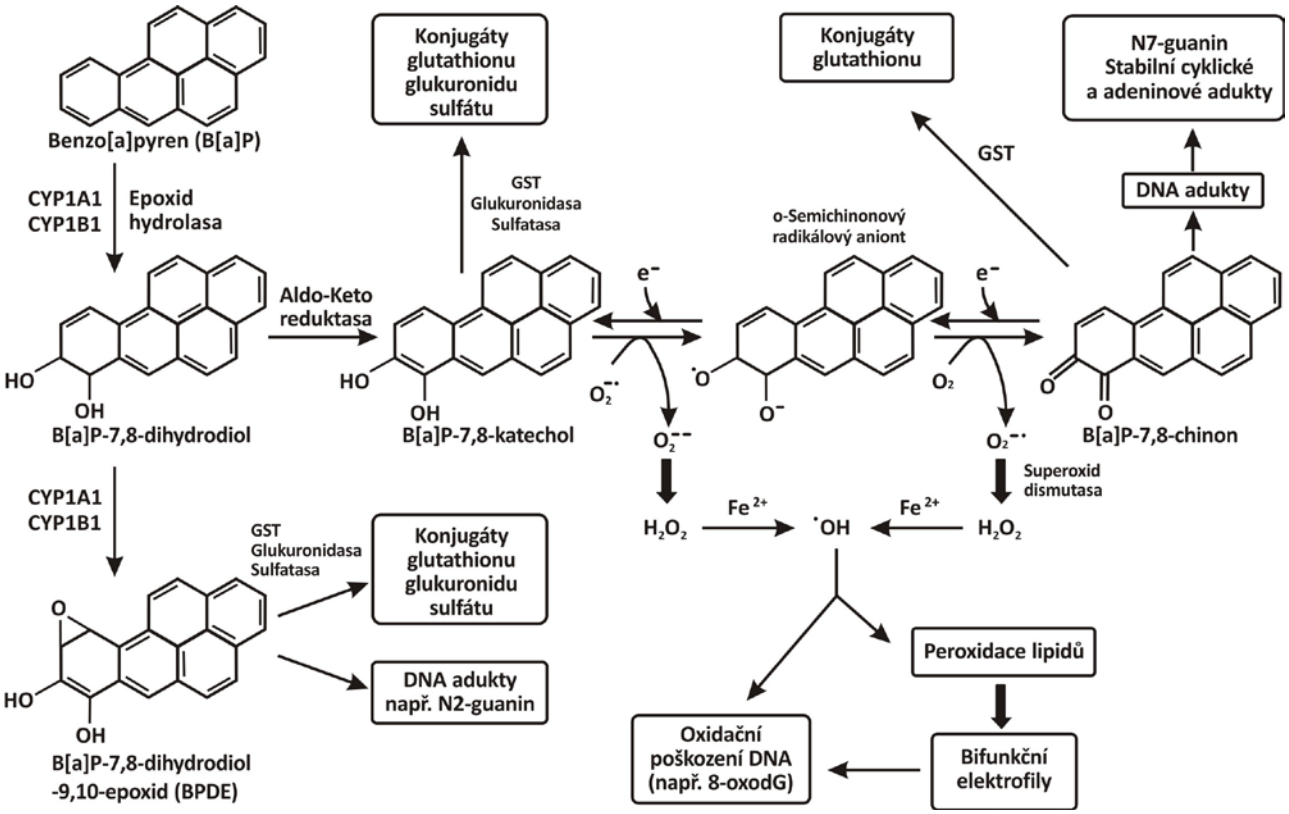
1.1. Karcinogenní polycyklické aromatické uhlovodíky

Mezi biologicky nejvýznamnější látky patří aerosolové částice PM2.5 (aerosolové částice $\leq$ 2,5 μm) a látky na ně navázané, jako jsou karcinogenní polycyklické aromatické uhlovodíky (k-PAU, obr. 1) (Binkova a kol., 1999). PM2.5 jsou významné vzhledem k jejich malé velikosti a relativně velkému povrchu, na který se zachytávají další látky, jež mohou být zdraví škodlivé. Díky své malé velikosti mají schopnost pronikat do lidského organismu respirační cestou. Frakce aerosolových částic do velikosti 2,5 μm je nejvíce zastoupenou frakcí v ovzduší, může reprezentovat i přes 90% prachových částic (Sugita a kol., 2004). Na tyto jemné částice se kromě k-PAU navazují i další látky (deriváty nitro- a alkyl PAU, toxické kovy apod.) a významně tím mění povahu, chování, toxicitu a vliv těchto částic na lidský organismus (Lewtas, 2007; Nishioka a Lewtas, 1992; Prokeš a kol., 2005; Sorensen a kol., 2003). Nejnovější studie ukazují, že jedněmi z nejvíce biologicky významných látek vázaných na PM2.5 jsou právě k-PAU (Binkova a kol., 1999; Topinka a kol., 2011). Některé PAU obsahující 4 a více benzenových jader jsou hodnoceny jako mutagenní a karcinogenní (Binkova a Sram, 2004; Lewtas, 2007). Mezinárodní agentura pro výzkum rakoviny (IARC) řadí 8 sloučenin, kterým jsme se věnovali, mezi karcinogeny skupiny 1 – prokázané lidské karcinogeny (benzo[a]pyren, B[a]P), 2A – pravděpodobné lidské karcinogeny (dibenz[a,h]anthracen), 2B – možné lidské karcinogeny (benz[a]anthracen, benzo[b]fluoranthen, benzo[k]fluoranthen, chrysen, indeno[1,2,3-c,d]pyren); a benzo[g,h,i]perylen je zařazen do skupiny 3 – neklasifikovaný jako lidský karcinogen (IARC, 2011).



Obr. 1: Jednotlivé PAU zařazené do skupiny k-PAU.

Inhalované PAU jsou schopny prostupovat skrze stěnu respiračního traktu. Absorpce může být ovlivňována lipofilitou jednotlivých PAU a také velikostí a charakterem částic, na které jsou adsorbovány (WHO, 2010). Karcinogenní a mutagenní účinky PAU se projevují až po jejich metabolické aktivaci (obr. 2). Jsou známy tři dráhy metabolické aktivace: dráha zahrnující tvorbu dihydrodiol epoxidu, dráha zahrnující tvorbu radikálových kationtů využívajících jednoelektronové oxidace a dráha vedoucí k tvorbě o-chinonů. Reaktivní intermediáty, které se při metabolické aktivaci PAU vytvářejí, se mohou vázat na DNA, vytvářet adukty, způsobovat vznik mutací, a tím zvyšovat riziko nádorových onemocnění. Dráha, při níž vznikají o-chinony, vede k redoxním reakcím, při nichž se vytvářejí reaktivní formy kyslíku (ROS), které pak způsobují oxidační poškození makromolekul (Rossner a kol., 2011).

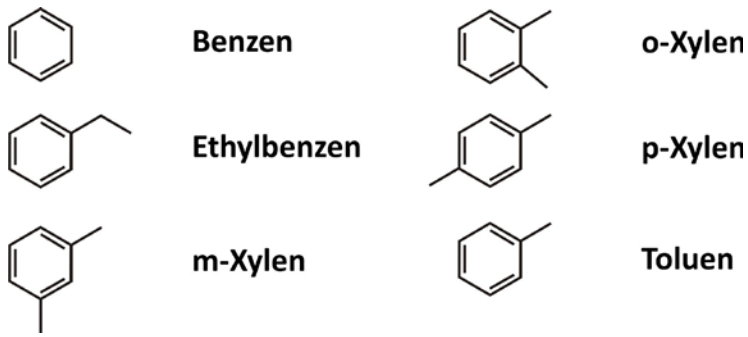


Obr. 2: Mechanismus metabolismu, aktivace a detoxifikace benzo[a]pyrenu (Singh a kol., 2007).

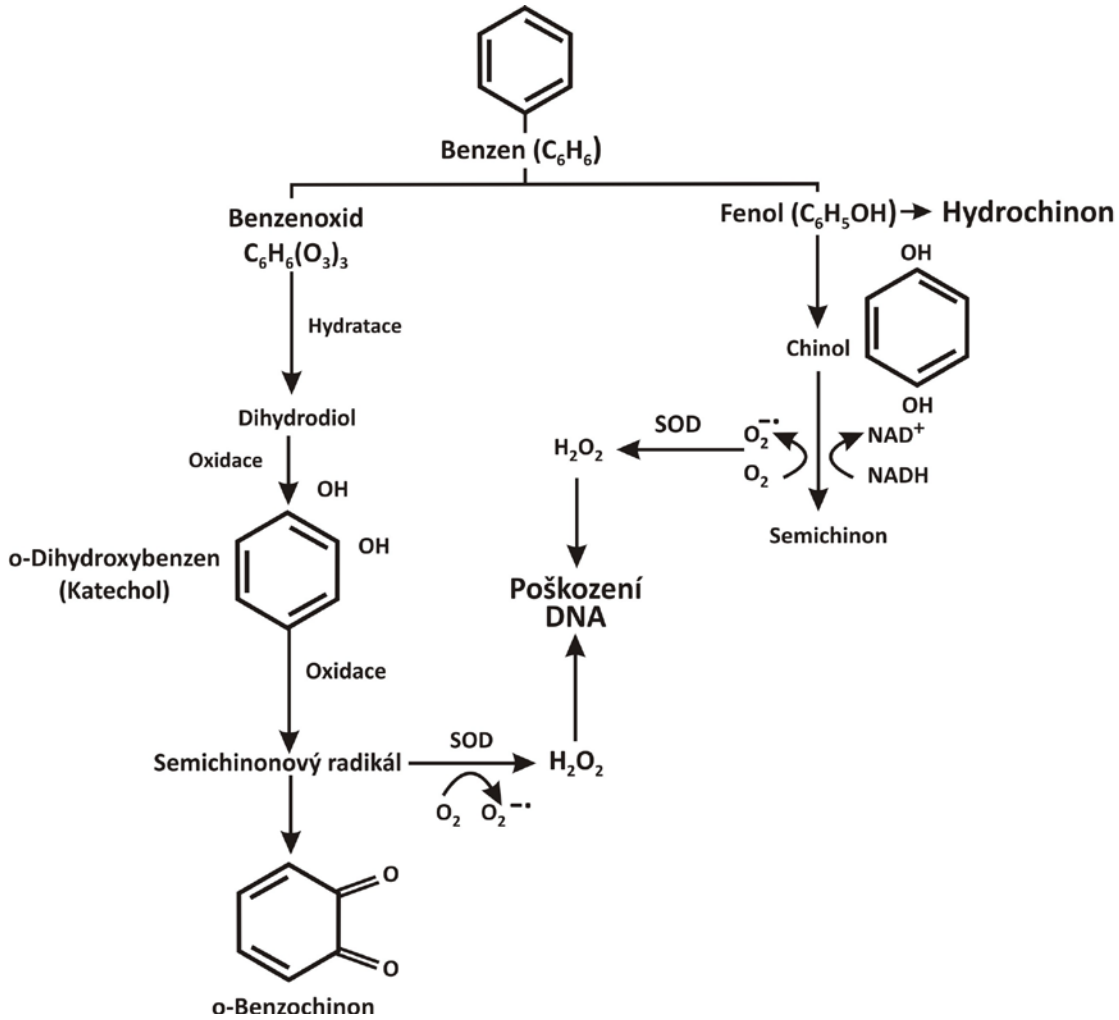
K hlavním zdrojům těchto látek patří nedokonalé spalovací procesy materiálů obsahujících uhlík, koksárenství, hutnictví, zplyňování a zkapalňování uhlí, výroba hliníku, ale i lokální topeniště a automobilová doprava. Vysoké expozice PM2.5 mohou mít mnoho nežádoucích účinků na lidské zdraví, od akutních srdečních onemocnění přes plicní a systémový oxidační stres, záněty, riziko ischemické choroby srdeční a mnohé další. Mohou vést ke zvýšení výskytu rakoviny plic i vyšší úmrtnosti (Brunekreef a Holgate, 2002; Künzli a Tager, 2005; Peng a kol., 2009; Pope a kol., 2006).

1.2. Těkavé organické látky

Dalšími významnými látkami jsou těkavé organické látky (Volatile Organic Compounds, VOC). Zákon č. 86/2002 Sb. definuje VOC jako jakoukoli organickou sloučeninu nebo směs organických sloučenin, s výjimkou methanu, jejíž počáteční bod varu je menší nebo roven 250°C, při normálním atmosférickém tlaku 101,3 kPa. Podle Úmluvy Evropské hospodářské komise Organizace spojených národů o dálkovém znečišťování ovzduší překračující hranice států jsou to všechny organické sloučeniny antropogenního původu, jiné než methan, které jsou schopné vytvářet fotochemické oxidanty reakcí s NO_x v přítomnosti slunečního záření. My jsme se zaměřili na benzen, toluen, ethylbenzen a m,p,o-xyleny (obr. 3). Podle IARC jsou tyto látky zařazeny do následujících kategorií: benzen, jako prokázaný lidský karcinogen patří do skupiny 1, ethylbenzen byl zařazen do skupiny 2B, toluen a xyleny do skupiny 3 (IARC, 2011). Benzen je metabolizován v játrech enzymem CYP2E1 na benzen oxid, který je přeměněn na několik produktů, včetně reaktivních chinonů, které mohou indukovat oxidační poškození, obrázek 4 (Rossner a kol., 2011; Stavridis, 2008). Tyto látky jsou běžnou součástí široce užívaných produktů, jako jsou rozpouštědla, paliva, barvy a nátěrové hmoty, čistící a kosmetické přípravky. Rozhodujícím zdrojem atmosférických emisí VOC ve městech se uvádí automobilová doprava (Crebelli a kol., 2001), avšak vysoké koncentrace lze také najít v blízkosti závodů na zpracování uhlí, rafinérií a chemických závodů (Topp a kol., 2004). Benzen, toluen, ethylbenzen a m,p,o-xyleny (BTEX) jsou spojovány s celou řadou negativních zdravotních dopadů od závratí, bolestí hlavy, podráždění očních spojivek, přes vlivy neurobehaviorální, hepatotoxické, nefrotoxické a karcinogenní (Hoxha a kol., 2009; Künzli a Tager, 2005; Sorensen a kol., 2003; WHO, 2000, 2010).



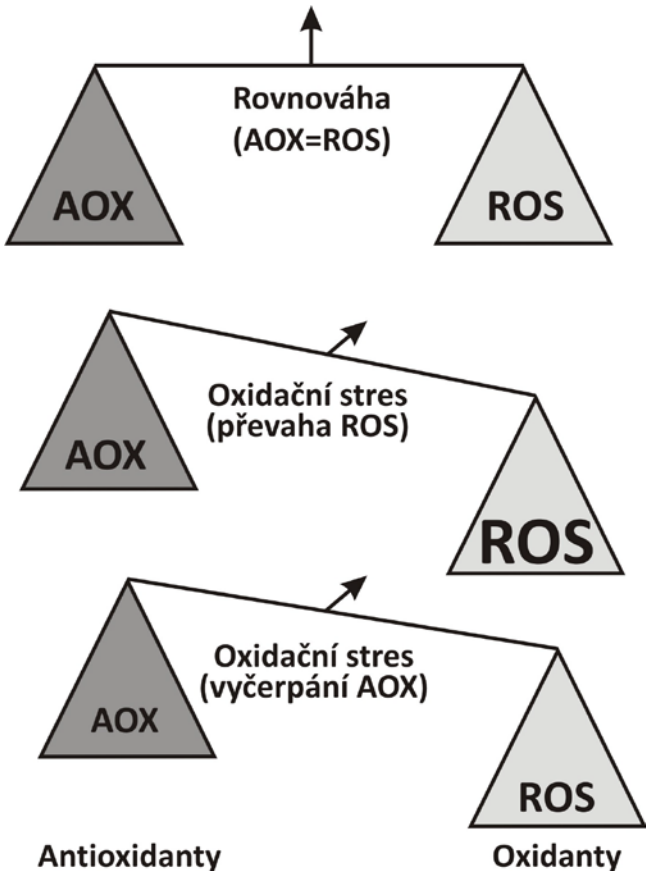
Obr. 3: Jednotlivé měřené BTEX.



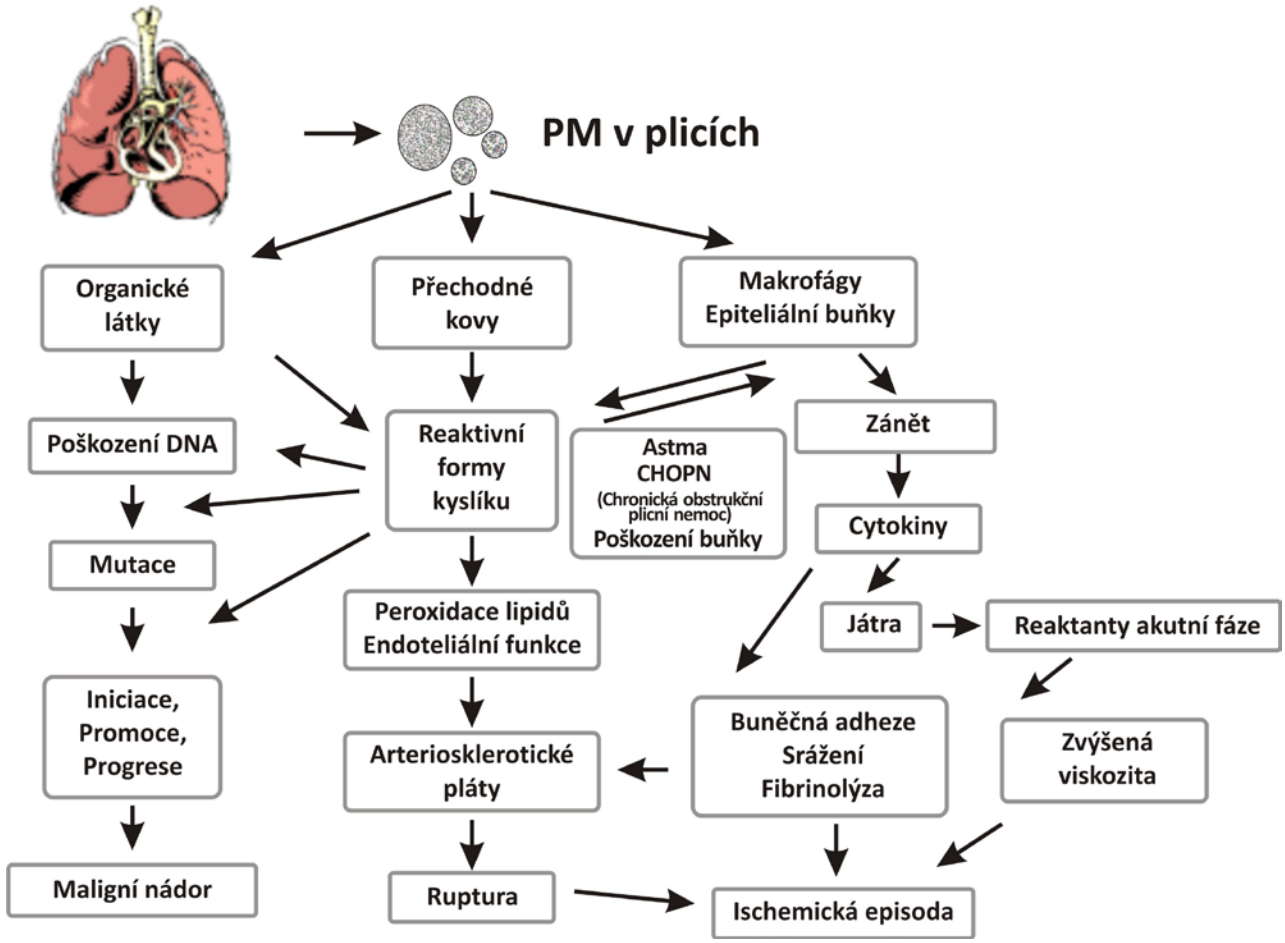
Obr. 4: Metabolismus benzenu a role H_2O_2 v poškození DNA (Stavridis, 2008).

1.3. Oxidační poškození

Aerosolové částice jsou schopné generovat či indukovat vznik volných radikálů v lidském organismu, které mohou vést ke zvýšení oxidačního stresu (Sorensen a kol., 2003). Oxidační stres je proces vyvolaný nerovnováhou mezi hladinami antioxidantů v organismu a faktorů působících jako oxidanty (obr. 5; Scandalios, 2002). U organismů využívajících aerobní metabolismus, tedy i u člověka, je oxidační poškození jevem přirozeným, proti němuž existují účinné obranné a reparační mechanismy. Oxidanty, působící prostřednictvím reaktivních forem kyslíku (ROS, Reactive Oxygen Species), můžeme podle jejich původu rozdělit na endogenní, vznikající v organismu, a exogenní, přítomné v zevním prostředí. ROS můžeme dělit podle chemické povahy na látky radikálové a neradikálové. Mezi nejvýznamnější ROS patří superoxid, hydroxylový radikál, peroxid vodíku, nebo peroxinitrit. Hlavním endogenním zdrojem oxidace v organismu je proces aerobní respirace, při němž elektrony unikající z membrán mitochondrií způsobují tvorbu superoxidu, který je velmi silným oxidačním činidlem. Oxidace doprovází také zánětlivé procesy a metabolické reakce, při nichž se účastní cytochrom P450. Mezi exogenní zdroje oxidantů řadíme faktory životního stylu (strava, kouření, tělesná aktivita) a kvalitu životního prostředí, zejména znečištění ovzduší. V optimálním případě je superoxid přeměněn superoxid dismutasami na peroxid vodíku, který je dále rozložen katalasami, nebo glutathion peroxidasami na vodu. Pokud je však expozice oxidantům příliš vysoká, nebo trvá delší dobu, antioxidační ochrana organismu selhává, dochází k oxidačnímu stresu a hromadí se oxidační poškození, které může postihnout všechny typy makromolekul, tj. DNA, lipidy i proteiny. Volné radikály se podílejí na vzniku a rozvoji četných onemocnění a chorobných stavů, mohou vyvolávat přímo vznik onemocnění anebo zhoršení či komplikaci průběhu onemocnění (Racek, 2003). Oxidační poškození makromolekul je spojováno např. se vznikem nádorových onemocnění, nemocemi dýchacího a kardiovaskulárního systému, obrázek 6 (Klaunig a Kamendulis, 2004; Rössner a kol., 2009).



Obr. 5: Oxidační stres je výsledkem nerovnováhy mezi hladinami reaktivních forem kyslíku (ROS) a antioxidanty. Za normálních podmínek je buňka schopná udržet rovnováhu. Oxidační stres vznikne je-li buňka vystavena zvýšeným hladinám ROS nebo v případě vyčerpání antioxidační ochrany (Scandalios, 2002).



Obr. 6: Ústřední role oxidačního stresu a zánětu v účincích aerosolových částic (PM) v procesech zapojených do rakoviny, kardiovaskulárních a plicních onemocnění (Wild a kol., 2008).

1.4. Biomarkery oxidačního poškození

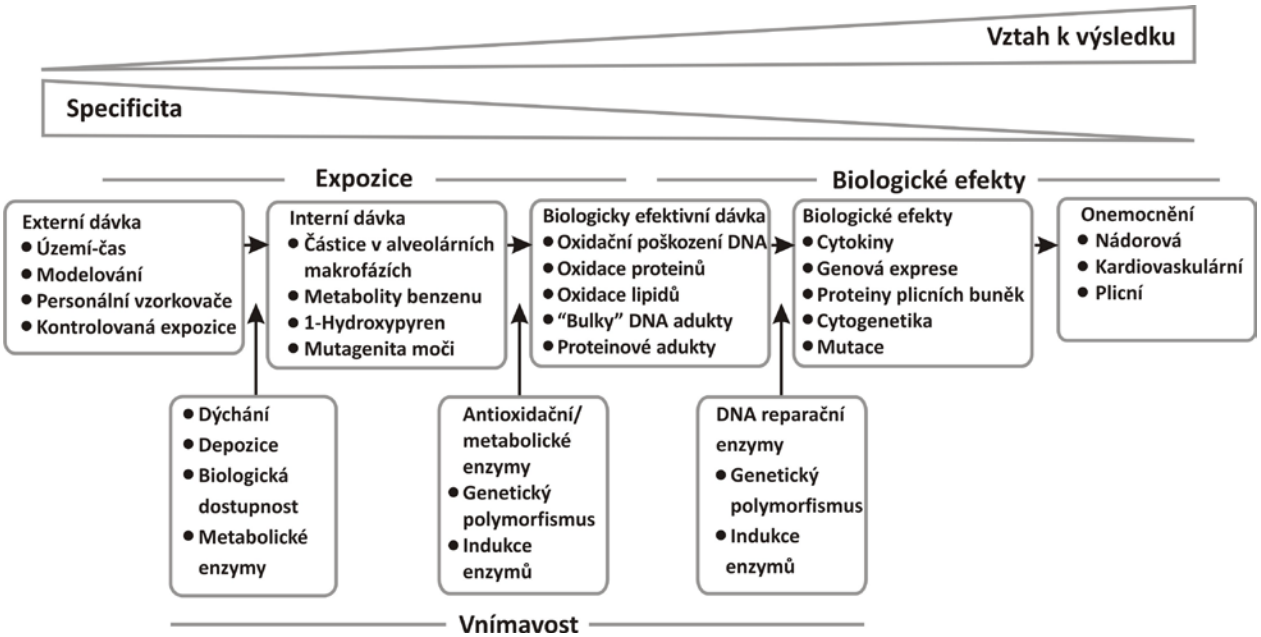
Epidemiologické studie stále spojují expozici znečištěnému ovzduší s dýchacími a kardiovaskulárními onemocněními a rakovinou (Brunekreef a kol., 2002; Pope a kol., 2002). Vzhledem ke komplexnímu složení znečištěného ovzduší je hodnocení expozice a prokázání kauzálního vztahu s biologickými efekty velmi složité. Molekulární epidemiologie může poskytnout silné nástroje použitím ukazatelů, resp. biomarkerů interní dávky, biologicky efektivní dávky, raného biologického efektu, biomarkerů individuální citlivosti, které se kombinují většinou s hodnocením externí dávky využívající personální monitory. Molekulární epidemiologické paradigma ve vztahu ke znečištění ovzduší je znázorněno na obrázku 7 (Wild a kol., 2008). My jsme si vybrali biomarkery biologicky efektivní dávky – biomarkery oxidačního

poškození. Z hlediska dopadu na lidské zdraví je poškození DNA působením ROS nejzávažnější. Vlivem ROS na DNA dochází ke změnám vlastností bází, konkrétně jejich schopnosti párovat se s komplementárními bázemi v druhém řetězci DNA (Cooke a kol., 2003). V konečném důsledku může tento fakt vést ke vzniku mutací. Kromě toho mohou ROS indukovat jedno- nebo dvouřetězcové zlomy DNA. Nejčastějším produktem oxidace bází v DNA je 8-oxodeoxyguanosin (8-oxodG), kterému se věnuje v odborné literatuře velká pozornost. 8-oxodG se páruje místo cytosinu s adeninem a pokud tato modifikovaná báze není včas rozpoznána a opravena reparačním systémem buňky, dojde v dalším replikačním cyklu k fixaci mutace. Důsledkem přítomnosti 8-oxodG v DNA je transverse GC-TA. Organismus má však vyvinutý mechanismus odstraňování poškozených bází – 8-oxodG je vyštěpen z DNA činností enzymu 8-oxogaunin DNA glykosylasy/AP lyasy a vylučován v moči, kde je možné jeho přítomnost změřit, a tak odhadnout míru oxidace DNA v organismu. Je však třeba zdůraznit, že hladiny 8-oxodG v moči jsou ovlivněny i dalšími faktory, zejména aktivitou reparačních systémů organismu.

Působením ROS na lipidy, konkrétně na nenasycené mastné kyseliny, vznikají reaktivní peroxidované lipidy, které reagují s dalšími molekulami v buňce a tím šíří oxidační poškození (Montuschi a kol., 2004). Peroxidované lipidy mohou ovlivnit i míru oxidace dalších makromolekul – DNA nebo proteinů. Kromě toho způsobuje přítomnost peroxidovaných lipidů v buněčných membránách změny jejich fluidity a inaktivaci membránově vázaných proteinů. Míra peroxidace lipidů se dá stanovit měřením produktů rozkladu lipid peroxidů; často sledovaným markerem je malondialdehyd (MDA), jehož přítomnost lze stanovit reakcí s kyselinou thiobarbiturovou. Nevýhodou tohoto druhu analýzy je relativně nízká specifita a také fakt, že MDA prezentuje jen malé množství (cca 1 %) produktů peroxidace lipidů. V průběhu 90.let minulého století se stále častěji začalo provádět měření isoprostanů jako ukazatelů peroxidace lipidů (Morrow a kol., 1990; Roberts a Morrow, 2000). Isoprostany vznikají v buněčných membránách působením ROS na arachidonovou kyselinu. Z membrán jsou odštěpovány působením fosfolipas a buď cirkulují v krevní plazmě, nebo jsou vylučovány močí. Obě tělní tekutiny je možno pro analýzu hladin isoprostanů použít. Nejlépe prostudovaným zástupcem isoprostanů je 15-F2t-isoprostan (15-F2t-IsoP), který je v současné době považován za nejspolehlivější ukazatel peroxidace lipidů. Byla prokázána souvislost mezi zvýšenými hladinami 15-F2t-IsoP a některými onemocněními (např. kardiovaskulární onemocnění, nemoci

plic, jater, ledvin, neurologické poruchy a mnohé další) (Montuschi a kol., 2004; Pratico a kol., 2001; Puthucheary a Nathan, 2008; Wu a kol., 2008). Na rozdíl od 8-oxodG reprezentuje 15-F2t-IsoP molekulu, jejíž přítomnost v krevní plazmě a moči je bezprostředně ovlivněna působením ROS na organismus. Výhodou stanovení 15-F2t-IsoP v moči je jeho stabilita a fakt, že se nevytváří *ex vivo* (Pratico a kol., 1998). Stanovení usnadňuje též existence komerčních kitů založených na použití metody ELISA (Enzyme-Linked ImmunoSorbent Assay).

Další skupinou makromolekul, na něž ROS mohou působit, jsou proteiny. Výsledkem reakce ROS s proteiny může být jak fragmentace proteinů, tak modifikace jejich funkčních skupin za tvorby hydroperoxidů a karbonylových skupin. Hladiny karbonylových skupin jsou tradičně používaným ukazatelem oxidace proteinů. Karbonylové skupiny jsou aldehydy a ketony vytvářející se na postranních řetězcích aminokyselin i na proteinových kostrách v průběhu jejich oxidace. Důsledkem jejich tvorby může být fragmentace proteinů, jejich nežádoucí propojování a v konečném důsledku ztráta katalytické a strukturní funkce. Karbonylové skupiny se v organismu hromadí jak v souvislosti s procesem stárnutí, tak jako doprovodný jev některých onemocnění (Beal, 2002; Shacter, 2000).



Obr. 7: Paradigma molekulární biologie aplikované na oblast znečištěného ovzduší (Wild a kol., 2008).

2. CÍLE

Cílem této studie bylo posoudit vliv znečištěného ovzduší na oxidační poškození DNA. Z látek, které znečišťují ovzduší, byly vybrány ty, které mají biologicky významný vliv na lidský organismus, PM2.5 a na ně navázané karcinogenní polycyklické aromatické uhlovodíky a BTEX, hlavně benzen. Jako ukazatele biologicky efektivní dávky byly studovány biomarkery oxidačního poškození DNA, proteinů a lipidů.

V rámci dizertační práce byly řešeny následující dílčí cíle:

- studovat personální expozici k-PAU a BTEX, změřit personální expozici studovaných osob personálními monitory;
- studovat koncentrace k-PAU ve venkovním ovzduší pomocí stacionárního měření, monitorovat k-PAU ve venkovním ovzduší v čase personálního monitoringu pro možnost porovnání těchto expozic;
- analyzovat biomarkery oxidačního poškození makromolekul (DNA, proteinů a lipidů) pro posouzení možného vlivu k-PAU a BTEX na lidský organismus;
- vyhodnotit vztah mezi koncentracemi k-PAU a BTEX a biomarkery oxidačního poškození;
- vyhodnotit nejvýznamnější zdroje personální expozice k-PAU a BTEX.

3. METODIKA

V této kapitole je ve stručnosti uvedena charakterizace studovaných populací a hlavních metodik k posouzení vlivu znečištěného ovzduší na oxidační poškození DNA, které k tomuto studiu využila autorka této práce. Úplné metodiky všech jednotlivých analýz použité v různých částech studie jsou uvedeny v daných publikacích v přílohách k této práci.

3.1. Studované populace

Ukazatele oxidačního poškození byly studovány v rámci velkých molekulárně epidemiologických studií na exponovaných populacích. První zvolenou skupinou bylo 50 řidičů autobusů Dopravního podniku hlavního města Prahy, a.s. Kontrolní skupinu tvořilo 50 mužů trávících většinu pracovní doby v uzavřených prostorách (administrativní pracovníci). Do studie byli vybráni jen nekuřáci, kteří nesměli v posledních 3 měsících před monitoringem podstoupit radiografické vyšetření či očkování. Každý účastník studie vyplnil dotazník o životním stylu a zdravotním stavu. Studie probíhala ve třech obdobích (14.11.-15.12.2005, 15.05.-16.06.2006, 13.11.-13.12.2006).

Hladiny 8-oxodG se dále studovaly na skupině 1007 6-10 letých dětí z Teplic a Prachatic. Byly to děti narozené v letech 1994-1999 ženám v okresech Teplice a Prachatice v rámci projektu Výsledky těhotenství (Šrám a kol., 1996). Od narození byly u žen a dětí dotazníkovým šetřením sledovány základní informace o zaměstnání, věku, vzdělání, životním stylu (kouření, alkoholu apod.), osobní anamnézy, nemoci apod. V roce 2002 studie pokračovala sledováním dětí ve věku 4,5 roku. Ve třetí etapě bylo prodlouženo sledování zdravotního stavu dětí až do konce roku 2004, kdy ve spolupráci s dětskými lékaři a sestrami byly doplněny výpisy onemocnění a získány podrobné údaje o alergických onemocněních, včetně výsledků kožního testování reakce na alergeny a odebrány vzorky moče, které se zejména využily na analýzu kotininu jako ukazatele expozice dětí tabákovému kouři a také na analýzy 8-oxodG (Dostál a kol., 2009).

Třetí studovanou skupinou a lokalitou v této práci je skupina úředníků v nejvíce zatíženém regionu České republiky – v Ostravě. Studie byla naplánována na modelovou skupinu městských strážníků, avšak Magistrát statutárního města Ostravy nepovolil jejich účast ve studii. Do projektu se zapojila skupina úředníků Krajského úřadu v Ostravě, sídlící v centru Ostravy.

Pro dodržení původně naplánovaných modelových skupin městských strážníků se do projektu připojili městští strážníci z přilehlých zatížených měst Karviná a Havířov. Kontrolní skupinu tvořili městští strážníci z Prahy. Všechny osoby byly nekuřáci. Každý účastník studie vyplnil dotazník o životním stylu a zdravotním stavu. K odhadu vlivu jednotlivých aktivit na personální expozici se využilo podrobných dotazníků o aktivitách studovaných osob, kteří je zapisovali v průběhu monitorovacího dne každou hodinu do zvláštního dotazníku aktivit (TLAD – time location activity diary). Osoby byly sledovány v zimě (únor, březen), v létě (květen, červen) roku 2009 a v zimě (leden, únor) roku 2010.

3.2. Personální monitoring

U sledovaných skupin se měřila personální expozice k-PAU vázaných na PM2.5 a BTEX. K měření k-PAU byl využit již zavedený monitoring k-PAU pomocí personálního vzorkovače PV1.7, viz. obrázek 8 (URG Corp., USA). k-PAU byly extrahovány z membránových filtrů a analyzovány vysokoúčinnou kapalinovou chromatografií (High Performance Liquid Chromatography, HPLC) s fluorescenční detekcí (chemickou analýzu prováděla akreditovaná firma ALS Czech Republic s.r.o., Praha, EN ISO CSN IEC 17025). Personální expozice BTEX byly měřeny pomocí pasivních vzorkovačů Radiello, obrázek 8 (Fondazione Salvatore Maugeri, Padova, Italy). BTEX zachycené na grafitizované uhlí byly získány termální desorpcí, analyzovány plynovou chromatografií s plameno-ionizační detekcí (chemickou analýzu prováděla firma ALS Czech Republic s.r.o.).

Obr. 8: Účastník personálního monitoringu s přístroji (k-PAU jsou vzorkovány přístrojem u pravé ruky účastníka, který je napojen na čerpadlo nasávající vzduch připojené k pasu; BTEX jsou vzorkovány pasivním přístrojem umístěným u levého ramene účastníka) *(foto Ludvík Hradilek, Aktuálně.cz)*.

3.3. Stacionární monitoring

Pro výpočet vztahu mezi hladinami ukazatelů oxidačního stresu a znečištěním venkovního ovzduší se použily hodnoty ze stacionárního monitoringu. Vzorkování probíhalo pomocí velkoobjemových odběrových zařízení High Volume Air vzorkovačů (obr. 9) umístěných přímo v měřených lokalitách. Aerosolové částice PM10 byly odebírány přístrojem HiVol (Anderson, USA) v Praze-Libuši a PM2.5 byly odebírány pomocí Hivol 3000 (model ECO-HVS3000, Ecotech, Australia) v ostatních měřených lokalitách. Vzorky byly odebírány většinou v průběhu kampaní personálního monitoringu denně, 24 hodin (pokud nedošlo k předčasnému zahlcení filtru). Byly využívány filtry Pallflex 20 x 25 cm T60A20 a TX40HI20WW. Filtry byly váženy před a po expozici pro obsah zachycených aerosolových částic a uchovávány při -18°C. Extrakci organické hmoty a chemickou analýzu prováděla firma ALS Czech Republic s.r.o. Pro chybějící data PM10, PM2.5, k-PAU či BTEX v daných lokalitách personálního monitoringu bylo dále využito měření Českého hydrometeorologického ústavu.

Obr. 9: High Volume Air vzorkovač s hlavicí nasávající aerosolové částice do velikosti 2,5 μm (Ecotech, Australia).

3.4. Stanovení ukazatelů oxidačního poškození

Hladina 8-oxodG v moči byla stanovena imunologickou metodou kompetitivní ELISA (Enzyme-Linked Immuno Sorbent Assay) podle publikované metodiky (Příloha 1, Rossner a kol., 2007; Yin a kol., 1995). Detekce je založena na použití primární protilátky specificky rozpoznávající 8-oxodG v biologickém materiálu (výrobce protilátky: JaICA, Japonsko). Vzorky moči byly analyzovány v triplikátech. Výsledky byly vztaženy na obsah kreatininu a vyjádřeny v nmol 8-oxodG/mmol kreatininu.

Pro analýzu 15-F2t-IsoP byl použit komerční kit od firmy Oxford Biomedical Reasearch (Oxford, MI, USA). Vzorky moči byly naředěny 7x pufrem dodaným s kitem a celý postup stanovení byl proveden podle doporučení výrobce. Každý vzorek byl analyzován v duplikátech. Koncentrace 15-F2t-IsoP byla vztažena na obsah kreatininu a vyjádřena v nmol 15-F2t-IsoP/mmol kreatininu.

Hladiny karbonylových skupin v proteinech krevní plasmy byly stanoveny metodou nekompetitivní ELISA podle publikovaného postupu (Buss a kol., 1997), s drobnými modifikacemi (viz. Příloha 1, Rossner a kol., 2007). Principem metody je tzv. derivatizace

analyzovaných vzorků – jejich inkubace s dinitrofenylhydrazinem, který se specificky váže na oxidovaná místa v proteinech, přičemž se mění na dinitrofenylhydrazon (DNP). Ten je stanoven pomocí specifické primární protilátky namířené proti DNP (výrobce Molecular Probes, OR, USA). Vzorky plasmy byly analyzovány v triplikátech, množství karbonylových skupin bylo vyjádřeno v nmol/ml plasmy.

3.5. Design studií

Hlavní design studií na dospělých populacích byl následující: po zvolení vhodných modelových skupin v každé lokalitě se přihlásili v daných organizacích dobrovolníci mužského pohlaví (bylo zvoleno jen jedno pohlaví vzhledem k možné proměnlivosti biomarkerů oxidačního stresu). Účastníci studie byli seznámeni s průběhem studie, jejími cíli a po skončení studie i s výsledky. Účastníci studie nosili po dobu 2x24 hod. personální měřící přístroje pro k-PAU a v rámci této doby i 1x24 hod. personální měřící přístroje pro BTEX. Ihned po skončení tohoto měření personální expozice, účastníci odevzdali (většinou ranní) moč a byla jim odebrána krev. Z těchto vzorků pak byly analyzovány biomarkery oxidačního stresu (spolu s dalšími ukazateli životního stylu jako jsou vitamíny A, C, E, hladiny cholesterolu a triglyceridů, koncentrace kotininu jako ukazatele expozice tabákovému kouři). Vždy šlo o zdravé muže nekuřáky. Pro odhad expozice venkovnímu ovzduší se pro výpočty vztahu jednotlivých ukazatelů využily i hodnoty ze stacionárního měření daných škodlivin ve venkovním ovzduší. Tyto se poté následně vztahovaly k hodnotám jednotlivých ukazatelů v různých periodách až do 2 měsíců před odběrem biologických vzorků, tedy čemu byly dané osoby v daných lokalitách vystaveny 2 měsíce před konáním samotného monitoringu a odběrem biologického materiálu.

3.6. Statistické vyhodnocení

Vzhledem k šíři různých použitých postupů a metodik k posouzení vlivu znečištěného ovzduší na oxidační poškození a vlivu jednotlivých možných faktorů tohoto působení bylo využito různých statistických postupů vhodných vždy pro daný soubor dat. Jednotlivé postupy jsou uvedeny v přílohách u každé studované práce.

4. VÝSLEDKY

4.1. Biomarkery oxidačního poškození u skupiny řidičů autobusů v Praze

Biomarkery oxidačního poškození byly prvně analyzovány u pražské skupiny řidičů autobusů. V článku Rossner a kol. (2007, Příloha 1) jsou prezentovány výsledky studie s řidiči autobusů a kontrolní skupinou, která trávila více než 90% času ve vnitřních prostorech. Byly změřeny aktuální personální expozice k-PAU a venkovní koncentrace PM2.5 a PM10 ze stacionárních monitorů. Kontrolní skupina měla s překvapením vyšší expozice k-PAU než řidiči autobusů, i když byla vzorkována podle hladin aerosolových částic (PM10 i PM2.5) v čistším období (skupina řidičů byla vzorkována na konci listopadu, kontrolní skupina na začátku prosince). Významně zvýšené hladiny měřených biomarkerů (8-oxodG, 15-F2t-IsoP, karbonylových skupin) byly zjištěny pro skupinu řidičů autobusů. U všech biomarkerů byl zjištěn vztah ke koncentracím polutantů v ovzduší (8-oxodG pozitivně koreloval s PM10; 15-F2t-IsoP pozitivně korelovaly s PM2.5 i PM10; karbonylové skupiny pozitivně korelovaly se všemi měřenými polutanty).

Zaměřili jsme se na oxidační poškození DNA ve třech různých měřených obdobích (zima roku 2005 a léto a zima roku 2006) (Rossner a kol., 2008, Příloha 2). K měřeným polutantům přibyly i personální expozice BTEX. Řidiči autobusů měli vyšší hladiny 8-oxodG ve všech měřených obdobích, i když hladiny personálních expozic i venkovního ovzduší kolísaly. Multivariátní analýzy potvrdily koncentrace PM2.5 i PM10 měřené stacionárními monitory v období 3 dny před odběrem biologického materiálu (moči) jako jediné faktory významně ovlivňující hladiny 8-oxodG.

Sezónní variabilita 15F2t-IsoP a karbonylových skupin byla také studována (Rossner a kol., 2008, Příloha 3). Také tyto biomarkery byly vyšší u řidičů autobusů v zimním období. Peroxidace lipidů byla nejvíce ovlivněna koncentracemi k-PAU a B[a]P z personálního monitoringu, významný efekt také vykazoval benzen či xyleny. Oxidace proteinů korelovala negativně s hladinami k-PAU, B[a]P, PM2.5 a některými BTEX (benzenem, ethylbenzenem, m,p-xyleny) a měla vyšší hladiny v letním období, poukazující na další faktor ovlivňující hladiny tohoto ukazatele.

4.2. 8-oxodeoxyguanosin u dětí z Teplic a Prachatic

V této části studie jsme se věnovali stanovení 8-oxodG u skupiny 6-10 letých dětí ze zatížené lokality Teplice a kontrolní oblasti Prachatice. Hladiny 8-oxodG byly u dětí významně ovlivněny věkem, příslušností k rómskému etniku a ETS (environmental tobacco smoke, tabákový kouř v prostředí). Multivariátní modely ukázaly vyšší hladiny markeru u dětí s nitroděložní růstovou retardací v Prachaticích. Vyšší hladiny byly zjištěny u dětí s alergickou rýmou v Teplicích. Hladiny PM2.5, PM10 i k-PAU ze stacionárního monitoringu týden před odběrem biologického materiálu významně ovlivňovaly hladiny 8-oxodG (Svecova a kol., 2009, Příloha 4).

4.3. Biomarkery oxidačního poškození makromolekul na Ostravsku

Personální expozice k-PAU a BTEX spolu s biomarkery oxidačního stresu byly změřeny v nejzatíženější lokalitě České republiky – na Ostravsku. Kontrolní skupinou byli městští strážníci v Praze. Osoby z Ostravska byly vystaveny výrazně vyšším hladinám škodlivin v ovzduší než osoby z Prahy ($p<0,001$). Měřilo se ve třech různých obdobích: v zimě a létě roku 2009 a v zimním období roku 2010. Markery oxidačního poškození byly poprvé testovány při vysokých koncentracích škodlivin. V zimě roku 2010, kdy v Ostravě probíhala silná inverze, průměrná personální expozice B[a]P až 15x přesáhla koncentraci 1 ng/m^3, která je považována za hraniční hodnotu nezpůsobující zásadní poškození genetického materiálu (WHO, 2010). Při analýze prvních dvou období monitoringu v Ostravě bylo s překvapením zjištěno, že hladiny 8-oxodG v moči osob z Ostravska se nelišily od pražských vzorků. Hladina markeru peroxidace lipidů 15-F2t-IsoP, byla významně zvýšena u osob žijících na Ostravsku. Při korelaci hladin markerů oxidačního stresu s personální expozicí znečišťujícím látkám z ovzduší, hladiny 8-oxodG nekorelovaly s žádnou ze sledovaných látek. Peroxidace lipidů byla zvýšena po expozici k-PAU, B[a]P, benzenu, toluenu a m,p-xylenu (Rossner a kol., 2009, Příloha 5). Z uvedených biomarkerů expozici polutantům z ovzduší nejlépe odrážely hladiny peroxidace lipidů. Další analýzy ukázaly, že nejlepší korelace byla zjištěna pro B[a]P ($R=0,573$, $p<0,001$). Pozitivní korelace mezi hladinami 15-F2t-IsoP a expozicemi B[a]P však platila jen do 83.percentilu koncentrací B[a]P, což odpovídá koncentraci asi 8,82 ng/m^3. Při vyšších dávkách B[a]P již nebyla závislost lineární (Rossner a kol., 2010, Příloha 6).

Pro další možné vysvětlení neočekávaných výsledků bylo využito analýz chromozomových aberací a exprese vybraných genů podílejících se na BER (bázové excizní reparaci; geny OGG1, APEX1, XRCC1) a NHEJ (nehomologickém spojování konců DNA; geny LIG4, XRCC4, XRCC5 a XRCC6) u osob z Prahy a Ostravy. Výsledky cytogenetických parametrů ani exprese vybraných genů z drah BER či NHEJ se mezi oběma lokalitami nelišily. Multivariátní analýzy však naznačily, že vyšší hladiny vitamínu C a E v krevní plazmě mohou chránit organismus proti oxidačnímu poškození DNA a lipidů. Vyšší hladiny 8-oxodG než medián byly asociovány se zvýšenou expresí XRCC1. Proti našemu očekávání byly hladiny karbonylových skupin pozitivně asociovány se zvýšenými koncentracemi vitaminu A. Expozice benzenu zvyšovala procento aberantních buněk. Byla zjištěna významně vyšší exprese genu XRCC5 u osob z Ostravy. Studie poukázala na možný vliv faktorů životního stylu na ukazatele oxidačního poškození (Rossner a kol., 2011, Příloha 7).

Pro odhad síly jednotlivých faktorů životního stylu ovlivňujících personální expozici k-PAU, byla vypracována studie využívající výsledky z Prahy a Ostravska. Multivariátní analýzy zahrnující podrobné aktivity účastníků monitoringu, stejně jako základní charakteristiky životního stylu vyhodnotily jako nejvýznamnější faktory venkovní ovzduší a vliv dopravy. Dalšími významnými faktory z bivariátních analýz byly: ETS, domácí topení uhlím, dřevem a plynem, frekvence využívání digestoře, vaření a cestování autem (Svecova a kol., 2011, Příloha 8). Podobná studie věnující se faktorům personální expozice BTEX poukázala na významné vlivy personální expozice benzenu, kterými byly vnitřní prostředí, ETS, vaření, domácí topení v krbu či plynem, cestování autem a čas trávený v restauraci. Expozice ostravské populace byla významně ovlivněna koncentracemi benzenu ve venkovním ovzduší. Z bivariátních analýz vyšlo více možných faktorů ovlivňujících personální expozice BTEX (Svecova a kol., 2012, Příloha 9).

5. DISKUZE

V této práci jsou diskutovány výsledky monitoringu k-PAU a BTEX z ovzduší spolu s výsledky analýz měřených biomarkerů oxidačního stresu na zvolených populacích. Výsledky byly získané v rámci velkých molekulárně epidemiologických studií na exponovaných populacích a jsou pouhou částí těchto studií.

5.1. Personální expozice k-PAU a BTEX a oxidační poškození makromolekul u řidičů autobusů v Praze

V roce 2006 byla zavedena metodika měření personální expozice BTEX u řidičů autobusů, která úspěšně pokračovala v měření expozic i pro další měřené populace (Švecová a kol., 2007). Výsledky personální expozice byly dále korelovány s výsledky oxidačního poškození makromolekul (Přílohy 1-3). Pro vztah mezi hladinami biomarkerů a znečištěním venkovního ovzduší se použily hodnoty ze stacionárního monitoringu. Pro většinu měřených polutantů platil vztah, kdy koncentrace polutantů v ovzduší byly vyšší díky meteorologickým podmínkám a topení v zimním období. Výjimku tvořily vysoké profesionální expozice například u pracovníků v garážích pražské MHD v létě roku 2006 (Švecová a kol., 2007). Při srovnání koncentrací z personálního monitoringu s koncentracemi měřenými stacionárními přístroji byly výsledky rozdílné pro různé druhy škodlivin. U k-PAU platil trend, kdy personální expozice byla nižší než stacionárně měřené koncentrace, zatímco personální expozice BTEX zejména benzenu, byla mnohokrát vyšší, vzhledem k tomu že lidé se pohybují blíže zdrojům znečištění a byli proto více exponováni, než jak ukazovaly hodnoty měřené stacionárními přístroji (Švecová a kol., 2009).

Pokud se korelovaly stacionárně měřené koncentrace polutantů ve venkovním ovzduší s biomarkerem 8-oxodG, ukázala se významná korelace pro PM2.5 i PM10, vztah však nevyšel pro B[a]P či k-PAU. U skupiny řidičů autobusů pražské MHD bylo pozorováno zvýšené oxidační poškození DNA, lipidů i proteinů ve srovnání s kontrolní skupinou. Zvýšení bylo pozorováno zejména v zimním období, kdy je vyšší i znečištění ovzduší. Znečištěné ovzduší se proto zdálo být faktorem ovlivňujícím oxidační poškození. Hladiny karbonylových skupin byly u této části studie jediným ukazatelem, který pozitivně významně koreloval s B[a]P i k-PAU. Řidiči autobusů však jsou také vystaveni vyššímu mentálnímu stresu, vycházejícího z rušného

silničního provozu ve městě a zodpovědnosti za spolucestující. Stres mohl také ovlivňovat hladiny měřených biomarkerů (Djuric a kol., 2008). Hladiny vitamínů bývají spojovány s antioxidačním účinkem a v této části studie se podařilo potvrdit významnou negativní korelaci vitamínu C a 15-F2t-IsoP. U vitamínu E jsme však zjistili pozitivní korelaci se všemi třemi markery oxidačního stresu. Tyto výsledky mohly být ovlivněny dalšími faktory, jako je životní styl či dieta účastníků studie, avšak také ochrannou funkcí organismu proti oxidačnímu stresu. Pozitivní asociace byla také nalezena mezi hladinou vitamínu A a karbonylovými skupinami (Rossner a kol., 2007, Příloha 1).

Srovnání hladin 8-oxodG v jednotlivých obdobích 2005-2006 nekorespondovalo s koncentracemi polutantů ve venkovním ovzduší. Výsledky poukazovaly na možnost dalšího faktoru odpovědného za změny hladin 8-oxodG (Rossner a kol., 2008, Příloha 2). Následná analýza dat a shrnutí většího počtu faktorů spolu s daty o znečištění ovzduší měsíc před odběrem biologického materiálu ukázala největší vliv polutantů z ovzduší (kromě benzenu) 3 dny před odběrem biologického materiálu. Hladiny 8-oxodG významně ovlivňovaly koncentrace PM2.5 a PM10 ze stacionárního monitoringu. Tato část studie ukázala, že i když k-PAU mají potenciál indukovat oxidační poškození DNA, budou zde pravděpodobně jiné faktory hrající významnou roli v odpovědi organismu na environmentální znečištění. Dalšími možnými faktory jsou mentální stres, ozón či ultrajemné částice (frakce 57-nm z automobilové dopravy, Bräuner a kol., 2007). Hlavním výsledkem v této části studie byl fakt že expozice PM2.5 a PM10 3 dny před odběrem pozitivně ovlivňovala hladiny 8-oxodG v moči. Výsledky vycházely konzistentně i pro další 3 denní intervaly do 42 dnů před odběrem. Vitamín C vykazoval protektivní efekt proti oxidačnímu poškození DNA.

Hladiny 15-F2t-IsoP v moči významně ovlivňovaly personální koncentrace k-PAU a B[a]P 48h před odběrem. Bivariátní lineární regrese ukázala vztah mezi personální expozicí benzenu a xylenů. Vliv kotininu, triglyceridů a vitamínu E na peroxidaci lipidů potvrdila bivariátní logistická regrese. Z této části studie vyšel poznatek, že oxidační poškození DNA a lipidů se liší. Ukazatel peroxidace lipidů byl významně pozitivně ovlivněn hladinami k-PAU z personálního i stacionárního monitoringu. Ze statistických analýz vyplynulo, že 15-F2t-IsoP není ukazatel okamžitého efektu PM2.5 jako 8-oxodG. Možné vysvětlení se nabízí, že existence opravných mechanismů DNA opravuje poškození DNA krátce po jejich vzniku. Poškození lipidů

se neopravuje; spíše se poškození akumulují a tudíž umožňují detekci polutantům delší období před vzorkováním.

Karbonylové skupiny byly neočekávaně negativně korelovány s personální expozicí k-PAU spolu s ethylbenzenem a m,p-xyleny. Negativní korelace byla také zjištěna pro vitamín C a E. Statistické analýzy ukázaly, že expozice k-PAU a benzenu 3 dny před odběrem významně snižovaly hladiny karbonylových skupin. Oxidační stres může být ovlivňován i dalšími polutanty. Korelace s koncentracemi ozónu v ovzduší ukázala významně pozitivní efekt na hladiny karbonylových skupin. V této části studie bylo zjištěno zvýšené oxidační poškození proteinů a lipidů u řidičů autobusů, které může být interpretováno jako ukazatel zvýšeného rizika k různým onemocněním, zahrnujícím kardiovaskulární onemocnění a rakovinu. Výsledky lidských studií ukazují, že mechanismus oxidačního poškození hraje významnou roli v kardiovaskulárních efektech v důsledku znečištění aerosolovými částicemi (PM2.5). Výsledky ukázaly rozdíly v odpovědi reakce organismu na polutanty z ovzduší a významnost měření několika ukazatelů oxidačního stresu najednou. Shrnujícím výsledkem bylo stanovení vyššího oxidačního poškození DNA, lipidů i proteinů u řidičů autobusů v zimním období (Rossner a kol., 2008, Příloha 3).

5.2. 8-oxodeoxyguanosin u dětí z Teplic a Prachatic

Děti se vyznačují vyšší zranitelností vůči škodlivinám, nejen ze znečištěného ovzduší, vzhledem k mnohým odlišnostem od dospělého organismu: nekompletní metabolismus, nevyzrálý imunitní systém, vysoká míra infekcí respiračními patogeny a rozdílný vzor chování a aktivit. Negativní vztah hladin 8-oxodG a věku dítěte by mohl být způsobován růstem a vývojem plic a zráním metabolismu a imunitního systému. Vyšší hladiny 8-oxodG u mladších dětí (6-7 let) by mohly odpovídat i vyšší citlivosti mladších dětí vůči ETS, či trávení většího času s kouřícími rodiči než starší děti (9-10 let). Oxidační poškození DNA bylo ovlivněno dosaženým vzděláním matky, poukazující na roli životního stylu bez ETS, kvalitu péče či diety. Vztah 8-oxodG k nitroděložní růstové retardaci může poukazovat na funkční deficit vzniklý při nitroděložním vývoji. Expozice dětí cigaretovému kouři má mnoho potvrzených negativních důsledků. V naší studii jsme potvrdili silnou korelaci kotininu (ukazatele ETS) z moči dětí s hladinami 8-oxodG. Zvýšený oxidační stres může být startovacím bodem pro respirační, alergickou či další nemocnost dětí. Výhodou této studie byla dostupnost dat o znečištění ovzduší

v průběhu sbírání biologického materiálu i před tímto obdobím. Mohli jsme tudíž individuálně korelovat data o 8-oxodG v různých intervalech před odběrem biologického materiálu. Multivariátní modely ukázaly největší ovlivnění hladin 8-oxodG do 9 dnů před odběrem biologického materiálu pro PM10, PM2.5, k-PAU i B[a]P, i když pro tuto analýzu byla využita data ze stacionárního monitoringu. Zajímavým zjištěním byla pozitivní korelace 8-oxodG u dětí s alergickou rýmou v Teplicích a negativní v Prachaticích. Tento rozdíl může spočívat v rozdílnosti těchto dvou měst, zatímco ve více zalesněných Prachaticích je více dětí citlivých na pylový alergen, ve více znečištěných Teplicích více dětí může reagovat na znečištění ovzduší. Výsledky této části studie naznačovaly, že 8-oxodG by mohl být komplexním biomarkerem znečištění ovzduší a životního stylu. Výsledky také poukazovaly na vyšší oxidační poškození u mladších dětí, tedy jejich vyšší citlivost a významnou roli znečištěného ovzduší, které může představovat významné zdravotní riziko hlavně pro předškolní děti. Dalo by se spekulovat, že expozice ETS stejně jako krátkodobá expozice jemným aerosolovým částicím a k-PAU jsou schopny indukovat oxidační stres (Svecova a kol., 2009, Příloha 4).

5.3. Biomarkery oxidačního poškození makromolekul na Ostravsku

Článek Rossner a kol. (2009, Příloha 5) ukazuje hodnoty personálního monitoringu k-PAU a BTEX na Ostravsku a v Praze. Potvrdilo se vysoké zatížení karcinogenním látkám obyvatel na Ostravsku. Několikanásobné překračování imisních limitů by se teoreticky mělo odrazit na měřených biomarkerech. I když se v Ostravě měřilo se skupinou úředníků, která tráví většinu svého času v uzavřených prostorech kanceláří, byla jejich expozice v porovnání s Prahou zvýšená (Švecová a kol., 2009). Zatížení v zimních měsících velmi komplikují inverzní stavy atmosféry, kdy koncentrace v ovzduší významně vzrůstají (Švecová a kol., 2010).

I přes vysokou expozici znečišťujícím látkám v ovzduší na Ostravsku byly hladiny 8-oxodG v moči porovnatelné s hodnotami nalezenými u pražské populace, a to nejen v letním, ale i v zimním období. Fakt, že ani velmi vysoká expozice znečištěnému ovzduší nemá za následek zvýšenou hladinu 8-oxodG v moči, lze možná vysvětlit původem této oxidované báze. 8-oxodG je sice produktem působení ROS na DNA, nicméně významným zdrojem 8-oxodG v moči jsou mechanismy reparace DNA. Navíc 8-oxodG nepochází jen z oxidace DNA, ale i z oxidace volných nukleotidů přítomných v buňkách. Tato skutečnost je nevýhodou

analýzy 8-oxodG v moči. Metoda je však neinvazivní a v průběhu zpracování nedochází k indukci oxidačního poškození. I když k-PAU neindukovaly zvýšení hladin 8-oxodG v moči ani v našich předchozích studiích, oxidační poškození DNA korelovalo s expozicí PM10 a PM2.5. Dalo by se tedy spekulovat, zda za indukci oxidačního poškození DNA projevujícího se zvýšením 8-oxodG v moči jsou zodpovědné jiné látky než k-PAU. Pro objasnění vlivu k-PAU na hladiny 8-oxodG v moči by bylo nutné provést další laboratorní analýzy: např. analýzu genové exprese a stanovení hladin enzymů zodpovědných za reparaci DNA.

Peroxidace lipidů byla výrazně zvýšena u osob na Ostravsku ve srovnání s pražskou populací, a to především v zimním období. Potvrdila se vysoce významná pozitivní korelace mezi personální expozicí k-PAU a B[a]P a hladinami 15-F2t-IsoP v krevní plazmě. Výsledek poukazuje na skutečnost, že přítomnost k-PAU v ovzduší má za následek zvýšení hladin peroxidovaných lipidů v organismu. Peroxidované lipidy nejen narušují strukturu buněčných membrán a funkci membránově vázaných proteinů, ale díky tvorbě reaktivních intermediátů způsobují šíření oxidačního poškození v organismu vedoucího k poškození DNA a proteinů. Peroxidace lipidů byla ovlivněna i některými BTEX (benzenem, toluenem a m,p-xyleny), avšak korelace byla výrazně slabší než v případě k-PAU, nicméně též signifikantní. Tyto výsledky ukázaly, že zvýšená expozice znečišťujícím látkám v ovzduší, především k-PAU, pozorovaná na Ostravsku, je doprovázena vyšší mírou peroxidace lipidů (Rossner a kol., 2009, Příloha 5).

V další analýze výsledků měření z Ostravska byla potvrzena významná korelace benzenu, B[a]P a k-PAU s hodnotami peroxidace lipidů, kde nejlepší korelace byla zjištěna pro B[a]P. Výsledky opět naznačily, že zvýšené hladiny benzenu a k-PAU, včetně B[a]P, nemají vliv na produkty oxidace DNA v moči, ani na hladiny oxidovaných proteinů v krevní plazmě. Z těchto studií lze shrnout, že hladiny 8-oxodG v moči neodráží krátkodobou expozici benzenu a k-PAU těsně před odběrem biologického materiálu, a to ani v případě velmi vysokých koncentrací látek (Rossner a kol., 2010, Příloha 6).

Článek Rossner a kol. (2011, Příloha 7) ukazuje rozdíl v životním stylu dvou porovnávaných populací strážníků a úředníků, kdy pražští strážníci byli více vystaveni ETS, měli zvýšený LDL a snížený HDL cholesterol. Ostravští úředníci měli vyšší hladiny vitamínů A, C, E. Z těchto údajů je vidět velký rozdíl mezi studovanými populacemi. Také pracovní stres u těchto skupin bude významně rozdílný, což se mohlo odrazit na hladinách biomarkerů. Ve studii v zimě

2010 se však neprojevil rozdíl u oxidačního poškození makromolekul, cytogenetických parametrů (F_G/100, %AB.B.) ani exprese BER či NHEJ genů. Rozdíl v genomické frekvenci translokací (F_G/100) byl na hranici významnosti (p=0,060), avšak u pražských strážníků. Exprese XRCC4 byla zvýšená u pražské skupiny (p=0,038). Vyšší hladiny vitamínu C a E ochraňují organismus před oxidačním poškozením DNA. Tento děj se potvrdil u účastníků s vyššími hladinami 8-oxodG v moči, kteří měli významně nižší hladiny vitamínu C a E. Vitamín C také ochraňuje před peroxidací lipidů. Účastníci s hodnotami 15-F2t-IsoP vyššími než medián měli nižší hladiny vitamínu C. Vyšší hladiny 8-oxodG asociovaly se zvýšenou expresí XRCC1. Vyšší hladiny karbonylových skupin byly asociovány se zvýšenými koncentracemi vitamínu A. Významně zvýšená exprese XRCC1 byla pozorována u účastníků s vyššími hladinami vitamínu C. Exprese XRCC5 byla zvýšena u ostravských úředníků. Z výsledků se dá usuzovat, že antioxidační vlastnosti vitamínů C a E snížily tvorbu 8-oxodG v buněčné DNA a tím i hladinu močí vylučovaných oxidovaných bází. Lze spekulovat, zda exprese XRCC1 je spojena s vyššími hladinami vitamínu C a podporuje práci BER, což by mohlo vést k rychlejšímu odstranění 8-oxodG z DNA a vyšší reparační účinnosti. Výsledky naznačují, že účinek vysokých koncentrací škodlivin v ovzduší mohou kompenzovat jiné vlivy, např. genová exprese či faktory životního stylu. Proto bylo žádoucí vyhodnotit i faktory personální expozice k-PAU a BTEX.

Příloha 8 (Svecova a kol., 2012a) se věnuje faktorům ovlivňujícím personální expozici k-PAU. K analýze byly využity nejen dotazníky životního stylu, ale také podrobné dotazníky aktivit během monitoringu, které účastníci studie vyplňovali každou hodinu. Z výsledků vyplývá, že ostravští úředníci tráví až 87% ve vnitřních prostorách budov, kdežto městští strážníci jen 46-60% a až 34% času tráví venku. Multivariátní modely vyhodnotily jako nejvýznamnější faktory ovlivňující personální expozici k-PAU venkovní ovzduší a vliv dopravy. Z bivariátních analýz dalšími významnými faktory byly: ETS, domácí topení uhlím, dřevem a plynem, frekvence využívání digestoře, vaření a cestování autem.

Příloha 9 (Svecova a kol., 2012b) se věnuje faktorům ovlivňujícím personální expozici BTEX. Personální expozice benzenu nejvíce ovlivňovaly: vnitřní prostředí, ETS, vaření, domácí topení v krbu či plynem, cestování autem a čas trávený v restauraci. Expozice ostravské populace byla významně ovlivněna koncentracemi benzenu ve venkovním ovzduší. Pro kvalitnější vyhodnocení faktorů ovlivňujících personální expozice by byla vhodná studie využívající

simultánního měření vnitřní, venkovní a personální expozice k odhadu hlavních zdrojů ovlivňující personální expozice a síly jednotlivých faktorů. Také by bylo vhodné analyzovat vlivy jednotlivých významných faktorů personální expozice s příslušnými biomarkery.

6. ZÁVĚR

Práce se zabývala studiem expozice škodlivým látkám v ovzduší (k-PAU a BTEX) a vyhodnocením ukazatelů oxidačního poškození makromolekul. Všechny dílčí cíle dizertační práce byly splněny a byly získány následující nové poznatky:

- Oxidační poškození DNA (hladiny 8-oxodG v moči) byly významně ovlivňovány hladinami aerosolových částic PM2.5 i PM10 ve venkovním ovzduší (významně hladiny 8-oxodG ovlivňovaly koncentrace PM2.5 a PM10 v období 3 dny před odběrem biologického materiálu až do 42 dnů; tedy více než měsíc před odběrem biologického materiálu).

- Hladiny 8-oxodG se liší u dospělého a dětského organismu. U 6-10 letých dětí hladiny 8-oxodG klesaly s věkem a vyšším vzděláním matek. Vyšší hladiny 8-oxodG byly zjištěny u rómského etnika, intrauterinní růstové retardace v souboru dětí v Prachaticích a u dětí s alergickou rýmou v Teplicích. Byl potvrzen silný vtah k ETS. U dětí mezi významné faktory ovlivňující hladiny 8-oxodG patřily koncentrace PM2.5, PM10, k-PAU i B[a]P ve venkovním ovzduší týden před odběrem biologického materiálu.

- Hladiny 15-F2t-IsoP v moči i krevní plazmě byly pozitivně ovlivňovány koncentracemi k-PAU i B[a]P z personálního i stacionárního měření. Hladiny tohoto ukazatele byly zvýšeny u osob v zatížených podmínkách hlavně v zimním období. Hladiny 15-F2t-IsoP v krevní plazmě byly také ovlivněny některými VOC (benzenem, toluenem a m,p-xyleny). Peroxidace lipidů se zdá být ukazatelem nejlépe asociovaným s expozicí znečišťujícím látkám v ovzduší (hlavně k-PAU i B[a]P), avšak jen do určité horní hranice. Předkládané studie ukázaly, že mezi dávkou (koncentrací B[a]P) a účinkem (hladinami 15-F2t-IsoP) existuje nelineární vztah: při nižších koncentracích B[a]P se peroxidace lipidů měnily lineárně, avšak po dosažení vyšších koncentrací B[a]P se již hladiny 15-F2t-IsoP téměř neměnily.

- Ukazatel oxidace proteinů – karbonylové skupiny nevykazovaly jednotný trend ani ve vztahu k polutantům v ovzduší ani u zatížených skupin. Předpokládáme, že hladiny tohoto ukazatele budou ovlivněny dalšími polutanty (např. ozón) či dalšími faktory.

- Vitamín C vykazoval negativní asociaci s hladinami 8-oxodG, 15-F2t-IsoP i karbonylovými skupinami. Vitamín C je molekula, která reguluje odpověď buňky na stres, působí na buněčnou diferenciaci, proliferaci, buněčnou smrt a reparaci DNA. Spolu s vitamínem E mohou mít vliv na expresi řady genů, které mohou ovlivňovat hladiny biomarkerů i reparační mechanismy.

- Hladiny měřených ukazatelů oxidačního stresu mohou být ovlivňovány mnohými dalšími faktory jako je: mentální stres, životní styl (kouření, dieta, fyzická aktivita), další polutanty v ovzduší (ozón, těžké kovy apod.).

- Faktory významně ovlivňující personální expozice k-PAU byly venkovní ovzduší, doprava, ETS, domácí topení uhlím, dřevem a plynem, frekvence využívání digestoře, vaření a cestování autem.

- Personální expozice benzenu byla nejvíce ovlivněna vnitřním prostředím, ETS, vařením, domácím topením v krbu či plynem, cestováním autem, časem tráveným v restauraci; a u ostravské populace i venkovním ovzduším.

7. VÝHLED DO BUDOUCNA

Při analýze personální expozice karcinogenním látkám a biomarkerů oxidačního poškození vyplynula nutnost analyzovat faktory, které personální expozici nejvíce ovlivňují. Tato oblast je u nás teprve na počátku poznání. V jiných zemích se však tomuto problému již věnují a dosahují velmi dobrých výsledku v regulaci a následné eliminaci zdrojů tuto expozici nejvíce ovlivňujících. Proto mohu další analýzy personální expozice toxickým látkám jen doporučit pro eliminaci následných rizik. Spojení s vyhodnocením biomarkerů expozice a účinku je velkou výhodou dalšího posuzování. V České republice podle mého názoru velice chybí měření personální expozice nejen u běžné populace, avšak i v zátěžových provozech, kde tyto expozice mohou dosahovat velmi vysokých hodnot. Tyto analýzy dávají nutné podklady pro předcházení zdravotních rizik, kde prevence je jedním z nejdůležitějších bodů. Co se týká zdrojů k-PAU a VOC v České republice, nejvýznamnějším zdrojem pro celou populaci bude patrně znečištění ovzduší v zimních měsících a ETS. ETS by měl být omezen na veřejných prostorách, aby nedocházelo k nežádoucí expozici zvláště citlivých skupin, kterými jsou děti, těhotné ženy a citliví (nemocní) jedinci. Tito jsou v dnešní době běžně exponováni na veřejných prostorech a expozici ETS se dnes prakticky nedá vyhnout. Spojením této expozice s polutanty z ovzduší velkých měst zatížených dopravou či v některých částech republiky i průmyslem tvoří velké zatížení oblastí, způsobující zdravotní potíže pro citlivé jedince. V dnešní době již existuje mnoho mezinárodních studií tyto poznatky potvrzujících. Bylo by vhodné u nás potvrdit tyto výzkumy měřením a upozornit na konkrétní ohrožení, aby mohlo být co nejvíce dodrženo jedno ze základních lidských práv podle Listiny základních práv a svobod, která je součástí ústavního pořádku České republiky, zaručující ve své hlavě čtvrté všem lidem na území ČR hospodářská, sociální a kulturní práva. Mezi ně podle článku 31 Listiny patří i právo na ochranu zdraví. Každý má také právo na příznivé životní prostředí podle článku 35 odstavec 1 Listiny.

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9. Přílohy 1-9

Příloha 1

Rossner P. Jr., Svecova V., Milcova A., Lnenickova Z.,

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Oxidative and nitrosative stress markers in bus drivers

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Abstract

Exposure to ambient air pollution is associated with many diseases. Oxidative and nitrosative stress are believed to be two of the major sources of particulate matter (PM)-mediated adverse health effects. PM in ambient air arises from industry, local heating, and vehicle emissions and poses a serious problem mainly in large cities. In the present study we analyzed the level of oxidative and nitrosative stress among 50 bus drivers from Prague, Czech Republic, and 50 matching controls. We assessed simultaneously the levels of 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) and 8-oxodeoxyguanosine (8-oxodG) in urine and protein carbonyl groups and 3-nitrotyrosine (NT) in blood plasma. For the analysis of all four markers we used ELISA techniques. We observed significantly increased levels of oxidative and nitrosative stress markers in bus drivers. The median levels (min, max) of individual markers in bus drivers versus controls were as follows: 8-oxodG: 7.79 (2.64–12.34) nmol/mmol versus 6.12 (0.70–11.38) nmol/mmol creatinine ($p < 0.01$); 15-F_{2t}-IsoP: 0.81 (0.38–1.55) nmol/mmol versus 0.68 (0.39–1.79) nmol/mmol creatinine ($p < 0.01$); carbonyl levels: 14.1 (11.8–19.0) nmol/ml versus 12.9 (9.8–16.6) nmol/ml plasma ($p < 0.001$); NT: 694 (471–3228) nmol/l versus 537 (268–13833) nmol/l plasma ($p < 0.001$). 15-F_{2t}-IsoP levels correlated with vitamin E ($R = 0.23$, $p < 0.05$), vitamin C ($R = -0.33$, $p < 0.01$) and cotinine ($R = 0.47$, $p < 0.001$) levels. Vitamin E levels also positively correlated with 8-oxodG ($R = 0.27$, $p = 0.01$) and protein carbonyl levels ($R = 0.32$, $p < 0.001$). Both oxidative and nitrosative stress markers positively correlated with PM2.5 and PM10 exposure. In conclusion, our study indicates that exposure to PM2.5 and PM10 results in increased oxidative and nitrosative stress.

Keywords: Oxidative stress; 15-F_{2t}-isoprostane; 8-Oxodeoxyguanosine; Carbonyl groups; 3-Nitrotyrosine; Bus drivers; Air pollution; Particulate matter

1. Introduction

Air pollution is associated with many negative health effects, including increased morbidity and mortality. Diseases in which ambient air particulate matter is believed to play a role include pulmonary and cardiovascular disorders and cancer [1,2]. PM is comprised of a mixture of various chemical compounds. Negative effects of PM on human health may relate both to the

Abbreviations: 15-F_{2t}-IsoP, 15-F_{2t}-isoprostane; 8-oxodG, 8-oxodeoxyguanosine; NT, 3-nitrotyrosine; PM, particulate matter; PM2.5, particulate matter <2.5 μm; PM10, particulate matter <10 μm; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; ROS, reactive oxygen species; TMB, tetramethyl benzidine

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content of carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) and chemicals and processes inducing oxidative and nitrosative damage to DNA, lipids and proteins due to the presence of reactive compound such as quinones, toxic metals, and benzene, as well as cellular processes like metabolism, or inflammation [3,4]. Effects have been observed at very low exposure levels, and it is not clear whether any threshold level exists below which no health effects occur [1].

Oxidative stress, mediated by reactive oxygen species (ROS) is a process induced by endogenous, as well as exogenous factors. Endogenous factors include normal physiologic processes, such as oxidative phosphorylation, P450 metabolism, including metabolism of estrogens, peroxisomes, and inflammatory cell activation. Various environmental factors, including, smoking, diet, or exposure to ambient air pollution, represent exogenous sources of ROS. Oxidative stress may result in direct DNA damage, lipid peroxidation, protein oxidation, mitochondrial damage, or membrane disruption [5]. Since the process is complex, it has been suggested that several oxidative stress markers should be observed at a time to get a better understanding of the reactions taking place within an organism [6].

8-Oxodeoxyguanosine (8-oxodG), the most abundant and most often studied product of oxidative DNA damage, is highly mutagenic and if not repaired its presence results in GC > TA transversions. If repaired, 8-oxodG is excreted in urine. Measurement of 8-oxodG levels in cells, tissues, or urine is considered a general biomarker of oxidative stress. Urinary 8-oxodG levels are also believed to correspond to total DNA excision repair capacity of an organism [7]. However, another significant pool of extracellular 8-oxodG may be oxidation of the nucleotide pool [8]. The most frequently used methods of 8-oxodG analysis include HPLC with electrochemical detection and competitive ELISA [9].

F_2-isoprostanes, compounds derived from arachidonic acid via a free radical-catalyzed mechanism, belong among extensively used markers of lipid peroxidation. While several groups of F_2-isoprostanes exist, most studies concentrated on the biological activity of 15-F_{2t}-isoprostane (15-F_{2t}-IsoP) [10]. Isoprostanes are initially generated from cell membrane-bound arachidonic acid by free radical attack. They are cleaved from the sites of their origin by phospholipases and then circulate in plasma and are excreted in urine [11,12]. F_2-isoprostanes can be detected in biological fluids, such as urine, blood plasma, bronchoalveolar lavage, or cerebrospinal fluid, as well as in tissues. The main advantage of urinary measurements of F_2-isoprostanes is that the compounds are very stable and are not formed ex vivo [13]. Methods used for 15-F_{2t}-isoprostane detection include gas chromatography–mass spectroscopy, liquid chromatography-mass spectroscopy, and a competitive ELISA [14] that is particularly suitable of the analysis of large number of samples.

Protein carbonyl levels are the most frequently used biomarker of protein oxidation. Their accumulation has been observed with aging and several human diseases, including cancer (reviewed in Refs. [15,16]). Several methods of protein carbonyl groups detection are available [15], including a colorimetric assay based on derivatization of the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH) [17]. The product of this reaction is 2,4-nitrophenylhydrazone (DNP), a stable compound that can be detected spectrophotometrically. An alternative ELISA method that is faster and requires less amount of biological sample was developed [18]. The assay uses anti-DNP antibodies; it is reproducible and correlates well with the classical colorimetric assay.

Nitric oxide (NO) is a reactive free radical synthesized in almost all vertebrate organ systems by the action of NO synthases. NO plays a key role in the regulation of airway functions. Nitrosative stress arises when NO is produced excessively in the presence of oxidative stress. In this environment, NO gives rise to highly reactive nitrogen species such as peroxynitrite that react with DNA, proteins, and lipids and cause their damage (reviewed in Ref. [3]). Reactive nitrogen species have extremely short half-time, thus to detect their formation and effect on an organism, stable products need to be analyzed. It has been shown that 3-nitrotyrosine (NT), the stable product of peroxynitrite attack on proteins, can be found in blood plasma [19]. However, other NO-independent pathways of 3-nitrotyrosine formation mediated by myeloperoxidase and eosinophil peroxidase exist. Various methods of NT detection are available including HPLC, GC–MS, or antibody-based assays such as ELISA, western blotting, or immunohistochemistry [20–22].

In the present study, we investigated the effect of ambient air pollution characterized by the concentrations of PM2.5, PM10, c-PAHs and BaP on oxidative and nitrosative stress markers in bus drivers. Oxidative stress markers included: urinary 8-oxodG as a marker of DNA damage and repair, urinary 15-F_{2t}-isoP as a marker of lipid peroxidation, and plasma carbonyl levels as a marker of protein oxidation. As a marker of nitrosative stress NT levels in blood plasma were assessed.

2. Material and methods

2.1. Subjects and sampling

The study population consisted of 50 exposed subjects, bus drivers working in the center of Prague, Czech Republic, presumably exposed to high levels of air pollution. The control subjects consisted of 50 healthy male volunteers spending >90% of daily time indoors. All subjects were non-smokers. Each subject completed a questionnaire on personal medical history and life-style. All participants were followed in winter due to higher air pollution levels during this season.

All participants signed an informed consent form and could cancel their participation at any time during the study according to the Helsinki II declaration. The study was approved by the ethical committee of the Institute of Experimental Medicine AS CR in Prague. Any person with medical treatment, radiography or vaccination up to 3 months before sampling was not included in the study.

The blood samples were collected by venipuncture into vacuettes containing sodium heparin; both blood and urine samples were coded and transported to the Laboratory of Genetic Ecotoxicology. The samples were processed within 2 h and were kept in aliquots at −80 °C.

2.2. Exposure assessment

Subjects' exposure to c-PAHs was carried out by personal monitors used by the study subjects during two consecutive days (48 h). The monitors were equipped with filters collecting particles of aerometric diameter 2.5 μM (PM2.5). Quantitative chemical analysis of c-PAHs (benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, benzo[*g*,*h*,*i*]perylene and indeno-[1,2,3-*cd*]pyrene) was performed by HPLC with fluorescence detection.

Ambient air quality during sampling periods was monitored using stationary versatile air pollution samplers (VAPS) at two locations in Prague with heavy and lighter traffic. The samplers measured continuously the levels of PM10 and PM2.5.

2.3. 8-OxodG ELISA

Urinary 8-oxodG levels were analyzed by competitive ELISA essentially as previously described [23]. Wells were coated with 5 ng of 8-oxoG conjugated with bovine serum albumin (BSA; total volume, 50 μl/well) by drying the plates overnight at 37 °C. Plates were washed with PBS/Tween (0.05% Tween 20 in PBS) and blocked with 200 μl/well of blocking buffer (1% FCS in PBS/Tween) for 1 h at 37 °C. After blocking, 50 μl of 8-oxodG standards (concentration range, 1.25–40 ng/ml) and urine samples (diluted 1:1 with PBS) were added followed by 50 μl of primary antibody (JaICA, Japan, clone N45.1, concentration 0.2 μg/ml). After incubation for 1.5 h at 37 °C and washing, 100 μl of secondary antibody conjugated with alkaline phosphatase (Sigma) were added. Another 1.5 h incubation at 37 °C was followed by washing with PBS/Tween and with 0.01% diethanolamine in water. The color was developed by adding 100 μl of p-nitrophenyl phosphate substrate (1 mg/ml of 1 mol/l diethanolamine) per well and incubating the plates for 30–60 min at 37 °C. The absorbance was measured with a microplate reader at 405 nm. Any samples with inhibition <20% or >80% were repeatedly analyzed either without dilution or with further dilution, respectively. Each sample was analyzed in triplicate. Urinary 8-oxodG concentration was expressed as nmol 8-oxodG/mmol creatinine.

2.4. 15-F_{2t}-IsoP immunoassay

Urinary 15-F_{2t}-IsoP levels were analyzed using immunoassay kits from Oxford Biomedical Research (Oxford, MI, USA). Urine samples were thawed to room temperature and diluted 6× using the dilution buffer provided with the kit. Analysis was done according to the recommendations of the manufacturer. Each sample was analyzed in duplicate. 15-F_{2t}-IsoP concentration was divided by creatinine levels and expressed as nmol 15-F_{2t}-IsoP/mmol creatinine.

2.5. Protein carbonyl assay

The levels of protein carbonyl groups were assessed using a noncompetitive ELISA, essentially as described in Ref. [18], with some modifications [24]. Briefly, the oxidized protein standards were prepared by incubation of BSA (50 mg/ml) with 0.73 M H_2O_2 and 0.42 mM Fe^{2+} for 1 h at 37 °C. The reaction was stopped with 40 μM butylated hydroxytoluene. The carbonyl content of the oxidized BSA standard was measured spectrophotometrically [17]. It was then diluted with native (unoxidized) BSA and PBS to give a final carbonyl content of 2.0 nmol/mg protein and protein concentration of 4 mg/ml. Total protein concentration in the plasma samples was measured using Bicinchoninic Acid Kit (Sigma) and the samples were diluted with PBS to a final protein concentration of 4 mg/ml. After the derivatization with DNPH, the plate was coated with 200 μl of sample and incubated overnight at 4 °C in the dark. The plate was washed with PBS/Tween (0.05% Tween 20 in PBS) and blocked with 0.1% BSA in PBS for 1.5 h. After another washing step, biotinylated primary anti-DNP antibody (Molecular Probes, OR, USA; diluted 1:1500 with 0.1% BSA, 0.1% Tween 20 in PBS) was added and the plate was incubated at 37 °C for 1 h. Another washing was followed by adding the streptavidin-biotynylated horseradish peroxidase conjugate (Amersham Biosciences, UK; diluted 1:4000 in 0.1% BSA, 0.1% Tween 20 in PBS) and incubation at room temperature for 1 h. Color was developed by adding the tetramethyl benzidine (TMB) liquid substrate system (Sigma) and the reaction was stopped with H_2SO_4 after 15–25 min incubation in the dark. The absorbance was measured with a microplate reader at 450 nm. Each sample was analyzed in triplicate. Plasma protein carbonyl concentration was expressed as nmol carbonyl/ml plasma.

2.6. 3-Nitrotyrosine assay

NT plasma levels were assessed using a noncompetitive ELISA with polyclonal anti-nitrotyrosine primary antibody (Sigma) and nitrated BSA (NT-BSA) as a standard. Nitrated BSA was prepared by the incubation of BSA solution (10 mg/ml in PBS, pH 7.4) with tetranitromethane, and NT concentration was estimated spectrophotometrically as described in Ref. [25]. Wells were coated with 50 μl NT-BSA (165 ng/ml in PBS), and incubated overnight at 37 °C. Plates were washed with PBS/Tween (0.05% Tween 20 in PBS) and blocked with 200 μl/well of blocking buffer (1% FCS in PBS/Tween) for 1 h at 37 °C. After blocking, 50 μl of NT-BSA standards (concentration range, 100–800 nM) and plasma samples (diluted 1:1.5 with PBS) were added followed by 50 μl of primary antibody (diluted 1:6000 in blocking buffer). After incubation for 1.5 h at 37 °C and washing, 100 μl of secondary biotin-SP-conjugated donkey anti-rabbit antibody (Jackson ImmunoResearch, PA, USA, diluted 1:10,000 in blocking buffer) was added and plates were incubated again for 1.5 h at 37 °C. After another washing step streptavidin-biotinylated horseradish peroxidase conjugate (Amersham Biosciences, UK; diluted 1:4000 in blocking buffer) was added. Plates were incubated for 1 h at 37 °C and washed. Color was developed by adding the TMB liquid substrate system and the reaction was stopped with H_2SO_4 after 50–60 min incubation in the dark. The absorbance was measured with a microplate reader at 450 nm. Any samples with inhibition <20% or >80% were repeatedly analyzed either without dilution or with further dilution, respectively. Each sample was analyzed in triplicate. Plasma nitrotyrosine concentration was expressed in nmol/l plasma.

2.7. Cotinine assay and vitamin analysis

Urinary cotinine levels as a marker of smoking were analyzed by radioimmunoassay [26]. The plasma levels of vitamins A and E were analyzed by HPLC with UV detection [27], vitamin C levels were assessed according to Tanishima and Kita [28].

2.8. Statistical analysis

Statistical calculations were performed using SPSS software. For the data that was not distributed normally, nonparametric methods were used: Mann–Whitney Rank Sum *U*-test for comparison of two groups and Spearman Rank correlation test for evaluating the relationship between biomarkers. Data with normal distribution was analyzed by *T*-test and Pearson correlation test. Multiple linear regression analysis was performed to identify factors affecting levels of oxidative and nitrosative stress markers.

3. Results

3.1. Study population characteristics

The information on the study population is shown in Table 1. Both groups did not differ by age ($p = 0.37$) and cotinine/creatinine levels ($p = 0.19$). Cotinine/creatinine levels were assessed as a marker of cigarette smoke exposure. Plasma levels of vitamin C were the same in both groups ($p = 0.30$), while both vitamin A and vitamin E levels were higher in bus drivers (vitamin A, $p < 0.01$; vitamin E, $p < 0.001$).

3.2. Exposure to environmental pollution

Table 2 shows the exposure to BaP, c-PAHs, PM2.5 and PM10 in bus drivers and controls. BaP and c-PAHs levels were assessed by personal monitors and show the actual exposure to environmental pollutants for individual subjects. Due to the very low amount of PM collected by personal monitors we were not able to assess the individual exposure to PM2.5 and PM10. To obtain information on PM levels in ambient air during the sampling periods we used stationary VAPS monitors that showed the air quality for each given period. Table 2 demonstrates that, unexpectedly, the exposure to BaP and c-PAHs was significantly higher in controls than in bus drivers (BaP, $p < 0.01$; c-PAHs, $p < 0.05$). Bus drivers were sampled from 15 to 29 November 2005, while the control samples were collected later, from 9 to 19 December 2005. The results from VAPS monitors show that the ambient air quality was better during the sampling of controls (PM2.5, $p < 0.001$, PM10, $p < 0.001$).

Table 1
Characteristics of study population

	Bus drivers $N = 50$		Controls $N = 50$		p
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
Age (years)	49.8 ± 9.5	48.9 (32.0, 62.0)	50.5 ± 10.5	53.0 (23.0, 66.0)	0.37
Cotinine/creatinine (ng/mg creatinine)	23.9 ± 51.5	14.4 (3.0, 358.1)	53.2 ± 158.4	11.4 (3.0, 781.7)	0.19
Vitamin A (mg/l)	1.0 ± 0.3	1.0 (0.5, 1.7)	0.9 ± 0.2	0.8 (0.4, 1.6)	<0.01
Vitamin E (mg/l)	13.9 ± 3.7	14.3 (7.8, 22.9)	9.8 ± 3.0	9.0 (4.0, 17.0)	<0.001
Vitamin C (mg/l)	9.2 ± 3.4	8.7 (3.6, 19.6)	10.0 ± 3.3	10.0 (3.6, 18.1)	0.30

Table 2
Exposure to BaP, c-PAHs and PM

	Bus drivers $N=50$		Controls $N=50$		p
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
BaP (ng/m^3)[a]	1.3 ± 0.7	1.1 (0.3, 3.3)	1.8 ± 1.0	1.7 (0.4, 6.7)	<0.01
c-PAHs (ng/m^3)[a]	7.1 ± 3.7	5.7 (1.9, 18.9)	9.4 ± 5.5	9.2 (2.1, 35.4)	<0.05
PM2.5 (μg/m^3)[b]	32.1 ± 8.1	31.7 (19.3, 43.3)	20.9 ± 6.8	20.3 (11.6, 29.8)	<0.001
PM10 (μg/m^3)[b]	38.6 ± 8.2	37.3 (25.1, 49.7)	24.1 ± 6.5	23.8 (13.9, 32.5)	<0.001

[a] Exposure to BaP and c-PAHs assessed by personal monitors in two consecutive days.
[b] PM2.5 and PM10 levels show the overall air quality in the period when bus drivers (15–29 November 2005) and controls (9–19 December 2005) were sampled.

3.3. Oxidative and nitrosative stress markers

Table 3 and Fig. 1 illustrate the comparison of oxidative and nitrosative stress markers between bus drivers and controls. Significantly elevated levels in bus drivers were observed for all three markers of oxidative stress. The median concentration of 8-oxodG in bus drivers was 7.79 nmol/mmol creatinine (min, max; 2.64–12.34), while the corresponding value in controls was 6.12 nmol/mmol (min, max; 0.70–11.38) ($p<0.01$). The concentration of 15-F_{2t}-IsoP among bus drivers reached the median value 0.81 nmol/mmol (min, max; 0.38–1.55), and in controls 0.68 nmol/mmol (min, max; 0.39–1.79) ($p<0.01$). The levels of protein carbonyl groups in bus drivers was 14.1 nmol/ml (min, max; 11.8–19.0), while in controls it was 12.9 nmol/ml (min, max; 9.8–16.6) ($p<0.001$). Similar to the oxidative stress markers, levels of NT were significantly higher in bus drivers (median levels of NT: 694 nmol/l (min, max; 471–3228) in bus drivers, and 537 nmol/l (min, max; 268–13833) in controls; $p<0.001$).

3.4. Correlation of oxidative and nitrosative stress markers with factors possibly affecting their levels

We analyzed possible correlations between 8-oxodG, 15-F_{2t}-IsoP, protein carbonyl, and NT levels and age, vitamins A, E, and C and cotinine levels, as well as markers of exposure to polluted air (Table 4). Urinary 8-oxodG correlated positively with vitamin E levels ($R=0.27$, $p=0.01$) and PM10 exposure ($R=0.20$, $p=0.05$). Negative correlations with BaP ($R=-0.24$, $p<0.05$), and c-PAHs ($R=-0.25$, $p=0.01$) exposure were also found. Levels of 15-F_{2t}-IsoP correlated positively with vitamin E levels ($R=0.23$, $p<0.05$), cotinine levels ($R=0.40$, $p=0.001$), PM2.5 ($R=0.20$, $p=0.05$) and PM10 ($R=0.23$, $p<0.05$) exposure; a negative correlation with vitamin C levels ($R=-0.33$, $p=0.001$) was observed. Plasma carbonyl levels correlated positively with vitamin A ($R=0.25$, $p<0.05$) and vitamin E ($R=0.32$, $p<0.001$) levels, and with the markers of air pollution: BaP ($R=0.28$, $p=0.01$), c-PAHs (0.29, $p=0.01$), PM2.5 ($R=0.30$, $p=0.01$), and PM10 ($R=0.31$, $p<0.001$). 3-Nitrotyrosine plasma levels correlated positively with levels of PM2.5 ($R=0.29$, $p=0.01$) and PM10 ($R=0.30$, $p=0.01$).

3.5. Multiple linear regression analysis of factors affecting levels of oxidative and nitrosative stress markers

The results are reported in Table 5. Urinary 15-F_{2t}-IsoP was negatively associated with vitamin C levels; each mg increase of vitamin C/ml plasma decreased 15-F_{2t}-IsoP levels of 0.022 nmol/mmol creatinine. Plasma carbonyl levels were positively affected by BaP exposure; each ng/m^3 increase of BaP increased plasma carbonyl levels of 0.586 nmol/ml. Plasma carbonyl lev-

Table 3
Levels of oxidative and nitrosative stress markers (8-oxodG, 15-F_{2t}-IsoP, protein carbonyl, NT) in bus drivers and controls

	Bus drivers			Controls			p
	N	Mean ± S.D.	Median (min, max)	N	Mean ± S.D.	Median (min, max)	
8-OxodG (nmol/mmol creatinine)	50	7.59 ± 2.25	7.79 (2.64–12.34)	50	6.29 ± 2.59	6.12 (0.70–11.38)	<0.01
15-F_{2t}-isoprostane (nmol/mmol creat)	50	0.85 ± 0.26	0.81 (0.38–1.55)	50	0.73 ± 0.33	0.68 (0.39–1.79)	<0.01
Carbonyl (nmol/ml)	40	14.4 ± 1.7	14.1 (11.8–19.0)	45	13.0 ± 1.6	12.9 (9.8–16.6)	<0.001
3-Nitrotyrosine (nmol/l)	40	815 ± 459	694 (471–3228)	45	856 ± 1989	537 (268–13833)	<0.001

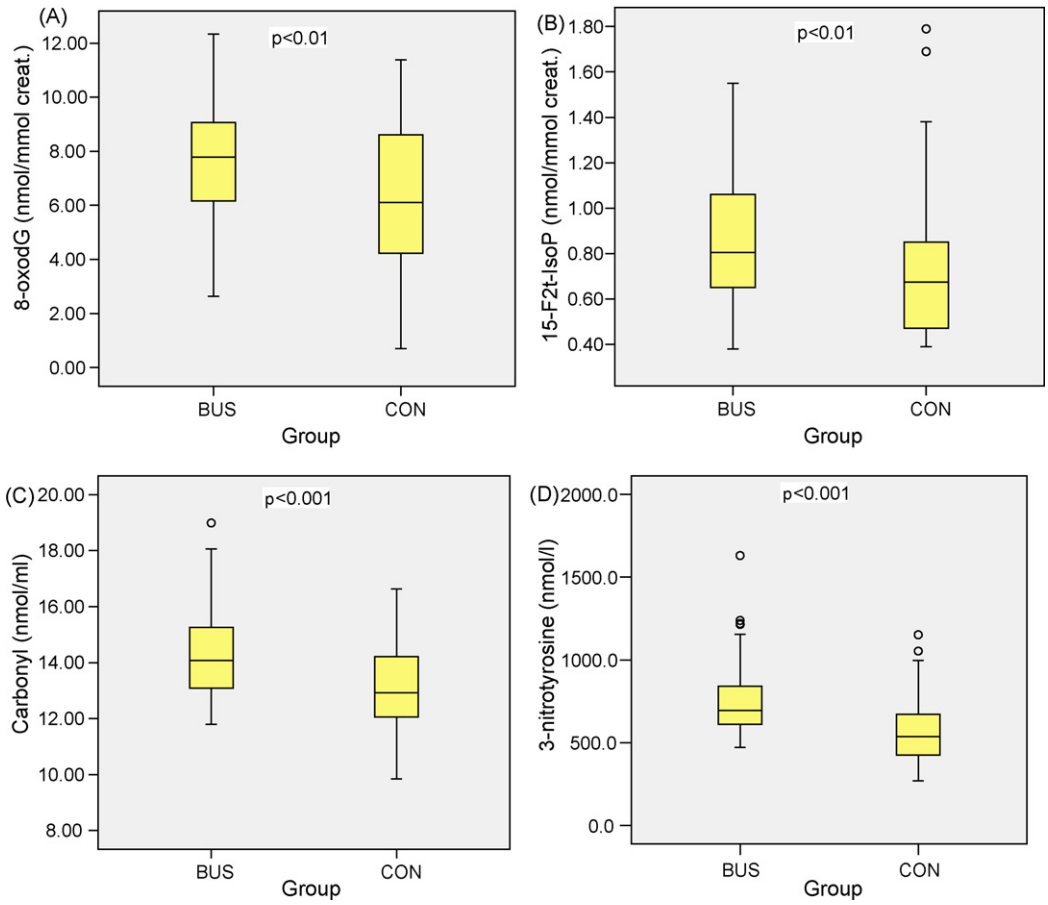

Fig. 1. Comparison of oxidative and nitrosative stress markers between bus drivers (BUS) and controls (CON)—graph A: urinary 8-oxodG levels ($p < 0.01$); graph B: urinary 15-F_{2t}-Iso levels ($p < 0.01$); graph C: plasma carbonyl levels ($p < 0.001$); graph D: plasma 3-nitrotyrosine levels ($p < 0.001$); an outlier (control sample, NT concentration: 13833 nM) is not shown in the graph.

els were also significantly higher in exposed subjects. Plasma 3-nitrotyrosine levels were negatively correlated with age; each additional year decreased 3-nitrotyrosine levels of 40 nmol/l.

4. Discussion

In the present study, we analyzed the effect of air pollution on markers of oxidative and nitrosative stress in bus drivers and controls. Bus drivers were selected as a group with a high risk of exposure to air pollution. Since they operate buses in Prague in places with heavy traffic, we expected them to have high levels of exposure to c-PAHs and BaP. Unexpectedly, the exposure to both c-PAHs and BaP was significantly lower then in the control group. First, we hypothesized the ambient air quality was worse in the period of bus drivers sampling due to atmospheric inversions that are common in this season. To check the air quality we analyzed the data from the stationary VAPS monitors. The levels of PM2.5 and PM10 measured by VAPS at two locations in Prague revealed that air pollution had been higher in the bus drivers' sampling period. Thus, we obtained unexpected results: bus drivers sampled in the period of higher air pollution had lower exposure to c-PAHs and BaP than the control group sampled in the period with lower air pollution. We hypothesize that the lower exposure levels of bus drivers could be caused by the fact that, due to the winter season, drivers spent most of their working shift in the closed bus cabin with closed windows. On the other hand, controls were selected among administrative workers of Transportation Authority of the City of Prague. Its offices are located in the city center; thus, the controls could also have been exposed to relatively high air pollution.

Despite the lower levels of BaP and c-PAHs exposure, all analyzed oxidative and nitrosative stress markers were increased in bus drivers, which is also in accordance

Table 4
Correlation between oxidative stress markers and factors that can affect oxidative stress among controls

	8-OxodG	15-F_{2t}-isoprostane	Carbonyl	3-Nitrotyrosine
Age				
R	0.09	0.10	−0.10	−0.09
Sig.	0.38	0.33	0.35	0.42
Vitamin A				
R	−0.07	0.07	0.25	0.01
Sig.	0.52	0.52	0.02	0.95
Vitamin E				
R	0.27	0.23	0.32	0.09
Sig.	0.01	0.03	0.0001	0.40
Vitamin C				
R	0.01	−0.33	−0.19	0.05
Sig.	0.97	0.001	0.09	0.67
Cotinine				
R	0.16	0.40	0.09	0.13
Sig.	0.13	0.001	0.43	0.25
BaP				
R	−0.24	0.07	0.28	−0.17
Sig.	0.02	0.50	0.01	0.12
c-PAHs				
R	−0.25	0.09	0.29	−0.12
Sig.	0.01	0.40	0.01	0.29
PM2.5				
R	0.17	0.20	0.30	0.29
Sig.	0.11	0.05	0.01	0.01
PM10				
R	0.20	0.23	0.31	0.30
Sig.	0.05	0.02	0.0001	0.01

with the worse ambient air quality measured by PM2.5 and PM10 levels in this sampling period. We speculate that the exposure to c-PAHs and BaP is not sufficient for increased oxidative and nitrosative stress in this group. Our results support the hypothesis that PM2.5 exposure is the actual cause of increased levels of oxidative stress markers [29].

We also have to take into account another possible explanation for the higher oxidative and nitrosative stress in bus drivers: bus drivers are exposed to mental stress that arises from their responsibility for passengers' health and safety. Theoretically, this kind of stress could contribute to increased levels of the analyzed markers.

8-OxodG is a widely used marker of oxidative DNA damage. It was assessed in several studies to analyze the effect of polluted urban air on bus drivers [30], taxi drivers [31], or people living in a city center [32]. While urinary levels of 8-oxodG were increased in city bus drivers [30] as compared with drivers from a suburban area, and higher in taxi drivers than in a control group [31], these studies do not give any information on the level of exposure to either c-PAHs and BaP, or PM. In the study of Sorensen et al. [32], personal exposure to PM2.5 in 50 students living and studying in Copenhagen was measured over a 1-year period and DNA damage markers were analyzed. At the same time ambient PM2.5 concentrations were measured. The results of this study indicate that only the personal exposure to PM2.5 was a predictor of 8-oxodG levels in lymphocyte DNA. No effect on urinary 8-oxodG levels was found. Ambient PM2.5 concentrations had no effect on any of the markers analyzed. The authors conclude that personal PM2.5 exposure is more important for the induction of oxidative DNA damage than ambient PM2.5 concentrations. In the present study, we analyzed personal exposure to c-PAHs and BaP, and ambient exposure to PM2.5 and PM10, but not personal exposure to PM2.5. There were technical differences between monitors used in our study and the study of Sorensen et al. [32] (lower flow, samples collected for 2× 24 h, instead of 48 h) that resulted in the fact that we were not able to measure personal exposure to PM2.5. However, our results indicate that, unlike the above-mentioned study, exposure to ambient levels of PM10 correlated well with 8-oxodG urinary levels. This discrepancy may be caused by differences in exposure to PM in Copenhagen and Prague, with the values in Prague being about three-fold higher. Also, the chemical composition of PM from both cities, that is crucial for DNA damage [33], probably differs.

Table 5
Multiple linear regression analysis of factors affecting levels of oxidative and nitrosative stress markers (8-oxodG, 15-F_{2t}-IsoP, protein carbonyl, NT) among all subjects; adjusted for age

	Intercept	Age (years)	BaP (ng/m^3)	Vitamin E (mg/l)	Vitamin C (mg/l)	Exposed/controls
8-OxodG	2.84	0.021	–	0.154	0.090	0.696
15-F_{2t}-isoprostane	0.68	0.004	–	–	−0.022*	–
Carbonyl	13.52	−0.029	0.586**	0.073	−0.078	1.195**
3-Nitrotyrosine	2861	−40*	–	–		–

* $p < 0.05$; ** $p < 0.01$.

We should also note that protein carbonyl levels were the only analyzed marker that significantly positively correlated with BaP and c-PAHs exposure; no such correlation was observed for 15-F_{2t}-IsoP, 8-oxodG, and NT.

Unexpectedly, we found negative correlations between 8-oxodG levels and c-PAHs and BaP exposure. While this may be surprising, we must bear in mind the fact that urinary 8-oxodG levels reflect not only DNA damage, but are also the result of DNA repair [34] which may have contributed to this result.

Studies on effect of PM on protein oxidation and lipid peroxidation are scarce. Among them, the Copenhagen study of Sorensen et al. [35] found a significant correlation between protein oxidation measured by plasma 2-amino-adipic semialdehyde and black smoke exposure. Plasma malondialdehyde levels, a marker of lipid peroxidation, were increased in women only. Similar results for both protein oxidation and lipid peroxidation were found in an earlier study in Copenhagen bus drivers [36]. To the best of our knowledge, no studies on protein carbonyl levels, as a marker of protein oxidation, and 15-F_{2t}-IsoP, as a marker of lipid peroxidation, in relation to PM exposure have been published so far.

The suitability of 3-nitrotyrosine as a marker of polluted air was studied primarily *in vitro*, or in animal models. In mice, both diesel exhaust particles [37] and exposure to cigarette smoke [38] resulted in nitrotyrosine formation. Diesel exhaust particles and cigarette smoking have also been proved to induce protein nitration *in vitro* [39–41]. Thus, our study is the first one analyzing the effect of c-PAHs, BaP, and PM exposure on plasma NT levels in humans.

Cigarette smoking results, among other side effects, in increased oxidative stress. It has been shown repeatedly that 15-F_{2t}-isoprostane levels positively correlate with smoking status [42]. In this regard, our study is in concordance with the results of other authors.

While vitamins in plasma serve as natural antioxidants, relationship between their levels and oxidative stress markers is often contradictory, with some studies finding no effect [43,44], others finding a negative association [45]. In our study, we found a significant negative correlation between vitamin C and 15-F_{2t}-IsoP levels. However, vitamin E levels were positively correlated with all three markers of oxidative stress. Similar results were obtained in the study of Bianchini et al. [46], where 8-oxodG levels in lymphocytes were positively associated with plasma alpha-tocopherol. These results may be caused by the differences in diet, but they may also reflect the protective response of organism to oxidative stress. We speculate that, as a consequence of stress, plasma levels of lipophilic antioxidants may increase to ensure better protection of organism. A positive association was also found between protein carbonyl and vitamin A plasma levels. The explanation for this result may be the same as for vitamin E levels.

Multiple linear regression analysis confirmed some of the results obtained with correlation tests. Thus, 15-F_{2t}-IsoP levels were significantly affected by plasma vitamin C levels and carbonyl levels were related to exposure to BaP. However, in general, the correlations between oxidative and nitrosative stress markers and BaP, c-PAHs, and PM exposure were not observed in the model used for multiple linear regression.

In conclusion, we found that higher exposure to PM2.5 and PM10 was associated with increased levels of a panel of oxidative and nitrosative stress markers. Exposure to c-PAHs and BaP had no effect on 15-F_{2t}-isoP and NT levels.

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Příloha 2

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Part I. Oxidative damage to DNA

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Seasonal variability of oxidative stress markers in city bus drivers Part I. Oxidative damage to DNA

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ABSTRACT

We investigated the seasonal variability of 8-oxodeoxyguanosine (8-oxodG), a marker of oxidative damage to DNA, in urine of 50 bus drivers and 50 controls in Prague, Czech Republic, in three seasons with different levels of air pollution: winter 2005, summer 2006 and winter 2006. The exposure to environmental pollutants (carcinogenic polycyclic aromatic hydrocarbons, c-PAHs, particulate matter (PM), and volatile organic compounds (VOC)) was monitored by personal and/or stationary monitors. For the analysis of 8-oxodG levels, the ELISA technique was used. Bus drivers were exposed to significantly higher levels of c-PAHs in winter 2006, while in the other two seasons the exposure of controls was unexpectedly higher than that of bus drivers. We did not see any difference in VOC exposure between both groups in summer 2006 and in winter 2006; VOC were not monitored in winter 2005. 8-OxodG levels were higher in bus drivers than in controls in all seasons. The median levels of 8-oxodG (nmol/mmol creatinine) in bus drivers vs. controls were as follows: winter 2005: 7.79 vs. 6.12 ($p = 0.01$); summer 2006: 6.91 vs. 5.11 ($p < 0.01$); winter 2006: 5.73 vs. 3.94 ($p < 0.001$). Multivariate logistic regression analysis identified PM2.5 and PM10 levels, measured by stationary monitors during a 3-day period before urine collection, as the only factors significantly affecting 8-oxodG levels, while the levels of c-PAHs had no significant influence.

1. Introduction

Ambient air pollution is a widespread problem that affects the human population in industrial countries, especially in large cities. Exposure to polluted air is associated with many diseases including cancer, as well as with increased mortality [1,2]. It is generally agreed that particulate matter (PM), a substantial part of polluted air, mediates its negative health effects via inflammation and/or oxidative stress induction [3]. PM consists of dust, soot and other solid, liquid and aerosol particles as well as various chemicals bound to them. In terms of the impact on human health, carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) are among the most important compounds present in PM [4]. c-PAHs are metabolized into reactive intermediates that bind to DNA and form PAH-DNA adducts. Unrepaired PAH-DNA adducts can lead to mutations; high PAH-DNA adduct levels have been correlated with an increased cancer risk [5]. Apart from their carcinogenicity, c-PAHs may also contribute to oxidative stress induction via their metabolism by CYP1A1 and the subsequent formation of reactive quinones [6]. However, PM contains other components with greater potency for inducing oxidative stress—transition metals, benzene and other volatile organic compounds (VOC), as well as solid particles causing inflammation [7,8].

VOC include almost all organic chemicals participating in atmospheric photochemical reactions. Traffic is the major source of VOC, but they are also used as paint thinners, metal degreasers, and cleaning and cosmetic products. Upon entering the organism in a gaseous, liquid or aerosol form, VOC can cause erythema, edema, increased skin blood flow, leukocyte infiltration, necrosis and increased levels of reactive oxygen species (ROS). VOC exposure is also linked with a higher incidence of asthma [9,10].

Oxidative stress is defined as an imbalance between the levels of pro-oxidants and/or ROS production and antioxidants in the organism [11]. ROS may arise from either exogenous or endogenous sources. While endogenous sources are mostly physiological and include various metabolic processes and inflammation, exogenous sources are represented by environmental factors, including smoking, diet, or exposure to environmental pollution. Oxidative stress causes damage to macromolecules, including DNA, proteins and lipids [12].

Oxidative damage to DNA is mediated mostly by the highly reactive hydroxyl radical that reacts with DNA by addition to double

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; ROS, reactive oxygen species; PM, particulate matter; PM2.5, particulate matter < 2.5 μm; PM10, particulate matter < 10 μm; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; B[*a*]P, benzo[*a*]pyrene; VOC, volatile organic compounds.

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bonds of DNA bases and by the abstraction of a hydrogen atom from the methyl group of thymine and C–H bonds of 2′-deoxyribose [13]. The attack of the hydroxyl radical on DNA yields a number of modified bases; however, as a biomarker of oxidative DNA damage 8-oxodeoxyguanosine (8-oxodG) is most often studied. The oxidized base is highly mutagenic and, if unrepaired, its presence in DNA causes GC > TA transversions. If repaired, 8-oxodGua is excreted in urine where it can be assessed by various techniques as a general biomarker of oxidative stress. It should be also noted that other sources of 8-oxodG, including oxidation of the nucleotide pool, may affect urinary 8-oxodG levels [14].

The aim of the present study was to analyze the effect of air pollution, particularly c-PAHs, benzo[*a*]pyrene (B[*a*]P, a model c-PAH), VOC and PM, on urinary 8-oxodG levels in 50 bus drivers and 50 controls working in Prague, Czech Republic, and to investigate seasonal variations in this marker of oxidative DNA damage. To meet this aim, we followed the study subjects in three seasons with different levels of air pollution: winter 2005, summer 2006 and winter 2006. We also investigated the effect of other factors that may affect the response of the organism to oxidative stress (vitamins A, C and E, HDL and LDL cholesterol and triglyceride levels, age, and cotinine as a marker of tobacco smoke exposure). Finally, we tried to identify an independent factor that affects oxidative stress levels. While some of the data gathered in the winter 2005 sampling period has already been published [15], the current work extends the previous results by adding VOC exposure and by adding two more sampling periods. This allowed us not only to analyze differences in oxidative stress markers between bus drivers and controls, but also to assess the seasonal variability of these biomarkers and to perform a detailed statistical analysis of factors affecting oxidative stress.

2. Material and methods

2.1. Subjects and sampling

The study population consisted of 50 exposed subjects (BUS), bus drivers working in the center of Prague, Czech Republic, presumably exposed to high levels of air pollution. The control subjects (CON) consisted of 50 healthy male volunteers spending >90% of daily time indoors. All subjects were non-smokers. Each subject completed a questionnaire on personal medical history and life-style. To compare the variations in air pollution and their impact on oxidative stress biomarkers, all participants were followed in three seasons: winter 2005 (groups denoted BUS1, CON1), summer 2006 (BUS2, CON2) and winter 2006 (BUS3, CON3).

All participants signed an informed consent form and could cancel their participation at any time during the study, according to the Helsinki II declaration. The study was approved by the ethical committee of the Institute of Experimental Medicine AS CR in Prague. Any person who underwent medical treatment, radiography or vaccination up to 3 months before sampling was not included in the study.

The spot urine samples and blood were collected at the end of the working shifts. For each subject, one urine sample and one blood sample was obtained for each season. The blood samples were collected by venipuncture into vacuettes containing sodium heparin. Samples were coded, transported to the Laboratory of Genetic Ecotoxicology, processed and kept in aliquots at −80 °C.

2.2. Exposure assessment—c-PAHs and PM

Subjects' exposure to c-PAHs was monitored by personal samplers used by the study subjects during 2 consecutive days (48 h). For each subject, the personal monitoring was performed once in each season. The samplers were equipped with filters collecting particles of aerometric diameter 2.5 μM (PM2.5) [16]. Quantitative chemical analysis of c-PAHs, (benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, dibenzo[*a*,*h*]anthracene, benzo[g,*h*,*i*]perylene and indeno[1,2,3-*c*,*d*]pyrene) was performed by HPLC with fluorescence detection according to the EPA method [17] in the certified laboratory ALS Czech Republic, Prague.

Ambient air quality during sampling periods was monitored using stationary versatile air pollution samplers (VAPS) at two locations in Prague with heavy and lighter traffic. The samplers continuously measured the levels of c-PAHs, PM10 and PM2.5 [18].

2.3. Exposure assessment—VOC

Traffic-related VOC (benzene, toluene, ethylbenzene, *m*-, *p*- and *o*-xylen) were collected on Radiello® radial diffusive samplers (Supelco, PA, USA) worn by study subjects for 24 h. VOC were trapped on graphitized charcoal by adsorption and recovered by thermal desorption. The analysis was performed by capillary gas chromatography with flame ionization detector technique detection in the certified laboratory ALS Czech Republic, Prague. The sorbent cartridges were preserved in carrier tubes at all times before and after each sampling. To reveal secondary contamination, blank samples were used with every sampling group.

Stationary monitoring of benzene, a representative VOC, was performed continually at the same locations as VAPS monitoring by gas chromatography with flame/photo ionization detection using analyzers by the Czech Hydrometeorological Institute, Prague.

2.4. 8-OxodG ELISA

Urinary 8-oxodG levels were analyzed by competitive ELISA essentially as previously described [15,19]. Each sample was analyzed in triplicate. Urinary 8-oxodG concentration was expressed as nmol 8-oxodG/mmol creatinine.

2.5. Cotinine assay, creatinine levels and vitamin analysis

Urinary cotinine levels as a marker of exposure to tobacco smoke were analyzed by radioimmunoassay [20]. Creatinine levels were measured in urine by the reaction with picric acid (the Jaffe method). The plasma levels of vitamins A, E and C were analyzed by HPLC with UV detection [21,22].

2.6. Plasma lipids

The plasma levels of LDL and HDL cholesterol and triglycerides were determined spectrophotometrically using Sigma diagnostics kits and appropriate standards.

2.7. Statistical analysis

Statistical analyses were performed using Statistica 7.1 (StatSoft, OK, USA) and SAS 9.1.3. (SAS Institute, NC, USA). For data that were not distributed normally, non-parametric methods were used: Mann–Whitney Rank Sum *U*-test for comparison of two groups or two sample periods. Multivariate logistic regression and linear regression analysis were performed to identify multifactorial impact affecting levels of 8-oxodG. For logistic regression estimates, continuous variables were transformed into a three level scale using tertiles, levels of 8-oxodG were transformed into a two level scale using medians.

3. Results

3.1. Characteristics of the study populations

The basic characteristics of the studied groups are shown in Table 1. The levels of parameters that may affect oxidative stress are presented. There were no age differences between the BUS and CON groups; cotinine/creatinine levels were comparable between both groups, also the levels of LDL, HDL cholesterol and triglycerides were mostly identical in both groups. We observed some differences in vitamin levels between the two groups in winter 2005 and summer 2006.

3.2. Exposure to environmental pollutants

Table 2 reports the levels of air pollutants measured by personal monitors. The monitors were used to assess the exposure to c-PAHs during 48 h preceding sample collection. Results for B[*a*]P, a model c-PAH, are also presented. Even though we expected the personal exposure to c-PAHs and B[*a*]P to be higher in bus drivers than in controls, we observed these results only in the winter 2006 sampling. In winter 2005, controls were exposed to significantly higher levels of c-PAHs and B[*a*]P, while in summer 2006 exposure to B[*a*]P did not differ and c-PAHs levels were higher in controls. Personal exposure to both c-PAHs and B[*a*]P differed significantly ($p < 0.001$) between sampling periods, with the highest levels observed in winter 2005 and lowest in summer 2006.

VOC exposure levels measured by personal monitors 24 h before the collection of biological material were available for the summer 2006 and winter 2006 sampling periods only; in winter 2005 the levels of these pollutants were not analyzed. Unexpectedly, there were essentially no differences between the BUS and CON groups.

Table 1
Characteristics of study subjects

	Winter 2005			Summer 2006			Winter 2006		
	BUS1[a] ($N=50$)	CON1[a] ($N=50$)	p	BUS2[a] ($N=50$)	CON2[a] ($N=50$)	p	BUS3[a] ($N=50$)	CON3[a] ($N=50$)	p
Age (years)	49.8 ± 9.5	50.5 ± 10.5	=0.37	49.6 ± 9.4	48.0 ± 11.9	=0.67	50.2 ± 9.4	48.6 ± 11.8	=0.64
Cotinine (ng/mg creatinine)	23.9 ± 51.5	53.2 ± 158.4	=0.19	23.0 ± 44.0	21.0 ± 33.0	=0.33	31.0 ± 66.0	35.0 ± 101.0	=0.18
Vitamin A (μmol/l)	3.0 ± 0.7	3.5 ± 1.0	<0.001	3.4 ± 0.7	2.6 ± 1.0	<0.05	3.3 ± 0.7	3.8 ± 3.6	=0.76
Vitamin C (μmol/l)	56.6 ± 18.8	52.2 ± 19.6	<0.01	52.9 ± 20.9	70.8 ± 20.8	<0.001	82.4 ± 21.1	83.7 ± 18.4	=0.48
Vitamin E (μmol/l)	22.8 ± 6.9	32.2 ± 8.6	=0.18	24.3 ± 7.5	22.4 ± 11.3	<0.001	29.3 ± 6.9	30.9 ± 12.2	=0.87
LDL cholesterol (mmol/l)	3.5 ± 0.8	3.2 ± 0.7	=0.15	3.5 ± 0.8	3.3 ± 0.8	=0.08	3.6 ± 0.7	3.3 ± 0.7	=0.06
HDL cholesterol (mmol/l)	1.4 ± 0.3	1.5 ± 0.2	=0.09	1.5 ± 0.2	1.5 ± 0.2	=0.42	1.4 ± 0.2	1.5 ± 0.2	<0.05
Triglycerides (mmol/l)	2.1 ± 1.1	2.1 ± 1.6	=0.52	1.8 ± 1.2	2.0 ± 1.7	=0.70	1.9 ± 0.8	1.9 ± 1.5	=0.22

[a] Mean ± S.D.

Table 2
Personal exposure to environmental pollutants

	Winter 2005			Summer 2006			Winter 2006		
	BUS1[a] ($N=50$)	CON1[a] ($N=50$)	p	BUS2[a] ($N=50$)	CON2[a] ($N=50$)	p	BUS3[a] ($N=50$)	CON3[a] ($N=50$)	p
Personal c-PAHs (ng/m^3)	7.1 ± 3.7	9.4 ± 5.5	<0.05	1.8 ± 0.5	2.0 ± 0.8	<0.01	5.4 ± 3.5	4.1 ± 1.7	=0.001
Personal B[a]P (ng/m^3)	1.3 ± 0.7	1.8 ± 1.02	<0.01	0.2 ± 0.1	0.3 ± 0.2	=0.18	1.0 ± 0.5	0.8 ± 0.4	<0.01
Personal VOC (μg/m^3)									
Benzene	N/A	N/A		6.2 ± 2.7	7.1 ± 6.9	=0.17	11.2 ± 15.4	6.9 ± 5.9	<0.001
Toluene	N/A	N/A		31.0 ± 60.2	93.7 ± 253.4	=0.22	44.1 ± 67.2	67.4 ± 139.6	=0.70
Ethylbenzene	N/A	N/A		3.9 ± 5.1	6.7 ± 9.1	=0.22	5.3 ± 4.2	6.7 ± 11.8	=0.59
m-, *p*-Xylene	N/A	N/A		9.8 ± 11.6	21.1 ± 29.6	<0.05	14.3 ± 11.0	22.5 ± 46.6	=0.85
o-Xylene	N/A	N/A		2.9 ± 1.9	5.6 ± 7.6	=0.08	4.1 ± 2.8	7.2 ± 19.8	=0.62

N/A: data not available.
[a] Mean ± S.D.

There were some exceptions, however: higher benzene exposure in bus drivers in winter 2006 and higher levels of *m*- and *p*-xylen in controls in summer 2006. A comparison of VOC levels in all subjects (bus drivers + controls) between both sampling seasons showed significantly ($p < 0.05$) higher levels of all pollutants in winter 2006, with the exception of toluene.

The lack of differences in personal exposure to air pollutants between bus drivers and controls, or the higher exposure in controls, may be explained by the fact that the offices where the control subjects were employed are located in the city center with high levels of air pollution.

3.3. Comparison of 8-oxodG levels between bus drivers and controls in individual seasons and between seasons

Table 3 shows the levels of 8-oxodG in bus drivers and controls in individual sampling periods. For all seasons, we consistently saw significantly higher 8-oxodG levels in bus drivers than in controls, suggesting possible deleterious effects of the environment on the bus drivers. A comparison of individual seasons revealed significant differences between sampling periods with some exceptions (BUS1 vs. BUS2, $p = 0.15$ and CON1 vs. CON2, $p = 0.06$). The highest 8-oxodG levels were detected in winter 2005, while the lowest levels were unexpectedly observed in winter 2006. This observation does not correspond with the levels of air pollutants measured by personal monitors and suggests that other factor(s) than c-PAHs and VOC may be responsible for increased oxidative damage to DNA.

3.4. Identification of factors affecting 8-oxodG levels

In order to identify factors that influence 8-oxodG levels, we pooled the data of exposure to c-PAHs, B[a]P and VOC obtained from personal monitors, information on plasma triglycerides, LDL and HDL cholesterol, vitamin and cotinine levels and age and analyzed their association with 8-oxodG levels obtained from all subjects in all sampling periods using bivariate linear regression, logistic regression and multivariate regression analysis. Due to the very low quantity of PM collected on the filters, we were not able to assess personal exposure to PM2.5 or PM10. However, since exposure to PM is believed to be very important in the process of oxidative stress induction, we used the PM2.5 and PM10 data from stationary monitors and analyzed their association with oxidative DNA damage. The levels of PM2.5, PM10 and c-PAHs were monitored continuously by VAPS systems at two locations in Prague, so that we were able to calculate PM and c-PAHs concentrations in the air within various time periods before sampling. VOC levels were also measured by stationary monitors, and benzene concentrations were added to the statistical analysis. To evaluate the effect of stationary PM, c-PAHs and benzene exposure, we analyzed the association between 8-oxodG levels in urine and the concentrations of air pollutants during 3-, 7- and 15-day intervals measured up to 2 months before

Table 3
Levels of 8-oxodG (in nmol/mmol creatinine) in the urine of bus drivers and controls in individual sampling periods

	BUS ($N=50$)		CON ($N=50$)		p
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
Winter 2005	7.59 ± 2.25	7.79 (2.64, 12.34)	6.29 ± 2.59	6.12 (0.70, 11.38)	=0.01
Summer 2006	6.73 ± 2.48	6.91 (1.30, 10.68)	5.51 ± 2.36	5.11 (2.34, 12.32)	<0.01
Winter 2006	5.67 ± 2.50	5.73 (1.54, 11.89)	3.82 ± 1.73	3.94 (0.45, 7.65)	<0.001

Table 4
Bivariate comparison by linear and logistic regression analysis between 8-oxodG levels and factors possibly affecting oxidative stress in all subjects ($N = 300$)

	Linear regression		Logistic regression	
	R (p-value)	p	OR (95% CI)	p
Personal B[a]P	0.10	=0.60	1.43 (0.90–2.27)	=0.13
Personal c-PAHs	0.03	=0.49	1.40 (0.88–2.22)	=0.16
Personal VOC				
Benzene	0.02	=0.39	1.64 (0.93–2.90)	=0.08
Toluene	−0.002	=0.13	0.86 (0.48–1.51)	=0.59
Ethylbenzene	−0.03	=0.18	1.14 (0.64–2.00)	=0.66
m-, p-Xylene	−0.01	=0.10	1.19 (0.68–2.10)	=0.54
o-Xylene	−0.03	=0.07	1.12 (0.63–1.97)	=0.70
Stationary B[a]P[a]	0.20	=0.10	1.15 (0.87–1.51)	=0.33
Stationary c-PAHs[a]	0.04	=0.07	1.31 (1.00–1.73)	=0.05
Stationary PM2.5[a]	0.06	<0.001	1.80 (1.36–2.39)	<0.001
Stationary PM10[a]	0.08	<0.001	2.05 (1.54–2.73)	<0.001
Stationary VOC-benzene[a]	0.06	=0.78	1.02 (0.76–1.38)	=0.87
Cotinine (> median)	0.03	=0.87	1.19 (0.76–1.88)	=0.45
LDL cholesterol	0.08	=0.68	1.16 (0.73–1.85)	=0.52
HDL cholesterol	−0.27	=0.68	0.96 (0.61–1.52)	=0.86
Triglycerides	0.04	=0.70	1.67 (1.05–2.66)	<0.05
Vitamin C	−0.02	<0.001	0.55 (0.34–0.88)	<0.05
Vitamin A	−0.09	=0.34	0.91 (0.57–1.44)	=0.68
Vitamin E	0.01	=0.47	1.59 (1.00–2.53)	=0.05
Age (>median)	0.03	=0.68	0.89 (0.56–1.41)	=0.89

[a] Results for stationary monitoring performed over a 3-day period, 3 days before sampling.

Table 5
Multivariate logistic regression analysis of environmental pollutants and their impact on 8-oxodG levels ($N = 300$)

	OR (95% CI)	p
Personal B[a]P	0.96 (0.71–1.29)	=0.78
Personal c-PAHs	1.03 (0.76–1.38)	=0.86
Personal VOC		
Benzene	1.02 (0.71–1.47)	=0.92
Toluene	0.95 (0.65–1.38)	=0.77
Ethylbenzene	1.05 (0.73–1.52)	=0.80
m-, p-Xylene	0.98 (0.68–1.41)	=0.92
o-Xylene	1.10 (0.76–1.59)	=0.63
Stationary B[a]P[a]	1.10 (0.83–1.47)	=0.51
Stationary c-PAHs[a]	1.28 (0.96–1.71)	=0.09
Stationary PM2.5[a]	1.64 (1.21–2.24)	<0.01
Stationary PM10[a]	2.16 (1.58–2.94)	<0.001
Stationary VOC-benzene[a]	0.99 (0.72–1.35)	=0.93

[a] Results for stationary monitoring performed over a 3-day period, 3 days before sampling.

urine collection. The analysis showed that exposure to these environmental pollutants, with the exception of benzene, in the 3-day period immediately preceding sample collection had the strongest effect on 8-oxodG levels. Thus, the data presented in Tables 4 and 5 are based on exposure to stationary c-PAHs and PM during this period. The mean, median, minimum, maximum and tertiles of stationary B[a]P, c-PAHs, PM2.5, PM10 and benzene for this period are presented in Table 6. The results for other 3-day periods and for longer periods (7 and 15 days) are not shown although they were also included in the statistical analysis.

Bivariate analysis of factors that may affect urinary 8-oxodG levels is presented in Table 4. The results indicate that personal exposure to either c-PAHs or B[a]P 48 h before the collection of biological material, or to any VOC 24 h before sampling, had no significant impact on 8-oxodG levels in the urine of any of the subjects. Exposure to c-PAHs, B[a]P and benzene measured by stationary monitors also did not correlate with oxidative damage to DNA. Only stationary PM2.5 and PM10 were factors significantly

Table 6
Levels of environmental pollutants assessed by stationary monitors

	Mean ± S.D.	Median (min, max)	Tertiles
Stationary B[a]P (ng/m^3)	1.6 ± 1.3	1.1 (0.4, 4.7)	0.8; 1.9
Stationary c-PAHs (ng/m^3)	8.6 ± 6.5	6.2 (2.1, 24.8)	4.3; 10.0
Stationary PM2.5 (μg/m^3)	16.4 ± 8.9	11.5 (5.3, 34.6)	10.8; 19.6
Stationary PM10 (μg/m^3)	20.7 ± 9.7	19.0 (6.2, 39.9)	17.0; 24.9
Stationary VOC-benzene (μg/m^3)	1.4 ± 0.7	1.4 (0.3, 3.2)	1.0; 1.7

Values are calculated as average concentrations from two locations in Prague, for all three sampling seasons, over an interval of 3 days before the collection of biological material.

affecting 8-oxodG levels. Among the other analyzed factors, vitamin C levels were found to be negatively correlated with 8-oxodG levels in urine. The results of linear regression were confirmed by logistic regression analysis (Table 4): among environmental pollutants, only stationary PM2.5 and PM10 affected 8-oxodG levels. Logistic regression also confirmed the negative effect of vitamin C and revealed the effect of triglycerides and the borderline influence of vitamin E in blood plasma.

Multivariate logistic regression analysis adjusted for all factors possibly affecting oxidative stress also showed that 8-oxodG levels in urine were influenced only by PM2.5 and PM10 concentrations. c-PAHs, B[a]P and VOC levels measured by either personal or stationary monitors did not correlate with oxidative damage to DNA (Table 5).

As mentioned above, the effects of stationary PM on 8-oxodG levels were most pronounced when the PM2.5 and PM10 concentrations measured over a 3-day period immediately before sampling were used in the analysis. The results for other 3-day periods up to 33 days before the collection of urine samples, showed a similar trend; however, the results were not significant in all periods (Table 7).

4. Discussion

In the present study we analyzed the effect of air pollution on urinary levels of 8-oxodG in a group of bus drivers and in controls in three seasons with different air pollution levels. Personal exposure to c-PAHs was highest in winter 2005 and lowest in summer 2006, while 8-oxodG levels in urine were unexpectedly lowest in winter 2006. This observation indicates that personal exposure to c-PAHs during 48 h before the collection of biological material is not a critical factor affecting oxidative damage to DNA.

PAHs may induce oxidative stress during their metabolism by the formation of reactive quinones. However, the results of epidemiological studies on 8-oxodG levels after occupational, as well as environmental, PAH exposure are contradictory. Several authors reported a positive correlation between PAH exposure, measured by urinary levels of 1-hydroxypyrene, and 8-oxodG levels, either in lymphocytes or in urine [23–26]. Others did not see any correlation between 8-oxodG levels and urinary 1-hydroxypyrene and/or PAH levels in the air [27–29]. Similarly, we did not find any effect of either personal or stationary c-PAH exposure on 8-oxodG levels in urine. These observations suggest that while c-PAHs may have the potential to induce oxidative DNA damage, other factors probably play a role in the response of the organism to environmental pollution. Mental stress caused by the drivers' responsibility for the passengers' health and safety may be one of the factors that cannot be measured exactly [30,31]. Ozone, an air pollutant known to induce oxidative damage to DNA [32,33], was measured by stationary monitors of the Czech Hydrometeorological Institute in Prague during all sampling periods. Its concentrations were included in the statistical analyses; however, no correlations with 8-oxodG lev-

Table 7
Effect of PM2.5 and PM10 levels measured by stationary monitors over various 3-day periods before sample collection on 8-oxodG levels in urine and the corresponding tertiles of PM2.5 and PM10

Period (days before sampling)	PM2.5			PM10		
	Tertiles ($\mu g/m^3$)	OR (95% CI)	*p*	Tertiles ($\mu g/m^3$)	OR (95% CI)	*p*
1–3	10.8; 19.6	1.64 (1.21–2.24)	<0.01	17.0; 24.9	2.16 (1.58–2.94)	<0.001
4–6	16.5; 22.5	0.98 (0.73–1.32)	=0.89	19.6; 27.2	1.18 (0.87–1.61)	=0.29
7–9	13.4; 21.6	1.41 (1.03–1.94)	<0.05	16.9; 26.3	1.66 (1.19–2.32)	<0.01
10–12	11.5; 21.8	1.19 (0.89–1.59)	=0.25	19.0; 24.2	1.26 (0.95–1.67)	=0.10
13–15	12.2; 21.6	1.40 (1.02–1.91)	<0.05	15.5; 27.2	1.49 (1.10–2.01)	<0.01
16–18	11.4; 19.4	1.26 (0.92–1.74)	=0.16	17.7; 24.2	0.96 (0.69–1.32)	=0.80
19–21	12.2; 21.8	1.42 (1.04–1.95)	<0.05	17.7; 26.3	1.94 (1.40–2.68)	=0.001
22–24	11.0; 19.6	2.15 (1.58–2.91)	<0.001	16.9; 24.1	2.91 (2.09–4.05)	<0.001
25–27	11.0; 23.9	2.70 (1.94–3.76)	<0.001	18.1; 33.0	2.68 (1.93–3.73)	<0.001
28–30	14.6; 23.9	1.73 (1.26–2.39)	<0.001	19.4; 28.6	1.71 (1.24–2.36)	<0.01
31–33	11.4; 30.0	2.12 (1.53–2.95)	<0.001	18.1; 33.0	3.35 (2.32–4.83)	<0.001

Analysis was performed by multivariate logistic regression.

els were observed (data not shown). There are other variables that we did not study due to technical limitations. Ultrafine particles (UFP), especially the 57-nm fraction from vehicle emissions, have been recently shown to induce systemic oxidative stress [34]. UFP, which were not specifically measured in our study, may have been responsible for the higher oxidative stress detected in the groups of bus drivers.

VOC, particularly benzene, are known to induce oxidative damage [35]. In occupational exposure studies, 8-oxodG levels in urine correlated with exposure to benzene [36,37]; another study [38] reported a positive correlation between air benzene and 8-oxodG in lymphocytes. In our study, VOC exposures calculated for all subjects (bus drivers and controls together) were higher in the winter season for most chemicals; however, the differences were mostly due to higher exposure among the controls. For bus drivers the differences in exposure were not significant. 8-OxodG levels were not affected by VOC, probably due to the relatively low environmental concentrations of these pollutants.

It is generally accepted that PM, particularly PM2.5 and ultrafine particles, plays a key role in oxidative damage caused by air pollution [3]. It has been shown that personal exposure to PM2.5 correlated well with 8-oxodG levels in lymphocyte DNA in a group of 50 Copenhagen students sampled repeatedly during a period of 1 year. No such correlation has been observed for stationary PM2.5 monitoring and 8-oxodG in lymphocyte DNA, though a weak association with urinary 8-oxodG was observed [39]. The authors conclude that for moderate exposure to PM, personal monitors are more informative than stationary monitors. It has been proposed that to show an association between PM exposure measured by stationary monitoring and 8-oxodG levels, high exposure levels are required [3]. In another study, personal exposure to PM2.5, black smoke and nitrogen dioxide measured in 2-day periods in 30 subjects was compared with bedroom, front door and background (stationary) levels of these pollutants. Personal PM2.5 concentrations were correlated only with bedroom and front door levels, but not with background concentrations [40]. In our study, we identified both PM10 and PM2.5 stationary levels as independent factors increasing urinary 8-oxodG concentrations. Due to technical reasons we were not able to measure personal PM exposure [15]; instead, we used the data on PM2.5 and PM10 concentrations measured by stationary monitors at two locations in Prague. Stationary PM2.5 exposure levels in Prague were almost threefold higher than in Copenhagen (median levels: 24.3 $\mu g/m^3$ vs. 9.2 $\mu g/m^3$). We may also speculate that the chemical composition of PM may differ between the two cities. Differences in the chemical composition of PM have been shown to be very important for the induction of DNA damage [41]. Thus, differences in PM quantities and composition may explain why the results of our study and the study of Sorensen et al. [39] differ.

The originality of our study lies in the fact that stationary monitoring was performed continuously during both years that the study was conducted. The collected air pollution data allowed us to correlate individual urinary 8-oxodG levels with the levels of pollutants measured at different times and for different periods before the collection of urine samples and thus identify the time frame that may be crucial for the induction of DNA oxidative damage. Our results indicate that exposure to PM2.5 and PM10 3 days before sampling positively affects 8-oxodG levels in urine. This result was also generally consistent for other 3-day intervals up to 42 days before urine collection (data not shown for the whole period).

Studies on the effect of nutrition and life-style factors on 8-oxodG levels show mostly inconsistent results. Vitamin levels did not correlate with urinary 8-oxodG in some studies [42,43], while other authors observed a significant positive correlation of alpha-tocopherol and carotenoids with 8-oxodG [44], or a significant negative correlation between vitamin C and 8-oxodG [45]. Similarly, in our study we observed a consistent protective effect of vitamin C on oxidative DNA damage; the significant results were confirmed in both bivariate and multivariate (data not shown) regression analysis.

The effects of triglycerides and LDL and HDL cholesterol on oxidative stress have been studied mostly in relation to obesity, diabetes and cardiovascular diseases. While triglycerides and LDL cholesterol in its oxidized form have been suggested to increase oxidative stress levels [46–48], HDL cholesterol acts as an antioxidant and may decrease oxidative stress [49]. In our study, we only observed a significant positive effect of triglycerides on 8-oxodG levels; neither LDL nor HDL modified levels of oxidative DNA damage.

Smoking was found to be a significant factor increasing 8-oxodG levels, though not all studies confirmed this observation (reviewed in [35]). The absence of any correlation between urinary cotinine and 8-oxodG levels observed in our study was probably caused by the fact that all subjects enrolled in the study were non-smokers and that their exposure to tobacco smoke through passive smoking was too low to significantly affect 8-oxodG levels.

An age-related increase of 8-oxodG in urine, as well as in lymphocyte DNA, was observed in a recent study [50], while another study found no relationship between urinary 8-oxodG and age over an age range of 35–65 years [42]. Similarly, we did not see any effect of age on 8-oxodG in urine; the mean age of all subjects in our study was 49 years.

In conclusion, we observed increased levels of urinary 8-oxodG in bus drivers in all three sampling periods. The levels of 8-oxodG

varied significantly between seasons, but they were not related to either personal or stationary exposure to c-PAHs, B[*a*]P or VOC. PM2.5 and PM10 levels measured by stationary monitors during a 3-day interval before sampling were identified as independent factors significantly correlating with 8-oxodG levels.

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Příloha 3

Rossner P. Jr., Svecova V., Milcova A., Lnenickova Z.,
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Seasonal variability of oxidative stress markers in city bus drivers
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Seasonal variability of oxidative stress markers in city bus drivers Part II. Oxidative damage to lipids and proteins

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ABSTRACT

The aim of the present study was to investigate the seasonal variability of markers of oxidative damage to lipids (15-F_{2t}-isoprostane, 15-F_{2t}-IsoP) and proteins (protein carbonyl levels) in 50 bus drivers and 50 controls from Prague, Czech Republic, and to identify factors affecting oxidative stress markers. The samples were collected in three seasons with different levels of air pollution. The exposure to environmental pollutants (carcinogenic polycyclic aromatic hydrocarbons, c-PAHs, particulate matter, PM2.5 and PM10, and volatile organic compounds, VOC) was monitored by personal and/or stationary monitors. For the analysis of both markers, ELISA techniques were used. The median levels of individual markers in bus drivers versus controls were as follows: 15-F_{2t}-IsoP (nmol/mmol creatinine): winter 2005, 0.81 versus 0.68 ($p < 0.01$); summer 2006, 0.62 versus 0.60 ($p = 0.90$); winter 2006, 0.76 versus 0.51 ($p < 0.001$); carbonyl levels (nmol/ml plasma): winter 2005, 14.1 versus 12.9 ($p = 0.001$); summer 2006, 17.5 versus 16.6 ($p = 0.26$); winter 2006, 13.5 versus 11.7 ($p < 0.001$). Multivariate logistic regression identified PM levels measured by stationary monitors over a period 25–27 days before urine collection as a factor positively associated with lipid peroxidation, while protein oxidation levels correlated negatively with both c-PAHs and PM levels. In conclusion, markers of oxidative damage to lipids and proteins were increased in bus drivers in winter seasons, but not in summer. Lipid peroxidation was positively correlated with c-PAHs and PM exposure; protein oxidation correlated negatively and was highest in summer suggesting another factor(s) affecting protein carbonyl levels.

1. Introduction

The fact that air pollution is associated with increased morbidity and mortality, mainly due to cardiovascular and respiratory diseases and cancer, has been described in numerous studies (reviewed, among others, in Refs. [1–4]). Although air pollution is a persistent problem, pollutants causing negative health effects have changed from sulfur dioxide, produced mainly by the combustion of coal, to particulate matter (PM), polycyclic aromatic hydrocarbons (PAHs), ozone and nitrogen dioxide that are related to increasing traffic [1]. Oxidative stress is one of the mechanisms that are believed to play a major role in the negative health consequences of exposure to polluted air [4]. While the first part of our study [5] concentrated on oxidative damage to DNA and its association with exposure to ambient pollutants, the present article focuses on oxidative damage to proteins and lipids.

After being attacked by reactive oxygen species (ROS), lipids are peroxidized, forming reactive intermediates that may further react with other molecules and propagate oxidative stress [6]. Apart from this process, oxidized lipids also change the properties of cellular membranes, including their fluidity and the inactivation of membrane-bound proteins. As a result, normal cellular functions are impaired. To monitor lipid peroxidation in vivo, several biomarkers have been proposed. Traditionally, measurement of malondialdehyde (MDA) using a reaction with thiobarbituric acid has been used as a marker of lipid peroxidation [7]. This marker, however, was proved to be a non-specific product of lipid peroxidation [8]. Since isoprostanes were described to be formed in vivo in humans almost two decades ago [9], they have become the most reliable marker of lipid peroxidation that was proved to be associated with oxidative damage in a number of human diseases [10]. Isoprostanes are prostaglandin-like compounds formed by a free-radical attack on arachidonic acid independently of cyclooxygenase

Abbreviations: 15-F_{2t}-IsoP, 15-F_{2t}-isoprostane; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; ROS, reactive oxygen species; PM, particulate matter; PM2.5, particulate matter < 2.5 μm; PM10, particulate matter < 10 μm; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; B[a]P, benzo[a]pyrene; VOC, volatile organic compounds; TMB, tetramethyl benzidine.

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enzymes. They include several groups, but F_2-isoprostanes, particularly 15-F_{2t}-isoprostane (15-F_{2t}-IsoP), are the best characterized and most often studied compounds.

Because proteins are major components of most biological systems, they are an important target for ROS, scavenging 50–75% of reactive intermediates [11]. Protein oxidation leads to the formation of a number of products, with protein carbonyls being the best characterized. Protein carbonylation is an irreversible, non-enzymatic process that results in the introduction of carbonyl groups into protein molecules in various ways. Proteins can react either directly with ROS or indirectly via a reaction with other oxidized macromolecules (lipids or sugars). Direct oxidation results in the formation of oxidation products of the side chains of the amino acids lysine, arginine, proline and threonine, in the oxidation of glutamyl residues, or in the cleavage of peptide bonds [11]. Indirect oxidation includes the reaction of a protein molecule with oxidized lipids (such as MDA reacting with lysine or 4-hydroxynonenal) or with the oxidation products of sugars. The oxidation of proteins results in changes in their conformation leading to their partial or total inactivation [12], their failure to fold correctly [11], or, in the case of heavy oxidation, to undergo proteolysis [13]. Protein oxidation has been linked with the progression of various diseases [14], although in many cases it remains unclear whether protein oxidation is the cause or the consequence of a particular illness. Analyses of protein carbonyl levels are generally accepted methods for the measurement of protein oxidation [15].

The present study concentrates on the effect of exposure to polluted air, measured by levels of carcinogenic PAHs (c-PAHs), PM2.5, PM10 and volatile organic compounds (VOC), on biomarkers of oxidative damage to lipids (15-F_{2t}-IsoP) and proteins (protein carbonyl levels). The study population consisted of 50 bus drivers and 50 controls, followed in winter 2005, summer 2006 and winter 2006. Apart from the personal exposure monitoring, stationary monitoring was conducted on a continual basis throughout all sampling periods, also including intervals up to several months before the study started. In addition to analyzing the levels of both markers of oxidative stress, we also assessed their association with other factors possibly affecting oxidative damage to macromolecules and looked for independent factors influencing the levels of oxidative stress markers in the study population, as described in Part I of the study [5]. Since oxidative damage to proteins, lipids and DNA after exposure to polluted air may follow different patterns, we believe that the parallel measurement of several oxidative stress markers is very important.

2. Materials and methods

2.1. Subjects, sampling and exposure assessment

Detailed information on the subjects' characteristics and their exposure to environmental pollutants was given in Part I of the study [5].

2.2. 15-F_{2t}-IsoP immunoassay

Urinary 15-F_{2t}-IsoP levels were analyzed using immunoassay kits from Oxford Biomedical Research (Oxford, MI, USA) as described in Ref. [16]. According to the manufacturer, results obtained with the kit correlate well with GC/MS following solid phase extraction ($R > 0.8$). Each sample was analyzed in duplicate. The 15-F_{2t}-IsoP concentration was divided by creatinine levels and expressed as nmol 15-F_{2t}-IsoP/mmol creatinine.

2.3. Protein carbonyl assay

The levels of protein carbonyl groups were assessed in blood plasma using a noncompetitive ELISA, essentially as described in Ref. [17], with some modifications [16,18]. Each sample was analyzed in triplicate. Plasma protein carbonyl concentration was expressed as nmol carbonyl/ml plasma.

2.4. Cotinine assay, creatinine levels and vitamin analysis

Urinary cotinine levels as a marker of exposure to tobacco smoke were analyzed by radioimmunoassay [19]. Creatinine levels were measured in urine by the reaction with picric acid (the Jaffe method). The plasma levels of vitamins A, E and C were analyzed by HPLC with UV detection [20,21].

2.5. Plasma lipids

The plasma levels of LDL and HDL cholesterol and triglycerides were determined spectrophotometrically using Sigma diagnostics kits and appropriate standards.

2.6. Statistical analysis

Statistical analyses were performed using Statistica 7.1 (StatSoft, OK, USA) and SAS 9.1.3. (SAS Institute, NC, USA). For data that were not distributed normally, non-parametric methods were used: Mann–Whitney Rank Sum *U*-test for comparison of two groups or two sample periods. Multivariate logistic regression and linear regression analysis were performed to identify multifactorial impact affecting levels of oxidative stress markers. For logistic regression estimates, continuous variables were transformed into a three level scale using tertiles; levels of oxidative stress markers were transformed into a two level scale using medians.

3. Results

3.1. 15-F_{2t}-IsoP and protein carbonyl levels in bus drivers and controls and in individual sampling periods

The results are reported in Table 1. Lipid peroxidation was significantly higher in bus drivers than in controls in both winter seasons. In summer 2006 we did not see any difference between the groups. With regards to the results of personal monitoring described in Part I of the study [5], the difference between the groups in the winter seasons cannot be attributed to their exposure to c-PAHs or VOC a short time (1–2 days) before sample collection. A comparison of 15-F_{2t}-IsoP levels between seasons revealed significant differences for bus drivers in winter 2005 and summer 2006 ($p < 0.001$), in summer 2006 and winter 2006 ($p < 0.001$) and for controls in winter 2005 and winter 2006 ($p < 0.05$). Other differences were not statistically significant.

Protein carbonyl levels followed the same pattern as 15-F_{2t}-IsoP: significant differences between bus drivers and controls in the winter seasons and the same levels in both groups in summer 2006. However, the comparison of individual seasons showed, unexpectedly, the highest carbonyl levels in the summer season, followed by winter 2005 and winter 2006. The differences between seasons for individual groups were statistically significant ($p < 0.001$), with the exception of bus drivers in winter 2005 and winter 2006 ($p = 0.58$). Since personal exposure to c-PAHs and VOC was lowest in summer, the result cannot be caused by exposure to these pollutants.

3.2. Factors affecting 15-F_{2t}-IsoP and protein carbonyl levels

We tried to identify independent factors affecting oxidative damage to lipids and proteins by pooling the exposure data from stationary and personal monitors and the biomarker data and performing a detailed statistical analysis that included bivariate linear and logistic regression and multivariate logistic regression analyses. The exposure to PM2.5 and PM10 measured by stationary monitors over 3-, 7- and 15-day periods up to 2 months before the collection of biological material was compared with the levels of a respective marker of oxidative stress. Other variables used in the analysis included stationary and personal c-PAHs, B[a]P and VOC exposures, cotinine, LDL and HDL cholesterol, triglycerides, vitamins C, A and E and age.

Personal c-PAHs and B[a]P exposure 48 h before sample collection significantly increased 15-F_{2t}-IsoP levels in urine; this result was observed consistently in all conducted statistical anal-

Table 1
Levels of 15-F_{2t}-IsoP in the urine and protein carbonyl groups in the plasma of bus drivers and controls in individual sampling periods

	15-F_{2t}-IsoP (nmol/mmol creatinine)					Protein carbonyl groups (nmol/ml plasma)				
	Bus (N = 50)		Con (N = 50)		p	BUS (N = 50)		CON (N = 50)		p
	Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)		Mean ± S.D.	Median (min, max)	Mean ± S.D.	Median (min, max)	
Winter 2005	0.84 ± 0.26	0.81 (0.38, 1.55)	0.73 ± 0.33	0.68 (0.39, 1.79)	<0.01	14.4 ± 1.7	14.1 (11.8, 19.0)	13.0 ± 1.6	12.9 (9.8, 16.6)	=0.001
Summer 2006	0.63 ± 0.21	0.62 (0.24, 1.14)	0.72 ± 0.52	0.60 (0.28, 3.40)	=0.90	17.8 ± 2.6	17.5 (12.0, 23.2)	17.2 ± 2.5	16.6 (13.0, 23.6)	=0.26
Winter 2006	0.85 ± 0.35	0.76 (0.31, 2.22)	0.60 ± 0.28	0.51 (0.25, 1.56)	<0.001	14.2 ± 3.0	13.5 (9.8, 19.4)	12.2 ± 3.2	11.7 (9.4, 31.1)	<0.001

Table 2
Bivariate comparison by linear and logistic regression analysis between 15-F_{2t}-IsoP levels and factors possibly affecting oxidative stress in all subjects (N = 300)

	Linear regression		Logistic regression	
	R	p	OR (95% CI)	p
Personal B[a]P	0.07	<0.05	1.72 (1.07, 2.74)	<0.05
Personal c-PAHs	0.02	<0.001	1.79 (1.12, 2.86)	<0.05
Personal VOC				
Benzene	0.007	<0.05	2.46 (1.39, 4.35)	<0.01
Toluene	0.0	=0.57	0.95 (0.54, 1.66)	=0.85
Ethylbenzene	0.006	=0.06	1.43 (0.82, 2.50)	=0.21
m-, *p*-Xylene	0.002	<0.05	1.62 (0.92, 2.83)	=0.09
o-Xylene	0.005	<0.05	1.55 (0.88, 2.72)	=0.13
Stationary B[a]P[a]	0.04	<0.05	1.70 (1.28, 2.62)	<0.001
Stationary c-PAHs[a]	0.008	<0.05	1.63 (1.23, 2.17)	<0.001
Stationary PM2.5[a]	0.004	=0.12	1.29 (0.98, 1.70)	=0.07
Stationary PM10[a]	0.002	=0.29	1.24 (0.94, 1.62)	=0.13
Stationary VOC				
Benzene[a]	0.06	=0.07	1.22 (0.91–1.65)	=0.19
Cotinine (>median)	0.009	=0.66	1.90 (1.19, 3.01)	<0.01
LDL cholesterol	0.02	0.49	0.97 (0.61, 1.54)	=0.89
HDL cholesterol	−0.05	=0.60	1.08 (0.68, 1.71)	=0.74
Triglycerides	0.007	=0.65	1.77 (1.11, 2.82)	<0.05
Vitamin C	−0.002	=0.06	0.83 (0.52, 1.32)	=0.43
Vitamin A	−0.02	=0.18	0.78 (0.49, 1.24)	=0.30
Vitamin E	0.002	=0.44	1.59 (1.00, 2.54)	=0.05
Age (>median)	0.09	=0.28	1.03 (0.65, 1.63)	=0.91

[a] Results for stationary monitoring performed over 3-day periods, 3 days before sampling.

yses (Tables 2 and 3). Bivariate linear regression also revealed a significant effect of personal exposure to benzene and xylene (Table 2); bivariate logistic regression confirmed the effect of personal exposure to benzene and identified cotinine, triglycerides and vitamin E levels as factors also associated with lipid peroxidation (Table 2).

The effects of pollutants measured by stationary monitors are presented for the 3-day period immediately preceding sample collection. While c-PAHs and B[a]P had a significant effect on 15-F_{2t}-IsoP levels, PM2.5, PM10 and benzene were not predictors of increased 15-F_{2t}-IsoP levels, or their influence was of borderline significance (i.e., PM2.5 analyzed by multivariate logistic regression) (Tables 2 and 3). Further analysis revealed that exposure to PM2.5 and PM10 at an earlier time before urine collection (3-day periods at least 25–27 days before sampling) was essential for observing a

Table 3
Multivariate logistic regression analysis of environmental pollutants and their impact on 15-F_{2t}-IsoP levels (N = 300)

	OR (95% CI)	p
Personal B[a]P	1.63 (1.19–2.23)	<0.05
Personal c-PAHs	1.62 (1.19–2.22)	<0.05
Personal VOC		
Benzene	1.44 (0.99–2.10)	=0.06
Toluene	1.02 (0.70–1.49)	=0.93
Ethylbenzene	1.16 (0.80–1.67)	=0.45
m-, *p*-Xylene	1.37 (0.95–1.97)	=0.09
o-Xylene	1.40 (0.95–2.05)	=0.09
Stationary B[a]P[a]	1.73 (1.28–2.33)	<0.001
Stationary c-PAHs[a]	1.65 (1.22–2.23)	=0.001
Stationary PM2.5[a]	1.34 (1.00–1.81)	=0.05
Stationary PM10[a]	1.32 (0.98–1.77)	=0.07
Stationary VOC		
Benzene[a]	1.24 (0.90–1.71)	=0.18

[a] Results for stationary monitoring performed over a 3-day period, 3 days before sampling.

Table 4
Effect of PM2.5 and PM10 levels measured by stationary monitors over various 3-day periods before sample collection on 15-F_{2t}-IsoP levels in urine and the corresponding tertiles of PM2.5 and PM10

Period (days before sampling)	PM2.5			PM10		
	Tertiles (μg/m³)	OR (95% CI)	*p*	Tertiles (μg/m³)	OR (95% CI)	*p*
1–3	10.8; 19.6	1.34 (1.00–1.81)	=0.05	17.0; 24.9	1.32 (0.98–1.77)	=0.07
4–6	16.5; 22.5	1.30 (0.96–1.76)	=0.09	19.6; 27.2	1.25 (0.92–1.69)	=0.16
7–9	13.4; 21.6	1.32 (0.96–1.80)	=0.09	16.9; 26.3	1.34 (0.97–1.84)	=0.08
10–12	11.5; 21.8	1.12 (0.84–1.51)	=0.43	19.0; 24.2	1.13 (0.85–1.50)	=0.39
13–15	12.2; 21.6	1.21 (0.89–1.65)	=0.22	15.5; 27.2	1.33 (0.99–1.79)	=0.06
16–18	11.4; 19.4	1.44 (1.04–2.01)	<0.05	17.7; 24.2	1.19 (0.87–1.63)	=0.29
19–21	12.2; 21.8	1.22 (0.89–1.66)	=0.22	17.7; 26.3	1.19 (0.86–1.63)	=0.29
22–24	11.0; 19.6	1.17 (0.87–1.57)	=0.30	16.9; 24.1	1.21 (0.90–1.63)	=0.20
25–27	11.0; 23.9	1.82 (1.33–2.50)	<0.001	18.1; 33.0	1.50 (1.10–2.04)	=0.01
28–30	14.6; 23.9	1.68 (1.22–2.31)	<0.001	19.4; 28.6	1.23 (0.91–1.67)	=0.18
31–33	11.4; 30.0	1.56 (1.14–2.15)	<0.01	18.1; 33.0	1.39 (1.01–1.92)	<0.05

Analysis was performed by multivariate logistic regression.

consistent significant effect of these pollutants on lipid peroxidation (Table 4).

Unexpectedly, protein carbonyl levels in plasma were negatively affected by personal c-PAHs and B[a]P exposure in bivariate linear regression analysis (Table 5). Again, this result was confirmed by bivariate and multivariate logistic regression analysis (Tables 5 and 6). Ethylbenzene and *m*- and *p*-xylene were the only VOC measured by personal monitors exhibiting a significant negative effect on protein carbonyl levels (Table 5, logistic regression); this observation, however, was not confirmed by multivariate regression (Table 6). We also found a negative influence of vitamin C (Table 5) and vitamin E (only when linear regression analysis was used) on protein carbonyl levels.

For analyzing the exposure to pollutants measured by stationary monitors, we used the same conditions as for 15-F_{2t}-IsoP. Exposure to c-PAHs, B[a]P and benzene for 3 days before plasma collection significantly decreased protein carbonyl levels in all statistical analyses. The same results were observed for exposure to PM2.5, while PM10 had no significant effect (Tables 5 and 6). PM2.5 had a similar effect on protein carbonyl levels regardless of the period of exposure before blood collection; to see the effect of PM10, exposure for a 3-day period 4–6 days before sampling was necessary. Similar results were found for most other 3-day periods (Table 7).

Table 5
Bivariate comparison by linear and logistic regression analysis between protein carbonyl levels and factors possibly affecting oxidative stress in all subjects (*N* = 300)

	Linear regression		Logistic regression	
	R	*p*	OR (95% CI)	*p*
Personal B[a]P	−1.09	<0.001	0.33 (0.20, 0.54)	<0.001
Personal c-PAHs	−0.17	<0.001	0.36 (0.22, 0.59)	<0.001
Personal VOC				
Benzene	−0.03	=0.34	1.12 (0.64, 1.98)	=0.69
Toluene	0.0	=0.78	0.93 (0.53, 1.64)	=0.80
Ethylbenzene	−0.03	=0.30	0.51 (0.29, 0.90)	<0.05
m-, *p*-Xylene	−0.01	=0.27	0.48 (0.27, 0.85)	<0.05
o-Xylene	−0.02	=0.45	0.66 (0.37, 1.16)	=0.15
Stationary B[a]P[a]	−0.81	<0.001	0.30 (0.22, 0.42)	<0.001
Stationary c-PAHs[a]	−0.16	<0.001	0.29 (0.20, 0.40)	<0.001
Stationary PM2.5[a]	−0.07	<0.01	0.66 (0.50, 0.88)	<0.01
Stationary PM10[a]	−0.008	=0.69	1.12 (0.84, 1.48)	=0.45
Stationary VOC				
Benzene[a]	−1.79	<0.001	0.23 (0.16–0.35)	<0.001
Cotinine (>median)	0.21	=0.31	0.88 (0.55, 1.40)	=0.60
LDL cholesterol	0.40	=0.12	1.37 (0.86, 2.19)	=0.19
HDL cholesterol	−1.29	=0.13	0.97 (0.61, 1.55)	=0.90
Triglycerides	0.21	=0.14	1.09 (0.68, 1.73)	=0.72
Vitamin C	−0.03	<0.001	0.44 (0.34, 0.88)	<0.05
Vitamin A	−0.18	=0.12	0.87 (0.55, 1.39)	=0.56
Vitamin E	−0.04	<0.05	0.71 (0.45, 1.14)	=0.16
Age (>median)	−0.001	=1.00	0.96 (0.60, 1.55)	=0.88

[a] Results for stationary monitoring performed over 3-day periods, 3 days before sampling.

Table 6
Multivariate logistic regression analysis of environmental pollutants and their impact on carbonyl levels (*N* = 300)

	OR (95% CI)	*p*
Personal B[a]P	0.35 (0.25–0.49)	<0.001
Personal c-PAHs	0.33 (0.23–0.47)	<0.001
Personal VOC		
Benzene	1.22 (0.82–1.83)	=0.33
Toluene	0.76 (0.50–1.51)	=0.20
Ethylbenzene	0.75 (0.50–1.13)	=0.17
m-, *p*-Xylene	0.77 (0.51–1.16)	=0.21
o-Xylene	0.68 (0.45–1.04)	=0.07
Stationary B[a]P[a]	0.27 (0.19–0.38)	<0.001
Stationary c-PAHs[a]	0.24 (0.17–0.35)	<0.001
Stationary PM2.5[a]	0.50 (0.36–0.70)	<0.001
Stationary PM10[a]	0.81 (0.58–1.13)	=0.21
Stationary VOC		
Benzene[a]	0.20 (0.13–0.31)	<0.001

[a] Results for stationary monitoring performed over a 3-day period, 3 days before sampling.

4. Discussion

In the second part of our study of city bus drivers, we analyzed the levels of oxidized proteins and lipids in the two study groups, as well as the seasonal variability of these markers. The levels of 15-F_{2t}-IsoP and protein carbonyls were significantly increased in bus drivers in both winter seasons, while no difference was observed in summer. In this regard the results differed from the 8-oxodeoxyguanosine (8-oxodG) levels measured in Part I of the study: oxidative damage to DNA in the bus drivers was higher in all sampling periods. We consistently saw significant seasonal variability only for protein carbonyls in controls. 15-F_{2t}-IsoP levels were generally higher in both winter seasons than in summer, but the differences between individual groups were not consistent. Protein carbonyl levels were unexpectedly highest in summer 2006.

In Part I of our study we showed that 8-oxodG levels are not affected by c-PAHs exposure measured by either personal or stationary monitoring; we have identified PM as a major independent predictor of urinary 8-oxodG levels [5]. Oxidative damage to proteins and lipids, however, did not show the same pattern. Urinary levels of 15-F_{2t}-IsoP were elevated in winter when exposure to

Table 7
Effect of PM2.5 and PM10 levels measured by stationary monitors over various 3-day periods before sample collection on plasma carbonyl levels and the corresponding tertiles of PM2.5 and PM10

Period (days before sampling)	PM2.5			PM10		
	Tertiles ($\mu g/m^3$)	OR (95% CI)	*p*	Tertiles ($\mu g/m^3$)	OR (95% CI)	*p*
1–3	10.8; 19.6	0.50 (0.36–0.70)	<0.001	17.0; 24.9	0.81 (0.58–1.13)	=0.21
4–6	16.5; 22.5	0.32 (0.23–0.46)	<0.001	19.6; 27.2	0.61 (0.44–0.85)	<0.01
7–9	13.4; 21.6	0.28 (0.19–0.42)	<0.001	16.9; 26.3	0.48 (0.34–0.69)	<0.001
10–12	11.5; 21.8	0.30 (0.21–0.43)	<0.001	19.0; 24.2	0.48 (0.35–0.65)	<0.001
13–15	12.2; 21.6	0.32 (0.22–0.46)	<0.001	15.5; 27.2	0.47 (0.34–0.66)	<0.001
16–18	11.4; 19.4	0.23 (0.15–0.36)	<0.001	17.7; 24.2	0.75 (0.54–1.04)	=0.08
19–21	12.2; 21.8	0.30 (0.20–0.44)	<0.001	17.7; 26.3	0.40 (0.27–0.59)	<0.001
22–24	11.0; 19.6	0.39 (0.28–0.55)	<0.001	16.9; 24.1	0.63 (0.45–0.87)	<0.01
25–27	11.0; 23.9	0.67 (0.49–0.93)	<0.05	18.1; 33.0	0.85 (0.61–1.16)	=0.30
28–30	14.6; 23.9	0.55 (0.39–0.76)	<0.001	19.4; 28.6	0.59 (0.42–0.83)	<0.01
31–33	11.4; 30.0	0.34 (0.23–0.48)	<0.001	18.1; 33.0	0.69 (0.49–0.98)	<0.05

Analysis was performed by multivariate logistic regression.

c-PAHs was higher. Statistical analysis revealed that both c-PAHs and B[a]P exposure levels from both personal and stationary monitors were significantly positively correlated with a marker of lipid peroxidation, suggesting that the mechanisms of oxidative stress induction in DNA and lipids differ.

PAHs may induce oxidative damage during their metabolism by the formation of reactive quinones [22]. These reactions might also explain our observations. Similarly to our results, Singh et al. [23] found significant correlations between PAHs exposure and lipid peroxidation, measured by MDA levels. However, measurements of MDA using a thiobarbituric acid assay do not represent a specific marker of lipid peroxidation [24]. In another study [25], urinary 15-F_{2t}-IsoP levels were not found to be increased in roofers applying asphalt. Thus, the effect of exposure to c-PAHs on lipid peroxidation is not conclusive. Unlike c-PAHs and B[a]P, we did not observe any effect of stationary PM2.5 and PM10 on levels of 15-F_{2t}-IsoP when PM concentrations over a 3-day period immediately preceding urine collection were used in the statistical analysis. Only when the multivariate model was applied did the effect of PM2.5 exposure reach borderline significance. Interestingly, when concentrations of PM2.5 and PM10 over various 3-day periods before sampling were included in the statistical analysis, we found significant effects of PM exposure on 15-F_{2t}-IsoP levels. This observation suggests that 15-F_{2t}-IsoP levels, unlike 8-oxodG, are not markers of immediate effect; rather, they reflect the exposure to pollutants that occurred at least 3–4 weeks before sampling. The possible explanation may be the existence of DNA repair mechanisms that remove damage to DNA shortly after it occurs. Damage to lipids is not repaired; rather the lesions accumulate and thus allow the detection of exposure to pollutants longer period before sampling.

Several studies have described a correlation between PM exposure and lipid peroxidation. Significant results were observed in an animal system for MDA levels [26] and after wood smoke exposure for 15-F_{2t}-IsoP levels [27]. A study of Sorensen et al. [28] measured PM2.5 and carbon black exposure in 50 students in Copenhagen four times in 1 year and analyzed, among others, also MDA levels. The authors found a significant effect of personal PM2.5 exposure on lipid peroxidation in women only; they did not see any significant correlation in men or when background (stationary) PM2.5 exposure levels were used. Unlike our study, the authors did not monitor stationary PM2.5 levels before the collection of biological samples and therefore did not perform analyses similar to ours that revealed a time factor to be very important in the induction of lipid peroxidation. Also, the median concentrations of background PM2.5 in Copenhagen were more than 2.6-fold lower than those in Prague (9.2 vs. 24.3 $\mu g/m^3$). Moreover, MDA measurements do not correlate with results obtained for 15-F_{2t}-IsoP [29,30].

The effect of c-PAHs and B[a]P exposure determined from both personal and stationary monitors on protein carbonyl levels was unexpectedly negative. The result was highly statistically significant and was confirmed in all statistical analyses that we conducted. The effect of PM2.5 studied over 3-day periods before sampling showed the same result, while exposure to PM10 revealed a similar time-dependent pattern as observed for 15-F_{2t}-IsoP levels. However, unlike lipid peroxidation the effect of PM10 exposure became significant already in a 3-day period 4–6 days before sampling. These findings, however, do not imply that increased exposure to c-PAHs and PM has a protective effect against protein oxidative damage. These observations are probably the result of the interaction of other pollutants (including ozone, as discussed below) with the organism. A few studies have analyzed the association between air pollution exposure and protein oxidation [31,32]; however, with regard to the design of the study and the analyzed subjects, only two are comparable with our data [28,33]. In both studies, protein oxidation was measured by using the levels of 2-aminoadipic semialdehyde and γ-glutamyl semialdehyde in hemoglobin. The first of these studies observed a negative association between γ-glutamyl semialdehyde and 1-hydroxypyrene, a urinary PAH metabolite, in a group of bus drivers and postal workers; the correlation with 2-aminoadipic semialdehyde was not significant. The authors suggested that a negative correlation may indicate the effect of additional pollutants that were not measured [33]. In the second study [28] already discussed above, no significant association between either marker of protein oxidation and personal or stationary exposure to PM2.5 was observed.

Although VOC, particularly benzene, induce oxidative stress [34] and several studies on the effect of benzene exposure on oxidative damage to DNA have been published (discussed in Ref. [5]), a Medline search did not yield any human studies analyzing the association between VOC (or benzene alone) exposure and lipid and/or protein oxidation. Contrary to our expectations we did not observe any consistent influence of VOC exposure measured by personal monitors on protein carbonyl or 15-F_{2t}-IsoP levels. Stationary benzene exposure showed a strong significantly negative correlation with protein carbonyl levels.

Oxidative stress may be also affected by other air pollutants and factors that were not measured as part of our study. Ozone is one such pollutant. Its concentrations, measured by the Czech Hydrometeorological Institute, were about 3-fold higher in the summer season than in winter; an average 24-h summer concentration was 75 $\mu g/m^3$. We tested the correlation of ozone concentrations with 15-F_{2t}-IsoP and protein carbonyl levels. We found that ozone levels were significantly positively associated with protein carbonyl levels in all the performed statistical analyses (data not shown). Ozone is

known to induce oxidative damage. In a recent study, ozone exposure correlated with oxidative damage to lipids [35], although in our study no effect on lipid peroxidation was found. It is possible that the negative correlation observed between protein carbonyl levels and c-PAHs and PM exposure reflects the seasonal variability of ozone, c-PAHs and PM and their effect on protein oxidation: the summer levels of c-PAHs and PM were low, but the ozone concentration was increased thus affecting protein carbonyl levels. However, there still may be other unidentified factors responsible for these surprising results.

Among other factors that seem to affect the levels of oxidative stress markers, we should note a positive association between cotinine (in our study considered as a marker of passive smoking) and 15-F_{2t}-IsoP levels. Similar results were seen by others [36–38], although negative reports have also been published [39]. Triglycerides positively correlated with 15-F_{2t}-IsoP in our study; similar results were noted by other authors [40–42]. Finally, we observed a significant negative correlation between protein carbonyl levels and vitamin C in both bivariate linear and logistic regression analyses. We found a similar effect of vitamin C on 8-oxodG levels in the first part of the present study. A protective effect of vitamin C on protein oxidation has also been described in in vitro [43] as well as in in vivo studies [44,45].

The increased oxidative damage to proteins and lipids in the group of bus drivers observed in the present study may be also interpreted as a marker of increased risk of various diseases, including cancer [46,47] and cardiovascular diseases [48]. The results of human studies consistently show that mechanisms of oxidative stress play a significant role in the cardiovascular effect of PM. Studies of the role of oxidative mechanisms associated with airborne particle toxicity are used as evidence of a mechanistic role of oxidative stress mediated by ROS as a mechanism of PM-induced inflammation and damage [49–52].

In conclusion, we found increased oxidative damage to proteins and lipids in bus drivers in both winter seasons, but not in summer. Protein carbonyl levels varied significantly between sampling periods and were highest in summer; the variability of 15-F_{2t}-IsoP levels was lower and the differences were mostly not significant. Lipid peroxidation levels positively correlated with c-PAHs exposure from both personal and stationary monitors; a significant effect of PM was detected only when its levels over a 3-day period 25–27 days before sample collection were analyzed. Protein oxidation was negatively associated with both c-PAHs and PM; ozone might be a factor causing high protein carbonyl levels in summer. The results of both of the presented studies point out the differences in the response of an organism to air pollutants and the importance of the simultaneous detection of several markers of oxidative stress. We may conclude that oxidative damage to DNA, lipids and proteins is increased in bus drivers in winter seasons. However, there does not seem to be a common factor or mechanism responsible for these observations.

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Příloha 4

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Urinary 8-oxodeoxyguanosine levels in children exposed to air pollutants

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ABSTRACT

Oxidative stress is believed to be one of the mechanisms of effects of air pollution to human health. We investigated levels of 8-oxodeoxyguanosine (8-oxodG), a marker of oxidative damage to DNA, in urine samples of 894 children from two districts in the Czech Republic: Teplice and Prachatice. We assessed the association between 8-oxodG levels and exposure to particulate matter of different size: ≤10 μm (PM10), ≤2.5 μm (PM2.5) and carcinogenic polycyclic aromatic hydrocarbons (c-PAHs); as well as between 8-oxodG levels and individual lifestyle, health and pregnancy outcomes. An ELISA technique was used for analysis of 8-oxodG levels. Median levels (range) of 8-oxodG in children from Teplice vs. Prachatice were as follows: 14.6 (3.1–326.5) nmol/mmol vs. 15.2 (3.0–180.8) nmol/mmol creatinine ($p = 0.34$). Levels of 8-oxodG were elevated in children exposed to environmental tobacco smoke (ETS) ($p < 0.05$) and among the Gypsy population ($p < 0.01$). Levels of 8-oxodG decreased with the child's age ($p < 0.001$) and increasing level of the mother's education ($p < 0.01$). Multivariate statistical analyses confirmed the effect of the child's age and ETS exposure on 8-oxodG levels. The exposure to PM10 and PM2.5 measured by stationary monitors during a 7-day period before urine collection, as well as the exposure to c-PAHs measured during 3-day periods 1–3 and 7–9 days before urine collection were identified as factors affecting 8-oxodG levels in multivariate models. The obtained results indicate that 8-oxodG is a sensitive biomarker for measuring the exposure of children to air pollution.

1. Introduction

There is a growing body of evidence linking serious health consequences with exposure to ambient air pollution. The complexity of exposure patterns, changes in the vulnerability of children at various stages of prenatal and postnatal development, and the practical limitations of research mean that understanding of the effect of air pollution on child health is incomplete [1].

The effect of exposure of ambient fine particles on child health is associated with intrauterine growth retardation (IUGR), infant mortality [2], and with increased bronchitis in pre-school children [3]. Exposure to respirable particulate matter of aerodynamic diameter ≤10 μm (PM10) is strongly and consistently associated with postnatal respiratory mortality, and less consistently associated with sudden infant death [4]. Respirable particulate matter of aerodynamic diameter ≤2.5 μm (PM2.5; fine PM) could be a major threat to children. This is because of their higher exposure to PM compared with adults, the immature state of lungs in childhood, and immune functions at birth. The mechanisms of air pollution effects are incompletely understood, but pregnant women, infants and children need specific protection against exposure to fine particles [5].

PM consists of dust, soot, other solid, liquid and aerosol particles, as well as various chemicals bound to them. Carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) are among the most important compounds in PM10 [6]. c-PAHs adsorbed onto PM2.5 are mainly derived from incomplete combustion, including mobile sources (e.g., motor vehicles) and stationary sources (e.g., residential heating or power plants). These compounds exhibit carcinogenic and/or mutagenic properties. c-PAHs are metabolized into reactive intermediates that bind to DNA and form PAH–DNA adducts that, if unrepaired, could lead to mutations. Apart from carcinogenicity, c-PAHs may contribute to induction of oxidative stress via their metabolism by CYP1A1 and subsequent formation of reactive quinones [7]. PM contains other components with higher potency to induce oxidative stress – transition metals, benzene and other volatile organic compounds (VOC)—as well as solid particles that cause inflammation [8,9]. Nitrated polycyclic aromatic hydrocarbons are another important component of PM, which have been shown to have carcinogenic/mutagenic activity [10].

Abbreviations: 8-oxodG, 8-oxodeoxyguanosine; APs, air pollutants; B[a]P, benzo[a]pyrene; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; ELISA, enzyme-linked immunosorbent assay; ETS, environmental tobacco smoke; IUGR, intrauterine growth retardation; LBW, low birth weight, <2500 g; PM, particulate matter; PM2.5, respirable particulate matter of aerodynamic diameter ≤2.5 μm; PM10, respirable particulate matter of aerodynamic diameter ≤10 μm; RNS, reactive nitrogen species; ROS, reactive oxygen species; VAPS, versatile air pollution samplers; VOC, volatile organic compounds.

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The exact mechanisms whereby particles exert their toxic effects at the cellular level are incompletely understood. There is accumulating evidence that particles can generate or induce generation of reactive oxygen species (ROS) in humans, leading to an increase in oxidative stress [8]. Excessive generation of ROS overwhelms the antioxidant defense system and can oxidize cellular biomolecules [11]. Oxidative stress represents a primary pathway leading to the respiratory and systemic inflammatory responses associated with PM exposure [12]. According to [13], long-term oxidative stress affects lung growth, and can lead to the development of chronic bronchitis, lung cancer, asthma and atherosclerosis.

ROS such as superoxide radicals, hydrogen peroxide and hydroxyl radicals may arise from exogenous or endogenous sources. The latter are mostly physiologic and include various metabolic processes and inflammation, whereas exogenous sources are environmental factors such as smoking, diet [14,15], ultraviolet radiation, ionizing radiation or exposure to environmental pollution [16]. In living cells, ROS are formed continuously as a consequence of metabolic reactions. Under normal physiologic conditions, a balance is maintained between endogenous oxidants and antioxidants. Excessive generation of oxidants or a decrease of antioxidants leads to an imbalance, and the abnormal oxidant system enters a state known as oxidative stress [17]. ROS can attack lipids, proteins and nucleic acids simultaneously [18]. The highly reactive hydroxyl radical reacts with DNA by addition to the double bonds of DNA bases, and by abstraction of a hydrogen atom from the methyl group of thymine and C–H bonds of 2′-deoxyribose [19]. The attack of the hydroxyl radical on DNA yields several modified bases but, as a biomarker of oxidative DNA damage, 8-oxodeoxyguanosine (8-oxodG) is most often studied. The oxidized base is highly mutagenic and, if unrepaired, its presence in DNA causes GC > TA transversions. If repaired, 8-oxodG is excreted in urine where it can be assessed by various techniques as a general biomarker of oxidative stress. Urinary levels of 8-oxodG are also believed to reflect the total DNA excision repair capacity of an organism [8]. Other sources of 8-oxodG, including oxidation of the nucleotide pool, may affect urinary levels of 8-oxodG [20]. Numerous reports have indicated that urinary 8-oxodG is not only a biomarker of generalized, cellular oxidative stress, but may also imply the risk of cancer, atherosclerosis and diabetes [17,18].

The effect of air pollution on child health in the Czech Republic is an ongoing research exercise. The aim is to understand the biologic significance of individual air pollutants (APs) for the purpose of risk assessment [21]. The objective of the present study was to analyse the effect of air pollution, particularly PM10, PM2.5, c-PAHs and B[a]P, on urinary levels of 8-oxodG in children from the districts of Teplice and Prachatice. Another objective was to investigate the relationship between oxidative stress, air pollution, lifestyle factors, pregnancy outcomes and child health. We tried to identify independent factors that affect levels of oxidative stress. We hypothesized that increased oxidative stress could affect morbidity in children, and therefore specifically analysed the relationship between 8-oxodG level and bronchial asthma, allergic rhinitis and atopic dermatitis.

2. Materials and methods

2.1. Subjects and sampling

All stages of the study were approved by the Ethical Committee of the Institute of Experimental Medicine AS CR, Prague, Czech Republic.

Between 1994 and 1999, all pregnant women giving birth in the districts of Teplice and Prachatice were asked to participate in the Pregnancy Outcome Study [22]. Only mothers who provided written informed consent were enrolled in the study. Teplice district (approximately 120,000 inhabitants and 1100 births per year; hereafter termed "Teplice") lies in the "brown-coal basin" of northern Bohemia and has a chemical industry, surface mining, and large coal power plants. Prachatice district (approximately 50,000 inhabitants and about 450 births per year; hereafter termed "Prachatice") is an agricultural region in southern Bohemia without heavy industry and moderate levels of air pollution.

The study group included all full-term singleton births in Teplice and Prachatice from women who had resided in the area for at least 1 year. The group was further restricted to the first delivery of the mother during the study period [23].

Personal and lifestyle data were obtained via self-administered questionnaires and medical records. These data included: occupational and other exposures; smoking and consumption of alcohol; reproductive history; health status; and use of medication. Questionnaires were completed in the hospital after delivery with the assistance of a trained nurse. In addition to questionnaire data, hospital staff used standardized forms specifically created for this project to collect medical and health care data on the course and outcome of the pregnancy. IUGR birth was defined as one in which birth weight fell below the tenth percentile, by sex and gestational week, for live births in the Czech Republic [23].

At delivery, 1492 mothers complied with sampling of cord blood, maternal peripheral venous blood and the placenta. These families were contacted again when children were 3 years or 4.5 years of age. The refusal rate was close to zero; most families agreed to take part in the follow-up of the health status of children. Pediatricians with whom children were registered abstracted medical records (based on informed consent) for all illnesses a child had since birth (see [3]). Pediatricians were contacted again in 2004 when 1007 children aged 6–10 years continued their participation in the study. Informed consent for each child was signed by his/her parents. Attention was paid to pediatric allergies, i.e., bronchial asthma, allergic or vasomotoric rhinitis and conjunctivitis (referred to as "allergic rhinitis" in the text), atopic dermatitis, and sensitization to inhalant allergens determined using skin tests. Urinary cotinine levels in children were used to determine the exposure of children to environmental tobacco smoke (ETS); 8-oxodG analysis was conducted in these urine samples. Finally, complete set of data was available for 894 children. These children form the cohort analysed in this study.

2.2. Monitoring of air pollution

The quality of ambient air during the sampling period was monitored using stationary Versatile Air Pollution Samplers (VAPS; URG-Carborro, NC, USA) [24]. Samplers continuously measured the levels of PM10, PM2.5, and c-PAHs. In winter (October to March), concentrations were measured daily, but the chemical analysis of c-PAHs was done for pooled samples from 3-day samplings and an average daily concentration level calculated.

2.3. Collection of urine samples

Mothers were instructed to bring the first morning urine samples of their child and to record the date and time of urine collection in the questionnaire. At pediatric offices, 5 mL of urine was transferred to polystyrene tubes and frozen at −18 °C. Tubes were transported to the Institute of Experimental Medicine and kept at −80 °C until analysis.

2.4. ELISA of 8-oxodG

Urinary 8-oxodG levels were analysed by competitive ELISA [25,26]. Wells were coated with 5 ng of 8-oxoG conjugated with bovine serum albumin (BSA; total volume, 50 μL/well) by drying the plates overnight at 37 °C. Plates were washed with phosphate-buffered saline (PBS)/Tween (0.05% Tween 20 in PBS) and blocked with 200 μL/well of blocking buffer (1% fetal calf serum (FCS) in PBS/Tween) for 1 h at 37 °C. After blocking, 50 μL of 8-oxodG standards (concentration range, 1.25–40 ng/mL) and urine samples (diluted 1:2 with PBS) were added, followed by 50 μL of primary antibody (clone N45.1, concentration 0.2 μg/mL; JaICA, Japan). After incubation for 1.5 h at 37 °C and washing, 100 μL of secondary antibody conjugated with alkaline phosphatase (Sigma–Aldrich) was added. Further incubation for 1.5 h at 37 °C was followed by washing with PBS/Tween and with 0.01% diethanolamine dissolved in water. Color was developed by adding 100 μL of p-nitrophenyl phosphate substrate (1 mg/mL of 1 mol/L diethanolamine) per well and incubating the plates for 30–60 min at 37 °C. Absorbance was measured with a microplate reader at 405 nm. Samples with inhibition <20% or >80% were repeatedly analysed without dilution or with further dilution, respectively. Each sample was analysed in triplicate. Concentration of urinary 8-oxodG was expressed as nmol 8-oxodG/mmol of creatinine.

2.5. Cotinine assay

Urinary cotinine levels were analysed by radioimmunoassay [27]. Results are expressed in ng cotinine/mg creatinine.

2.6. Statistical analyses

2.6.1. Statistical instruments and methods

Statistical analyses were done using Statistica 7.1 (StatSoft, OK, USA) and SAS 9.1.3. (SAS Institute, NC, USA). The nonparametric method for bivariate analyses was used for data that were not distributed normally: the Mann–Whitney Rank

Table 1
Characteristics of the study subjects.

	All		Teplice		Prachatice		
	N	Median (range)	*N*	Median (range)	*N*	Median (range)	*p*
Child's age (years)	894	7.5 (5.9–10.5)	495	7.6 (5.9–10.5)	399	7.4 (5.9–10.5)	0.30
Mother's age at delivery (years)	894	24.6 (17.1–46.0)	495	24.3 (17.1–46.0)	399	24.8 (17.1–40.7)	<0.01
Child cotinine/creatinine (ng/mg)	894	12.1 (1.5–3744.1)	495	17.4 (1.5–3744.1)	399	8.6 (1.6–2030.3)	<0.001

Sum *U*-test for comparison of two groups. More complex analysis was done using multivariate linear regression models and multivariate logistic models.

2.6.2. Data preparation

Analysis variables were constructed using data from several sources: the collection of urine samples, medical information on pediatric allergies, pregnancy outcome medical and maternal questionnaires, and the monitoring of air pollution. All files were cleaned, with checks for range and consistency. All variables were examined for missing data, outliers and deviations from the expected distribution. The distributions of all data were examined for plausibility, skewness, normality, etc., and analysis variables were then constructed. Air pollution data from regular air pollution monitoring were accumulated into means of 3-, 7- and 15-day periods over a period of 2 months before the urine sampling date.

2.6.3. Analysis plan and progress

The addressed hypothesis was that 8-oxodG levels were affected by air pollution, lifestyle and health factors. The analysis proceeded with the testing of bivariable relationships between 8-oxodG levels and independent variables in order to select variables for multivariate models. Since 8-oxodG follows an approximately normal distribution, multiple linear regression (a special case of generalized linear models with an independent correlation structure, identity link, and normal distribution) was used to adjust for potentially confounding covariates. To verify the results and because of the presence of many binary parameters multiple logistic regression was used. For the logistic regression, the air pollution data were transformed from continuous values into categorical variables using medians or tertiles.

The stepwise model building algorithm (SAS) was used to obtain individual models for the association of each air pollutant with 8-oxodG levels in both districts and in all periods examined (entry p value = 0.2 and stay p value = 0.1). In all stepwise model building algorithms, the child's age, the mother's age, cotinine levels, the mother's and father's smoking status, the mother's education, IUGR, LBW and the premature birth of the child were tested as potential confounders.

A set of social parameters of the parents obtained from maternal and medical questionnaires at delivery (mother's education, exposure to environmental tobacco smoke, IUGR and LBW syndromes), cotinine levels in urine and the diagnoses of pediatric bronchial asthma, allergic rhinitis and atopic dermatitis were tested as possible confounders. Confounding variables identified as important or quasi-important in individual models with single pollutants were included into the final multivariable model.

3. Results

3.1. Characteristics of study populations

The basic characteristics of studied groups are shown in Table 1. Mothers giving birth to children in the Teplice region were younger than mothers in the Prachatice region and their children had higher cotinine levels. Table 2 summarizes various factors affecting 8-oxodG levels. There were no differences between 8-oxodG levels in children from the Teplice district vs. children from the Prachatice district: 14.6 (3.1–326.5) nmol/mmol vs. 15.2 (3.0–180.8) nmol/mmol creatinine ($p = 0.34$). Factors positively associated with 8-oxodG levels among all children were Gypsy ethnicity ($p < 0.01$), age ($p < 0.001$) and ETS exposure indicated by cotinine levels ($p < 0.05$). Lower 8-oxodG levels were observed among children in the district Prachatice diagnosed with allergic rhinitis ($p < 0.01$).

3.2. Exposure to air pollutants

Sampling of urine was done from October 2004 until January 2005. Levels of PM10, PM2.5, and B[a]P in Teplice and Prachatice during this period are shown in Figs. 1 and 2. The highest values of PM were observed in November 26 in Teplice: 116.2 $\mu g/m^3$ of PM10 and 93.8 $\mu g/m^3$ of PM2.5. Similar concentrations were found in Prachatice on December 15: 103.2 $\mu g/m^3$ of PM10 and 92.6 $\mu g/m^3$ of PM2.5. Median values for the entire sampling period were: 30 $\mu g/m^3$ of PM10 and 22.7 $\mu g/m^3$ of PM2.5 in Teplice; 20.4 $\mu g/m^3$ of PM10 and 16.8 $\mu g/m^3$ of PM2.5 in Prachatice. Median B[a]P values for Teplice and Prachatice were 1.8 ng/m^3 vs. 0.9 ng/m^3, respectively. For statistical analysis, we distributed APs, including concentrations of PM10, PM2.5, c-PAHs and B[a]P, into 3-, 7- and 15-day intervals measured up to 1 month before urine collection.

3.3. Identification of factors affecting 8-oxodG levels

We pooled samples from both locations and analysed the association of 8-oxodG levels with variables that may affect oxidative stress using bivariate and multivariate regression analyses.

Multivariate linear regression analysis showed that the child's age, cotinine levels and IUGR were the most important factors affecting 8-oxodG levels (Table 3). Multivariate linear regression revealed the difference between Teplice and Prachatice; 8-oxodG levels were higher in children with allergic rhinitis from Teplice,

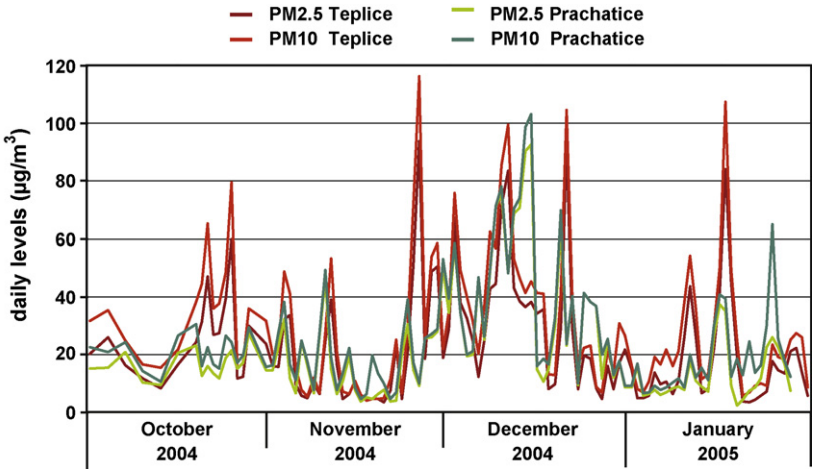

Fig. 1. Daily concentrations of PM10 and PM2.5 in Teplice and Prachatice districts during collection of urine samples (October 2004–January 2005).

Table 2
Factors associated with 8-oxodG levels (nmol/mmol of creatinine).

	N[a]	All median (range)[b]	p	N	Teplice median (range)	p	N	Prachatice median (range)	p
Region	894	14.7 (3.0–326.5)	0.34	495	14.6 (3.1–326.5)	–	399	15.2 (3.0–180.8)	–
Ethnicity									
European	817	14.6 (3.0–326.5)	<0.01	427	14.2 (3.1–326.5)	<0.01	390	15.1 (3.0–180.8)	0.14
Gypsy	72	17.1 (3.2–62.3)		63	17.3 (3.2–42.2)		9	18.5 (11.5–62.3)	
Mother's age									
<25	488	14.6 (3.1–326.5)	0.52	281	14.5 (3.1–326.5)	0.60	207	14.7 (3.7–51.7)	0.68
25–29	271	15.0 (3.0–180.8)		154	14.5 (3.2–119.8)		117	16.4 (3.0–180.8)	
30–34	101	14.8 (3.4–62.3)		48	14.5 (3.4–32.2)		53	16.1 (3.6–62.3)	
>=35	34	15.4 (4.2–45.3)		12	17.0 (7.5–43.7)		22	14.8 (4.2–45.3)	
Education									
University and/or high school	330	13.6 (3.2–45.3)	<0.01	163	13.7 (3.2–40.1)	0.05	167	13.5 (3.6–45.3)	<0.01
Lower	561	15.4 (3.0–326.5)		329	15.1 (3.1–326.5)		232	16.3 (3.0–180.8)	
Mother's smoking									
Yes	320	14.7 (3.1–180.8)	0.32	200	15.0 (3.1–119.8)	<0.05	120	14.6 (3.6–180.8)	0.51
No	574	14.8 (3.0–326.5)		295	14.2 (3.4–326.5)		279	15.4 (3.0–51.7)	
Pregnancy outcome									
LBW	65	13.1 (3.2–43.4)	0.60	53	13.5 (3.2–43.4)	0.82	12	12.4 (7.6–37.1)	0.68
Non-LBW	829	14.8 (3.0–326.5)		442	14.6 (3.1–326.5)		387	15.3 (3.0–180.8)	
IUGR	80	14.7 (3.7–180.8)	0.76	51	14.4 (5.5–43.4)	0.81	29	14.8 (3.7–180.8)	0.80
Non-IUGR	813	14.7 (3.0–326.5)		444	14.6 (3.1–326.5)		369	15.2 (3.0–62.3)	
Child's age									
<7	330	18.7 (3.1–119.8)	<0.001	175	18.0 (3.1–119.8)	<0.001	155	19.3 (4.3–62.3)	<0.001
7	227	15.9 (3.0–326.5)		132	16.2 (3.2–326.5)		95	14.9 (3.0–180.8)	
8	187	13.1 (4.0–113.0)		108	13.4 (4.0–113.0)		79	12.8 (4.3–38.6)	
9	105	9.1 (3.6–25.6)		56	8.8 (4.5–25.6)		49	9.3 (3.6–22.8)	
≥10	45	10.1 (3.4–20.4)		24	10.9 (3.4–20.4)		21	9.7 (6.0–18.7)	
Sex									
Male	442	14.5 (3.0–119.8)	0.57	240	14.5 (3.2–119.8)	0.76	202	14.5 (3.0–62.3)	0.24
Female	452	15.1 (3.1–326.5)		255	14.6 (3.1–326.5)		197	16.2 (3.9–180.8)	
Cotinine/creatinine (ng/mg)									
≥20	339	15.4 (3.1–326.5)	<0.05	230	15.4 (3.1–326.5)	0.01	109	16.3 (4.8–180.8)	0.49
<20	555	14.5 (3.0–51.7)		265	13.9 (3.2–43.4)		290	15.1 (3.0–51.7)	
Bronchial asthma									
+	57	14.6 (3.7–62.3)	0.72	38	14.7 (5.6–56.9)	0.86	19	13.4 (3.7–62.3)	0.47
–	831	14.8 (3.0–326.5)		452	14.5 (3.1–326.5)		379	15.2 (3.0–180.8)	
Allergic rhinitis									
+	92	12.8 (3.2–326.5)	<0.01	33	12.9 (3.2–326.5)	0.12	59	12.8 (3.6–38.5)	0.01
–	796	14.9 (3.0–180.8)		457	14.6 (3.1–119.8)		339	15.4 (3.0–180.8)	
Atopic dermatitis									
+	76	13.0 (3.2–56.9)	0.07	43	12.4 (3.2–56.9)	0.24	33	13.4 (4.6–51.7)	0.13
–	812	14.9 (3.0–326.5)		447	14.6 (3.1–326.5)		365	15.4 (3.0–180.8)	

[a] Data for some parameters not available for all participants.
[b] 8-OxodG levels (nmol/mmol creat.); data do not have normal distribution, nonparametric Mann–Whitney Rank Sum *U*-test was used.

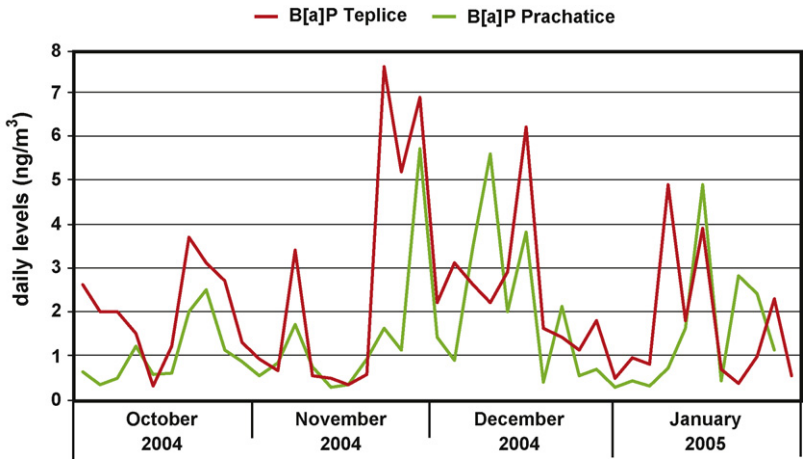

Fig. 2. Daily concentrations of B[a]P in Teplice and Prachatice districts during collection of urine samples (October 2004–January 2005).

Table 3
Effect of selected parameters on 8-oxodG levels by multivariate linear regression.

Intercept	All (37.3)		Teplice (35.6)		Prachatice (39.4)	
	B[a]	p	B	p	B	p
Child's age (years)	−2.9	<0.001	−2.8	<0.001	−3.0	<0.001
Cotinine/creatinine levels (above/below 20 ng/mg)	2.6	<0.02	3.9	0.02	0.7	0.63
Allergic rhinitis	1.6	0.34	9.1	<0.005	−2.8	0.08
IUGR (IUGR vs. non-IUGR)	1.3	0.47	−1.5	0.58	5.3	<0.02
Education level (secondary with exam vs. lower)	−1.9	0.09	−2.2	0.21	−2.1	0.09
Ethnicity (Gypsy vs. European)	0.1	0.96	−0.3	0.91	5.0	0.20

[a] Beta (standardized regression coefficient).

but no significant changes were observed in children from Prachatice (when adjusted for cotinine, IUGR and the child's age). The effect of IUGR was observed only in Prachatice (Table 3).

We used APs data from stationary monitors and analysed their association with oxidative DNA damage because PM exposure is believed to be very important in the induction of oxidative stress. We assessed the association between 8-oxodG levels in urine and concentrations of PM10, PM2.5, c-PAHs and B[a]P at different periods before urine collection. Bivariate and multivariate linear regression models identified the following periods to be correlated with 8-oxodG levels: a 3-day period (4–6 days before urine collection) for PM10 and PM2.5 (Table 4); and a 7-day period (1–7 days before urine collection) for PM10, PM2.5 (results similar to the other period; data not shown). Significant results were also observed for c-PAHs and B[a]P in the 3-day interval 1–3 days (Table 5) and 7–9 days before urine collection (results similar to the other period; data not shown). Significant results observed among all children were driven by higher APs exposure in Teplice; the effect of APs on 8-oxodG levels in Prachatice alone was not significant (Tables 4 and 5). The effect of age and ETS on oxidative stress marker and a positive association of 8-oxodG levels with allergic rhinitis in children living in the Teplice district were statistically significant even after adjustment to previous exposure to PM10, PM2.5, c-PAHs and B[a]P (Tables 4 and 5). Results for other periods (3-, 7-, 15-day intervals up to 1 month before urine collection) were also included in the statistical analysis, but no significant effect on oxidative stress was observed (data not shown).

We conducted a separate analysis of subjects with cotinine levels <20 ng/mg creatinine to analyse the effect of APs (PM10, PM2.5, c-PAHs and B[a]P) on 8-oxodG levels among children not exposed to passive smoking. According to Dostal et al. [28], these children are considered not to be exposed to ETS. We did not find a correlation with APs in this group (data not presented).

4. Discussion

In our study we analysed the effect of air pollution and other selected variables on oxidative stress measured as urinary levels of 8-oxodG in children. It should be noted that 8-oxodG levels in urine do not reflect only oxidative damage to DNA. 8-OxodG is removed from DNA by base excision repair and excreted into the urine, thus its urinary levels are believed to correspond to the total DNA excision repair capacity of the organism [8]. Oxidation of the nucleotide pool is another significant source of 8-oxodG in urine [20].

The higher vulnerability of children to exposure to air pollution is perhaps related to several differences between children and adults: incomplete metabolic systems, immature host defenses, high rates of infection by respiratory pathogens, and activity patterns [1].

Levels of 8-oxodG correlated negatively with the child's age. The growth and development of the lungs and the maturing of the metabolic systems and host defenses may explain this observation. The levels of 8-oxodG in adults observed in our recent study [14]

Table 4
Multivariate model of the effect of PM10 and PM2.5 on 8-oxodG levels (linear regression).

Region/pollutant	Period (days before sampling) 3-day (-4;-6)			
	PM10		PM2.5	
	R[a]	p	R	p
All				
Intercept	33.43		33.56	
APs[b]	0.14	<0.01	0.16	<0.05
Child's age	−2.75	<0.001	−2.74	<0.001
Cotinine (above/below 20 ng/mg)	3.13	<0.005	3.2	0.001
Allergic rhinitis	1.39	0.4	1.34	0.41
Teplice				
Intercept	28.78		28.57	
APs	0.18	<0.01	0.24	<0.01
Child's age	−2.5	<0.001	−2.51	<0.001
Cotinine (above/below 20 ng/mg)	4.4	<0.005	4.41	<0.005
Allergic rhinitis	7.5	0.01	7.59	0.01
Prachatice				
Intercept	43.51		43.71	
APs	−0.1	0.48	−0.13	0.30
Child's age	−3.07	<0.001	−3.07	<0.001
Allergic rhinitis	−3.04	0.06	−3.03	0.06

[a] R (regression coefficient).
[b] APs (air pollutants) specified by description of respective column.

Table 5
Multivariate model of the effect of c-PAHs and B[a]P on 8-oxodG levels (linear regression).

Region/pollutant	Period (days before sampling) 3-day (-1;-3)			
	c-PAHs		B[a]P	
	R[a]	p	R	p
All				
Intercept	34.27		34.01	
APs[b]	0.23	0.07	1.44	0.03
Child's age	−2.66	<0.001	−2.65	<0.001
Cotinine (above/below 20 ng/mg)	3.08	<0.005	3.04	<0.005
Allergic rhinitis	2.81	0.11	2.72	0.12
Teplice				
Intercept	31.53		31.15	
APs	0.32	0.06	2.11	<0.05
Child's age	−2.52	<0.001	−2.54	<0.001
Cotinine (above/below 20 ng/mg)	4.54	<0.005	4.42	<0.005
Allergic rhinitis	8.95	0.05	8.91	<0.005
Prachatice				
Intercept	40.09		40.25	
APs	0.02	0.88	0.02	0.98
Child's age	−3.05	<0.001	−3.05	<0.001
Allergic rhinitis	−1.64	0.21	−1.62	0.22

[a] R (regression coefficient).
[b] APs (air pollutants) specified by description of respective column.

were significantly lower than those in children. Higher oxidative damage in children was also observed by Tamura et al. [29]. However, no association of 8-oxodG levels with age was observed in our studies in adults [14,26]. The highest values of 8-oxodG in younger children (6–7 years) could also correspond to the higher sensitivity of young children to ETS, or to spending more time with smoking parents than older children (9–10 years).

Oxidative damage to DNA was affected by the educational level of mothers. It may indicate a healthier lifestyle (avoiding ETS exposure, quality of diet) and more thorough care for children. Higher oxidative damage to DNA was observed in Gypsy children, who have different standards of living as well as lower education of mothers.

The relationship between IUGR and increased oxidative damage, observed among children from Prachatice, may correspond to a functional deficit induced during intrauterine development. This observation is unexpected due to the lower air pollution in Prachatice, which should be associated with less adverse effects during pregnancy.

Many constituents of ambient air pollution from manufacturing, motor vehicles, and home heating are also components of cigarette smoke, including PM and many c-PAHs. Exposure to ETS therefore places children at greater risk for many health outcomes, including low birth weight, prenatal mortality, deficits in childhood growth, sudden infant death syndrome, middle-ear disease, bronchitis, pneumonia, cough, asthma, and wheezing [3,30–33]. Our study is the first report focused specifically on the association between oxidative damage to DNA and ETS exposure in a large cohort of children. We observed a strong correlation between urinary cotinine (marker of ETS exposure) and 8-oxodG levels. Such oxidative stress may be a starting point for respiratory and allergic morbidity [3].

Exposure to PM2.5 has been associated with impaired growth of lung function. Most studies reported increased prevalence of symptoms with increased exposure to fine PM [1]. A study done in Mexico City showed that in children constantly exposed to ROS and reactive nitrogen species (RNS) from exogenous and endogenous sources, 8-oxodG levels were 2.3–3-fold higher in nasal epithelial cells then in controls ($p < 0.05$) [34]. It is generally accepted that PM, particularly PM2.5 and ultrafine particles, has a key role in oxidative damage caused by air pollution [11].

The originality of our study is that stationary monitoring of PM2.5, PM10 and c-PAHs was done continually during the entire sampling period as well as before this period. Collected data on air pollution allowed us to correlate individual urinary 8-oxodG levels with levels of pollutants measured at different times and for different periods before collection of urine samples. We could therefore identify the time frame that may be crucial for induction of oxidative damage to DNA. In multivariate models, we found that exposure to PM10 and PM2.5 measured in a 3-day interval 4–6 days before sampling, PM10, PM2.5, and B[a]P in a 7-day period before sampling, c-PAHs and B[a]P in a 3-day interval 1–3 and 7–9 days before urine collection were significant factors positively affecting 8-oxodG levels in urine. We may generalize that APs (PM10, PM2.5, c-PAHs) increase oxidative damage within 1 week of exposure. However, the use of stationary monitoring could have possible drawbacks. The levels of pollutants are measured at selected permanent locations that do not necessarily correspond with the places where the study subjects were living. Therefore, concentrations obtained from stationary monitors represent the overall quality of the ambient air rather than actual exposure to air pollutants. For that, personal monitors would be more appropriate; however, their use on such a large scale would be technically very difficult.

Among all subjects, multivariate analysis confirmed a negative association of 8-oxodG levels with the child's age and increased 8-oxodG levels associated with ETS exposure. A positive association of allergic rhinitis with 8-oxodG levels was observed in Teplice; a negative one in Prachatice. A different cause of nasal allergies in the two districts may be one of the reasons for this difference. In the more "green" district of Prachatice, there is a significantly higher proportion of children sensitized to pollen, whereas there is significantly higher air pollution in Teplice. The information on diagnosed allergies describes the overall health status of each child rather than the health status at the time of sample collection. Preliminary results of longitudinal follow-up study show that morbidity due to respiratory diseases in most children significantly decreases with their age [35]. However, our results on allergic rhinitis indicate that the incidence increases with age.

Multivariate models of the effect of air pollutants, the child's age, ETS exposure and allergic rhinitis on 8-oxodG levels indicate that oxidative damage is more pronounced in the district of Teplice, which has higher levels of air pollution as well as ETS than the district of Prachatice. We may speculate that the level of 8-oxodG is a complex biomarker summing up the effect of air pollution and life style.

We may further speculate that a higher level of oxidative damage in younger children simultaneously indicates a higher vulnerability of this group to fine particle's exposure. It may be one more reason why air pollution represents a significant health risk especially for pre-school children.

5. Conclusion

We observed increased levels of urinary 8-oxodG in children exposed to ETS. Levels of 8-oxodG decreased significantly with age. Levels of PM10 and PM2.5 measured by stationary monitors during a 7-day interval before sampling, as well as c-PAHs and B[a]P in a 3-day interval 1–3 and 7–9 days before sampling, were identified as independent factors significantly correlating with 8-oxodG levels. We hypothesize that ETS exposure and short-term exposure to fine particles and c-PAHs induce oxidative stress, and therefore may be starting point for respiratory and allergic morbidity in children.

Conflict of interest

There is no conflict of financial interest for publication of this paper.

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Příloha 5

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Oxidační poškození u osob žijících v Praze a na Ostravsku

Ochrana ovzduší 5-6, 2009, 32-36

BIOMARKERY

OXIDAČNÍ POŠKOZENÍ U OSOB ŽIJÍCÍCH V PRAZE A NA OSTRAVSKU

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ABSTRAKT

Oxidační stres je proces vyvolaný nerovnováhou mezi hladinami antioxidantů a oxidantů. Může být indukován faktory endogenními i exogenními, včetně znečištěného ovzduší. Cílem naší studie bylo analyzovat vliv znečištěného ovzduší na markery oxidace DNA (8-oxodeoxyguanosin, 8-oxodG) a peroxidace lipidů (15-F2t-isoprostan, 15-F2t-IsoP) ve skupině 60 městských strážníků z Prahy a 100 městských strážníků a úředníků z Ostravska. Vzorky byly odebrány ve dvou obdobích s odlišnými hladinami znečištění ovzduší – v únoru 2009 a v červnu 2009. Expozice polycyklickým aromatickým uhlovodíkům (k-PAU), ani volatilním organickým látkám (VOC) neměla vliv na hladiny 8-oxodG. Hladiny 15-F2t-IsoP byly signifikantně zvýšeny u osob z Ostravska ve vzorcích odebraných v únoru 2009. Při společné analýze všech výsledků jsme zjistili pozitivní korelaci mezi expozicí k-PAU a hladinami 15-F2t-IsoP.
Klíčová slova: znečištění ovzduší, oxidační poškození, DNA, lipidy

OXIDATIVE DAMAGE IN SUBJECTS LIVING IN PRAGUE AND THE OSTRAVA REGION

Oxidative stress is a process caused by the imbalance between the levels of antioxidants and oxidants. It can be induced by various endogenous, as well as exogenous factors, including air pollution. The aim of our study was to analyze the effect of air pollution on markers of DNA oxidation (8-oxodeoxyguanosine, 8-oxodG) and lipid peroxidation (15-F2t-isoprostane, 15-F2t-IsoP) in a group of 60 policemen from Prague and 100 policemen and office workers from the Ostrava region. The samples were collected in two seasons with different levels of air pollution – February 2009 and June 2009. We found no effect of exposure to polycyclic aromatic hydrocarbons (c-PAHs) and volatile organic compounds (VOC) on 8-oxodG levels. Levels of 15-F2t-IsoP were significantly increased in subjects from the Ostrava region in samples collected in February 2009. Among all subjects we observed a significant positive correlation between exposure to c-PAHs and 15-F2t-IsoP levels.
Key words: *air pollution, oxidative damage, DNA, lipids*

ÚVOD

Oxidační stres je proces vyvolaný nerovnováhou mezi hladinami antioxidantů v organismu a faktorů působících jako oxidanty [1]. U organismů využívajících aerobní metabolismus, tedy i u člověka, je oxidační poškození jevem přirozeným, proti němuž existují účinné obranné a reparační mechanismy. Oxidanty, působící prostřednictvím reaktivních forem kyslíku (ROS, reactive oxygen species), můžeme podle jejich původu rozdělit na endogenní, vznikající v organismu, a exogenní, přítomné v zevním prostředí. ROS dělíme podle chemické povahy na látky radikálové a neradikálové. Mezi nejvýznamnější ROS patří superoxid, hydroxylový radikál, peroxid vodíku, nebo peroxinitrit. Hlavním endogenním zdrojem oxidace v organismu je proces aerobní respirace, při němž elektrony unikající z membrán mitochondrií způsobují tvorbu superoxidu, který je velmi silným oxidačním činidlem. Oxidace doprovází také zánětlivé procesy a metabolické reakce, při nichž se účastní cytochrom P450. Mezi exogenní zdroje oxidantů řadíme faktory životního stylu (strava, kouření, tělesná aktivita) a kvalitu životního prostředí, zejména znečištění ovzduší. V optimálním případě je superoxid přeměněn superoxid dismutázami na peroxid vodíku, který je dále rozložen katalázami, nebo glutation peroxidázami na vodu. Pokud je však expozice oxidantům příliš vysoká, nebo trvá delší dobu, antioxidační ochrana organismu selhává, dochází k oxidačnímu stresu a hromadí se oxidační poškození, které může postihnout všechny typy makromolekul, tj. DNA, lipidy i proteiny. Oxidační poškození makromolekul je spojováno se vznikem nádorových onemocnění i nemocemi dýchacího a kardiovaskulárního systému [2].

Z hlediska dopadu na lidské zdraví je poškození DNA působením ROS nejzávažnější. Vlivem ROS na DNA dochází ke změnám vlastností bází, konkrétně jejich schopnosti párovat se s komplementárními bázemi v druhém řetězci DNA [3]. V konečném důsledku může tento fakt vést ke vzniku mutací. Kromě toho mohou ROS indukovat jedno- nebo dvouřetězcové zlomy DNA. Nejčastějším produktem oxidace bází v DNA je 8-oxodeoxyguanosin (8-oxodG), kterému se věnuje v odborné literatuře velká pozornost. 8-oxodG se páruje místo cytosinu s adeninem a pokud tato modifikovaná báze není včas rozpoznána a opravena reparačním systémem buňky, dojde v dalším replikačním cyklu k fixaci mutace. Důsledkem přítomnosti 8-oxodG v DNA je tedy transverze GC-TA. Organismus má však vyvinutý mechanismus odstraňování poškozených bází – 8-oxodG je vyštěpen z DNA činností enzymu 8-oxoguanin DNA glykosylázy/AP lyázy a vylučován v moči, kde je možné jeho přítomnost změřit, a tak odhadnout míru oxidace DNA v organismu. Je však třeba zdůraznit, že hladiny 8-oxodG v moči jsou ovlivněny i dalšími faktory, zejména aktivitou reparačních systémů organismu.

Důsledkem působení ROS na lipidy, konkrétně nenasycené mastné kyseliny, je tvorba reaktivních lipid peroxidů, které reagují s dalšími molekulami v buňce a tím šíří oxidační poškození [4]. Peroxidované lipidy mohou tedy ovlivnit i míru oxidace dalších makromolekul – DNA, nebo proteinů. Kromě toho způsobuje přítomnost peroxidovaných lipidů v buněčných membránách změny jejich fluidity a inaktivaci membránově vázaných proteinů. Míra peroxidace lipidů se dá stanovit měřením produktů rozkladu lipid peroxidů; často sledovaným markerem je malondialdehyd (MDA), jehož přítomnost se dá stanovit reakcí s kyselinou thiobarbiturovou. Nevýhodou tohoto druhu analýzy je relativně nízká specifita a také fakt, že MDA prezentuje jen malé množství (cca 1 %) produktů peroxidace lipidů. V průběhu 90. let minulého století se stále častěji začalo provádět měření isoprostanů

jako markerů peroxidace lipidů [5, 6]. Isoprostany vznikají v buněčných membránách působením ROS na arachidonovou kyselinu. Z membrán jsou odštěpovány působením fosfolipáz a buď cirkulují v krevní plazmě, nebo jsou vylučovány močí. Obě tělní tekutiny je možno pro analýzu hladin isoprostanů použít. Nejlépe prostudovaným zástupcem isoprostanů je 15-F2t-isoprostan (15-F2t-IsoP), který je v současné době považován za nejspolehlivější marker peroxidace lipidů. Byla prokázána souvislost mezi zvýšenými hladinami 15-F2t-IsoP a některými onemocněními. Na rozdíl od 8-oxodG reprezentuje 15-F2t-IsoP molekulu, jejíž přítomnost v krevní plazmě a moči je bezprostředně ovlivněna působením ROS na organismus.

Negativní vliv znečištěného ovzduší na lidské zdraví byl popsán v řadě studií. Kromě jiného bylo znečištěné ovzduší spojováno s rozvojem alergií, astmatu, se vznikem chorob kardiovaskulárního a dýchacího systému a s nádorovými onemocněními [7, 8]. Prachové částice (PM, particulate matter), zvláště pak částice o aerodynamickém průměru menším než 10 μm (PM_{10}), jsou významnou složkou přítomnou v ovzduší, která je zodpovědná za negativní vliv znečištění na lidské zdraví. Tento vliv je dán samotnými částicemi, dále pak látkami na částice vázanými. Po vdechnutí putují částice do plic a v případě částic malé velikosti dále do krevního řečiště, jednotlivých orgánů, případně buněk. Jejich přítomnost vyvolá imunitní reakce organismu doprovázené tvorbou ROS, které mohou vést k oxidačnímu stresu. Z látek adsorbovaných na povrch PM jsou z hlediska lidského zdraví důležité zejména karcinogenní polycyklické aromatické uhlovodíky (k-PAU), volatilní organické látky (VOC) a reaktivní kovy.

PAU vznikají nedokonalým spalováním organické hmoty a jsou v životním prostředí široce rozšířené. Řada z nich je prokázanými lidskými karcinogeny. Jejich škodlivý efekt na organismus se projevuje až po metabolické aktivaci působením cytochromů P450, enzymů, které PAU přeměňují na reaktivní elektrofily, které se mohou vázat na DNA a pokud nejsou odstraněny reparačními systémy, mohou způsobovat mutace. Existuje však i další mechanismus metabolické aktivace PAU, při němž dochází k tvorbě PAU o-chinonů, které jsou elektrofilní a svojí přítomností v buňce způsobují tvorbu ROS a následně indukují oxidační poškození makromolekul [9]. Expozice lidského organismu k-PAU může mít tedy za následek nejen tvorbu mutací v DNA, ale i indukci oxidačního stresu se všemi důsledky, popsanými výše.

Mezi VOC řadíme většinu organických látek přítomných v atmosféře, které se účastní fotochemických reakcí. Jejich hlavním zdrojem je doprava, ale jsou přítomné i v řadě výrobků chemického průmyslu, např. v rozpouštědlech a v čisticích prostředcích, nebo v kosmetických přípravcích. V organismu, kam mohou vstoupit ve formě plynné, tekuté, nebo jako aerosol, způsobují otoky, zvýšené prokrvení kůže, infiltraci leukocytů a nekrózu v místě expozice, a zvýšení hladiny ROS. Expozice ROS je také spojována s vyšším výskytem astmatu [10, 11].

Cílem naší studie bylo ověřit, zda expozice k-PAU a VOC ovlivňuje oxidační poškození DNA a peroxidaci lipidů v lidském organismu. Sledovali jsme dvě skupiny osob žijících v oblastech s výrazně odlišnými hladinami znečištění ovzduší: 60 příslušníků městské policie Praha a 100 osob (příslušníků městské policie, úředníků magistrátu) z Ostravska (Karviná, Havířov, Ostrava). Obě oblasti patří k výrazně znečištěným lokalitám ČR, avšak hladiny škodlivin na Ostravsku několikanásobně převyšují hodnoty zjišťované v Praze. Proto byla v naší studii zvolena pražská skupina jako kontrolní. Obě skupiny byly sledovány ve dvou obdobích, v únoru a červnu 2009; v zimních měsících je expozice škodlivinám z ovzduší zvýšená ve srovnání s letním obdobím. U sledovaných osob byla měřena expozice k-PAU a VOC pomocí personálních monitorů a následně analyzováno oxidační poškození DNA a peroxidace lipidů. Předpokládali jsme, že expozice škodlivinám bude vyšší u ostravské než u pražské skupiny a současně, že zimní hodnoty budou vyšší než letní měření. Teoreticky by těmto trendům mělo odpovídat i oxidační poškození makromolekul, zejména peroxidace lipidů, která, jak je uvedeno výše, odráží přímo vliv ROS na lipidy a není ovlivněna dalšími buněčnými procesy.

MATERIÁL A METODY

Osoby zahrnuté do studie a sběr vzorků

Do studie bylo zahrnuto 160 osob: 60 příslušníků městské policie Praha tvořilo kontrolní skupinu, zatímco 100 osob z Ostravska (příslušníků městské policie Karviná a Havířov a úředníků Krajského úřadu Ostrava) představovalo skupinu zvýšeně exponovanou znečištěnému ovzduší. Všechny osoby byly nekuřáci. Každý účastník studie vyplnil dotazník o životním stylu a zdravotním stavu. Všechny osoby byly sledovány ve dvou odběrových obdobích: v zimě (únor) a v létě (červen) 2009.

Před zahájením studie byly vybrané osoby seznámeny s jejími cíli a podepsaly informovaný souhlas. Do studie nebyly zahrnuty osoby, které podstoupily v posledních 3 měsících radiografické vyšetření, nebo byly v tomto období očkovány.

V rámci studie byly odebrány vzorky krve a moči. Krev byla odebírána do zkumavek obsahujících heparin. Vzorky byly okamžitě po odběru dopraveny do laboratoře Oddělení genetické ekotoxikologie ÚEM AV ČR v Praze, kde byly zpracovány a uchovány při -80 °C. Vzorky odebrané na Ostravsku byly uloženy do 4 °C a okamžitě transportovány kurýrní službou do pražské laboratoře, kde byly ihned zpracovány. Doba od odběru vzorků do jejich uložení při -80 °C nepřesáhla 8 hodin.

Stanovení expozice k-PAU

Expozice k-PAU byla zjišťována pomocí personálních monitorů, které používali účastníci studie dva po sobě následující dny (celkem 48 hodin). Personální monitory byly vybaveny filtry umožňujícími sběr částic o velikosti 2,5 μm ($PM_{2,5}$). Kvantitativní chemická analýza k-PAU (benz[*a*]antracen, chrysen, benzo[*b*]fluoranten, benzo[*k*]fluoranten, benzo[*a*]pyren (B[*a*]P), dibenzo[*a,h*]antracen, benzo[*g,h,i*]perylen and indeno-[*1,2,3-cd*]pyren) byla provedena pomocí HPLC s fluorescenční detekcí.

Stanovení expozice VOC

Expozice VOC (benzen, toluen, etylbenzen, m,p-xylen a o-xylen) byla stanovena pomocí pasivních vzorkovačů Radiello®, které nosili účastníci studie 24 hodin. VOC zachycené na grafitizované uhlí byly před analýzou uvolněny termální desorpcí a následně analyzovány plynovou chromatografií s plamenoionizační detekcí. Analýzy k-PAU i VOC byly prováděny akreditovaným pracovištěm ALS Laboratory Group, ALS Czech Republic, s. r. o., Praha.

Tab. 1: Porovnání personální expozice k-PAU v Praze a na Ostravsku v únoru a červnu 2009

	Lokalita			
	Praha (N = 60)		Ostravsko (N = 100)	
	Únor 2009	Červen 2009	Únor 2009	Červen 2009
k-PAU (ng/m³)				
Průměr	4,27	1,03	20,56	3,19
Směrodatná odchylka	2,95	0,61	18,55	1,70
Median	3,57	0,85	11,48	2,95
Minimum	0,82	0,36	1,70	0,81
Maximum	16,43	3,64	86,58	9,80
Porovnání lokalit (Praha vs. Ostravsko) v rámci období (p)	< 0,001	< 0,001		
Porovnání období (únor vs. červen 2009) v rámci lokality (p)	< 0,001		< 0,001	

Tab. 2: Porovnání personální expozice B[a]P v Praze a na Ostravsku v únoru a červnu 2009

	Lokalita			
	Praha (N = 60)		Ostravsko (N = 100)	
	Únor 2009	Červen 2009	Únor 2009	Červen 2009
B[*a*]P (ng/m³)				
Průměr	0,80	0,12	3,50	0,43
Směrodatná odchylka	0,55	0,14	3,03	0,24
Median	0,66	0,08	1,95	0,37
Minimum	0,10	0,04	0,33	0,08
Maximum	3,37	1,00	12,60	1,24
Porovnání lokalit (Praha vs. Ostravsko) v rámci období (p)	< 0,001	< 0,001		
Porovnání období (únor vs. červen 2009) v rámci lokality (p)	< 0,001		< 0,001	

Statistická analýza

Pro statistickou analýzu byl použit software SPSS. Data, která neměla normální rozložení, byla analyzovaná neparametrickými testy – Mann-Whitney testem pro porovnání dvou skupin vzájemně a Spearmanovým korelačním testem pro zjištění vzájemných vztahů mezi biomarkery. Data s normální distribucí byla analyzována T-testem a Pearsonovým korelačním testem.

VÝSLEDKY

Expozice znečištěnému ovzduší

Personální expozice znečišťujícím látkám (k-PAU a VOC) jsou podrobně popsány v článku Švecová et al.: Personální monitoring polycyklických aromatických uhlovodíků a volatilních organických látek [15], proto uvádíme jen hodnoty expozice osmi k-PAU a specificky B[*a*]P jako modelové látky, která je v odborné literatuře nejčastěji studovaným k-PAU.

Jak vyplývá z **tabulek 1 a 2**, expozice k-PAU i B[*a*]P byla výrazně vyšší na Ostravsku než v Praze, a to v obou odběrových obdobích. Všechny zjištěné rozdíly byly vysoce statisticky signifikantní ($p < 0,001$). Podle očekávání byly zimní expozice v obou oblastech vyšší než letní hodnoty ($p < 0,001$).

Stanovení 8-oxodG

Hladina 8-oxodG v moči byla stanovena metodou kompetitivní ELISA podle publikované metodiky [12, 13]. Detekce je založena na použití primární protilátky specificky rozpoznávající 8-oxodG v biologickém materiálu (výrobce protilátky: JaICA, Japonsko). Vzorky moči byly analyzovány v triplikátech. Výsledky byly vztaženy na obsah kreatininu a vyjádřeny v nmol 8-oxodG/mmol kreatininu.

Stanovení 15-F2t-IsoP

Hladiny 15-F2t-IsoP byly stanoveny v krevní plazmě komerčním kitem od firmy Cayman Chemical Company (Ann Arbor, MI, USA) podle doporučení výrobce. Pro analýzu bylo použito 125 µl krevní plazmy, která byla nejprve hydrolyzována, aby došlo k uvolnění isoprostanů vázaných na lipoproteiny, a dále purifikována pomocí afinitního sorbentu dodaného výrobcem kitu. Vzorky byly naředěny v poměru 1:2 v dodaném pufru a následně analyzovány v duplikátech. Koncentrace 15-F2t-IsoP byla vyjádřena v pg 15-F2t-IsoP/ml plazmy.

Měření hladin kotininu

Hladiny kotininu v moči, které sloužily jako marker expozice tabákovému kouři, byly stanoveny radioimunochemicky [14].

Oxidační poškození DNA a lipidů

Výsledky analýz hladin markerů oxidačního poškození jsou uvedeny v **tabulkách 3 a 4.** Hladiny 8-oxodG v moči se u osob z Ostravska nelišily od pražských vzorků, a to ani v zimním, ani v letním období. Mezi oběma obdobími nebyl pozorován žádný rozdíl. Hladina markeru peroxidace lipidů, 15-F2t-IsoP, byla signifikantně zvýšena u osob žijících na Ostravsku, u vzorků odebraných v zimním období. V letním období byl trend stejný, avšak rozdíl nebyl statisticky signifikantní. Při porovnání odběrových období byly zjištěny signifikantně vyšší hladiny 15-F2t-IsoP v zimě 2009.

V **tabulce 5** jsou prezentovány výsledky korelační analýzy mezi hladinami markerů oxidačního stresu a expozicí znečišťujícím látkám z ovzduší stanovenou personálním monitory. Výpočet byl proveden pro všechny osoby zahrnuté do studie pro obě odběrová období (celkový počet vzorků – 320). Hladiny 8-oxodG v moči nekorelovaly s žádnou ze sledovaných látek. Peroxidace lipidů byla zvýšena po expozici některým VOC (benzen, toluen, m,p-xylen) a zejména po expozici k-PAU a B[*a*]P. Korelace mezi expozicí B[*a*]P a hladinami 15-F2t-IsoP v krevní plazmě je graficky znázorněna na **obrázku 1** (korelační koeficient $R = 0,53$, $p < 0,001$; v grafu není znázorněno sedm odlehlých vzorků s hladinami 15-F2t-IsoP vyššími než 500 pg/ml).

DISKUZE

Cílem naší studie bylo zjistit, jak se odráží vysoká expozice znečišťujícím látkám v ovzduší v hladinách markerů oxidačního poškození DNA a lipidů a tím stanovit případnou možnost ohrožení zdravotního stavu obyvatelstva. V našich předchozích studiích [13, 16, 17], v nichž jsme sledovali oxidační poškození u řidičů pražských autobusů a u administrativních pracovníků, korelovala hladina peroxidace lipidů s personálních expozicí k-PAU; tento výsledek však nebyl pozorován pro oxidaci DNA. Současná studie je unikátní především velmi vysokou hladinou expozice škodlivým látkám u osob žijících na Ostravsku, která v zimním období téměř 5krát převyšuje koncentrace zjištěné v Praze. Předpokládali jsme, že extrémní zátěž organismu se projeví nejen ve zvýšené peroxidaci lipidů, ale i ve zvýšených hladinách 8-oxodG v moči.

Výsledky laboratorních analýz potvrdily závěry předchozích studií. I přes extrémní znečištění ovzduší na Ostravsku byly hladiny 8-oxodG v moči porovnatelné s hodnotami nalezenými u pražské populace, a to nejen v letním, ale i v zimním období. Je třeba zdůraznit, že např. expozice k-PAU jsou na Ostravsku tak vysoké, že i jejich letní koncentrace jsou zhruba jen o 25 % nižší než koncentrace k-PAU v Praze v zimním období, které jsou samy o sobě nadlimitní. Fakt, že ani velmi vysoká expozice znečištěnému ovzduší nemá za následek zvýšenou hladinu 8-oxodG v moči, lze vysvětlit původem této oxidované báze. Jak již bylo zmíněno v úvodu, 8-oxodG je sice produktem působení ROS na DNA, nicméně významným zdrojem 8-oxodG v moči jsou mechanismy reparace DNA [18]. Navíc 8-oxodG nepochází jen z oxidace DNA, ale i z oxidace volných nukleotidů přítomných v buňkách [19]. Tato skutečnost je nevýhodou analýzy 8-oxodG v moči. Na druhé straně jde o metodu, při níž je získávání vzorků neinvazivní a v průběhu jejich zpracování nedochází k indukci oxidačního poškození [20]. Alternativou je analýza 8-oxodG v genomové DNA lymfocytů. Při použití této metody nejsou výsledky ovlivněny existencí dalších buněčných procesů, které mohou hladiny oxidované báze pozměnit. Materiálem pro izolaci DNA je ale většinou žilní krev, jde tedy o invazivní metodu, navíc komplikovanou tím, že v průběhu izolace DNA může dojít k indukci oxidačního poškození, a tedy k získání falešně pozitivních výsledků [21]. V případě analýzy metodou ELISA je nevýhodou též nutnost použít relativně velké množství DNA, pro jejíž izolaci je potřeba několik desítek mililitrů krve. I když k-PAU neindukovaly zvýšení hladin 8-oxodG v moči ani v našich předchozích studiích, oxidační poškození DNA korelovalo s expozicí PM_{10} i $PM_{2,5}$. Důvodem negativního výsledku může tedy být i fakt, že za indukci oxidačního poškození DNA projevujícího se zvýšením 8-oxodG v moči jsou zodpovědné jiné látky než k-PAU. Pro objasnění vlivu k-PAU na hladiny 8-oxodG v moči by bylo nutné provést další laboratorní analýzy,

Tab. 3: Hladiny 8-oxodG naměřené v moči odebrané od sledovaných osob v zimním a letním období 2009 v Praze a na Ostravsku

	Lokalita			
	Praha (N = 60)		Ostravsko (N = 100)	
	Únor 2009	Červen 2009	Únor 2009	Červen 2009
8-oxodG (nmol/mmnol kreat.)				
Průměr	5,24	5,28	5,51	5,27
Směrodatná odchylka	2,43	2,88	2,78	2,68
Median	5,11	5,21	5,54	5,11
Minimum	0,20	0,10	0,04	0,10
Maximum	14,37	20,39	12,92	18,40
Porovnání lokalit (Praha vs. Ostravsko) v rámci období (p)	0,52	0,99		
Porovnání období (únor vs. červen 2009) v rámci lokality (p)	0,94		0,53	

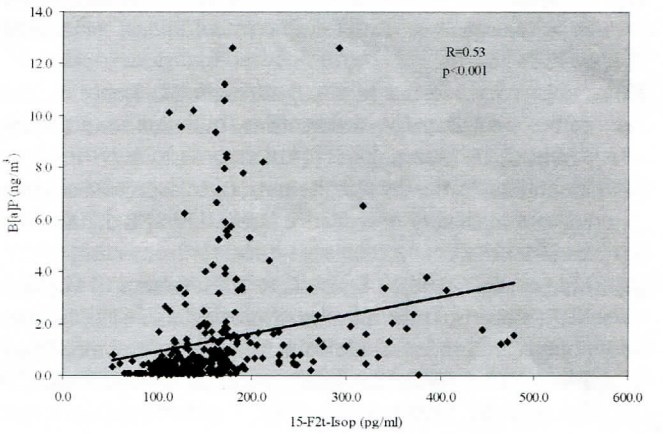
Tab. 4: Hladiny 15-F2t-IsoP naměřené v krevní plazmě odebrané sledovaným osobám v zimním a letním období 2009 v Praze a na Ostravsku

	Lokalita			
	Praha (N = 60)		Ostravsko (N = 100)	
	Únor 2009	Červen 2009	Únor 2009	Červen 2009
15-F2t-IsoP (pg/ml plazmy)				
Průměr	165,87	124,87	279,32	134,08
Směrodatná odchylka	41,67	43,60	303,56	37,03
Median	158,41	116,36	179,23	129,51
Minimum	108,74	64,81	101,25	53,02
Maximum	321,20	376,47	2410,27	262,11
Porovnání lokalit (Praha vs. Ostravsko) v rámci období (p)	< 0,001	0,16		
Porovnání období (únor vs. červen 2009) v rámci lokality (p)	< 0,001		< 0,001	

Tab. 5: Korelace mezi expozicemi k-PAU a VOC a hladinami markerů oxidačního poškození.

	8-oxodG (N = 320)		15-F2t-IsoP (N = 320)	
	Korelační koeficient	Signifikance	Korelační koeficient	Signifikace
B[*a*]P	0,075	0,179	0,530	0,000
k-PAU	0,066	0,240	0,490	0,000
Benzen	0,095	0,089	0,260	0,000
Toluen	0,038	0,498	0,280	0,000
Etylbenzen	0,027	0,628	0,009	0,876
m,p-xylen	0,061	0,275	0,220	0,000
o-xylen	0,015	0,785	-0,001	0,980

Obr. 1: Korelace mezi personální expozicí B[*a*]P a hladinami 15-F2t-IsoP v krevní plazmě



zahrnující například analýzu genové exprese a stanovení hladin enzymů zodpovědných za reparaci DNA.
Peroxidace lipidů byla výrazně zvýšena u osob žijících na Ostravsku ve srovnání s pražskou populací, a to především v zimním období. V letním období byl trend obdobný, avšak rozdíl mezi lokalitami nebyl statisticky signifikantní. Při porovnání období v rámci jedné lokality byla v obou případech peroxidace lipidů vyšší v únoru 2009. Nejvýznamnějším zjištěním naší studie je vysoce signifikantní pozitivní korelace mezi personální expozicí k-PAU a B[*a*]P a hladinami 15-F2t-IsoP v krevní plazmě. Výsledek lze interpretovat tak, že přítomnost k-PAU v ovzduší má za následek zvýšení hladin peroxidovaných lipidů v organismu. Jak již bylo uvedeno, peroxidované lipidy nejen narušují strukturu buněčných membrán a funkci membránově vázaných proteinů, ale díky tvorbě reaktivních intermediátů způsobují šíření oxidačního poškození v organismu vedoucí k poškození DNA a proteinů. Důsledkem je zvýšené riziko řady onemocnění, při jejichž vzniku hraje oxidační poškození roli: kardiovaskulární nemocnění, nemoci plic, jater, ledvin i neurologické poruchy [4, 22]. Peroxidace lipidů je ovlivněna i některými VOC (benzen, toluen, m,p-xylen), avšak korelace je výrazně slabší než v případě k-PAU, i když též signifikantní.

ZÁVĚR

Naše výsledky ukazují, že zvýšená expozice znečišťujícím látkám z ovzduší, především k-PAU, pozorovaná na Ostravsku, je doprovázena vyšší mírou peroxidace lipidů. Vzhledem k tomu, že byla prokázána spojitost mezi peroxidací lipidů a řadou onemocnění, obyvatelsko této oblasti má zvýšené riziko zhoršení zdravotního stavu jako důsledek dlouhodobé expozice znečištěnému ovzduší. Cílem odpovědných orgánů by tedy měla být snaha o snížení koncentrací škodlivin na Ostravsku, které jsou v současné době nejvyšší v ČR.

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Příloha 6

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Vliv znečištěného ovzduší na hladiny markerů oxidačního poškození makromolekul

Ochrana ovzduší 5-6, 2010, 38-43

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VLIV ZNEČIŠTĚNÉHO OVZDUŠÍ NA HLADINY MARKERŮ OXIDAČNÍHO POŠKOZENÍ MAKROMOLEKUL

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ABSTRAKT

Znečištění ovzduší je vážný problém ovlivňující životy milionů lidí na celém světě. Expozice znečištěnému ovzduší je spojována se vznikem celé řady onemocnění. Oxidační poškození makromolekul je jedním z důsledků vysokých koncentrací škodlivin v ovzduší. Cílem naší studie byla analýza hladin markerů oxidačního poškození DNA (8-oxo-7,8-dihydro-2'-deoxyguanosinu, 8-oxodG), lipidů (15-F2t-isoprostanu, 15-F2t-IsoP) a proteinů (karbonylových skupin) u skupiny městských strážníků z Prahy a administrativních pracovníků a městských strážníků z Ostravska. Vzorky byly odebírány ve třech obdobích (zima 2009, léto 2009, zima 2010) s odlišnými hladinami škodlivin v ovzduší. Koncentrace polutantů se též lišily mezi oblastmi: Ostravsko bylo signifikantně více znečištěné než Praha ve všech třech obdobích. Rozdíl byl nejvýraznější v zimě 2010, kdy během inverze byly personální expozice karcinogenním polycyklickým aromatickým uhlovodíkům na Ostravsku zhruba 5krát vyšší než v Praze. Markery oxidačního poškození byly poprvé testovány při takto vysokých koncentracích škodlivin. Jako nejvhodnější marker se ukázala peroxidace lipidů, která byla signifikantně zvýšena u osob z Ostravska v zimě 2009 a v létě 2009. Při vyšších koncentracích polutantů v zimě 2010 již nedocházelo k lineárnímu růstu hladin 15-F2t-IsoP.

Klíčová slova: znečištění ovzduší, oxidační poškození, makromolekuly, peroxidace lipidů

THE IMPACT OF AIR POLLUTION ON THE LEVELS OF MARKERS OF OXIDATIVE STRESS

Air pollution is a serious problem affecting lives of millions of people worldwide. Exposure to polluted air has been associated with a number of negative health effects. Oxidative damage to macromolecules is one of the consequences of high concentrations of pollutants in the ambient air. The aim of our study was to analyze the levels of markers of oxidative damage to DNA (8-oxo-7,8-dihydro-2'-deoxyguanosine, 8-oxodG), lipids (15-F2t-isoprostane, 15-F2t-IsoP) and proteins (carbonyl groups) in a group of city policemen from Prague and office workers and city policemen from the Ostrava region. The samples were collected in three seasons (winter 2009, summer 2009 and winter 2010) with different levels of air pollution. Also, the concentrations of air pollutants differed between regions: the Ostrava region was significantly more polluted than Prague in all three sampling seasons. The difference was particularly pronounced in winter 2010 when during inversion personal exposures to carcinogenic polycyclic aromatic hydrocarbons air pollutants in the Ostrava region were about five-fold higher than in Prague. Oxidative stress markers were tested for the first time at conditions of very high concentrations of pollutants. Lipid peroxidation was found to be the most suitable marker of exposure to air pollution: the levels were increased in subjects from the Ostrava region in winter 2009 and summer 2009. In winter 2010 when concentrations of air pollutants were higher no further linear increase of 15-F2t-IsoP levels was observed.

Key words: *air pollution, oxidative damage, macromolecules, lipid peroxidation*

ÚVOD

Znečištění ovzduší je jedním z faktorů významně přispívajícím k negativním vlivům moderní civilizace na lidské zdraví. Uvádí se, že environmentální polutanty v ovzduší jsou zodpovědné za vznik řady onemocnění a v konečném důsledku za zkracování délky života obyvatel žijících v nejvíce znečištěných oblastech. Mezi nemoci nejčastěji spojované se znečištěním ovzduší patří plicní onemocnění včetně astmatu, kardiovaskulární a nádorová onemocnění, alergie i záněty [1, 2]. Prachové částice (PM, particulate matter), zejména ty o aerodynamickém průměru < 2,5 μm, a látky na ně navázané patří mezi polutanty, které jsou z hlediska dopadu na lidské zdraví nejvýznamnější [3]. PM vznikají převážně spalováním organické hmoty, větší částice též mechanickými procesy rozpadu materiálů na prach. Chemické složení PM, které také určuje škodlivost PM pro lidský orga-

nismus, záleží na mnoha faktorech, včetně zdrojů spalování (motory, topeniště, průmyslové zdroje), klimatu, nebo na ročním období. PM nejčastěji obsahují rozličné organické látky (karcinogenní polycyklické aromatické uhlovodíky (k-PAU), nitrované PAU, chinony), přechodné kovy, ionty, reaktivní plyny, materiály biologického původu a minerály [3]. Z hlediska lidského zdraví patří k-PAU mezi nejvýznamnější složky PM, zejména pro jejich karcinogenitu a fakt, že vznikají při nedokonalém spalování fosilních paliv a jsou tedy v prostředí prakticky všudypřítomné [4]. Benzo[*a*]pyren (B[*a*]P), modelový k-PAU, je současně i prvním objeveným chemickým karcinogenem. Po vstupu do organismu jsou PAU metabolizovány, přičemž vytvářejí dihydrodioly a následně reaktivní diol-epoxidy. Karcinogenita PAU je dána schopností diol-epoxidů vázat se na DNA. Další mechanismus metabolismu PAU vede ke vzniku reaktivních forem kyslíku (reactive oxygen species, ROS) a následně k oxidačnímu poškození makromolekul. V tomto případě jsou PAU dihydrodioly metabolizovány na PAU o-chinony. Tyto látky vstupují do redoxních cyklů, přičemž se vytvářejí ROS [5]. Volatilní organické látky (volatile organic compounds, VOC) jsou další skupinou polutantů negativně ovlivňujících lidské zdraví. Benzen, reprezentant VOC, přítomný především v emisích z dopravy, cigaretovém kouři a výrobcích chemického průmyslu, je karcinogenní látka, jejíž expozice má za následek zvýšení rizika akutní myeloidní leukemie [6].

Oxidační poškození makromolekul je definováno jako změny v organismu způsobené vlivem oxidačního stresu. Oxidační stres je vyvolán nerovnováhou mezi hladinami oxidantů a antioxidantů. Může postihnout všechny buněčné makromolekuly: DNA, lipidy i proteiny [7]. Oxidanty jsou látky nebo procesy vyvolávající tvorbu ROS, nebo ROS samotné. Podle původu je lze rozdělit na endogenní (procesy související s buněčným metabolismem – oxidativní fosforylace, zánětlivé procesy) a exogenní (vlivy životosprávy, působení znečištěného prostředí, infekce apod.). Antioxidanty zahrnují jednak enzymy snižující hladiny oxidačního poškození, dále vitamíny, glutation a další látky snižující hladiny ROS, případně oxidačního poškození. V malé míře je oxidační stres přirozeným jevem a organismus je vybaven mechanismy, které mu umožňují se s ním vyrovnat. Fyziologické hladiny ROS jsou důležité, protože ROS plní roli signálních molekul a jsou nezbytné v embryonálním vývoji [8]. Avšak vysoké hladiny ROS, jež není organismus schopen účinně likvidovat, způsobují závažné škody.

Pro lidské zdraví jsou nejzávažnější změny vyvolané na úrovni DNA. ROS mohou působit na jednotlivé báze, které jsou složkami DNA. V důsledku toho dochází ke vzniku mutací [9]. Nejčastější modifikovanou bází indukovanou působením ROS je 8-oxo-7,8-dihydro-2'-deoxyguanosin (8-oxodG). Pokud není tato báze včas rozpoznána a odstraněna reparačními mechanismy buňky, dojde k chybnému párování bází a ke vzniku mutace. Kromě změn bází mohou ROS též poškozovat genetický materiál tím, že způsobují zlomy řetězců DNA. Oxidační poškození DNA může být snadno měřeno metodami ELISA jako hladiny 8-oxodG v moči, kam je oxidovaná báze vylučovaná. Je však nutno si uvědomit, že přítomnost 8-oxodG v moči je dána nejen mírou oxidačního poškození DNA, ale též účinností reparačních mechanismů organismu. Dalším významným cílem pro ROS jsou lipidy. Působením ROS na molekuly lipidů dochází k peroxidaci lipidů, což je proces, který v organismu probíhá opakovaně, dokud nedojde k jeho ukončení antioxidantem [10]. Primárním cílem pro ROS jsou membránové lipidy. Jejich peroxidace mění vlastnosti buněčných membrán, což narušuje normální buněčné funkce. V současnosti jsou za nejspolehlivější markery peroxidace lipidů považovány isoprostany, konkrétně pak 15-F2t-isoprostan (15-F2t-IsoP) [11]. Isoprostany vznikají působením ROS na arachidonovou kyselinu vázanou v membránách. Z membrán jsou isoprostany odštěpovány a buď cirkulují v krevní plazmě, nebo jsou z těla vylučovány močí. V obou tělních tekutinách jsou isoprostany relativně stabilní a podobně jako v případě 8-oxodG je možné je zde stanovit imunochemickými metodami (ELISA). Ovšem na rozdíl od 8-oxodG nejsou hladiny isoprostanů ovlivňovány účinností reparačních mechanismů, a tak představují přesnější ukazatel expozice organismu oxidačnímu stresu.

Vlivem ROS na proteiny dochází k tvorbě karbonylových skupin na postranních řetězcích aminokyselin, zejména prolinu, argininu, lysinu a treoninu, ke zlomům a chybnému skládání molekul proteinů. Oxidované proteiny mají narušenou funkci a transport. Buňka nedisponuje žádným reparačním systémem určeným pro proteiny, je pouze schopna poškozené proteiny rozpoznat, označit a následně degradovat [12, 13]. Vzhledem k vysoké koncentraci proteinů v krevní plazmě je tento materiál zvláště vhodný pro stanovování oxidace proteinů. Nejčastější metoda detekce je založena na vazbě dinitrofenylhydrazinu na karbonylové skupiny proteinů a použití protilátky proti dinitrofenylhydrazonu, který se navázáním v místě karbonylové skupiny vytvoří.

Cílem naší práce, která je rozšířením studie publikované v loňském roce [14], bylo porovnat hladiny oxidačního poškození DNA (8-oxodG), lipidů (15-F2t-IsoP) a proteinů (karbonylové skupiny) u subjektů ze dvou lokalit: městských strážníků z Prahy a administrativních pracovníků a městských strážníků z Ostravska. Pražská skupina byla zvolena jako kontrolní, jelikož hladiny znečištění ovzduší jsou v Praze v porovnání s Ostravskem výrazně nižší. Měření bylo provedeno opakovaně ve třech obdobích též s odlišnými hladinami znečištění: zima 2009 (střední hladiny), léto 2009 (nízké hladiny), zima 2010 (vysoké až extrémní hladiny znečištění). Expozice škodlivinám (k-PAU, B[*a*]P, benzenu) byla stanovena personálními monitory. Následně bylo stanoveno oxidační poškození v moči, resp. v krevní plazmě. Naším předpokladem bylo, že oxidační poškození makromolekul, na základě výsledků předchozí práce především peroxidace lipidů, bude vyšší u skupin exponovaných vyšším koncentracím škodlivin, tj. u skupiny z Ostravska a dále u skupin sledovaných v zimních obdobích.

MATERIÁL A METODY

Osoby zahrnuté do studie a sběr vzorků

Počet osob zahrnutých do studie se pohyboval v závislosti na odběrovém období od 161 do 214: kontrolní skupinu tvořilo 61 – 65 příslušníků městské policie Praha, skupinu exponovanou znečištěnému ovzduší představovalo 98 – 149 osob z Ostravska (příslušníků městské policie Karviná a Havířov a úředníků Krajského úřadu MSK v Ostravě a dobrovolníků z Bartovic a Radvanic). Všechny osoby byly nekuřáci. Každý účastník studie vyplnil dotazník o životním stylu a zdravotním stavu. Všechny osoby byly sledovány ve třech odběrových obdobích: v zimě (únor – březen) 2009, v létě (květen – červen) 2009 a v zimě (leden – únor) 2010.

Tab. 1: Personální expozice benzenu v Praze a na Ostravsku pro jednotlivá odběrová období v letech 2009–2010

	Období					
Benzen (μg/m³)	Zima 2009		Léto 2009		Zima 2010	
	Praha (N=65)	Ostravsko (N=98)	Praha (N=61)	Ostravsko (N=100)	Praha (N=65)	Ostravsko (N=149)
Průměr	5,28	8,39	3,36	8,20	5,66	14,9
Směrodatná odchylka	9,80	10,0	1,90	14,5	1,67	10,7
Median	3,39	6,77	2,97	5,11	5,50	9,97
Minimum	2,14	2,48	1,21	1,52	2,28	5,30
Maximum	76,20	99,7	15,9	111	10,8	59,6
Porovnání lokalit (p)	<0,001		<0,001		<0,001	

Tab. 2: Personální expozice B[a]P v Praze a na Ostravsku pro jednotlivá odběrová období v letech 2009–2010

	Období					
B[*a*]P (ng/m³)	Zima 2009		Léto 2009		Zima 2010	
	Praha (N=65)	Ostravsko (N=98)	Praha (N=61)	Ostravsko (N=100)	Praha (N=65)	Ostravsko (N=149)
Průměr	0,80	3,50	0,12	0,43	2,80	14,8
Směrodatná odchylka	0,55	3,03	0,14	0,24	1,87	13,3
Median	0,66	1,95	0,08	0,37	2,43	9,01
Minimum	0,10	0,33	0,04	0,08	0,28	2,19
Maximum	3,37	12,6	1,00	1,24	11,5	74,2
Porovnání lokalit (p)	<0,001		<0,001		<0,001	

Tab. 3: Personální expozice k-PAU v Praze a na Ostravsku pro jednotlivá odběrová období v letech 2009–2010

	Období					
k-PAU (ng/m³)	Zima 2009		Léto 2009		Zima 2010	
	Praha (N=65)	Ostravsko (N=98)	Praha (N=61)	Ostravsko (N=100)	Praha (N=65)	Ostravsko (N=149)
Průměr	4,27	20,6	1,03	3,19	19,6	100,2
Směrodatná odchylka	2,95	18,6	0,61	1,70	14,5	92,9
Median	3,57	11,5	0,85	2,95	17,8	59,6
Minimum	0,82	1,70	0,36	0,81	2,14	14,7
Maximum	16,4	86,6	3,64	9,80	103,9	513,0
Porovnání lokalit (p)	<0,001		<0,001		<0,001	

Před zahájením studie byly vybrané osoby seznámeny s cíli studie a podepsaly informovaný souhlas. Do studie nebyly zahrnuty osoby, které podstoupily v posledních 3 měsících radiografické vyšetření, nebo byly v tomto období očkovány.

V rámci studie byly odebrány vzorky krve a moče. Krev byla odebírána do zkumavek obsahujících heparin. Vzorky byly okamžitě po odběru dopraveny do laboratoře Oddělení genetické ekotoxikologie ÚEM AV ČR v Praze, kde byly zpracovány a uchovány při -80° C. Vzorky odebrané na Ostravsku byly uloženy do 4° C a okamžitě transportovány kurýrní službou do pražské laboratoře, kde byly ihned zpracovány. Doba od odběru vzorků do jejich uložení při -80 °C nepřesáhla 8 hodin.

Stanovení expozice benzenu

Expozice benzenu byla stanovena pomocí pasivních vzorkovačů Radiello®, které účastníci studie nosili 24 hodin. Benzen i další VOC zachycené na grafitizovaném uhlí byly před analýzou uvolněny termální desorpcí a následně analyzovány plynovou chromatografií s plameno-ionizační detekcí.

Stanovení expozice k-PAU

Expozice k-PAU byla zjišťována pomocí personálních monitorů, které používali účastníci studie dva po sobě následující dny (celkem 48 hodin). Personální monitory byly vybaveny filtry umožňujícími sběr částic o velikosti <2,5 μm ($PM_{2,5}$). Kvantitativní chemická analýza k-PAU, mezi něž patří benz[*a*]antracen, chrysen, benzo[*b*]fluoranten, benzo[*k*]fluoranten, benzo[*a*]pyren, dibenzo[*a,h*]antracen, benzo[*g,h,i*]perylen a indeno-[*1,2,3-cd*]pyren, byla provedena pomocí HPLC s fluorescenční detekcí. Analýzy k-PAU i VOC byly prováděny akreditovaným pracovištěm ALS Laboratory Group, ALS Czech Republic, s. r. o., Praha.

Stanovení 8-oxodG

Hladina 8-oxodG v moči byla stanovena metodou kompetitivní ELISA podle publikované metodiky [15, 16]. Detekce je založena na použití primární protilátky specificky rozpoznávající 8-oxodG v biologickém materiálu (výrobce protilátky: JaICA, Japonsko). Vzorky moči byly analyzovány v triplikátech. Výsledky byly vztaženy na obsah kreatininu a vyjádřeny v nmol 8-oxodG/mmol kreatininu.

Stanovení 15-F2t-IsoP

Hladiny 15-F2t-IsoP byly stanoveny v krevní plazmě komerčním kitem od firmy Cayman Chemical Company (Ann Arbor, MI, USA) podle doporučení výrobce. Pro analýzu bylo použito 125 μl krevní plazmy, která byla nejprve hydrolyzována, aby došlo k uvolnění isoprostanů vázaných na lipoproteiny, a dále purifikována pomocí afinitního sorbentu dodaného výrobcem kitu. Vzorky byly naředěny v poměru 1:2 v dodaném pufru a následně analyzovány v duplikátech. Koncentrace 15-F2t-IsoP byla vyjádřena v pg 15-F2t-IsoP/ml plazmy.

Stanovení karbonylových skupin v proteinech

Karbonylové skupiny v proteinech krevní plazmy byly analyzovány metodou nekompetitivní ELISA podle dříve publikovaných protokolů modifikovaných v naší laboratoři [16]. Vzorky byly analyzovány v triplikátech a hladiny oxidovaných proteinů byly vyjádřeny v nmol/ml krevní plazmy.

Měření hladin kotininu

Hladiny kotininu v moči, které sloužily jako marker expozice tabákovému kouři, byly stanoveny radioimunochemicky [17].

Statistická analýza

Pro statistickou analýzu byl použit software SPSS, verze 18,0. Data, která neměla normální rozložení, byla analyzovaná neparametrickými testy – Mann-Whitney testem pro porovnání dvou

skupin vzájemně a Spearmanovým korelačním testem pro zjištění vzájemných vztahů mezi biomarkery. Data s normální distribucí byla analyzována t-testem a Pearsonovým korelačním testem.

VÝSLEDKY

Personální expozice environmentálním polutantům

Výsledky stanovení personální expozice benzenu, B[*a*]P a k-PAU s použitím personálních monitorů jsou uvedeny v **tabulkách 1 – 3.** Pro všechny sledované látky a všechna tři testovaná období platí, že osoby z Ostravska byly vystaveny výrazně vyšším hladinám škodlivin než osoby z Prahy ($p < 0{,}001$). Podle očekávání byly nejnižší hodnoty expozice polutantům v letním období 2009, následované zimním obdobím 2009. Nejvyšší expozice byly zaznamenány v obou oblastech v zimním období 2010: hodnoty naměřené v Praze zhruba odpovídaly koncentracím zjištěným na Ostravsku v zimním období 2009, zatímco ostravské hodnoty činily přibližně 2 – 3násobek hodnot pražských. Je třeba zdůraznit, že koncentrace polutantů byly zvláště na Ostravsku extrémně vysoké v důsledku inverze; průměrná koncentrace B[*a*]P přesáhla téměř 15krát doporučenou koncentraci 1 ng/m^3, která je považována za hraniční hodnotu nezpůsobující zásadní poškození genetického materiálu. Další informace o expozici znečišťujícím látkám jsou uvedeny v článcích Švecové a kol. [18, 19].

Tab. 4: Peroxidace lipidů u kontrolních a exponovaných subjektů v letech 2009 – 2010

15-F2t-IsoP (pg/ml plazmy)	Období					
	Zima 2009		Léto 2009		Zima 2010	
	Praha (N = 65)	Ostravsko (N = 98)	Praha (N = 61)	Ostravsko (N = 100)	Praha (N = 65)	Ostravsko (N = 149)
Průměr	165,9	279,3	124,9	134,1	256,5	279,5
Směrodatná odchylka	41,7	303,6	43,6	37,0	104,7	124,5
Median	158,4	179,2	116,4	129,5	236,9	252,3
Minimum	108,7	101,3	64,8	53,0	119,6	90,6
Maximum	321,2	2410,3	376,5	262,1	647,8	814,6
Porovnání lokalit (p)	< 0,001		= 0,034		= 0,258	

Tab. 5: Hladiny oxidačního poškození DNA v jednotlivých skupinách v letech 2009 – 2010

8-oxodG (nmol/mmol kreatininu)	Období					
	Zima 2009		Léto 2009		Zima 2010	
	Praha (N = 65)	Ostravsko (N = 98)	Praha (N = 61)	Ostravsko (N = 100)	Praha (N = 65)	Ostravsko (N = 149)
Průměr	5,24	5,51	5,28	5,27	4,79	4,69
Směrodatná odchylka	2,43	2,78	2,88	2,68	1,65	2,14
Median	5,11	5,54	5,21	5,11	4,95	4,77
Minimum	0,20	0,04	0,10	0,10	0,52	0,19
Maximum	14,4	12,9	20,4	18,4	9,87	10,4
Porovnání lokalit (p)	= 0,520		= 0,987		= 0,742	

Tab. 6: Oxidace proteinů v analyzovaných skupinách v letech 2009 – 2010

karbonylové skupiny (nmol/ml plazmy)	Období					
	Zima 2009		Léto 2009		Zima 2010	
	Praha (N = 65)	Ostravsko (N = 98)	Praha (N = 61)	Ostravsko (N = 100)	Praha (N = 65)	Ostravsko (N = 149)
Průměr	23,1	23,2	22,3	19,8	23,6	22,0
Směrodatná odchylka	4,63	3,17	2,75	2,89	6,43	5,49
Median	21,7	23,3	22,4	20,0	22,8	21,3
Minimum	16,4	16,2	16,7	13,7	10,0	11,3
Maximum	35,4	34,9	27,8	32,7	39,1	43,3
Porovnání lokalit (p)	= 0,202		< 0,001		0,149	

Oxidační poškození makromolekul

V **tabulkách 4 – 6** jsou uvedeny hodnoty markerů oxidačního poškození lipidů, DNA a proteinů. Expozici polutantům z ovzduší nejlépe odrážely hladiny peroxidace lipidů **(tab. 4)**. Hodnoty měřené na Ostravsku byly výrazně vyšší než u pražské skupiny v zimě 2009 ($p < 0{,}001$). Menší, avšak stále signifikantní rozdíl byl zjištěn mezi oběma oblastmi v létě 2009 ($p = 0{,}034$). V zimním období 2010 byly hodnoty peroxidace lipidů též vyšší u ostravské než u pražské skupiny, avšak rozdíly nebyly statisticky signifikantní ($p = 0{,}258$). Žádné rozdíly mezi lokalitami ani mezi obdobími nebyly nalezeny pro hladiny 8-oxodG **(tab. 5)** a pro většinu výsledků stanovení karbonylových skupin proteinů **(tab. 6)**. Zde byly proti očekávání zaznamenány vyšší hladiny oxidačního poškození proteinů v pražské skupině v letním období 2009.

V **tabulce 7** jsou uvedeny výsledky korelační analýzy mezi koncentracemi polutantů měřenými personálními monitory a hladinami 15-F2t-IsoP v krevní plazmě. Všechny sledované látky (benzen, B[*a*]P, k-PAU) signifikantně korelují s naměřenými hodnotami peroxidace lipidů; nejlepší korelace byla zjištěna pro B[*a*]P (korelační koeficient $R = 0{,}573$, $p < 0{,}001$). Tento výsledek dále ilustruje **obrázek 1**, v němž je vynesena závislost hladin 15-F2t-IsoP na expozici B[*a*]P (dva vzorky s odlehlými hodnotami 15-F2t-IsoP > 1000 pg/ml nejsou na obrázku znázorněny). Další analýzou bylo zjištěno, že pozitivní korelace mezi hladinami 15-F2t-IsoP a expozicemi B[*a*]P platí jen do 83. percentilu koncentrací B[*a*]P, což odpovídá koncentraci asi 8,82 ng/m^3. Při vyšších dávkách B[*a*]P není již závislost lineární **(obr. 2)**.

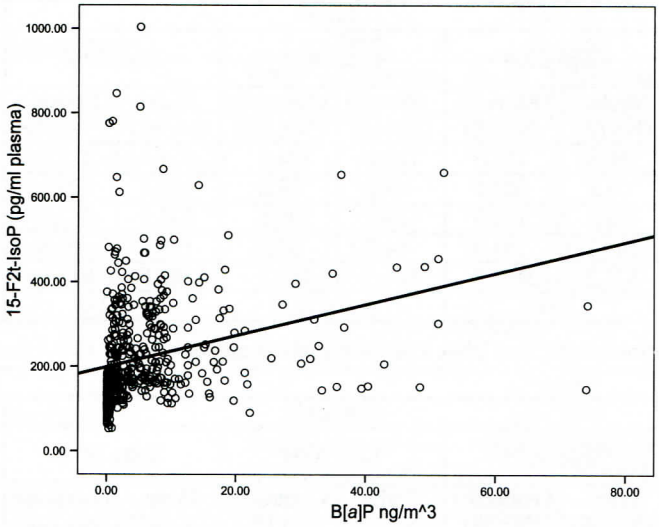
Tab. 7: Korelační analýza hladin environmentálních polutantů a peroxidace lipidů

15-F2t-IsoP (N=534)	Korelační koeficient	Signifikance
Benzen	0,368	< 0,001
B[*a*]P	0,573	< 0,001
k-PAU	0,567	< 0,001

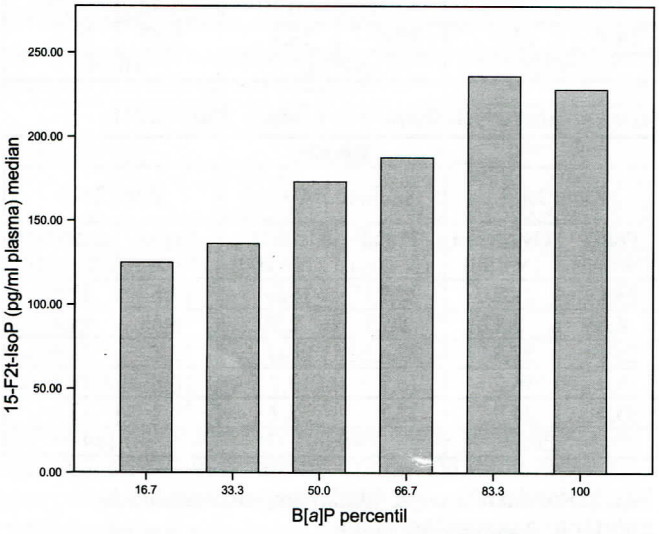
DISKUZE

Cílem naší studie bylo analyzovat hladiny oxidačního poškození makromolekul u osob žijících v oblastech lišících se koncentracemi polutantů v ovzduší. V naší laboratoři byly obdobně zaměřené studie již publikovány [20, 21], avšak současná práce je výjimečná velmi vysokými hladinami polutantů v ovzduší v době sledování studovaných skupin. Zvláště na Ostravsku

Obr. 1: Korelace mezi personální expozicí benzo[*a*]pyrenu a hladinami peroxidovaných lipidů v krevní plazmě



Obr. 2: Vztah mezi hladinami peroxidace lipidů a koncentracemi benzo[*a*]pyrenu rozdělenými do percentilů



v zimním období 2010 byly v důsledku inverze zaznamenány extrémní koncentrace polutantů v ovzduší, kdy personální expozice více než 10krát převyšovaly hodnoty naměřené u osob z našich předchozích studií prováděných u řidičů autobusů a administrativních pracovníků v Praze [20, 21].

Peroxidace lipidů se zdá být markerem nejlépe asociovaným s expozicí znečišťujícím látkám v ovzduší, avšak jen do určité horní hranice. V zimě 2009 byl zjištěn statisticky vysoce významný rozdíl mezi hladinami 15-F2t-IsoP v Praze a na Ostravsku. Tomu odpovídal rozdíl v průměrné personální expozici B[*a*]P v obou lokalitách (Praha – 0,80 ng/m^3, Ostravsko – 3,50 ng/m^3). V létě 2009 byly naměřené koncentrace polutantů několikanásobně nižší (B[*a*]P: Praha – 0,12 ng/m^3, Ostravsko – 0,43 ng/m^3), nicméně peroxidace lipidů byla stále signifikantně zvýšena u ostravské skupiny. V zimním období 2010 byly zaznamenány velmi vysoké personální expozice B[*a*]P: Praha – 2,80 ng/m^3, Ostravsko – 14,80 ng/m^3. I přes vysoký rozdíl koncentrací polutantů nebyly mezi hladinami 15-F2t-IsoP u obou skupin signifikantní rozdíly. Naše další analýzy ukázaly, že mezi dávkou (koncentrací B[*a*]P) a účinkem (hladinami 15-F2t-IsoP) existuje nelineární vztah: při nižších koncentracích B[*a*]P se peroxidace lipidů mění lineárně, avšak po dosažení vyšších koncentrací B[*a*]P se již hladina 15-F2t-IsoP téměř nemění. Toto zjištění a s ním související popsaná pozitivní korelace mezi expozicí B[*a*]P a hladinami 15-F2t-IsoP v krevní plazmě jsou nejvýznamnějšími výsledky naší práce.

V předchozích studiích byl též pozorován vztah mezi expozicí znečištěnému ovzduší a peroxidací lipidů [16, 21, 22], ale uvedená nelineární závislost je popisována poprvé.

Získané výsledky mají velký význam z hlediska lidského zdraví. Peroxidace lipidů není jednorázový proces, ale jak již bylo uvedeno, vznikají při něm reaktivní intermediáty, jejichž prostřednictvím se oxidační poškození šíří, dokud nedojde k jejich inaktivaci působením antioxidantů. Při nedostatečných hladinách antioxidantů a vysokých koncentracích peroxidovaných lipidů pak může docházet k poškození dalších makromolekul (DNA, proteinů) a buněčných struktur. Tato poškození se pak mohou projevit vyšším rizikem řady onemocnění [23]. Lze říci, že expozice znečištěnému ovzduší vyvolává peroxidaci lipidů, která pak v delším horizontu může vyústit k poškození celého organismu. Snížení znečištění ovzduší především na Ostravsku by tedy mělo být primárním cílem pro odpovědné orgány.

Naše výsledky naznačují, že zvýšené hladiny benzenu a k-PAU, včetně B[*a*]P nemají vliv na produkty oxidace DNA v moči, ani na hladiny oxidovaných proteinů v krevní plazmě. Výsledky získané pro 8-oxodG doplňují a potvrzují data z naší předešlé studie ostravské a pražské populace, v níž nebyly zjištěny žádné rozdíly v hladinách 8-oxodG mezi porovnávanými skupinami [14]. Obdobné výsledky byly popsány v již zmíněné studii u řidičů autobusů a administrativních pracovníků [20]. Lze tedy shrnout, že hladiny 8-oxodG v moči neodráží krátkodobou expozici benzenu a k-PAU těsně před odběrem biologického materiálu, a to ani v případě velmi vysokých koncentrací látek. Výsledky mohou být dány faktem, že uvedené látky neindukují oxidační poškození DNA (což se zvláště v případě k-PAU zdá málo pravděpodobné), nebo zde hrají roli mechanismy, kterými se 8-oxodG do moči uvolňuje. Oxidovaná báze vznikne primárně buď oxidací volných nukleotidů v buňce [24] nebo, a to především, jako výsledek oxidace deoxyguanosinu, který je součástí DNA, a do moči se pak uvolní po rozpoznání a vyštěpení reparačními mechanismy [25]. Výsledná hladina 8-oxodG v moči je tedy závislá jak na výchozí míře oxidace DNA, tak na schopnosti organismu oxidované báze z těla vyloučit, tj. na účinnosti reparačních mechanismů. Lze se domnívat, že vysoké koncentrace znečišťujících látek na Ostravsku stimulují reparační mechanismy osob žijících v této oblasti. V důsledku toho je oprava poškozené DNA účinnější a rychlejší než u osob žijících v méně znečištěných oblastech, což se odrazí v neočekávaně nízkých hladinách markerů oxidačního poškození v moči.

Oxidace proteinů se mezi sledovanými skupinami většinou též nelišila, s výjimkou letního období 2009, kdy hladiny karbonylových skupin byly signifikantně nižší u osob žijících na Ostravsku i přesto, že znečištění ovzduší bylo v této oblasti vyšší. Porovnání hladin karbonylových skupin pro danou oblast v různých obdobích ukazuje prakticky stejné hodnoty pro subjekty z Prahy, zatímco u ostravské skupiny je zřetelný signifikantní pokles koncentrací karbonylových skupin v létě 2009 ve srovnání se zimou 2009 a následně opět vzestup v zimním období 2010. Tento trend odpovídá našim předpo-

kladům. Fakt, že hladiny karbonylových skupin u pražských subjektů jsou téměř konstantní, i když se koncentrace polutantů v ovzduší měnily, lze vysvětlit změnami koncentrací jiných, nemonitorovaných látek, jejichž vyšší hladiny v létě 2009 v Praze by mohly indukovat relativně vysoké hladiny karbonylových skupin. Obdobné výsledky jsme obdrželi v naší předcházející studii [21], v níž v letním období byly hladiny karbonylových skupin výrazně vyšší než v zimních obdobích. Předpokládali jsme, že zodpovědná za to může být vyšší hladina ozonu v letních měsících, která může působit jako oxidant.

ZÁVĚR

Naše studie ukazuje, že expozice znečištěnému ovzduší vede ke zvýšení hladin peroxidovaných lipidů. Vzhledem k tomu, že je znám vztah mezi peroxidací lipidů a vznikem řady onemocnění, je obyvatelstvo postižených oblastí bezprostředně ohroženo zhoršením zdravotního stavu v dlouhodobém horizontu. Proto by měla být učiněna opatření vedoucí ke snížení koncentrací škodlivin (zejména k-PAU) na Ostravsku. Pro ověření pozitivního efektu snížení zátěže populace Moravskoslezského kraje $PM_{2,5}$ a k-PAU by bylo vhodné po realizaci těchto opatření studii měření hladin biomarkerů opakovat.

Poděkování
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Příloha 7

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Expression of XRCC5 in peripheral blood lymphocytes is upregulated in subjects from a heavily polluted region in the Czech Republic

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Expression of *XRCC5* in peripheral blood lymphocytes is upregulated in subjects from a heavily polluted region in the Czech Republic

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ABSTRACT

Air pollution causes oxidative damage to macromolecules, chromosomal aberrations and changes in gene expression. We investigated the levels of oxidative stress markers [8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), 15-F_{2t}-isoprostane (15-F2t-IsoP), protein carbonyls] and cytogenetic parameters [genomic frequency of translocations (F_G/100), percentage of aberrant cells (%AB.C.) and acentric fragments (ace)] in subjects living in Prague and in the heavily polluted Ostrava region. We also compared the expression of genes participating in base excision repair (BER) and non-homologous end-joining (NHEJ). We analyzed 64 subjects from Prague and 75 subjects from Ostrava. We measured oxidative stress markers by ELISA, cytogenetic parameters by fluorescence in situ hybridization and gene expression by quantitative PCR. The levels of air pollutants (benzo[a]pyrene, B[a]P; carcinogenic polycyclic aromatic hydrocarbons, c-PAHs; benzene) measured by personal monitors were significantly elevated in Ostrava compared to Prague ($p < 0.001$). Despite this fact, we observed no differences in biomarkers of oxidative stress between the two locations. Moreover, subjects from Ostrava were less likely to have above-median levels of %AB.C. (OR; 95% CI: 0.18; 0.05–0.67; $p = 0.010$). Multivariate analyses revealed that subjects living in Ostrava had increased odds of having above-median levels of *XRCC5* expression (OR; 95% CI: 3.33; 1.03–10.8; $q = 0.046$). Above-median levels of 8-oxodG were associated with decreased levels of vitamins C (OR; 95% CI: 0.37; 0.16–0.83; $p = 0.016$) and E (OR; 95% CI: 0.25; 0.08–0.75; $p = 0.013$), which were elevated in subjects from Ostrava. We suggest that air pollution by c-PAHs affects *XRCC5* gene expression, which probably protects subjects from Ostrava against the induction of a higher frequency of translocations; elevated vitamin C and E levels in the Ostrava subjects decrease the levels of 8-oxodG.

1. Introduction

Ambient air pollution poses a problem to millions of people worldwide. Exposure to polluted air is associated with increased mortality and a higher incidence of cardiovascular and pulmonary diseases, as well as cancer [1]. Among many chemical compounds present in the ambient air, polycyclic aromatic hydrocarbons (PAHs) and benzene are notable because of their carcinogenicity and their abundance in the environment [2]. PAHs require metabolic activation to exert their mutagenic and/or carcinogenic effects. Three principal pathways are currently proposed for the metabolic activation of PAHs: the pathway via dihydrodiol epoxide, the pathway via radical cation by one-electron oxidation and the *o*-quinone pathway [3]. Reactive intermediates formed as a result of the metabolic activation of PAHs may bind to DNA, form PAH-DNA adducts, cause mutations and thus increase cancer risk [4]. The *o*-quinone pathway of PAH metabolism leads to redox cycling, the formation of reactive oxygen species (ROS) and thus oxidative damage of cellular macromolecules [5]. Benzene is metabolized in the liver by CYP2E1 into benzene oxide, which is further metabolized into several products, including reactive quinones [6] that again may induce oxidative damage.

Oxidative damage affects all macromolecules: DNA, lipids and proteins [7]. Damage caused to DNA results in single- or double

Abbreviations: 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; ACTB, actin β; APE1/APEX1, apurinic/apyrimidinic endonuclease; B[a]P, benzo[a]pyrene; BER, base excision repair; c-PAHs, carcinogenic polycyclic aromatic hydrocarbons; Cq, quantification cycle; DSB, double strand break; ELISA, enzyme-linked immunosorbent assay; ETS, environmental tobacco smoke; FISH, fluorescence in situ hybridization; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; NHEJ, non-homologous end-joining; OGG1, 8-oxoguanine DNA glycosylase; 15-F2t-IsoP, 15-F_{2t}-isoprostane; LIG4, ligase 4; qPCR, quantitative PCR; XRCC, X-ray repair complementing defective repair in Chinese hamster cells.

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strand breaks (DSB) of the nucleic acid chain or base modifications leading to mutations [8,9]. 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is the most abundant and most frequently studied oxidatively modified DNA base. If unrepaired, its presence in DNA leads to GC>TA transversions. Oxidized bases in DNA, including 8-oxodG, are repaired by the action of the short-patch or long-patch base excision repair pathway (BER) [10]. The first step of BER involves a DNA glycosylase that cleaves the modified base, which results in an apurinic/apyrimidinic (AP) site formation. Mammalian cells contain eleven different glycosylases, each with a specialized function [11]; 8-oxoguanine DNA glycosylase (OGG1) was reported to be responsible for 90% of glycosylase activity in human cell extracts [12]. The AP site is recognized and cleaved by apurinic/apyrimidinic endonuclease (APE1/APEX1), the missing nucleotide is replaced by the activity of DNA polymerase β (Pol β) and DNA is sealed by the DNA ligase III/XRCC1 complex. In the event of long-patch repair, several nucleotides are replaced by the action of the proteins Pol β, PCNA, Fen 1 and ligase 1 [10].

DNA strand breaks are considered one of the most dangerous DNA lesions [13]. They can induce apoptosis, cause gene inactivation, or lead to chromosomal aberrations [14]. DNA strand breaks are repaired by two mechanisms: DNA ends that share long homologous regions are joined by homologous recombination, while non-homologous end-joining repair (NHEJ) occurs when DNA strands with as few as 2 bp of homology are ligated. NHEJ is believed to be a pathway for the repair of most double-strand DNA breaks involved in chromosomal translocations [15]. A number of proteins take part in the recognition of double-strand breaks and their repair [15]. The first protein that binds at DNA breaks in the nucleus is Ku [a complex of Ku70 (XRCC6) and Ku80 (XRCC5) proteins]; it forms Ku-DNA complexes that serve as a structure binding other proteins. The next protein involved is DNA-PKcs, which binds to Ku-DNA and forms the DNA-PK complex, a nuclear serine/threonine protein kinase. Another enzyme associated with DNA-PKcs is a nuclease called Artemis with both endo- and exonuclease activities. Polymerases mu and lambda, members of the Pol X family, synthesize missing DNA nucleotides and the XRCC4-DNA ligase IV complex ligates DNA strands.

In the present study we investigated the levels of markers of oxidative damage to DNA (8-oxodG), lipids (15-F_{2t}-isoprostane, 15-F2t-IsoP) and proteins (protein carbonyl groups), chromosomal aberrations (F_G/100, %AB.C., ace) and the expression of selected genes participating in BER (including *OGG1*, *APEX1*, *XRCC1*) and NHEJ (including *LIG4*, *XRCC4*, *XRCC5* and *XRCC6*) in 64 subjects living and working in Prague and 75 subjects from the heavily polluted city of Ostrava. The genes were selected based on the fact that they participate in the repair of 8-oxodG and affect the frequency of chromosomal aberrations. Our study is a part of a large molecular epidemiological study that investigates the effect of air pollution on human health in two regions of the Czech Republic, Prague and Ostrava. The Ostrava region is currently the most polluted part of the country. We hypothesized that due to the air pollution, the subjects from Ostrava would exhibit significantly higher levels of oxidative damage to macromolecules, as well as an elevated frequency of chromosomal aberrations. We also expected the expression of selected genes to be upregulated in subjects from the Ostrava region. Our study is unique because it measures for the first time the levels of biomarkers in subjects exposed to air pollutants in concentrations that are unprecedented in the entire European Union. In the past we analyzed the frequency of chromosomal aberrations in policemen from Prague [16–19] and the levels of oxidative stress markers in Prague's bus drivers [20–22] and policemen. However, the present study is our first in which the assessment of oxidative stress markers and chromosomal aberrations is accompanied by the measurement of DNA repair gene expression.

2. Materials and methods

2.1. Subjects and sampling

The study population consisted of 64 city policemen working in Prague, Czech Republic, and 75 office workers working in Ostrava, Czech Republic. The study subjects were followed in the winter season of 2010. Each participant completed a questionnaire on their personal medical history and lifestyle. All participants signed an informed consent form and could cancel their participation at any time during the study, according to the Helsinki II declaration. The study was approved by the Ethical Committee of the Institute of Experimental Medicine AS CR in Prague. Any person who underwent medical treatment, radiography or vaccination up to 3 months before sampling was not included in the study.

From each subject, a spot urine sample was obtained. The blood samples were collected by venipuncture into vacuettes containing sodium heparin (for cytogenetic analysis) or EDTA (for oxidative stress markers and gene expression analysis). Samples were coded, transported to the Laboratory of Genetic Ecotoxicology and processed. Samples to be analyzed for levels of oxidative stress markers were kept in aliquots at −80 °C.

2.2. Exposure assessment

The subjects' exposure to c-PAHs was monitored by personal samplers during 48 h. The samplers were equipped with filters collecting particles of aerometric diameter ≤ 2.5 μm (PM2.5) [23]. Quantitative chemical analysis of c-PAHs (benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenzo[a,h]anthracene, benzo[g,h,i]perylene and indeno[1,2,3-cd]pyrene) was performed by HPLC with fluorescence detection according to the EPA method [24] in the certified laboratory ALS Czech Republic, Prague.

Benzene was collected on Radiello® radial diffusive samplers (Supelco, Park Bellefonte, PA, USA) worn by study subjects for 24 h. It was adsorbed on graphitized charcoal and recovered by thermal desorption. The analysis was performed by capillary gas chromatography with flame ionization technique detection in the certified laboratory ALS Czech Republic, Prague.

2.3. Analysis of oxidative stress markers

8-OxodG ELISA. Urinary 8-oxodG levels were analyzed by competitive ELISA essentially as previously described [25,20]. Urinary 8-oxodG concentration was expressed as nmol 8-oxodG/mmol creatinine.

15-F2t-IsoP ELISA. Plasma 15-F2t-IsoP levels were analyzed using immunoassay kits from Cayman Chemical Company (Ann Arbor, MI, USA) as previously described [26]. The 15-F2t-IsoP concentrations were expressed as pg 15-F2t-IsoP/ml plasma.

Protein carbonyl assay. The levels of protein carbonyl groups were assessed in blood plasma using a noncompetitive ELISA, as previously described [27], with some modifications [20,28]. Plasma protein carbonyl concentration was expressed as nmol carbonyl/ml plasma.

2.4. Fluorescence in situ hybridization (FISH)

Whole venous blood cultures were established within 24 h after blood collection, according to the method described by Rossner et al. [29]. The protocol used to perform FISH with whole chromosome probes for chromosomes #1 and #4 was described in detail by Beskid et al. [19].

2.5. Gene expression analysis

Sample processing. To separate the leukocytes from the whole blood samples and isolate RNA, the LeukoLOCK™ Total RNA Isolation System (Ambion Inc., Austin, TX, USA) was used. The cells were then stored at −20 °C for several weeks until RNA extraction was performed.

RNA extraction. The extraction of leukocyte RNA was conducted according to the manufacturer's recommendations. RNA was quantified spectrophotometrically using a Nanodrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The integrity of RNA was determined using an Agilent 2100 Bioanalyzer (Agilent Technologies Inc., Santa Clara, CA, USA). The vast majority of samples (85%) had a RNA integrity number (RIN) between 6.0 and 9.0. Only ten samples had a RIN lower than 5.0.

Reverse transcription. The Transcriptor High Fidelity cDNA synthesis Kit (ROCHE, Mannheim, Germany) was used. cDNA was produced starting with 0.5 or 1 μg of total RNA. The original protocol was modified by using 2.5 μM oligo$(dT)_{18}$ and 10 μM random hexamers for priming and setting the incubation time and temperature to 30 min and 50 °C, respectively. Total reaction volume was 20 μl.

Quantitative PCR (qPCR). For all qPCR measurements, the 7900HT Fast Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) was used. Each qPCR reaction was carried out in a final volume of 14 μl containing 3.5 μl of diluted cDNA, 2.8 μl of water and 7 μl of TaqMan 2× Universal PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). For determining the expression of the genes *APEX1*, *GAPDH*, *OGG1*, *XRCC1*, *XRCC4*, *XRCC5* and *XRCC6*, the reaction mixture contained an additional 200 nM forward and 200 nM reverse primers and 100 nM probe (Universal ProbeLi-

brary; Roche, Mannheim, Germany). The expression of *ACTB* and *LIG4* was assessed using 0.7 μl of RealTime Ready assays per reaction. Cycling conditions were: 10 min at 95 °C followed by 40 cycles of amplification (15 s at 95 °C and 60 s at 60 °C).

Data analysis. Raw data were analyzed with SDS Relative Quantification Software version 2.3 (Applied Biosystems, USA) to assign the baseline and threshold for Cq determination. Cq values were further analyzed using GenEx software version 5.2.7 (MultiD Analyses AB, Goteborg, Sweden). The expression levels of the target genes were normalized to the expression levels of the reference genes *GAPDH* and *ACTB*.

Further details about the gene expression analysis are given in the Supplementary material.

2.6. Plasma lipids, vitamins and cotinine assay

The plasma levels of cholesterol, LDL- and HDL-cholesterol and triglycerides were determined spectrophotometrically using diagnostic kits (BioVendor, Brno, Czech Republic) and appropriate standards. Levels of vitamins A, E and C in plasma were analyzed by HPLC [30,31]. Urinary cotinine levels as a marker of exposure to tobacco smoke were analyzed by radioimmunoassay [32].

2.7. Statistical analysis

We first compared the basic characteristics of the study groups (age, exposure to air pollutants, levels of cotinine, plasma lipids and vitamins) and the levels of biomarkers of oxidative stress, cytogenetic parameters and gene expression data. We used the Mann–Whitney *U*-test for those variables that did not follow a normal distribution and the *t*-test for data distributed normally. We further transformed the investigated variables into a two-level scale using medians and analyzed by multivariate logistic regression associations between: (a) oxidative stress markers and age, sampling location, exposure to benzene and environmental tobacco smoke (ETS; cotinine levels), plasma lipids and vitamins, and genes participating in BER; (b) cytogenetic parameters and age, sampling location, benzene and ETS exposure, plasma lipids and vitamins and genes participating in NHEJ. Exposure to c-PAHs, B[a]P and education level were found to be collinear with the sampling location and therefore these parameters were omitted in multivariate models. Using logistic regression we further analyzed the association between BER genes and vitamin levels and sampling locations and the expression of BER and NHEJ genes.

To correct for multiple comparisons, we used the FDR method (QVALUE software [33]). All other analyses were performed using SPSS 19.0 software.

3. Results

The basic characteristics of the study population are presented in Table 1. The age of the subjects was the same in both locations ($p = 0.338$). The exposure to air pollutants measured by personal monitors was significantly lower in subjects from Prague than in subjects from Ostrava (mean values ± SD: benzene: 5.64 ± 1.68 vs. 17.81 ± 11.78 μg/m^3, $p < 0.001$; B[a]P: 2.81 ± 1.88 vs. 17.09 ± 16.85 ng/m^3, $p < 0.001$; c-PAHs: 19.60 ± 14.56 vs. 118.7 ± 117.2 ng/m^3, $p < 0.001$ for subjects from Prague and Ostrava, respectively). Subjects from Prague were exposed to higher levels of ETS than subjects from Ostrava (mean values of cotinine ± SD: 65.12 ± 223.3 vs. 8.44 ± 6.51 ng/mg creatinine for subjects from Prague and Ostrava, respectively). We further analyzed the parameters related to nutrition that may influence oxidative stress and/or cytogenetic markers: plasma lipids and vitamins A, C and E. Our results indicate that subjects from Ostrava had a healthier lifestyle. While the levels of triglycerides and LDL cholesterol were lower in the Ostrava subjects, HDL cholesterol levels were lower among the subjects from Prague (mean ± SD: triglycerides: 1.51 ± 0.83 vs. 1.24 ± 0.69 mmol/l, $p = 0.016$; LDL cholesterol: 2.64 ± 0.64 vs. 2.39 ± 0.51 mmol/l, $p = 0.010$; HDL cholesterol: 1.14 ± 0.27 vs. 1.35 ± 0.27 mmol/l, $p < 0.001$ for subjects from Prague and Ostrava, respectively). Vitamin levels were significantly higher in the subjects from Ostrava (mean ± SD: vitamin A: 0.80 ± 0.34 vs. 1.09 ± 0.42 mg/l, $p < 0.001$; vitamin C: 8.39 ± 3.15 vs. 11.85 ± 4.14 mg/l, $p < 0.001$; vitamin E: 9.83 ± 3.96 vs. 17.09 ± 8.81 mg/l, $p < 0.001$, for subjects from Prague and Ostrava, respectively).

Although the exposure to benzene, B[a]P and c-PAHs was substantially higher in the Ostrava region, the levels of the analyzed biomarkers did not correspond with this trend (Table 2). Oxidative damage to macromolecules, cytogenetic parameters, as well as the expression of selected BER and NHEJ genes did not differ between the two locations. The difference in the genomic frequency of translocations (F_G/100) was close to the borderline of significance ($p = 0.060$), but contrary to our expectations, the values were higher in subjects from Prague. The expression of *XRCC4* was also higher in subjects from Prague ($p = 0.038$). After correction for multiple comparisons, the difference was no longer statistically significant ($q = 0.152$).

The results of multivariate logistic regression analyses investigating associations between oxidative stress markers in all subjects and other studied parameters are reported in Table 3. Higher levels of plasma vitamins C and E protect the organism against oxidative DNA damage: subjects with above-median levels of 8-oxodG in the urine had significantly lower plasma levels of vitamin C (odds ratio (OR), 95% CI: 0.37, 0.16–0.83), $p = 0.016$) and E (OR, 95% CI: 0.25, 0.08–0.75, $p = 0.013$). Vitamin C further protects the organism against lipid peroxidation: subjects with above-median levels of 15-F2t-IsoP had lower vitamin C levels (OR, 95% CI: 0.45, 0.20–1.00, $p = 0.049$). Above-median levels of 8-oxodG were further associated with the elevated expression of *XRCC1* (OR, 95% CI: 2.53, 1.05–6.09, $q = 0.048$). Associations between 15-F2t-IsoP and protein carbonyls with the expression of BER genes were not calculated, because BER does not repair oxidatively damaged lipids and proteins. Unexpectedly, above-median levels of protein carbonyls were associated with elevated plasma concentrations of vitamin A (OR, 95% CI: 3.39, 1.25–9.16, $p = 0.016$). In Table 4 the associations between the cytogenetic parameters and other variables are shown. Both F_G/100 and the percentage of aberrant cells (%AB.C.) were positively associated with age (OR, 95% CI: 3.94, 1.78–8.71, $p = 0.001$; 4.09, 1.84–9.12, $p = 0.001$, for F_G/100 and %AB.C., respectively). Exposure to benzene was positively associated with %AB.C. (OR, 95% CI: 3.50, 1.12–10.97; $p = 0.031$). Contrary to our expectations, subjects from Ostrava were less likely to have above-median levels of %AB.C. (OR, 95% CI: 0.18, 0.05–0.67, $p = 0.010$). The expression levels of none of the selected

Table 1
Characteristics of the study groups.

Variable	Prague ($N = 64$)		Ostrava ($N = 75$)		p
	Mean ± SD	Median (min, max)	Mean ± SD	Median (min, max)	
Age (years)	39.0 ± 8.5	37.5 (25.1, 61.9)	38.3 ± 9.9	35.7 (26.7, 63.7)	0.338
Benzene (μg/m^3)	5.64 ± 1.68	5.49 (2.28, 10.80)	17.81 ± 11.78	12.90 (5.34, 49.7)	<0.001
B[a]P (ng/m^3)	2.81 ± 1.88	2.44 (0.3, 11.5)	17.09 ± 16.85	8.42 (2.2, 74.2)	<0.001
c-PAHs (ng/m^3)	19.60 ± 14.56	17.78 (2.14, 103.9)	118.7 ± 117.2	54.72 (14.74, 513)	<0.001
Cotinine (ng/mg creatinine)	65.12 ± 223.3	12.26 (4.33, 1272)	8.44 ± 6.51	6.67 (1.97, 38.8)	<0.001
Triglycerides (mmol/l)	1.51 ± 0.83	1.35 (0.53, 5.63)	1.24 ± 0.69	1.09 (0.43, 4.29)	0.016
Total cholesterol (mmol/l)	4.57 ± 0.99	4.49 (1.59, 8.28)	4.60 ± 0.71	4.50 (3.33, 6.39)	0.812
HDL cholesterol (mmol/l)	1.14 ± 0.27	1.11 (0.56, 1.94)	1.35 ± 0.27	1.34 (0.89, 2.26)	<0.001
LDL cholesterol (mmol/l)	2.64 ± 0.64	2.57 (0.86, 5.11)	2.39 ± 0.51	2.28 (1.64, 4.35)	0.010
Vitamin A (mg/l)	0.80 ± 0.34	0.80 (0.22, 1.80)	1.09 ± 0.42	1.09 (0.24, 2.30)	<0.001
Vitamin C (mg/l)	8.39 ± 3.15	8.30 (1.5, 21.7)	11.85 ± 4.14	11.80 (4.2, 22.6)	<0.001
Vitamin E (mg/l)	9.83 ± 3.96	9.90 (2.4, 19.0)	17.09 ± 8.81	16.80 (3.0, 39.1)	<0.001

Table 2
Parameters of oxidative stress, DNA damage and gene expression in study subjects.

Variable	Prague (N = 64)		Ostrava (N = 75)		p	q
	Mean ± SD	Median (min, max)	Mean ± SD	Median (min, max)		
8-oxodG (nmol/mmol creat.)	4.84 ± 1.61	4.99 (0.52, 9.87)	4.28 ± 2.27	4.16 (0.19, 10.44)	0.102	
15-F_{2t}-isoprostane (pg/ml)	257.0 ± 105.4	238.6 (119.6, 647.8)	282.5 ± 120.8	273.5 (90.6, 814.6)	0.192	
Protein carbonyls (nmol/ml)	23.6 ± 6.48	22.4 (10.0, 39.1)	22.6 ± 6.79	21.3 (11.3, 43.3)	0.348	
F_G/100	1.37 ± 1.03	1.12 (0, 4.11)	1.11 ± 1.09	0.75 (0, 5.23)	0.060	
%AB.C.	0.23 ± 0.15	0.20 (0, 0.60)	0.20 ± 0.16	0.12 (0, 0.70)	0.120	
Ace	0.29 ± 0.68	0 (0, 4.0)	0.29 ± 0.62	0 (0, 3.0)	0.701	
OGG1	1.04 ± 0.33	0.99 (0.44, 2.84)	1.04 ± 0.28	0.94 (0.57, 1.94)	0.923	0.923
APEX1	1.02 ± 0.31	0.97 (0.46, 2.22)	1.04 ± 0.24	1.00 (0.64, 1.75)	0.701	0.923
XRCC1	1.08 ± 0.36	1.09 (0.51, 1.96)	1.02 ± 0.29	0.92 (0.51, 1.92)	0.235	0.705
LIG4	1.06 ± 0.39	1.00 (0.40, 2.13)	1.05 ± 0.29	1.01 (0.47, 1.80)	0.925	0.925
XRCC4	1.09 ± 0.31	1.03 (0.39, 1.92)	0.99 ± 0.26	0.99 (0.49, 1.81)	0.038	0.152
XRCC5	0.99 ± 0.19	0.99 (0.67, 1.63)	1.04 ± 0.19	1.04 (0.63, 1.45)	0.084	0.168
XRCC6	1.03 ± 0.25	1.04 (0.50, 1.79)	1.02 ± 0.20	1.00 (0.61, 1.63)	0.641	0.855

q—adjusted for multiple comparisons.

Table 3
Associations of selected parameters with oxidative stress markers.

Variable	8-oxodG (OR[a] (95% CI, p; q))	15-F2t-IsoP (OR[a] (95% CI, p))	Carbonyl (OR[a] (95% CI, p))
Age (below/above median)	1.42 (0.64, 3.13), 0.384	0.58 (0.27, 1.27), 0.174	1.60 (0.76, 3.39), 0.217
Location (Prague/Ostrava)	0.49 (0.14, 1.68), 0.258	1.96 (0.59, 6.49), 0.271	0.52 (0.16, 1.68), 0.274
Benzene (below/above median)	1.80 (0.62, 5.18), 0.277	0.61 (0.23, 1.64), 0.329	12.13 (0.79, 5.70), 0.134
Cotinine (below/above median)	0.64 (0.27, 1.56), 0.327	1.51 (0.655 3.45), 0.337	1.06 (0.48, 2.36), 0.891
Total cholesterol (below/above median)	1.30 (0.42, 3.99), 0.650	2.54 (0.84, 7.61), 0.095	2.21 (0.77, 6.35), 0.141
Triglycerides (below/above median)	2.17 (0.87, 5.43), 0.098	0.96 (0.40, 2.29), 0.924	1.33 (0.57, 3.09), 0.506
HDL cholesterol (below/above median)	0.72 (0.27, 1.92), 0.512	1.99 (0.80, 4.98), 0.139	0.62 (0.25, 1.53), 0.297
LDL cholesterol (below/above median)	1.15 (0.36, 3.65), 0.808	0.82 (0.26, 2.55), 0.731	0.52 (0.17, 1.56), 0.244
Vitamin C (below/above median)	0.37 (0.16, 0.83), 0.016	0.45 (0.20, 1.00), 0.049	0.86 (0.40, 1.84), 0.693
Vitamin A (below/above median)	2.16 (0.76,6.18), 0.150	1.25 (0.46, 3.37), 0.664	3.39 (1.25, 9.16), 0.016
Vitamin E (below/above median)	0.25 (0.08, 0.75), 0.013	1.46 (0.53, 3.99), 0.463	0.71 (0.26, 1.93), 0.496
APEX (below/above median)	0.46 (0.16, 1.34), 0.155; 0.097	N/A	N/A
OGG1 (below/above median)	1.13 (0.39, 3.29), 0.826; 0.344	N/A	N/A
XRCC1 (below/above median)	2.53 (1.05, 6.09), 0.038; 0.048	N/A	N/A

q—adjusted for multiple comparisons; N/A—not applicable.
[a] Adjusted for all variables in the table.

NHEJ genes showed any association with the levels of F_G/100. For %AB.C. and ace, these associations were not calculated because NHEJ does not participate in their formation and/or repair.

Since vitamins C and E seem to prevent oxidative DNA damage and the levels of both vitamins were elevated in subjects from Ostrava, we investigated the associations between the expression of the BER genes and vitamin levels. The results of multivariate logistic regression are reported in Supplementary Table 3. We observed a significant increase in the expression of *XRCC1* in subjects with above-median levels of vitamin C (OR, 95% CI: 2.25, 1.00–5.02, p = 0.048).

Univariate and multivariate analyses of gene expression levels in both studied regions are reported in Table 5. The table summarizes the odds of expression of BER and NHEJ genes in subjects from Prague/Ostrava to be above/below median values. *XRCC5* was found to have above-median expression in subjects in the more polluted Ostrava region (OR, 95% CI: 3.33, 1.03–10.8, q = 0.046). The expression of other NHEJ or BER genes was not significantly changed.

Table 4
Associations of selected parameters with cytogenetic biomarkers.

Variable	F_G/100 (OR[a] (95% CI, p; q))	%AB.C. (OR[a] (95% CI, p))	ace (OR[a] (95% CI, p))
Age (below/above median)	3.94 (1.78, 8.71), 0.001	4.09 (1.84, 9.12), 0.001	1.03 (0.43, 2.48), 0.954
Location (Prague/Ostrava)	0.54 (0.16, 1.82), 0.319	0.18 (0.05, 0.67), 0.010	2.32 (0.52, 10.34), 0.269
Benzene (below/above median)	2.56 (0.90, 7.34), 0.079	3.50 (1.12, 10.97), 0.031	1.07 (0.33, 3.52), 0.906
Cotinine (below/above median)	1.31 (0.57,2.99), 0.525	1.55 (0.66, 3.64), 0.316	1.89 (0.73, 4.88), 0.190
Total cholesterol (below/above median)	0.84 (0.28, 2.53), 0.757	2.21 (0.72, 6.80), 0.168	0.86 (0.24, 3.09), 0.819
Triglycerides (below/above median)	0.93 (0.39, 2.22), 0.878	1.01 (0.41, 2.51), 0.978	1.96 (0.71, 5.44), 0.196
HDL cholesterol (below/above median)	0.70 (0.27, 1.82), 0.467	0.91 (0.35, 2.40), 0.845	1.81 (0.65, 5.07), 0.259
LDL cholesterol (below/above median)	1.90 (0.62, 5.81), 0.261	0.83 (0.26, 2.62), 0.749	1.01 (0.28, 3.72), 0.987
Vitamin C (below/above median)	1.37 (0.62, 3.05), 0.440	1.65 (0.73, 3.75), 0.233	0.46 (0.18, 1.15), 0.097
Vitamin A (below/above median)	1.41 (0.50, 4.03), 0.519	0.94 (0.33, 2.70), 0.913	0.34 (0.10, 1.12), 0.076
Vitamin E (below/above median)	0.73 (0.25, 2.14), 0.571	1.02 (0.35, 2.98), 0.975	1.67 (0.51, 5.49), 0.397
LIG4 (below/above median)	0.87 (0.37, 2.01), 0.735; 0.980	N/A	N/A
XRCC4 (below/above median)	1.01 (0.47, 2.18), 0.980; 0.980	N/A	N/A
XRCC5 (below/above median)	0.58 (0.23, 1.42), 0.233; 0.932	N/A	N/A
XRCC6 (below/above median)	1.05 (0.46, 2.40), 0.917; 0.980	N/A	N/A

q—adjusted for multiple comparisons; N/A—not applicable.
[a] Adjusted for all variables in the table.

Table 5
Univariate and multivariate estimates of associations between gene expression and sampling locations.

Variable	Univariate analysis			Multivariate analysis		
(Prague/Ostrava)	Crude OR (95% CI)	*p*	*q*	OR (95% CI)	*p*	*q*
OGG1	0.65 (0.33, 1.26)	0.200	0.127	0.35[a] (0.11, 1.12)	0.077	0.171
APEX	1.15 (0.59, 2.25)	0.675	0.285	0.74[a] (0.24, 2.30)	0.603	0.504
XRCC1	0.57 (0.29, 1.13)	0.106	0.127	0.79[a] (0.25, 2.46)	0.680	0.504
LIG4	1.15 (0.59, 2.25)	0.675	0.165	1.35[b] (0.44, 4.16)	0.606	0.170
XRCC4	0.92 (0.47, 1.78)	0.793	0.165	0.78[b] (0.26,2.40)	0.670	0.170
XRCC5	2.07 (1.05, 4.09)	0.035	0.029	3.33[b] (1.03,10.8)	0.045	0.046
XRCC6	0.73 (0.37, 1.42)	0.346	0.144	0.74[b] (0.24, 2.28)	0.604	0.170

q—Adjusted for multiple comparisons.
[a] Adjusted for location, exposure to benzene, levels of cotinine, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, vitamin C, A, E, age, 8-oxodG.
[b] Adjusted for location, exposure to benzene, levels of cotinine, total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, vitamin C, A, E, age, F_G/100.

4. Discussion

In the present study we investigated the levels of markers of oxidative stress, cytogenetic parameters and the expression of selected genes participating in DNA repair pathways in subjects living in the heavily polluted city of Ostrava and compared them with subjects living in a relatively clean location, the city of Prague. Despite high levels of air pollution in Ostrava, no analyzed biomarker was elevated in subjects from this region. However, our results suggest that other factors, namely gene expression and lifestyle factors, may compensate for high levels of air pollution.

In our previous studies we analyzed oxidative damage in environmentally exposed populations: in bus drivers [21,22] and city policemen [18] from Prague. In these studies oxidative damage to DNA and proteins was not elevated in subjects exposed to above-median levels of c-PAHs, B[a]P and/or benzene. Only lipid peroxidation was significantly higher in subjects of the bus drivers study exposed to above-median levels of c-PAHs and B[a]P. Subjects exposed to above-median benzene concentrations had non-significantly increased lipid peroxidation levels. In the study of city policemen, lipid peroxidation was non-significantly elevated in the winter sampling period when the ambient air was more polluted. There is, however, a substantial difference between the above-mentioned results and the present data. In the previous studies the levels of air pollution were significantly lower (mean B[a]P concentrations ranged from 0.18 to 2.12 ng/m^3), while the actual levels of B[a]P in Ostrava reached up to 74.2 ng/m^3. Due to these extreme concentrations of air pollutants, we expected to observe differences in the levels of the analyzed biomarkers between the two locations. Another reason for our assumption was that other authors have reported increased oxidative stress after environmental [34–36] and occupational [37–40] exposure to PAHs. Similarly, exposure to benzene was found to be associated with oxidative stress by some authors [41,42].

It is well known that vitamins, particularly vitamins C and E, act as antioxidants in the organism. The ability of vitamin A to scavenge free radicals seems to be limited [43]. In a number of studies the effect of vitamin C alone, or vitamin C supplemented with vitamin E and/or other vitamins on oxidative damage in the DNA of blood cells has been studied (reviewed in [44]). In most, but not all of these studies, the levels of 8-oxodG or other measured markers of oxidative stress decreased after supplementation of the study subjects with vitamins. Urinary excretion of 8-oxodG was also found to be negatively correlated with plasma vitamin C levels [45]. In our study the plasma levels of vitamins A, C and E were significantly higher in subjects living in the more polluted Ostrava region. We observed a negative association between the levels of vitamins C and E and the urinary excretion of 8-oxodG. We hypothesize that the antioxidant properties of both vitamins decreased the formation of 8-oxodG in cellular DNA and thus the urinary excretion of the oxidized base.

Another aspect that may play a role in the unexpectedly low levels of oxidative stress biomarkers in the Ostrava group is the effect of vitamins C and E on the expression of various genes that may influence the studied biomarkers. Vitamin C serves as a molecule affecting cellular stress responses, cell differentiation, proliferation, cell death and DNA repair [44,46]. There is even a report showing that vitamin C supplementation results in increased concentrations of OGG1 mRNA and a decrease in 8-oxodG levels in lymphocyte DNA [47]. Vitamin E modulates the expression of a number of genes that belong to two signal transduction pathways: a pathway centered on protein kinase C and a pathway centered on phosphatidylinositol 3-kinase [48]. We may assume that the elevated vitamin C and E levels in the Ostrava group upregulated certain genes that affected repair mechanisms, resulting in lower than expected urinary 8-oxodG concentrations. Our data indicate that *XRCC1* may be one of these genes. XRCC1 is a scaffold protein that interacts with most components of the short patch BER pathway [49]. It is essential for the coordination of all BER steps. It has been shown that the interaction of XRCC1 with OGG1 results in a 2- to 3-fold stimulation of the DNA glycosylase activity of OGG1. XRCC1 also passes on the DNA intermediate from OGG1 to APEX1, which leads to the acceleration of BER [50]. Thus, we can speculate that elevated *XRCC1* expression associated with above-median levels of vitamin C results in better orchestration of BER, which in turn leads to the faster removal of 8-oxodG from DNA and a higher repair efficiency. The fact that we collected spot urine samples may explain why we did not see any increase in 8-oxodG levels in the Ostrava subjects: due to the faster and more efficient DNA repair, some (a substantial amount of) 8-oxodG was already excreted from the body before the collection of the urine.

We have already analyzed chromosomal aberrations by FISH in several environmentally exposed populations, but none from a heavily polluted region comparable with Ostrava [16,18,19,51]. The frequency of chromosomal aberrations was affected by air pollution in groups exposed to B[a]P concentrations of at least 1.0 ng/m^3. Thus, DNA damage was elevated in subjects exposed to an average B[a]P concentration of 1.6 ng/m^3 vs. those exposed to 0.4 ng/m^3 B[a]P [16], in city policemen exposed to 1.6 ng/m^3 vs. controls exposed to 0.8 ng/m^3 B[a]P [51] and in subjects exposed up to 3.8 ng/m^3 B[a]P vs. controls exposed to an average B[a]P concentration up to 2.0 ng/m^3 [19]. It is noteworthy that in the last study, no linear dose-response relationship between F_G/100 and exposure to B[a]P was observed for B[a]P concentrations above 3.6 ng/m^3. On the other hand, no effect of environmental pollution on F_G/100 was observed in city policemen with an average B[a]P exposure of 1.0 ng/m^3 vs. those exposed to 0.2 ng/m^3 [18]. Our present data show that despite extreme exposure to B[a]P in Ostrava, the subjects from this region had comparable or lower levels of transloca-

tions than subjects from Prague. No difference in the levels of acentric fragments between the two locations was observed. Further, our results indicate that changes in the gene expression of *XRCC5* in the Ostrava subjects may be one of the factors responsible for this paradoxical observation. Other factors not analyzed in the present study may include the effectiveness of mRNA translation and the subsequent formation of DNA repair proteins and their post-translational modifications. Currently, we have no explanation as to why only the expression of *XRCC5* mRNA, but not other studied DNA repair genes, was upregulated in the subjects from Ostrava.

NHEJ is one of the pathways repairing DNA double strand breaks (DSB). The repair may be carried out directly by re-ligation of strand breaks; in this case the repair is precise, and no errors are introduced into the DNA strands. If, however, the sequences surrounding the break are lost, a part of the DNA sequence is deleted, the repair is not error-free and chromosomal translocations may be induced [52]. XRCC5 (Ku80), together with XRCC6 (Ku70) form a heterodimer complex Ku, which is the first protein recognizing DSBs and binding to the ends of DNA. Ku is abundant in cells; it is estimated that the average distance between two Ku molecules is only 4–6 times the Ku diameter [53]. Thus, early detection of DSB is probably not a factor limiting the efficiency of NHEJ [53]. Surprisingly, according to an *in vitro* study in rat cells, overexpression of the human Ku complex leads to the reduced repair capacity of DSB [54]. Although we are well aware of the limitations of comparisons between *in vitro* and *in vivo* conditions, this observation may partly explain our unexpected results: the elevated expression of *XRCC5* reduces the activity of the NHEJ pathway, which in turn results in a lower than expected frequency of chromosomal aberrations. Another explanation is related to the mechanism of the precise NHEJ. It has been shown that BRCA1 protein participates in the precise end-joining by suppressing the activity of the MRN complex, a complex of the proteins RAD50/MRE11/NBS1 that removes sequences flanking a DSB and allows NHEJ to repair the break by an error-prone mechanism [55]. We may speculate that in the Ostrava subjects the precise end-joining mechanism was preferentially activated leading to DNA repair without the induction of chromosomal aberrations. These hypotheses, however, do not explain why there were no differences observed between the two locations for acentric fragments. It is possible that better vitamin supplementation, supposedly leading to higher ROS scavenging, may result in the lower than expected ace frequency in the Ostrava group. This statement is further supported by the fact that in our study, lipid peroxidation was non-significantly ($p = 0.068$, data not shown) associated with the frequency of ace.

5. Conclusions

Subjects living in the heavily polluted Ostrava region had comparable levels of oxidative stress markers and cytogenetic parameters as subjects from the substantially cleaner city of Prague. Our results suggest that the elevated expression of *XRCC5* and higher levels of vitamins C and E in the Ostrava group compensate for the negative effect of air pollution on human health.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.mrfmmm.2011.06.001.

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Příloha 8

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Personal exposure to carcinogenic polycyclic aromatic hydrocarbons in the Czech Republic

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PERSONAL EXPOSURE TO CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN THE CZECH REPUBLIC

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Abstract

Personal exposures to carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) bound to airborne $PM_{2.5}$ (particulate matter <2.5μm) were measured in the context of a large-scale molecular epidemiological study in order to identify the impacts of air pollution on human health. Sampling was carried out in 3 industrial cities in the Czech Republic: Ostrava, Karvina and Havirov. The city of Prague, exhibiting much lower industrial air pollution but a high level of traffic, served as a control. The first monitoring campaigns were held in winter and were repeated in the summer of 2009. The active personal monitors PV1.7 for $PM_{2.5}$ bound c-PAHs were used. Non-smoking city policemen from Prague, Karvina and Havirov, and office workers from Ostrava, participated in the study. All participants completed a personal questionnaire and a time-location-activity diary (TLAD). The average personal winter exposure to c-PAHs (sum of the eight PAHs - benz[a]anthracene, benzo[a]pyrene, benzo[b]fluoranthene, benzo[g,h,i]perylene, benzo[k]fluoranthene, chrysene, dibenz[a,h]anthracene, indeno[1,2,3-c,d]pyrene) was highest in Karvina, 39.1, followed by Ostrava at 15.1 and Prague at 4.3 ng/m^3. The winter levels were significantly higher than the summer values ($p<0.001$): 4.3 in Karvina, 3.0 in Ostrava, 1.6 in Havirov and 1.0 ng/m^3 in Prague. The average personal benzo[a]pyrene winter/summer exposures were: 6.9/0.6 in Karvina, 2.5/0.4 in Ostrava, 0.8/0.1 in Prague and 0.2 ng/m^3 in summer in Havirov. In this study, we examined personal exposure to c-PAHs and tested it for associations with potential predictor variables collected from questionnaires addressing life style factors and day-to-day activities. We found outdoor concentration, ETS exposure, home heating fuel of coal, wood or gas, frequency of exhaust fan use, cooking, and commuting by a car to be the main determinants of personal exposure.

Introduction

The adverse effects of air pollution on human health have been well documented in many studies (Brunekreef and Holgate, 2002; Brunekreef et al., 2009; Künzli and Tager, 2005; Peng et al., 2009; Pope et al., 2002; Pope et al., 2004; Sorensen et al., 2003; Sram et al., 2005). The main sources of air pollution in the Czech Republic are industry, traffic and local heating (CENIA, 2009). The most important biologically effective components of air pollution are fine aerosol particles and compounds bound to them, particularly carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) (Binkova et al., 1999). The aerosol particles having a diameter smaller than 2.5 μm ($PM_{2.5}$) are most important because of their ability to reach the lower respiratory tract in humans. From the retention point of view and the deposition of aerosols in the lungs, fine particles are the most dangerous because of their penetration through the bronchioles to the air sacks (Sugita et al., 2004). High $PM_{2.5}$ air pollution can have acute effects on human health from short-term exposure contributing to acute coronary events, especially among patients with underlying coronary artery disease. It may also contribute to complications of atherosclerosis and may play a role in triggering acute ischemic heart disease events. Higher long-term exposures of $PM_{2.5}$ contributes to pulmonary and systemic oxidative stress, inflammation, progression of atherosclerosis, risk of ischemic heart disease, could increase total mortality as well as lung carcinoma (Brunekreef and Holgate, 2002;

Künzli and Tager, 2005; Peng et al., 2009, Pope, et al., 2006). Along with B[a]P and other c-PAHs, other toxic substances (such as nitro- and alkyl PAH derivatives) could be bound on $PM_{2.5}$ and could change their composition, toxicity and impact on human health (Lewtas, 2007; Nishioka and Lewtas 1992). However, recent data suggest that the most biologically important $PM_{2.5}$ components are PAHs (Topinka, et al., 2011). Some of these are classified as mutagenic and carcinogenic (compounds with 4 and more aromatic rings) (Binkova and Sram, 2004, Lewtas, 2007). We measured eight most often reported (carcinogenic) 4-6 ring PAHs which are only a subset of the group of potentially carcinogenic polycyclic aromatic compounds (4-6 ring PAHs, their sulfur- and nitrogen-containing analogues and their alkylated homologues). But selected eight c-PAHs can be used as a marker of exposure for the whole set because generally the concentrations of heterocyclic PAHs show proportionality to the concentrations of the corresponding PAHs (Brandt and Watson, 2003). The International Agency for Research on Cancer (IARC) classifies 8 compounds that were monitored as carcinogens in group 1 – carcinogens to humans (benzo[a]pyrene), group 2A – probable carcinogens to humans (dibenz[a,h]anthracene), group 2B – possible carcinogens to humans (benz[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, chrysene, indeno[c,d]pyrene) and group 3 - not classifiable as to its carcinogenicity to humans (benzo[g,h,i]perylene) (IARC, 2009).

Previous studies have demonstrated that indoor air contains 50-70% of the pollution present in the outdoor air (Monn et al., 1997), but this depends strongly on the extent of ventilation and the type of housing. When assessing personal exposure, we need to consider that people spend on average 80-90% of their daily time indoors (Adgate et al., 2004; Brunekreef and Holgate, 2002; Jedrychowski et al., 2007). Personal exposure to air pollution is substantially affected by the distance from the source of pollution (proximity of busy roads, industrial sources), environmental tobacco smoke (ETS), oven cooking or frying. Personal exposure to air pollution depends strongly on individual activities (Devi et al., 2009; Gerharz et al., 2009). Together with a long-term increase in carcinogenic risk, human exposure to $PM_{2.5}$ can have significant adverse effects on the respiratory and cardiovascular systems (Pope et al., 2002; Pope et al., 2004).

In the context of a large-scale molecular epidemiology study, we analyzed the impact of air pollution on the population of industrial cities within the Moravian-Silesian region (MSR) of the Czech Republic: Ostrava, Karvina and Havirov. The capital city of Prague served as a control. We investigated personal exposure to c-PAHs and the impact on biomarkers of exposure, effect and susceptibility that could demonstrate possible damage to important biomolecules (DNA, proteins, lipids) and changes in the human transcriptome. This paper describes individual exposures to c-PAHs and their relationships to some exposure-modifying parameters. The analysis of the relationship of these exposures to various biomarkers is in progress.

Methods

Sampling Localities

The MSR is an industrial and heavily populated area situated in the easternmost part of the Czech Republic. The region has been characterized by coal-mining, the processing of coal and metallurgy from the second half of the 18^{th} century. It is bordered on the west, east and partially the south by mountains, forming a basin with frequent temperature inversions in winter. The MSR covers 5 427 km^2 with 1.25 million inhabitants (Czech Statistical Office, 2010). The MSR administrative structure

consists of six districts (from the west: Bruntal, Opava, Novy Jicin, Ostrava city, Karvina and Frydek-Mistek). The Karvina district is one of the most densely populated districts of the Czech Republic (789 inhabitants/km^2). There are approximately 30 thousands industrial facilities in the MSR. The most important industrial sources of air pollution are metallurgy, steel, coke ovens, coal-mining and power generation facilities. The population density in the MSR increases vehicle emissions and energy consumption, which are important factors decreasing air quality in this area. Fifty percent of the inhabitants use central heating, 34% natural gas, 10% coal, 3.3% electricity and 3% wood (Czech Hydrometeorological Institute, CHMI, 2008). With respect to the rising prices of energy, there could also be a "supplemental" kind of fuel that could significantly contribute to the air pollution in winter.

Prague is the capital and largest city in the Czech Republic, situated in central Bohemia. The city is spread over nine hills with 1.3 million inhabitants (Czech Statistical Office, 2010). Traffic and home heating are considered to be the most important sources of air pollution in winter. Most of the households (51.5%) use central heating, 40% natural gas, 5.7% electricity, 2.4% coal and 0.2% wood (CHMI, 2008).

Study Population

The study was planned to include three monitoring campaigns: winter 2009, summer 2009 and winter 2010. We are presenting the results from the first two campaigns. Non-smoking city policemen from Prague, Karvina and Havirov, and office workers from Ostrava, 22-63 years old, participated in the study. The exact days of the monitoring campaigns, the number of persons and the average age in different locations are shown in Table 1. The same subjects participated in both campaigns. In an attempt to exclude unreported smoking, cotinine levels (a major nicotine metabolite) were measured in urine samples. Participants were informed prior to the field study that their smoking status would be checked biochemically (Langone and Van Vunakis, 1982). All participants completed a personal history questionnaire giving information on their place of residence, dietary habits, health history, and activities that might influence their exposure to the agents of interest for the study. Exposure and behavior pattern of the cohorts in different localities and seasons are included in Supplemental Material, Table 1. In addition, during a 2-day observation period, when they carried the personal monitors, they kept a detailed time-location-activity diary (TLAD) in which they recorded information about their location and activities every hour. Time distribution of activities for individual cohorts in both seasons are presented in Supplemental Material, Table 2. All the volunteers received information about the purpose of the study and signed an informed consent form prior to entering the study. The ethical committee of the Institute of Experimental Medicine AS CR in Prague approved the study.

Personal Exposure Monitoring

Personal exposure to 8 c-PAHs was measured by personal monitors used by the study subjects during two consecutive days (48h). The active PV 1.7 monitors (URG Corp, Chapel Hill, NC, USA) were equipped with Teflon-impregnated glass fiber filters T60A20 (Pallflex) collecting $PM_{2.5}$ particles (Binkova et al., 1995). The sampler was connected to a pump operating at 1.7 L/min. powered by batteries with inlet attached to the individual's breathing zone and was located by his bed during the night (total weight of the sampler 1kg) (Williams, et al., 1999). Quantitative chemical analysis of

8 c-PAHs (benz[a]anthracene (B[a]A), benzo[a]pyrene (B[a]P), benzo[b]fluoranthene (B[b]F), benzo[g,h,i]perylene (B[ghi]P), benzo[k]fluoranthene (B[k]F), chrysene (Chry), dibenz[a,h]anthracene (DB[ah]A), indeno[1,2,3-c,d]pyrene (I[cd]P) was performed using a High Performance Liquid Chromatograph (HPLC) instrument with fluorescence detection in the laboratories of the certified company ALS Czech Republic, Prague (EN ISO CSN IEC 17025).

Statistical Analysis

Statistical analyses were done using Statistica 7.1 (StatSoft, OK, USA), SAS 9.1.3. (SAS Institute, NC, USA) and SPSS 17.0 software. The nonparametric method for bivariate analyses was used for data that did not follow a normal distribution: the Mann–Whitney Rank Sum U-test for comparison of two groups. The impact of TLAD activities to individual exposition values was estimated by splitting up all cases according to activity lengths into two groups by medians: below median (BM) and above median (AM) values of activity length. If in some cases median of distribution of individual TLAD activity was equal to zero, instead of median was used as splitting criterion condition equal to zero (BM) respective greater than zero (AM). Relations to personal exposure of this AM vs. BM group were tested by U-test. Multivariate models of estimating impact to personal exposures was completed by multivariate linear regression using forward stepwise mode building option.

Results

Personal Exposure to c-PAHs

The average winter c-PAHs and B[a]P personal exposure values were 15.2 and 2.5 ng/m^3 in Ostrava, 39.1 and 6.9 ng/m^3 in Karvina and 4.3 and 0.8 ng/m^3 in Prague, respectively ($p<0.001$) (Table 2). There was a similar rank in the concentrations of all monitored c-PAHs in the winter period – the highest concentrations in Karvina, followed by Ostrava and the lowest in Prague. A seasonal variation was clearly evident, as the values of c-PAHs and B[a]P exposure were significantly higher during the winter than during the summer period in all locations ($p<0.001$). The highest exposures were confirmed in Karvina city where the levels in summer were similar to the winter levels of c-PAHs in Prague (Figure 1).

The Environmental and Life Style Factors Affecting Personal Exposure

There were higher concentrations of c-PAHs in households that used coal and wood indoors for heating, but only 15% of respondents used this type of heating and the results were not significant. There were significantly higher concentrations of c-PAHs ($p<0.001$) when using a gas stove instead of an electric stove for cooking. Also, significantly higher concentrations of c-PAHs were found for subjects that cooked frequently. Using an exhaust fan in the kitchen decreased the cooking fumes and thus the concentration of pollutants (data not shown).

The smoking status (tobacco smoke exposure) is always very important in the case of personal exposure (Table 3). The effect of passive smoking reported in the questionnaires was an important factor contributing to the personal exposure to c-PAHs. Being in the company of smokers outdoors appeared as a significant factor for c-PAHs exposure in winter ($p<0.05$) and summer ($p<0.01$). Being in the company of smokers indoors also elevated exposure. Smoking at home increased personal exposure (significant in the summer season, $p<0.05$).

The results from the time activity questionnaire (TLAD) reflecting the period of personal exposure monitoring (48 hours) are shown in Table 4. The results from bivariate analyses indicate that using public transport lowered personal exposure to c-PAHs (significant in winter $p<0.01$), while using a car increased exposure to c-PAHs in winter ($p<0.05$). The results also showed that time spent at home significantly increased exposure to c-PAHs. Passive smoking as well as activities performed with smokers increased personal exposure (only significant data shown). Travelling significantly decreased exposure, which could be connected with travelling by public transport as that lowers exposure as mentioned above. It seems that using windows for ventilation increased exposure to c-PAHs mainly in the summer. Time spent indoors significantly increased exposure, while time spent at work decreased c-PAHs exposure. Time spent outdoors decreased exposure to c-PAHs, significantly in winter period ($p<0.01$). Time spent in restaurants showed an increasing trend, significant for B[a]P exposure in winter ($p<0.05$). Stratified time activity results by different localities show decreasing impact of travelling in Karvina in winter ($p<0.01$), decreasing trend for being outdoor in summer in Ostrava for c-PAHs ($p<0.05$) and decreased exposure as being in work in Ostrava in winter ($p<0.05$) (data shown in Supplemental Material, Table 3). Linear models of relation between outdoor concentrations and personal exposures revealed strong correlation of c-PAHs ($p<0.001$) in all groups ($B=0.31$, $p<0.0001$), in Prague ($B=0.48$, $p<0.001$) and in Ostrava ($B=0.29$, $p<0.0001$). Multivariate regression model estimates strong significant relation between outdoor concentration of c-PAHs and personal exposures ($B=0.31$, $p<0.0001$) in winter period. Many confounders from questionnaires were tested and as significant were found: being in work, at home or elsewhere (indoor) estimated as significant decreasing of relation outdoor concentration and personal exposure ($B=-0.56$, $p<0.001$, $B=-0.34$, $p<0.05$, $B=-0.59$, $p<0.05$ per hour of activity) for the Prague and Ostrava group together. When controlled for the individual locality, data had the same trend but were not significant (data not shown).

Discussion

Personal exposure to c-PAHs in industrial parts of the Ostrava region was measured. The city of Prague was used as a control locality. Previous studies (Peluso, et al., 1998, Taioli, et al., 2007, Topinka et al., 2007) have shown that city policemen are a representative group reflecting the extent of air pollution in cities. They spend most of their work-time moving through the city, breathing its ambient air. In our study, city policemen from Prague, Karvina and Havirov were employed. The office workers enrolled in the study in Ostrava represented the majority of the population who spend most of their time indoors. Considering the high air pollution levels in Ostrava, personal exposure in this city is increased even in the population of subjects spending most of their time indoors.

Since 2004 (as we had data for B[a]P) Ostrava-Bartovice has been the most polluted locality in the Czech Republic (annual average 9.2 ng/m^3 in 2009, CHMI 2010). These concentrations are much higher by far than the EU (WHO standard) limit of 1 ng/m^3 of B[a]P, which is expected to be reached by the end of 2012 (DIRECTIVE 2004/107/EC). It is very likely that the more than nine-fold higher B[a]P levels pose a long-term increased health risk for the local inhabitants (Hertz-Picciotto et al., 2007, Rossner et al., 2010, Sram et al., 2007, Svecova et al., 2009, Topinka et al., 2007). In comparison to other studies dealing with personal exposure to B[a]P, Prague levels (winter/summer 0.8/0.1 ng/m^3) are similar to the levels

observed in pregnant women in New York City - winter/summer 0.63/0.2 ng/m^3 (Tonne et al., 2004). Zmirou et al. (2000), measured the personal exposure of adult non-smoking volunteers in the Grenoble, France, metropolitan area and found an average B[a]P value of 0.67 ng/m^3, even though the winter exposures were indicated to be 3 to 25 times greater than summer exposures. Both occupational and environmental personal exposures to B[a]P were measured in taxi drivers in Genoa, Italy (Piccardo et al., 2004). The summer 1998/winter 1999/summer 1999 mean exposures were 1.4/1.23/1.22 ng/m^3, respectively, with significantly lower values in controls (0.16 ng/m^3). The personal B[a]P exposures in Ostrava city (winter/summer 2.5/0.4 ng/m^3) were highest for a non-occupationally exposed urban population.

Based on the questionnaire data, more than half of the study participants in Ostrava city used public transport and less than half used a car. According to bivariate analyses subjects using public transport had significantly lower exposure to c-PAHs in the winter season than the other groups ($p<0.05$), but this finding was not observed in the Prague participants. Bivariate models also showed being indoor as increasing personal exposure, but multivariate models of relation between outdoor concentration and personal exposure revealed being indoor as decreasing confounder indicating outdoor concentration as more important factor. Personal exposure is also determined by other factors such as ETS, the type of home heating, fumes encountered in the cooking process (e.g. frying or broiling), time spent in restaurants, and other activities. Detailed information about the subject`s life style and activities during the period of personal monitoring was obtained through a personal history questionnaire and TLAD. Statistical analyses suggest that factors increasing personal exposure include: outdoor concentration, ETS exposure, home heating fuel of coal, wood or gas, frequency of exhaust fan use, cooking, and commuting by car. In a study of personal exposures to PAHs in Krakow, the authors considered the following variables as short-term predictors of personal exposure to c-PAHs: residence in the city center, ambient temperature, wind speed, ETS exposure, frequency of exhaust fan use, residence near an industrial plant, commuting by tram, apartment height (floor), home heating fuel of coal or wood, time spent outdoors (hour/day), and simultaneously monitored outdoor concentration of c-PAHs (Choi et al., 2008).

The personal exposures to c-PAHs in Ostrava and Karvina show high levels in the winter period. This region, together with the Polish part of Silesia, is probably the most polluted area in the EU by c-PAHs. B[a]P poses one of the major health risks from polluted air in urban and industrial areas. The Ostrava region is, due to its composition of sources, especially coke production and metallurgy, the most polluted region in the Czech Republic. Development over the last 12 years has resulted in a lasting adverse situation in this region that poses a long-term health risk for local residents.

Conclusion

Our study analyzed personal exposure to c-PAHs in inhabitants of Ostrava, Karvina, Havirov and Prague. As the main determinants of exposure, we observed outdoor concentration, ETS exposure, home heating fuel of coal, wood or gas, frequency of exhaust fan use, cooking, and commuting by car. The high biological activity and adverse effects of c-PAHs on human health are major reasons to monitor, regulate and decrease the prolonged and unsustainable high levels of these air pollution components in the Moravian-Silesian region. This goal might be reached by the use of the latest technology for major industrial sources and home heating. Our results from

personal monitoring confirmed the high personal exposure to c-PAHs of Ostrava region residents. It is recommended to repeat the personal monitoring of c-PAHs after 2-4 years of attempts to improve air quality in the region in order to determine whether they have been effective. A detailed measurement of outdoor concentration is needed to evaluate the effect on personal exposure as we are planning in our ongoing research. A further important task would be to measure personal occupational exposure in industrial workers or residents that use solid fuels for heating their households.

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Figure legends:

Figure 1. Personal B[a]P exposures by location and season. The horizontal lines in the box indicate the median value. The box boundaries indicate the 25th and 75th percentiles; the capped bars indicate the 5th and 95th percentiles.

Table 1. Periods of the monitoring campaigns, the number of participants and the average age in all locations.

2009	Locality	N	Age
2/08 - 2/20	Prague	65	37.9
3/06 – 3/13	Ostrava	74	37.7
3/02 – 3/06	Karvina	24	36.4
5/17 – 5/27	Prague	61	38.6
6/10 – 6/19	Ostrava	64	40.2
6/06 – 6/10	Karvina	24	36.6
6/08 – 6/10	Havirov	12	37.6

N - number of participants, age - average age of participants

Table 2. Personal c-PAHs and B[a]P exposures at all locations by season.

	Period	2-3/2009				5-6/2009			
	Region	N	Mean ± SD	Median (Range)	P	N	Mean ± SD	Median (Range)	P
c-PAHs	Prague	65	4.27 ± 2.95	3.57 (0.82-16.43)	-	61	1.03 ± 0.61	0.85 (0.36-3.64)	-
	Havirov	0	-	-	-	12	1.59 ± 0.57	1.32 (1.02-2.46)	<0.001
	Karvina	23	39.08 ± 17.33	42.39 (13.85-67.18)	<0.001	23	4.27 ± 1.99	4.09 (1.13-9.80)	<0.001
	Ostrava	72	15.19 ± 15.15	9.75 (1.70-86.58)	<0.001	64	3.04 ± 1.38	2.93 (0.81-6.67)	<0.001
B[a]P	Prague	65	0.80 ± 0.55	0.66 (0.10-3.37)	-	61	0.12 ± 0.14	0.08 (0.04-1.00)	-
	Havirov	0	-	-	-	12	0.23 ± 0.08	0.21 (0.08-0.34)	<0.001
	Karvina	23	6.85 ± 2.89	6.54 (3.28-12.60)	<0.001	23	0.59 ± 0.30	0.57 (0.08-1.24)	<0.001
	Ostrava	72	2.53 ± 2.26	1.69 (0.33-12.60)	<0.001	64	0.41 ± 0.19	0.37 (0.08-0.97)	<0.001

P-values refer to comparisons between other localities and Prague and were obtained with the Mann-Whitney U-test.

Table 3. Personal exposures determined by ETS in all groups by season.

Period		2-3/2009				5-6/2009			
Pollutant	Characteristic	N	Mean ± SD	Median (Range)	P	N	Mean ± SD	Median (Range)	P
c-PAHs	Work time indoors with smokers	10	21.64 ± 20.15	12.48 (1.83-61.43)	0.14	8	3.84 ± 3.22	2.45 (0.98-9.80)	0.17
	Work time indoors without smokers	119	14.55 ± 16.41	8.19 (0.91-86.58)	-	99	2.54 ± 1.59	2.25 (0.36-7.09)	-
	Work time outdoors with smokers	19	21.35 ± 20.76	17.79 (0.82-64.77)	<0.05	26	3.46 ± 2.21	3.08 (0.82-9.80)	<0.01
	Work time outdoors without smokers	74	11.02 ± 16.08	5.00 (0.91-86.58)	-	78	1.60 ± 1.24	1.10 (0.36-6.53)	-
	Smoking at home	6	18.65 ± 12.73	22.50 (2.58-31.51)	0.32	3	5.47 ± 3.75	3.32 (3.31-9.80)	<0.05
	No smoking at home	157	13.89 ± 16.68	7.39 (0.82-86.58)	-	158	2.31 ± 1.65	1.91 (0.36-7.09)	-
B[a]P	Work time indoors with smokers	10	3.98 ± 3.62	2.65 (0.39-11.20)	0.10	8	0.50 ± 0.46	0.31 (0.08-1.24)	0.29
	Work time indoors without smokers	119	2.47 ± 2.65	1.56 (0.18-12.60)	-	99	0.33 ± 0.23	0.30 (0.04-0.98)	-
	Work time outdoors with smokers	19	3.69 ± 3.31	3.31 (0.10-10.20)	<0.05	26	0.50 ± 0.34	0.44 (0.08-1.24)	<0.05
	Work time outdoors without smokers	74	1.95 ± 2.72	0.88 (0.18-12.60)	-	78	0.19 ± 0.19	0.08 (0.04-0.92)	-
	Smoking at home	6	3.62 ± 2.43	4.49 (0.48-5.92)	0.24	3	0.72 ± 0.45	0.47 (0.44-1.24)	<0.05
	No smoking at home	157	2.38 ± 2.72	1.35 (0.10-12.60)	-	158	0.31 ± 0.25	0.26 (0.04-1.02)	-

P-values were obtained with the Mann-Whitney U-test.

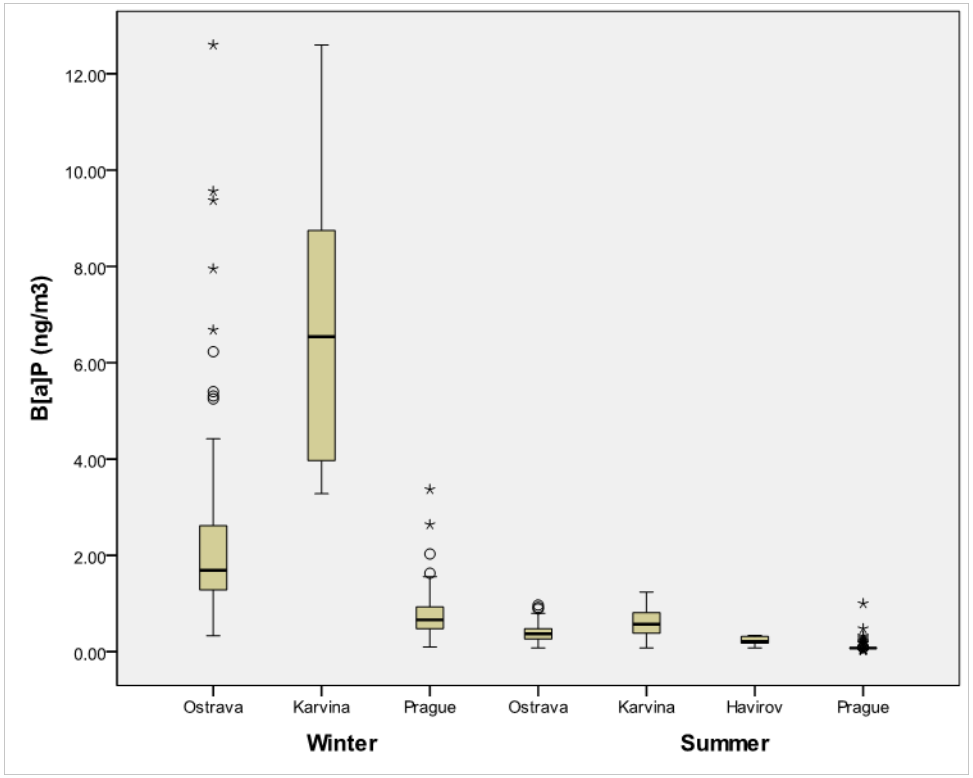
Table 4. Personal exposures according to time activity questionaires in all groups by season.

	Period		2-3/2009				5-6/2009		
	Variable		N	Mean ± SD	Median (Range)	P	N	Mean ± SD	Median (Range)	P
c-PAHs	Public transport	BM	82	17.84 ± 17.98	10.80 (0.91-67.18)	-	87	2.57 ± 1.90	2.01 (0.43-9.80)	-
		AM	81	10.24 ± 14.05	6.25 (0.82-86.58)	<0.01	74	2.14 ± 1.51	1.86 (0.36-6.53)	0.14
	Car	BM	78	12.54 ± 17.75	5.90 (0.82-86.58)	-	78	2.32 ± 1.59	2.07 (0.36-6.98)	-
		AM	85	15.46 ± 15.32	9.93 (1.34-61.43)	<0.05	83	2.43 ± 1.88	1.80 (0.43-9.80)	0.90
	At home	BM	81	13.84 ± 17.81	5.48 (0.82-67.18)	-	73	2.21 ± 2.07	1.09 (0.36-9.80)	-
		AM	82	14.29 ± 15.29	8.91 (0.91-86.58)	<0.05	88	2.51 ± 1.40	2.24 (0.41-6.47)	<0.01
	Elsewhere	BM	96	11.79 ± 15.12	5.69 (0.82-86.58)	-	88	2.09 ± 1.71	1.28 (0.36-9.80)	-
		AM	67	17.31 ± 18.01	10.40 (1.34-67.18)	<0.01	73	2.72 ± 1.72	2.25 (0.43-7.09)	<0.01
	Passive smoking	0	140	12.30 ± 15.39	6.52 (0.82-86.58)	-	139	2.29 ± 1.64	1.84 (0.36-7.09)	-
		above 0	23	24.80 ± 19.44	18.64 (3.66-63.54)	<0.001	22	2.90 ± 2.25	2.39 (0.79-9.80)	0.18
	Travelling	BM	63	16.75 ± 17.68	10.01 (0.91-67.18)	-	70	2.72 ± 1.88	2.24 (0.41-9.80)	-
		AM	100	12.37 ± 15.63	6.51 (0.82-86.58)	<0.1	91	2.11 ± 1.58	1.44 (0.36-6.98)	<0.01
	Ventilation by window	BM	77	13.78 ± 16.01	7.08 (0.91-64.77)	-	73	2.17 ± 1.79	1.44 (0.36-9.80)	-
		AM	86	14.32 ± 17.09	7.93 (0.82-86.58)	0.31	88	2.54 ± 1.68	2.26 (0.48-6.98)	0.06
	Indoor	BM	81	12.37 ± 16.96	4.46 (0.82-67.18)	-	85	2.24 ± 1.99	1.28 (0.36-9.80)	-
		AM	82	15.73 ± 16.05	9.75 (1.70-86.58)	<0.001	76	2.52 ± 1.41	2.23 (0.49-6.53)	<0.01
	Outdoor	BM	76	14.19 ± 16.06	9.21 (1.53-86.58)	-	73	2.50 ± 1.52	2.17 (0.43-6.67)	-
		AM	87	13.95 ± 17.04	5.38 (0.82-67.18)	<0.01	88	2.27 ± 1.90	1.39 (0.36-9.80)	0.06
	In work	BM	80	17.39 ± 17.59	9.53 (1.70-86.58)	-	76	2.90 ± 1.55	2.77 (0.69-7.09)	-
		AM	83	10.86 ± 14.88	4.34 (0.82-67.18)	<0.001	85	1.90 ± 1.77	1.05 (0.36-9.80)	<0.001
	Restaurant	0	132	13.18 ± 16.27	7.16 (0.82-86.58)	-	114	2.35 ± 1.57	2.10 (0.41-6.67)	-
		above 0	31	17.84 ± 17.43	10.40 (0.91-63.54)	0.06	47	2.42 ± 2.10	1.67 (0.36-9.80)	0.60
B[a]P	Public transport	BM	82	3.12 ± 3.11	1.82 (0.18-12.60)	-	87	0.35 ± 0.28	0.26 (0.04-1.24)	-
		AM	81	1.71 ± 2.04	1.20 (0.10-12.60)	<0.01	74	0.28 ± 0.23	0.26 (0.04-1.00)	0.17
	Car	BM	78	2.11 ± 2.76	1.16 (0.10-12.60)	-	78	0.31 ± 0.24	0.26 (0.04-1.02)	-
		AM	85	2.71 ± 2.66	1.69 (0.27-11.20)	<0.05	83	0.32 ± 0.28	0.24 (0.04-1.24)	0.66
	At home	BM	81	2.42 ± 2.98	1.03 (0.10-12.60)	-	73	0.29 ± 0.32	0.09 (0.04-1.24)	-
		AM	82	2.43 ± 2.45	1.60 (0.18-12.60)	<0.05	88	0.33 ± 0.20	0.30 (0.04-0.92)	<0.01
	Elsewhere	BM	96	2.06 ± 2.48	1.13 (0.10-12.60)	-	88	0.27 ± 0.26	0.18 (0.04-1.24)	-
		AM	67	2.95 ± 2.96	1.70 (0.31-12.60)	<0.01	73	0.36 ± 0.25	0.30 (0.04-1.02)	<0.01
	Passive smoking	0	140	2.13 ± 2.54	1.26 (0.10-12.60)	-	139	0.30 ± 0.24	0.25 (0.04-0.98)	-
		above 0	23	4.22 ± 3.09	3.28 (0.55-10.60)	<0.001	22	0.41 ± 0.34	0.31 (0.08-1.24)	0.14

Travelling	BM	63	2.93 ± 3.04	1.69 (0.18-12.60)	-	70	0.36 ± 0.27	0.29 (0.04-1.24)	-
	AM	100	2.10 ± 2.45	1.28 (0.10-12.60)	<0.01	91	0.28 ± 0.25	0.20 (0.04-1.02)	<0.05
Ventilation by window	BM	77	2.38 ± 2.63	1.28 (0.18-11.20)	-	73	0.28 ± 0.27	0.20 (0.04-1.24)	-
	AM	86	2.46 ± 2.80	1.44 (0.10-12.60)	0.39	88	0.34 ± 0.25	0.30 (0.04-1.02)	<0.05
Indoor	BM	81	2.21 ± 2.95	0.82 (0.10-12.60)	-	85	0.30 ± 0.30	0.18 (0.04-1.24)	-
	AM	82	2.63 ± 2.46	1.69 (0.33-12.60)	<0.001	76	0.33 ± 0.20	0.30 (0.04-0.92)	<0.01
Outdoor	BM	76	2.37 ± 2.43	1.60 (0.29-12.60)	-	73	0.32 ± 0.23	0.28 (0.04-0.97)	-
	AM	87	2.47 ± 2.96	0.94 (0.10-12.60)	<0.05	88	0.30 ± 0.29	0.21 (0.04-1.24)	0.16
In work	BM	80	2.95 ± 2.81	1.69 (0.33-12.60)	-	76	0.39 ± 0.22	0.35 (0.04-1.02)	-
	AM	83	1.92 ± 2.54	0.82 (0.10-12.60)	<0.001	85	0.24 ± 0.28	0.08 (0.04-1.24)	<0.001
Restaurant	0	132	2.26 ± 2.67	1.28 (0.10-12.60)	-	114	0.31 ± 0.24	0.28 (0.04-1.00)	-
	above 0	31	3.11 ± 2.86	1.90 (0.18-10.60)	<0.05	47	0.32 ± 0.31	0.25 (0.04-1.24)	0.46

BM-below median; AM-above median.
Elsewhere-other activity than work or home; indoor-hours spent indoors; outdoor-hours spent outdoors.
P-values were obtained with the Mann-Whitney U-test.

Figure 1.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Prague
Winter
Summer

Supplemental Material

PERSONAL EXPOSURE TO CARCINOGENIC POLYCYCLIC AROMATIC HYDROCARBONS IN THE CZECH REPUBLIC

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Supplemental Tables

Supplemental Material, Table 1. Exposure and behavior pattern of the cohorts in different localities and seasons; N - number of participants.

participant information	winter						summer							
	Prague		Ostrava		Karvina		Prague		Ostrava		Karvina		Havirov	
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
with partner	64	(98 %)	70	(95 %)	21	(88 %)	59	(97 %)	59	(92 %)	23	(96 %)	11	(92 %)
without partner	1	(2 %)	4	(5 %)	3	(13 %)	2	(3 %)	5	(8 %)	1	(4 %)	1	(8 %)
education														
primary					1	(4 %)								
secondary	57	(88 %)	6	(8 %)	21	(88 %)	54	(89 %)	6	(9 %)	22	(92 %)	9	(75 %)
university	8	(12 %)	68	(92 %)	2	(8 %)	7	(11 %)	58	(91 %)	2	(8 %)	3	(25 %)
heating														
central heating outdoors	39	(65 %)	44	(70 %)	6	(55 %)	38	(66 %)	48	(83 %)	10	(59 %)	10	(77 %)
gas stove indoors	9	(15 %)	6	(10 %)	3	(27 %)	8	(14 %)	4	(7 %)	3	(18 %)		
electric stove indoors	3	(5 %)	3	(5 %)			3	(5 %)						
fireplace indoors	5	(8 %)	4	(6 %)	2	(18 %)	3	(5 %)	4	(7 %)	3	(18 %)	2	(15 %)
cole stove indoors	1	(2 %)	2	(3 %)			2	(3 %)						
wood stove indoors	3	(5 %)	4	(6 %)			4	(7 %)	2	(3 %)	1	(6 %)	1	(8 %)
sport activities														
daily	9	(14 %)	11	(15 %)	8	(33 %)	13	(19 %)	11	(14 %)	8	(31 %)	4	(33 %)
weekly	35	(55 %)	46	(63 %)	13	(54 %)	36	(54 %)	50	(66 %)	15	(58 %)	7	(58 %)
monthly	12	(19 %)	7	(10 %)	1	(4 %)	7	(10 %)	4	(5 %)	1	(4 %)	1	(8 %)
no	8	(13 %)	9	(12 %)	2	(8 %)	11	(16 %)	11	(14 %)	2	(8 %)		
rest in nature (hours)														
0	13	(22 %)	4	(6 %)	2	(10 %)	10	(15 %)	3	(5 %)	4	(19 %)	1	(8 %)
<5	12	(21 %)	26	(40 %)	4	(20 %)	11	(17 %)	25	(39 %)	7	(33 %)		
<10	13	(22 %)	18	(28 %)	3	(15 %)	12	(18 %)	15	(23 %)	4	(19 %)	2	(17 %)
<15	7	(12 %)	10	(15 %)	7	(35 %)	12	(18 %)	11	(17 %)	1	(5 %)	4	(33 %)
<20	2	(3 %)	2	(3 %)	2	(10 %)	3	(5 %)	2	(3 %)	1	(5 %)	2	(17 %)
<25	7	(12 %)	4	(6 %)	1	(5 %)	7	(11 %)	4	(6 %)	2	(10 %)	1	(8 %)
<30	1	(2 %)	1	(2 %)			2	(3 %)	2	(3 %)	1	(5 %)		
>=30	3	(5 %)			1	(5 %)	8	(12 %)	2	(3 %)	1	(5 %)	2	(17 %)

Supplemental Material, Table 2. Time distribution of activities in hours during the 48h monitoring period for individual cohorts in both seasons according to TLAD (time-location-activity diary).

Participant information	Winter						Summer							
	Prague		Ostrava		Karvina		Prague		Ostrava		Karvina		Havirov	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
At home	17,9	4,5	**23,9**	**4,8**	18,0	5,1	18,5	2,9	**22,6**	**5,4**	20,3	7,4	**25,2**	**3,2**
Travelling	3,8	2,0	**3,1**	**1,4**	**2,5**	**1,3**	3,9	2,1	**3,0**	**1,4**	**2,7**	**3,2**	**2,0**	**1,0**
In work	24,1	3,2	**17,1**	**4,7**	22,4	5,1	24,4	2,5	**18,0**	**3,9**	**20,6**	**7,5**	**10,8**	**6,5**
Restaurant	0,2	0,5	0,4	1,1	**0,8**	**1,7**	0,5	0,8	0,4	1,1	1,0	1,5	1,6	2,0
Elsewhere	1,4	3,1	1,4	2,2	**1,9**	**2,8**	0,6	1,5	**2,1**	**3,3**	1,9	3,4	**6,0**	**6,1**
Outdoor	16,6	8,9	**1,0**	**1,3**	**10,3**	**7,1**	13,8	9,5	**2,6**	**2,7**	13,4	9,8	10,1	5,1
Indoor	23,1	6,9	**42,4**	**6,6**	26,9	10,0	24,9	8,4	**39,6**	**7,2**	21,8	10,8	25,0	9,2
Ventilation by window	4,8	9,9	**6,7**	**8,7**	6,7	9,1	10,1	12,9	**18,4**	**13,9**	8,2	7,9	**17,8**	**9,9**
Car	5,4	8,6	1,7	1,6	**6,7**	**5,0**	5,9	9,1	1,8	1,8	4,7	6,2	2,8	4,4
Public transport	2,5	2,8	**1,4**	**1,6**	**0,4**	**1,2**	2,1	2,5	1,3	1,5	**0,3**	**0,8**	**0,2**	**0,6**
Passive smoking	0,1	0,2	0,3	0,8	**1,5**	**2,9**	0,4	1,6	0,1	0,4	**1,4**	**2,7**	0,8	1,4

Significant differences are highlighted in bold. The reference group is Prague. Comparison done by Mann-Whitney U-test.

Supplemental Material, Table 3. Personal exposures according to time activity questionaires stratified by locality and season.

	Period			2-3/2009				5-6/2009			
	Variable			N	Mean ± SD	Median (Range)	P	N	Mean ± SD	Median (Range)	P
c-PAH	Public transport	Prague	BM	29	3.90 ± 3.29	3.08 (0.81-14.03)	0.66	26	0.81 ± 0.27	0.70 (0.36-1.37)	0.70
			AM	36	3.59 ± 1.97	3.05 (0.74-9.25)	-	35	0.95 ± 0.65	0.70 (0.36-2.87)	-
		Havirov	BM	0	-	-	-	11	1.29 ± 0.49	1.10 (0.85-2.10)	0.47
			AM	0	-	-	-	1	1.91	1.91 (1.91-1.91)	-
		Karvina	BM	21	34.56 ± 16.12	38.44 (5.25-58.10)	0.15	22	3.60 ± 1.75	3.17 (1.03-8.25)	0.68
			AM	3	20.25 ± 8.25	17.14 (14.00-29.60)	-	2	3.59 ± 0.37	3.59 (3.33-3.85)	-
		Ostrava	BM	36	12.71 ± 9.29	9.81 (3.19-38.77)	0.17	28	2.63 ± 1.17	2.56 (0.70-5.71)	0.82
			AM	38	13.53 ± 16.45	7.36 (1.46-77.07)	-	36	2.61 ± 1.20	2.46 (1.01-5.63)	-
	Car	Prague	BM	33	3.39 ± 1.70	3.20 (0.74-8.13)	1.00	28	0.89 ± 0.66	0.70 (0.36-2.87)	0.07
			AM	32	4.08 ± 3.32	2.88 (1.13-14.03)	-	33	0.89 ± 0.38	0.80 (0.36-2.01)	-
		Havirov	BM	0	-	-	-	6	1.36 ± 0.45	1.13 (0.96-1.97)	0.52
			AM	0	-	-	-	6	1.33 ± 0.58	1.02 (0.85-2.10)	-
		Karvina	BM	12	33.59 ± 17.97	39.57 (5.25-58.10)	0.77	11	3.24 ± 1.48	2.76 (1.29-5.63)	0.34
			AM	12	31.96 ± 14.46	28.46 (14.00-52.66)	-	13	3.89 ± 1.82	3.66 (1.03-8.25)	-
		Ostrava	BM	33	14.40 ± 17.53	7.30 (1.46-77.07)	0.28	22	2.71 ± 1.21	2.67 (1.04-5.53)	0.63
			AM	41	12.11 ± 8.81	9.79 (3.19-38.77)	-	42	2.57 ± 1.18	2.30 (0.70-5.71)	-
	At home	Prague	BM	31	3.41 ± 2.14	3.08 (0.74-9.25)	0.46	22	0.87 ± 0.66	0.70 (0.36-2.87)	0.10
			AM	34	4.02 ± 3.01	3.12 (0.81-14.03)	-	39	0.90 ± 0.43	0.70 (0.36-2.37)	-
		Havirov	BM	0	-	-	-	6	1.20 ± 0.39	1.09 (0.94-1.97)	0.52
			AM	0	-	-	-	6	1.49 ± 0.59	1.50 (0.85-2.10)	-
		Karvina	BM	12	35.05 ± 15.64	35.15 (15.32-58.10)	0.39	11	3.56 ± 2.04	3.27 (1.03-8.25)	0.79
			AM	12	30.50 ± 16.66	32.48 (5.25-52.66)	-	13	3.62 ± 1.37	3.66 (1.65-5.70)	-
		Ostrava	BM	35	13.40 ± 12.17	8.59 (1.46-56.06)	0.66	33	2.67 ± 1.26	2.35 (1.01-5.71)	0.90
			AM	39	12.89 ± 14.51	7.96 (3.19-77.07)	-	31	2.57 ± 1.11	2.55 (0.70-5.53)	-
	Elsewhere	Prague	BM	47	3.48 ± 1.94	3.20 (0.74-10.79)	0.79	48	0.86 ± 0.47	0.70 (0.36-2.75)	0.52
			AM	18	4.37 ± 3.89	2.60 (1.15-14.03)	-	13	1.02 ± 0.67	0.85 (0.36-2.87)	-
		Havirov	BM	0	-	-	-	6	1.35 ± 0.49	1.13 (0.95-2.05)	0.63
			AM	0	-	-	-	6	1.34 ± 0.55	1.09 (0.85-2.10)	-
		Karvina	BM	11	31.75 ± 13.54	29.60 (14.00-52.66)	0.75	15	3.51 ± 1.78	3.33 (1.03-8.25)	0.65
			AM	13	33.63 ± 18.29	38.44 (5.25-58.10)	-	9	3.73 ± 1.56	3.27 (1.65-5.70)	-
		Ostrava	BM	38	12.54 ± 14.07	7.36 (1.46-77.07)	0.26	30	2.59 ± 1.09	2.56 (0.70-5.52)	0.73
			AM	36	13.75 ± 12.75	9.12 (3.08-56.06)	-	34	2.65 ± 1.27	2.27 (1.01-5.71)	-

Passive smoking	Prague	0	61	3.61 ± 2.63	2.91 (0.74-14.03)	0.06	52	0.84 ± 0.48	0.70 (0.36-2.87)	0.06
		above 0	4	5.47 ± 2.15	5.29 (3.18-8.13)	-	9	1.21 ± 0.67	0.84 (0.70-2.37)	-
	Havirov	0	0	-	-	-	9	1.33 ± 0.51	1.10 (0.85-2.10)	0.52
		above 0	0	-	-	-	3	1.41 ± 0.56	1.10 (1.08-2.05)	-
	Karvina	0	14	32.54 ± 17.93	32.88 (5.25-58.10)	1.00	16	3.31 ± 1.50	2.92 (1.03-5.70)	0.27
		above 0	10	33.10 ± 13.69	33.62 (14.00-51.66)	-	8	4.18 ± 1.94	3.69 (1.93-8.25)	-
	Ostrava	0	65	12.67 ± 13.00	8.24 (1.46-77.07)	0.33	62	2.63 ± 1.20	2.53 (0.70-5.71)	0.89
		above 0	9	16.46 ± 16.28	9.83 (4.83-56.06)	-	2	2.37 ± 0.26	2.37 (2.18-2.55)	-
Travelling	Prague	BM	29	4.00 ± 3.11	3.18 (0.74-14.03)	0.66	25	0.89 ± 0.40	0.80 (0.36-2.01)	0.15
		AM	36	3.51 ± 2.19	2.99 (1.13-10.79)	-	36	0.89 ± 0.60	0.70 (0.36-2.87)	-
	Havirov	BM	0	-	-	-	6	1.24 ± 0.40	1.10 (0.96-2.05)	0.75
		AM	0	-	-	-	6	1.45 ± 0.60	1.43 (0.85-2.10)	-
	Karvina	BM	12	41.25 ± 13.89	45.72 (20.08-58.10)	<0.01	10	3.24 ± 1.51	2.74 (1.29-5.70)	0.38
		AM	12	24.30 ± 13.56	16.51 (5.25-46.01)	-	14	3.85 ± 1.79	3.50 (1.03-8.25)	-
	Ostrava	BM	33	10.50 ± 6.84	9.12 (3.88-37.46)	0.84	27	2.51 ± 1.13	2.30 (1.01-5.71)	0.52
		AM	41	15.25 ± 16.68	7.96 (1.46-77.07)	-	37	2.71 ± 1.22	2.79 (0.70-5.63)	-
Ventilation by window	Prague	BM	31	3.34 ± 2.33	3.20 (0.81-12.40)	0.25	28	0.93 ± 0.58	0.78 (0.36-2.87)	0.47
		AM	34	4.08 ± 2.86	3.06 (0.74-14.03)	-	33	0.86 ± 0.47	0.70 (0.40-2.75)	-
	Havirov	BM	0	-	-	-	6	1.04 ± 0.11	1.09 (0.85-1.16)	0.20
		AM	0	-	-	-	6	1.65 ± 0.55	1.94 (0.94-2.10)	-
	Karvina	BM	12	33.89 ± 14.54	33.62 (14.00-57.50)	0.91	13	3.54 ± 1.88	3.27 (1.03-8.25)	0.71
		AM	12	31.66 ± 17.87	32.46 (5.25-58.10)	-	11	3.66 ± 1.48	3.85 (1.29-5.63)	-
	Ostrava	BM	35	13.98 ± 12.14	8.77 (1.46-51.37)	0.45	29	2.55 ± 1.07	2.55 (0.70-5.71)	0.99
		AM	39	12.37 ± 14.49	8.23 (3.88-77.07)	-	35	2.68 ± 1.27	2.41 (1.01-5.63)	-
Indoor	Prague	BM	26	3.48 ± 2.37	2.76 (1.13-10.79)	0.43	29	0.87 ± 0.48	0.70 (0.36-2.37)	0.58
		AM	39	3.89 ± 2.80	3.20 (0.74-14.03)	-	32	0.91 ± 0.56	0.72 (0.36-2.87)	-
	Havirov	BM	0	-	-	-	6	1.55 ± 0.54	1.56 (0.95-2.10)	0.08
		AM	0	-	-	-	6	1.14 ± 0.39	1.02 (0.85-1.91)	-
	Karvina	BM	12	35.76 ± 14.03	38.04 (14.00-57.50)	0.45	13	3.61 ± 1.36	3.33 (1.03-5.70)	0.66
		AM	12	29.78 ± 17.81	22.16 (5.25-58.10)	-	11	3.58 ± 2.05	3.27 (1.29-8.25)	-
	Ostrava	BM	33	13.67 ± 13.77	7.30 (1.46-56.06)	0.95	32	2.72 ± 1.31	2.55 (0.70-5.71)	0.76
		AM	41	12.70 ± 13.19	8.33 (3.59-77.07)	-	32	2.53 ± 1.04	2.38 (1.06-5.05)	-
Outdoor	Prague	BM	30	3.62 ± 2.23	3.22 (0.74-10.79)	0.74	32	0.81 ± 0.31	0.70 (0.36-1.87)	0.87
		AM	35	3.82 ± 2.95	2.88 (0.81-14.03)	-	29	0.98 ± 0.67	0.70 (0.36-2.87)	-
	Havirov	BM	0	-	-	-	6	1.01 ± 0.12	1.02 (0.85-1.16)	0.05
		AM	0	-	-	-	6	1.68 ± 0.52	1.94 (0.96-2.10)	-

		Karvina	BM	11	33.90 ± 19.45	41.85 (5.25-58.10)	0.79	11	3.40 ± 1.97	2.76 (1.29-8.25)	0.28
			AM	13	31.82 ± 13.10	29.60 (14.00-52.66)	-	13	3.76 ± 1.43	3.72 (1.03-5.70)	-
		Ostrava	BM	36	11.85 ± 12.93	8.32 (3.59-77.07)	0.62	25	2.85 ± 0.86	2.87 (1.53-5.05)	<0.05
			AM	38	14.34 ± 13.83	8.01 (1.46-56.06)	-	39	2.48 ± 1.34	2.04 (0.70-5.71)	-
	In work	Prague	BM	30	3.83 ± 2.60	3.12 (0.81-12.40)	0.60	27	0.81 ± 0.33	0.70 (0.36-2.01)	0.91
			AM	35	3.64 ± 2.69	3.08 (0.74-14.03)	-	34	0.95 ± 0.63	0.70 (0.36-2.87)	-
		Havirov	BM	0	-	-	-	5	1.42 ± 0.54	1.10 (0.94-2.10)	0.81
			AM	0	-	-	-	7	1.29 ± 0.50	1.10 (0.85-2.05)	-
		Karvina	BM	11	35.29 ± 16.93	40.69 (5.25-57.50)	0.43	12	3.42 ± 1.72	2.82 (1.03-5.70)	0.56
			AM	13	30.64 ± 15.47	27.32 (14.00-58.10)	-	12	3.77 ± 1.68	3.50 (1.29-8.25)	-
		Ostrava	BM	29	15.50 ± 14.00	9.83 (4.29-56.06)	<0.05	20	2.60 ± 1.21	2.30 (1.06-5.53)	0.71
			AM	45	11.60 ± 12.87	6.94 (1.46-77.07)	-	44	2.63 ± 1.18	2.56 (0.70-5.71)	-
	Restaurant	Prague	0	56	3.67 ± 2.47	3.13 (0.74-14.03)	0.86	43	0.94 ± 0.59	0.70 (0.36-2.87)	0.58
			above 0	9	4.11 ± 3.62	2.63 (0.81-12.40)	-	18	0.77 ± 0.26	0.70 (0.36-1.37)	-
		Havirov	0	0	-	-	-	7	1.29 ± 0.50	1.10 (0.85-2.10)	0.57
			above 0	0	-	-	-	5	1.43 ± 0.53	1.10 (0.95-2.05)	-
		Karvina	0	16	33.72 ± 17.27	38.04 (5.25-58.10)	0.76	12	3.00 ± 1.03	2.90 (1.03-5.24)	0.09
			above 0	8	30.88 ± 13.88	26.92 (15.07-51.66)	-	12	4.19 ± 2.00	4.45 (1.29-8.25)	-
		Ostrava	0	60	12.91 ± 13.38	8.24 (1.46-77.07)	0.64	52	2.76 ± 1.24	2.74 (0.70-5.71)	<0.05
			above 0	14	14.09 ± 13.75	8.86 (4.83-56.06)	-	12	2.01 ± 0.58	1.99 (1.01-3.16)	-
B[a]P	Public transport	Prague	BM	29	0.83 ± 0.72	0.64 (0.18-3.37)	0.50	26	0.09 ± 0.05	0.08 (0.04-0.24)	0.90
			AM	36	0.77 ± 0.38	0.69 (0.10-1.63)	-	35	0.13 ± 0.18	0.08 (0.04-1.00)	-
		Havirov	BM	0	-	-	-	11	0.23 ± 0.08	0.20 (0.08-0.34)	0.47
			AM	0	-	-	-	1	0.30	0.30 (0.30-0.30)	-
		Karvina	BM	21	6.93 ± 3.14	7.18 (0.88-12.60)	0.15	22	0.60 ± 0.32	0.57 (0.08-1.24)	0.75
			AM	3	4.34 ± 1.26	4.01 (3.28-5.73)	-	2	0.65 ± 0.11	0.65 (0.57-0.72)	-
		Ostrava	BM	36	2.55 ± 1.83	1.86 (0.74-9.56)	0.07	28	0.42 ± 0.21	0.40 (0.08-0.97)	0.52
			AM	38	2.45 ± 2.60	1.60 (0.33-12.60)	-	36	0.39 ± 0.18	0.37 (0.08-0.92)	-
	Car	Prague	BM	33	0.71 ± 0.34	0.69 (0.10-1.63)	0.89	28	0.14 ± 0.19	0.08 (0.04-1.00)	0.49
			AM	32	0.89 ± 0.70	0.62 (0.27-3.37)	-	33	0.10 ± 0.07	0.08 (0.04-0.30)	-
		Havirov	BM	0	-	-	-	6	0.24 ± 0.06	0.24 (0.17-0.33)	0.87
			AM	0	-	-	-	6	0.22 ± 0.10	0.20 (0.08-0.34)	-
		Karvina	BM	12	6.66 ± 3.44	7.70 (0.88-12.60)	0.77	11	0.54 ± 0.32	0.44 (0.08-1.02)	0.38
			AM	12	6.55 ± 2.82	5.83 (3.28-11.20)	-	13	0.66 ± 0.30	0.63 (0.08-1.24)	-
		Ostrava	BM	33	2.64 ± 2.78	1.59 (0.33-12.60)	0.17	22	0.41 ± 0.17	0.38 (0.18-0.89)	0.77
			AM	41	2.38 ± 1.71	1.83 (0.74-9.56)	-	42	0.41 ± 0.21	0.36 (0.08-0.97)	-

At home	Prague	BM	31	0.72 ± 0.38	0.69 (0.10-1.63)	0.66	22	0.10 ± 0.10	0.08 (0.04-0.48)	0.92
		AM	34	0.87 ± 0.67	0.65 (0.18-3.37)	-	39	0.12 ± 0.16	0.08 (0.04-1.00)	-
	Havirov	BM	0	-	-	-	6	0.23 ± 0.06	0.21 (0.18-0.33)	0.81
		AM	0	-	-	-	6	0.24 ± 0.11	0.25 (0.08-0.34)	-
	Karvina	BM	12	6.88 ± 2.79	6.55 (3.36-12.60)	0.60	11	0.59 ± 0.36	0.57 (0.08-1.24)	0.84
		AM	12	6.33 ± 3.44	6.05 (0.88-11.20)	-	13	0.62 ± 0.27	0.63 (0.25-0.98)	-
	Ostrava	BM	35	2.52 ± 2.01	1.76 (0.33-9.37)	0.74	33	0.42 ± 0.22	0.36 (0.08-0.97)	0.88
		AM	39	2.48 ± 2.46	1.68 (0.74-12.60)	-	31	0.40 ± 0.17	0.37 (0.08-0.76)	-
Elsewhere	Prague	BM	47	0.74 ± 0.38	0.69 (0.10-2.03)	0.81	48	0.12 ± 0.15	0.08 (0.04-1.00)	0.49
		AM	18	0.95 ± 0.85	0.58 (0.31-3.37)	-	13	0.12 ± 0.12	0.08 (0.04-0.48)	-
	Havirov	BM	0	-	-	-	6	0.24 ± 0.06	0.24 (0.17-0.33)	0.87
		AM	0	-	-	-	6	0.22 ± 0.10	0.20 (0.08-0.34)	-
	Karvina	BM	11	6.36 ± 2.51	5.73 (3.28-11.20)	0.75	15	0.58 ± 0.33	0.57 (0.08-1.24)	0.57
		AM	13	6.81 ± 3.57	7.18 (0.88-12.60)	-	9	0.65 ± 0.29	0.59 (0.26-1.02)	-
	Ostrava	BM	38	2.45 ± 2.46	1.60 (0.33-12.60)	0.35	30	0.41 ± 0.18	0.40 (0.08-0.89)	0.83
		AM	36	2.55 ± 2.01	1.79 (0.70-9.37)	-	34	0.41 ± 0.21	0.34 (0.08-0.97)	-
Passive smoking	Prague	0	61	0.78 ± 0.55	0.64 (0.10-3.37)	0.10	52	0.10 ± 0.08	0.08 (0.04-0.48)	0.10
		above 0	4	1.13 ± 0.51	1.16 (0.55-1.63)	-	9	0.23 ± 0.30	0.08 (0.08-1.00)	-
	Havirov	0	0	-	-	-	9	0.22 ± 0.09	0.20 (0.08-0.34)	0.64
		above 0	0	-	-	-	3	0.26 ± 0.08	0.26 (0.18-0.33)	-
	Karvina	0	14	6.63 ± 3.62	6.37 (0.88-12.60)	1.00	16	0.55 ± 0.31	0.50 (0.08-0.98)	0.30
		above 0	10	6.57 ± 2.28	6.23 (3.28-10.60)	-	8	0.71 ± 0.30	0.66 (0.25-1.24)	-
	Ostrava	0	65	2.43 ± 2.20	1.68 (0.33-12.60)	0.23	62	0.41 ± 0.20	0.38 (0.08-0.97)	0.66
		above 0	9	2.98 ± 2.61	1.91 (0.90-9.37)	-	2	0.33 ± 0.01	0.33 (0.32-0.33)	-
Travelling	Prague	BM	29	0.84 ± 0.70	0.66 (0.10-3.37)	1.00	25	0.10 ± 0.07	0.08 (0.04-0.30)	0.47
		AM	36	0.76 ± 0.42	0.67 (0.27-2.03)	-	36	0.12 ± 0.17	0.08 (0.04-1.00)	-
	Havirov	BM	0	-	-	-	6	0.23 ± 0.06	0.21 (0.17-0.33)	0.75
		AM	0	-	-	-	6	0.24 ± 0.10	0.25 (0.08-0.34)	-
	Karvina	BM	12	8.24 ± 2.67	8.45 (3.93-12.60)	<0.01	10	0.54 ± 0.31	0.42 (0.08-0.98)	0.33
		AM	12	4.97 ± 2.61	3.87 (0.88-10.10)	-	14	0.65 ± 0.31	0.61 (0.08-1.24)	-
	Ostrava	BM	33	2.11 ± 1.26	1.83 (0.89-6.68)	0.84	27	0.38 ± 0.18	0.35 (0.08-0.97)	0.38
		AM	41	2.81 ± 2.77	1.64 (0.33-12.60)	-	37	0.43 ± 0.20	0.42 (0.08-0.92)	-
Ventilation by window	Prague	BM	31	0.77 ± 0.59	0.68 (0.18-3.37)	0.45	28	0.14 ± 0.19	0.08 (0.04-1.00)	0.83
		AM	34	0.83 ± 0.53	0.65 (0.10-2.64)	-	33	0.10 ± 0.07	0.08 (0.04-0.33)	-
	Havirov	BM	0	-	-	-	6	0.18 ± 0.06	0.19 (0.08-0.26)	0.06
		AM	0	-	-	-	6	0.28 ± 0.07	0.32 (0.18-0.34)	-

	Karvina	BM	12	6.85 ± 2.81	6.14 (3.28-11.20)	0.82	13	0.58 ± 0.32	0.57 (0.08-1.24)	0.64
		AM	12	6.36 ± 3.43	6.55 (0.88-12.60)	-	11	0.63 ± 0.31	0.72 (0.08-1.02)	-
	Ostrava	BM	35	2.59 ± 1.93	1.75 (0.33-7.95)	0.56	29	0.40 ± 0.19	0.38 (0.08-0.97)	0.90
		AM	39	2.42 ± 2.51	1.68 (0.84-12.60)	-	35	0.41 ± 0.20	0.37 (0.08-0.92)	-
Indoor	Prague	BM	26	0.74 ± 0.45	0.58 (0.27-2.03)	0.49	29	0.13 ± 0.18	0.08 (0.04-1.00)	0.97
		AM	39	0.84 ± 0.62	0.69 (0.10-3.37)	-	32	0.11 ± 0.09	0.08 (0.04-0.48)	-
	Havirov	BM	0	-	-	-	6	0.27 ± 0.07	0.28 (0.18-0.34)	0.09
		AM	0	-	-	-	6	0.20 ± 0.08	0.19 (0.08-0.30)	-
	Karvina	BM	12	7.13 ± 2.62	7.17 (3.28-11.20)	0.49	13	0.62 ± 0.27	0.57 (0.08-1.02)	0.69
		AM	12	6.08 ± 3.51	4.97 (0.88-12.60)	-	11	0.59 ± 0.36	0.59 (0.08-1.24)	-
	Ostrava	BM	33	2.52 ± 2.19	1.75 (0.33-9.37)	0.90	32	0.41 ± 0.21	0.37 (0.08-0.97)	0.82
		AM	41	2.48 ± 2.31	1.69 (0.78-12.60)	-	32	0.40 ± 0.18	0.38 (0.18-0.80)	-
Outdoor	Prague	BM	30	0.75 ± 0.41	0.69 (0.10-2.03)	0.76	32	0.08 ± 0.05	0.08 (0.04-0.27)	0.08
		AM	35	0.84 ± 0.66	0.59 (0.18-3.37)	-	29	0.15 ± 0.19	0.08 (0.04-1.00)	-
	Havirov	BM	0	-	-	-	6	0.19 ± 0.06	0.20 (0.08-0.26)	0.17
		AM	0	-	-	-	6	0.28 ± 0.08	0.32 (0.17-0.34)	-
	Karvina	BM	11	6.86 ± 3.85	7.18 (0.88-12.60)	0.66	11	0.55 ± 0.34	0.44 (0.08-1.24)	0.30
		AM	13	6.38 ± 2.38	5.92 (3.28-11.20)	-	13	0.66 ± 0.29	0.69 (0.08-1.02)	-
	Ostrava	BM	36	2.26 ± 2.08	1.67 (0.78-12.60)	0.60	25	0.44 ± 0.15	0.42 (0.20-0.80)	0.07
		AM	38	2.72 ± 2.39	1.76 (0.33-9.56)	-	39	0.39 ± 0.21	0.33 (0.08-0.97)	-
In work	Prague	BM	30	0.84 ± 0.62	0.66 (0.18-3.37)	0.77	27	0.09 ± 0.06	0.08 (0.04-0.30)	0.09
		AM	35	0.76 ± 0.49	0.66 (0.10-2.64)	-	34	0.14 ± 0.18	0.08 (0.04-1.00)	-
	Havirov	BM	0	-	-	-	5	0.24 ± 0.07	0.20 (0.18-0.34)	0.62
		AM	0	-	-	-	7	0.22 ± 0.09	0.22 (0.08-0.33)	-
	Karvina	BM	11	7.13 ± 3.19	8.22 (0.88-10.60)	0.31	12	0.58 ± 0.34	0.49 (0.08-1.02)	0.66
		AM	13	6.16 ± 3.03	5.55 (3.28-12.60)	-	12	0.63 ± 0.29	0.61 (0.08-1.24)	-
	Ostrava	BM	29	2.82 ± 2.17	1.98 (0.84-9.37)	<0.05	20	0.39 ± 0.18	0.34 (0.18-0.89)	0.57
		AM	45	2.29 ± 2.29	1.56 (0.33-12.60)	-	44	0.41 ± 0.20	0.38 (0.08-0.97)	-
Restaurant	Prague	0	56	0.77 ± 0.47	0.65 (0.10-2.64)	0.94	43	0.13 ± 0.16	0.08 (0.04-1.00)	0.32
		above 0	9	0.96 ± 0.97	0.69 (0.18-3.37)	-	18	0.08 ± 0.04	0.08 (0.04-0.19)	-
	Havirov	0	0	-	-	-	7	0.21 ± 0.09	0.20 (0.08-0.34)	0.57
		above 0	0	-	-	-	5	0.26 ± 0.08	0.26 (0.18-0.33)	-
	Karvina	0	16	6.77 ± 3.38	6.86 (0.88-12.60)	0.67	12	0.50 ± 0.22	0.49 (0.08-0.95)	0.11
		above 0	8	6.28 ± 2.52	5.83 (3.31-10.60)	-	12	0.71 ± 0.36	0.81 (0.08-1.24)	-
	Ostrava	0	60	2.45 ± 2.24	1.67 (0.33-12.60)	0.44	52	0.43 ± 0.20	0.42 (0.08-0.97)	0.06
		above 0	14	2.69 ± 2.31	1.83 (0.84-9.37)	-	12	0.31 ± 0.12	0.30 (0.08-0.53)	-

BM-below median; AM-above median.
Elsewhere-other activity than work or home; indoor-hours spent indoors; outdoor-hours spent outdoors.
P-values were obtained with the Mann-Whitney U-test.

Příloha 9

Svecova V., Topinka J., Solansky I., Sram R. J.

Personal exposure to volatile organic compounds in the Czech Republic

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PERSONAL EXPOSURE TO VOLATILE ORGANIC COMPOUNDS IN THE CZECH REPUBLIC

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Abstract

Personal exposures to volatile organic compounds (VOCs) were measured in three industrial cities in the Czech Republic, Ostrava, Karvina and Havirov, while the city of Prague served as a control in a large-scale molecular epidemiological study identifying the impacts of air pollution on human health. Office workers from Ostrava and city policemen from Karvina, Havirov and Prague were monitored in the winter and summer of 2009. Only adult non-smokers participated in the study (N=160). Radiello® diffusive passive samplers were used to measure the exposure to benzene, toluene, ethylbenzene, meta- plus para-xylene and ortho-xylene (BTEX). All participants completed a personal questionnaire and a time-location-activity diary (TLAD). The average personal BTEX exposure levels in both seasons were 7.2/34.3/4.4/16.1 μg/m^3, respectively. The benzene levels were highest in winter in Karvina, Ostrava and Prague: 8.5, 7.2 and 5.3 μg/m^3, respectively. The personal exposures to BTEX were higher than the corresponding stationary monitoring levels detected in the individual localities ($p<0.001$; except m,p-xylene in summer). The indoor environment, ETS (environmental tobacco smoke), cooking, a home heating fireplace or gas stove, automobile use and being in a restaurant were important predictors for benzene personal exposure. Ostrava`s outdoor benzene pollution was a significant factor increasing the exposure of the Ostrava study participants in winter ($p<0.05$).

Introduction

The contamination of urban air by toxic organic pollutants causes concern about adverse health effects. Volatile organic compounds (VOCs) including benzene, toluene, ethylbenzene, meta- plus para-xylene and ortho-xylene (BTEX) are associated with a wide range of human health effects, from headaches and eye irritation to central nervous system depression and cancer (Hoxha, et al., 2009; Künzli and Tager, 2005; Sorensen, et al., 2003; WHO, 2000, 2010). BTEX plays a vital role in the troposphere chemistry; together with NO_x, they are precursors required for the photochemical production of ozone and other components of photochemical smog (Jurvelin, 2003).

Traffic is believed to be the critical source of atmospheric BTEX emissions in cities (Crebelli, et al., 2001), but high concentrations can also be found near coal processing plants or refineries and chemical plants (Topp et al., 2004). This research focused on aromatic hydrocarbons with respect to their high toxicity. Another source of these hydrocarbons are losses by evaporation during the manipulation, storage and distribution of petrol (Wallace, 2001). Important indoor sources are building materials and furniture, attached garages, heating and cooking systems, stored solvents, paints and organic coatings, cleaning and cosmetic products and various human activities (WHO, 2010).

One of the most important representatives of VOCs is benzene, which is characterized by the International Agency for Research on Cancer (IARC) as a human carcinogen. Ethylbenzene is classified as a possible carcinogen to humans and toluene and xylenes are categorized as not classifiable as to their carcinogenicity to humans (IARC, 2009).

Continuous stationary monitoring has already become a part of air pollution monitoring systems, but these data cannot provide a real personal exposure profile. There is large spatial variation in the distribution of BTEX in urban areas (Han and Naeher, 2006). Factors such as wind, temperature, traffic density, city buildings and industrial sources greatly increase spatial variation (Upmanis, et al., 2001). Personal exposure is determined by outdoor and indoor concentrations, as well as personal activities. Time spent outdoors, distance from the source of pollution (proximity of busy roads, industrial sources), the impact of environmental tobacco smoke (ETS) as well as indoor sources are very important factors. International studies have reported that people spend 80-93% of their time indoors, 1-7% in enclosed transit and 2-7% outdoors. Thus, an individual's exposure to air pollution is dependent on individual activity patterns, reflecting time spent in different microenvironments: indoors, outdoors, as well as commuting (Adgate et al., 2004, Brunekreef and Holgate, 2002, Cocheo et al., 2000; Delgado-Saborit, et al., 2011).

Because there are not data about the personal exposure to BTEX and potential sources of such exposure in the Czech Republic we tried to measure this exposure and analyze the main determinants of exposure in the context of a large-scale molecular epidemiological project studied the impact of air pollution on the population of industrial cities within the Moravian-Silesian Region (MSR) of the Czech Republic: Ostrava, Karvina and Havirov. The capital city of Prague served as a control. During last years (2002-2009) the concentrations of benzene were in Prague et least four times lower than in the Ostrava city. Personal exposure to BTEX was investigated with its impact on biomarkers of exposure, effect and susceptibility that could demonstrate relevant damage of genetic material. This report presents results on personal exposure to BTEX and its relationship to some exposure-modifying parameters.

Methods

Study Area

The MSR is situated in the easternmost part of the Czech Republic (Supplemental Material, Figure 1). It is an industrial and heavily populated area. The MSR covers 5,427 km^2 with 1.25 million inhabitants (Czech Statistical Office, 2010). The Ostrava, Karvina and Havirov lie in the north-eastern part of MSR with 1411.8, 1037.2 and 2484.5 inhabitants/km^2, respectively. The most important industrial sources of air pollution are metallurgy, steel, coke ovens, coal-mining and power generation facilities. Fifty percent of the inhabitants use central heating, 34% natural gas, 10% coal, 3% wood and 3.3% electricity. The VOC emissions have been calculated to be 17,466 tons per year (Czech Hydrometeorological Institute, CHMI, 2008).

Prague is the capital and largest city of the Czech Republic, situated in central Bohemia. The city is spread over nine hills on 496 km^2 with 1.3 million inhabitants (Czech Statistical Office, 2010). Traffic and home heating are considered to be the most important sources of air pollution in winter. 51.5% of households use central heating, 40% natural gas, 5.7% electricity, 2.4% coal and 0.2% wood. VOC emissions have been calculated to be 13,159 tons per year in 2008 (CHMI, 2008).

Study Population

Ostrava city policemen were originally planned for this study, but the Ostrava City Authority did not approve their inclusion, so they were replaced by office workers from Ostrava city and policemen from the neighbouring towns of Karvina and

Havirov. City policemen from Prague served as a control group as long-term followed group in the city without industry. Prague had the advantage that long-term biomonitoring data were available and good monitoring conditions for such a big project were experienced. Even though there is a big difference in exposure patterns between these two groups (office workers and policemen), we think that comparison of exposure data of subjects from industrial and non-industrial cities are of great importance (these data have not been previously measured in CR). The study was planned to include three monitoring campaigns: winter 2009, summer 2009 and winter 2010. Here, we are presenting the results from the first two campaigns. The participants were recruited on a voluntary basis using a recruitment letter distributed to all male employees. The same participants took part in both campaigns, and all of them were non-smokers. Because of high number of participants and lack of personal monitors we were not able to monitor all localities simultaneously. Localities were monitored after each other in same season by this schedule: February 2009 in Prague and March 2009 in Ostrava and Karvina. The summer campaigns were held at the end of May in Prague, whereas Ostrava, Karvina and Havirov were measured at the beginning of June 2009. Seventy-four office workers joined the study in Ostrava in winter and 64 in summer (average age 37.7±9.8). Sixty-five city policemen participated in Prague in winter and 60 in summer (average age 38.3±8.7), 24 city policemen in Karvina (average age 36.5±7.2) and 12 city policemen in Havirov in summer (average age 40.2±9.6). All participants gave their written consent prior to the study after having received written and oral information about the study. The participants answered a personal history questionnaire giving information on their place of residence, transportation mode, type of housing, life style, and activities that might influence their exposure to agents of interest for the study. In addition, during an observation period when they carried the personal monitors, they kept a detailed time-location-activity diary (TLAD) in which they recorded information about their location and activities every hour. The personal sampler used was attached to the volunteer's lapel and during the night was set on the bedside table. All stages of the study were approved by the Ethical Committee of the Institute of Experimental Medicine AS CR, Prague, Czech Republic.

Personal Exposure Monitoring

Benzene, toluene, ethylbenzene and m,p,o-xylenes were measured by Radiello® passive diffusive samplers (Fondazione Salvatore Maugeri, Padova, Italy) for 24-h (24.2 ± 2.1 h). A yellow diffusive body with an adsorbing cartridge of graphitised charcoal (Carbograph 4) was used. The cartridges were stored in a refrigerator (4-8 ^{o}C). BTEX were trapped by adsorption and recovered by thermal desorption, while analysis was performed by capillary gas chromatography with Flame Ionization Detection (FID) in the laboratories of the certified company ALS Czech Republic, Prague (EN ISO CSN IEC 17025). The method allowed detection limits as low as 0.5; 0.1; 0.1; 0.2; 0.1 μg/m^3 for BTEX, respectively. Analytical method has been described in details elsewhere (Pennequin-Cardinal et al., 2005) and the sampler has been widely used (Chatzis et al., 2005; Crebelli et al., 2001; Strandberg et al., 2005; Strandberg et al., 2006).

The monitoring campaigns were held according to working shifts of participants. Office workers and policemen in Havirov were monitored during the weekdays; policemen in Prague and Karvina were monitored during whole week. The monitoring period usually started in the morning 8 am, only in Karvina group there were 2 working shifts started monitoring at 7 am and 7 pm. All participants in every location

started and finished monitoring at work in central location. For quality control 8% of samples were taken as blanks parallel with the samples in all experiments in this study. 88% of blanks were under detection limits. Duplicate samples were run at the workplace of office workers with the deviation less than 15%.

Stationary Monitoring

Information about outdoor BTEX was used from automated measuring program of the Czech Hydrometeorological Institute. The concentrations in relevant days of personal monitoring were compared and data were used from Prague Smichov and Ostrava Fifejdy and Ceskobratrska stations. The instrument Environnement SA, VOC71M measured in Prague and Syntech Spectras, GC855 in Ostrava. BTEX were analyzed by gas chromatography with photo-ionization detection.

Statistical Analysis

To determine the effect of life style and subject behavior we have tested data from 2 types of questionnaires, which could help to detect additional individual sources of BTEX or effect of outdoor concentration which was assigned to individual subject and it`s personal exposure. From the personal history questionnaire we used information mainly about: heating – central heating out of flat/gas heating in flat/electric heating/coal stove in flat/wood stove in flat/fireplace; cooking information – cooking on gas stove/electric stove/other stove, cooker hood using often/sometimes/rarely, cooking yes/no, cooking daily/every other day/weekly/sometimes/never; ventilation – in work/at home/elsewhere and way of ventilation by air-conditioner/by short window opening/by long window opening/no ventilation; traffic subjective perception - by density in both localities – work/home; passive smoking – smoking in subject's presence – in work/in leisure, smoking of living partner - yes/no, smoking in flat – yes/no. The time-location-activity-diary included information about description of activities during monitoring day: home/traveling/at work/in restaurant/elsewhere; and specified the presence of participants: indoor/outdoor/car/public transport/actual passive smoking.

Statistical analyses were done using Statistica 7.1 (StatSoft, OK, USA). The nonparametric method for bivariate analyses was used to compare data samples that were not distributed normally: the Mann–Whitney Rank Sum *U*-test for comparison of two groups. For each individual activity from the TLAD questionnaire were calculated the median of distribution of this activity. According to the median was sample divided into two groups: below median (BM) and above median (AM). If value of median was equal to zero, split was done equal to zero and above zero. Distribution was done independently in both followed periods (winter/summer 2009). To assess the effect of outdoor concentrations to personal exposures of individuals measured by personal monitoring models of multivariate linear regression were used. Models were built up with forward stepwise model building option. From the reason of big volume of the models we chose and published only the most important parameters.

Results

Characteristics of the Participants

Depending on the locality, 8-25% of policemen had a university degree in contrast to 92% of the office workers. Table 1 shows the time distribution of the activities of individual groups in both seasons according to the time activity questionnaires during

the 24-h monitoring period (not all subject fulfill the questionnaire correctly, 92% in the first campaign and 83% in the second completed the questionnaires in 22-26 hours). The group of Ostrava office workers spent 75-87% of their time indoors from which around 50% at home. The group of policemen in Karvina spent 49-60% of their time indoors and 37-43% at home, while the Prague policemen spent less time indoors (46-49%) and also at home (only 36%), while spending 34% of their time outdoors, which is around 8 hours compared to the 18 minutes reported by the Ostrava participants in winter. The difference in the time spent at work is due to the 12 hour working shifts of the policemen. The Karvina participants were the group spent the highest time exposed to passive smoking ($p<0.01$, Table 1). More information about the subjects‘ demographic characteristics can be found in Svecova at al. (2012).

Personal Exposure

Table 2 shows the average BTEX personal exposure levels in both seasons. The benzene levels were highest in Karvina in both seasons and greater in the summer ($p<0.001$) (winter/summer 8.5/16.4 $\mu g/m^3$). In the other locations the benzene levels were significantly higher in winter ($p<0.01$). Exposures to toluene, ethylbenzene and the xylenes were the highest in Karvina followed by Prague and Ostrava, due to the fact that city policemen spend their work time in the streets, in contrast to office workers. The levels were higher in winter. Personal exposures to xylenes were significantly higher in Prague policemen than in Ostrava office workers in winter ($p<0.001$) as well as to m,p-xylene in summer ($p<0.01$). Also, higher concentrations of o-xylene were found in Karvina policemen in summer ($p<0.01$). These results probably reflect the greater traffic-related exposure of the groups of policemen.

Environmental and Life Style Factors Affecting Personal Exposure

Bivariate analysis, which used information from the personal history questionnaires, showed the influence of heating systems on personal exposure. A significant effect for increased exposure to ethylbenzene and m,p,o-xylenes ($p<0.01$) was observed for an indoor fireplace in summer, but no significant effect was seen for benzene or toluene when analyzing all groups together. In Karvina there were significantly higher concentrations of benzene, ethylbenzene and xylenes in summer because of the use of an indoor gas fire (could reflect using gas fire during cold nights even in this period or using boiler for hot water or cooking) and of o-xylene due to the use of a fireplace ($p<0.05$). Analyzing effect of cooking in all groups together, there were significantly higher concentrations of benzene when using a gas stove instead of an electric stove for cooking (7.39±9.56 vs. 6.57±10.20, $p<0.01$). Using an extractor hood in the kitchen lowered the concentrations of pollutants. This factor was significant for benzene in the summer (5.50±7.11 vs. 7.33±15.78, $p<0.01$). Smoking status is always very important in the case of personal exposure. Analyzing all groups together significant effects were: being with smokers outdoors during the working hours appeared as a significant factor for benzene in winter (6.39±5.59 vs. 5.23±3.29, $p<0.01$; 11% of respondents indicate this option) and summer (11.19±22.90 vs. 5.53±10.32, $p<0.05$; 13% of respondents), also for toluene in summer (23.42±43.20 vs. 19.23±21.34, $p<0.01$). Being in the company of smokers outside of working hours (reported 5% of respondents) significantly elevated exposure to toluene (274.23±639.83 vs. 36.94±171.08, $p<0.01$), ethylbenzene (4.84±3.80 vs. 3.95±4.35, $p<0.01$), m,p-xylene (15.13±12.09 vs. 12.53±15.18, $p<0.01$) and o-xylene (5.39±4.25 vs. 4.02±3.91, $p<0.01$) in winter.

The results from the time-activity questionnaires are shown in Supplemental Material, Table 1. Analyzing all groups together, the results indicated that using public transport lowered personal exposure to benzene in both seasons (5.53±3.28 vs. 8.28±12.87 $p<0.05$ in winter and 4.44±2.79 vs. 7.62±14.76 in summer) and other pollutants significantly in summer (toluene 11.50±13.00 vs. 20.59±27.71 $p<0.01$; ethylbenzene 2.30±2.56 vs. 3.75±4.62, $p<0.001$; m,p-xylene 7.40±6.96 vs. 11.16±12.05, $p<0.01$; o-xylene 2.43±2.15 vs 3.66±4.10, $p<0.01$). Using a car significantly increased exposure to benzene in winter 8.76±13.47 vs. 5.33±3.07, $p<0.01$ and summer 8.11±16.51 vs. 4.87±4.09, $p<0.05$; ethylbenzene in summer 3.59±3.83 vs. 2.83±4.15, $p<0.05$; m,p-xylene in summer 10.91±10.32 vs. 8.63±10.63; and o-xylene in summer 3.46±2.79 vs. 2.93±4.06, $p<0.05$. The results also suggest that time spent at home significantly increased exposure to benzene in winter (7.49±10.06 vs. 6.40±9.86, $p<0.01$) but decreased personal exposure in summer (5.81±5.70 vs. 7.18±16.82, $p<0.01$). Due to the elevated levels of benzene indoors, time spent outdoors lowered personal exposure to benzene in winter (6.56±8.98 vs 7.65±11.01, $p<0.01$). In contrast, there were significantly higher exposures to toluene, ethylbenzene and xylenes outdoors (toluene 58.46±231.79 vs. 15.65±17.35, $p<0.01$; ethylbenzene 4.54±5.22 vs. 3.20±2.85, $p<0.05$ in winter and 3.73±4.78 vs. 2.47±2.53, $p<0.05$ in summer; m,p-xylene 14.84±17.89 vs. 9.63±10.33, $p<0.01$ in winter and 11.02±12.43 vs. 7.97±6.98, $p<0.05$ in summer; o-xylene 4.79±4.82 vs. 3.13±2.24, $p<0.01$ in winter and 3.62±4.32 vs. 2.59±1.92, $p<0.05$). Passive smoking as well as activities performed in the company of smokers increased exposure to all pollutants (only significant data shown: benzene in winter 7.34±2.00 vs. 7.05±10.37, $p<0.01$; toluene in summer 35.22±58.54 vs. 15.33±16.25, $p<0.05$). The results indicated that ventilation by windows significantly decreased the levels of toluene (11.94±9.37 vs. 23.33±32.53, $p<0.01$), ethylbenzene (2.96±4.43 vs. 3.46±3.43, $p<0.01$), m,p-xylene (8.49±10.90 vs. 11.19±9.90, $p<0.001$) and o-xylene (2.78±3.78 vs. 3.67±3.14, $p<0.001$) exposures in summer. Time spent indoors increased exposure to benzene in winter (8.29±10.86 vs. 5.79±8.82, $p<0.001$), but decreased exposure to m,p-xylene (10.38±12.43 vs. 14.50±17.13, $p<0.01$ in winter and 8.57±8.13 vs. 10.96±12.58, $p<0.01$ in summer) and o-xylene (3.21±2.29 vs. 4.85±4.96, $p<0.01$ in winter and 2.75±2.19 vs. 3.66±4.54, $p<0.01$ in summer). In the group of Ostrava office workers, time spent outdoors had a positive effect on personal exposure to all BTEX (significant for benzene in summer 7.39±4.01 vs. 5.17±2.48, $p<0.01$; ethylbenzene in summer 3.03±2.04 vs. 2.06±1.26, $p<0.05$; m,p-xylene in summer 9.33±6.69 vs. 6.44±3.78, $p<0.05$; o-xylene in winter 3.65±2.02 vs. 2.92±2.12, $p<0.01$ and o-xylene in summer 3.11±2.10 vs. 2.22±1.32, $p<0.05$). Time spent at work was negatively associated with exposure to benzene in all groups analyzed together (6.31±8.67 vs. 8.04±11.39, $p<0.001$ in winter and 5.59±10.57 vs. 7.31±13.02, $p<0.001$ in summer), while a positive association was found in all groups for m,p-xylene in winter (12.92±15.38 vs. 11.70±14.56, $p<0.05$). In Prague policemen, time spent at work was positively associated with benzene exposure in summer (3.48±2.00 vs. 2.61±0.67, $p<0.01$). Time spent in restaurants increased exposure to toluene in summer (18.85±15.27 vs. 16.60±25.25, $p<0.01$).

When comparing the measured personal BTEX concentrations with outdoor levels in the same period and location, we found that the personal exposures were much higher $p<0.001$ (Supplemental Material, Table 2). Linear multiple regression models of effect of outdoor concentrations and personal exposures in Prague and Ostrava revealed a significant relation for benzene in both groups together (row regression coefficient B=1.13, $p<0.01$ in winter, B=1.26, $p<0.001$ in summer).

Significant effects were also found in the Ostrava participants (B=1.66, p<0.001 in winter and B=0.83, p<0.05 in summer). For other pollutants, a significant impact was found in summer in the Prague and Ostrava groups together, except for m,p-xylene, which was significant in the Ostrava participants only (Supplemental Material, Table 3).

In this paragraph selected parts of multivariate linear model are presented. They were compiled to describe relation between individual BTEX according to outdoor concentration data and personal exposure data influenced by time activity profile from TLAD and other parameters from questionnaires. From the reasons of big volume and lucidity whole models could not be included. For benzene exposure in winter in Prague policemen, significant factors were a smoking partner (B=7.54, p<0.05) and cooking (B=6.79, p<0.05). In Karvina policemen, significant factors for benzene exposure in winter were a smoking partner (B=10.97, p<0.001), cooking on a gas stove (B=28.26, p<0.001), a fireplace in the home (B=23.27, p<0.001) and being at work (B=0.27, p<0.01), while in summer additional significant factors were identified: using a car (B=8.97, p<0.001), being in a restaurant (B=7.62, p<0.001) and using public transport (B=10.41, p<0.01). In the Ostrava office workers, the models identified as significant determinants of benzene exposure influence of outdoor pollution (B=2.49, p<0.05) and frequent use of an extractor hood (B=7.93, p<0.05) in winter and traveling (B=2.32, p<0.001), cooking on an electric stove (B=1.66, p<0.05), being outdoors (B=0.43, p<0.05) and also being indoors in summer (B=0.17, p<0.01). Factors significantly influencing personal exposure to toluene in Prague were passive smoking (B=408.20, p<0.001), being in a restaurant (B=92.21, p<0.01) in winter, a smoking partner (B=8.89, p<0.001), residence in the city (B=4.64, p<0.01), being at work (B=1.23, p<0.01) and traveling (B=1.60, p<0.01) in the summer. In Ostrava the significant factors of toluene were residence in the city (B=8.42, p<0.05) and cooking (B=11.43, p<0.01), while in Karvina outdoor concentrations of toluene (B=280.77, p<0.001) were important in winter. Personal exposure to ethylbenzene was significantly affected by a fireplace at home (B=2.78, p<0.05) in winter and cooking on electric stove (B=3.46, p<0.05) and heating with gas stove (B=5.02, p<0.001) in summer in all groups. Residing in Ostrava city was a significant factor for ethylbenzene exposure in summer (B=0.76, p<0.01). Cooking on electric stove (B=5.06, p<0.05), cooking on gas stove (B=7.13, p<0.01), fireplace in flat (B=5.76, p<0.05) and smoking (B=41.27, p<0.01) were important factors for m-xylene in all groups in summer. o-xylene had cooking (B=1.46, p<0.05), heating with gas stove (B=2.71, p<0.01) and fireplace at home (B=3.95, p<0.001) as significant determinants of exposure in winter and outdoor concentration (B=9.20, p<0.05), residence in the city (B=4.12, p<0.05), cooking on electric stove (B=5.52, p<0.01) and travelling by car (B=1.64, p<0.001) in all groups in summer. Residency in the city was a significant influence in Ostrava in the summer on the concentration of m,p-xylene (B=1.89, p<0.05) and in Karvina in the summer on o-xylene exposure (B=7.12, p<0.001).

Discussion

The average personal benzene exposure levels in this study ranged from 3.4 to 16.4 $\mu g/m^3$. Higher exposures (13.1–24.6 $\mu g/m^3$) were found in Athens, Greece (Chatzis, et al., 2005), and also in Milan, Italy in 1999/2000, where exposure to benzene was 31.8 $\mu g/m^3$ in traffic officers and 13.0 $\mu g/m^3$ in indoor office workers during the work shift and toluene levels were 128.7 $\mu g/m^3$ and 43.4 $\mu g/m^3$, respectively (Hoxha, et al.,

2009). Average personal toluene levels in our study ranged from 11.0 to 115.1 μg/m^3, whereas personal exposure in Athens varied between 53 - 80 μg/m^3. The higher values were observed mainly in winter when the wind speed did not exceed 0.5 m/s (Alexopoulos, et al., 2006). We have not studied the impact of wind speed, but the toluene values in our study were also higher in winter ($p<0.01$), which could be explained by differences in climate parameters. Personal exposures to VOCs within this study are higher than exposures in UK cities such as London or Birmingham, also higher than in New York City or Helsinki (Delgado-Saborit, et al., 2009; Han and Naeher, 2006; Kinney, et al., 2002).

Personal exposure to VOCs is determined by many factors such as ETS, the type of home heating, fumes encountered in the cooking process (e.g., frying or broiling), time spent in restaurants, "do-it-yourself" (DIY) activities and many others. Therefore, detailed information about the subject`s life style and activities during the period of personal monitoring are of great importance. In this study detailed information was obtained through a personal history questionnaire and a TLAD. As we measured different groups in different cities, diverse factors influenced the personal exposures of individual groups. As the main determinants of benzene exposure we assessed the indoor environment, ETS, cooking, and the use of a gas stove or fireplace in winter, and automobile use and being in a restaurant in summer. Ostrava`s outdoor pollution was an important factor for the Ostrava participants. For toluene exposure, cooking and the home environment, ETS, being in a restaurant, living in the city of Ostrava and the outdoor concentration of toluene in Karvina were significantly important. Personal exposure to ethylbenzene was mainly determined by the home environment, cooking, the outdoor concentration of the pollutant, a fireplace or gas stove in the home, residence in Ostrava city, and ETS. Factors significantly affecting the concentrations of m,p-xylene were the home environment, cooking, ETS, a fireplace or gas stove in the home, travelling and residence in Ostrava city in the summer. Exposure to o-xylene was influenced mainly by the home environment, a fireplace or gas stove in the home, ETS, residence in Karvina in summer and a garage inside the house. The study in Greece (Chatzis, et al., 2005) revealed that population exposure to benzene depends mainly on the time spent outdoors, indoor pollution and the method of transportation. The authors also suggested that the strategy of fixed point monitoring is insufficient to estimate exposure in highly exposed groups, which is in agreement with our findings. The impact of ETS was clearly seen in other studies in low pollution regions such as Helsinki, where exposures to BTEX-compounds were 1.2-1.5 times higher for the population of ETS-exposed participants than for those not exposed. The strongest source factors for participants not exposed to ETS were traffic related sources (Jurvelin, 2003). Comparisons of outdoor and personal BTEX values in our study show that for all BTEX compounds, people are closer to the sources of pollution and exposed more than the outdoor numbers would indicate as showed also other studies (Cocheo et al., 2000; Crebelli et al., 2001).

Personal exposure to benzene and other VOCs together with other pollutants such as PM2.5 and PAHs contribute to a high air pollution burden in Ostrava and Karvina. Benzene is one of the significant health risks posed by the polluted air of urban and industrial areas (Bagryantseva, et al., 2010, Hoxha, et al., 2009, Rossner, et al., 2008, Rossnerova, et al., 2009). Our study tried to assess the main determinants of exposure to VOCs in large cities in the Czech Republic where the Ostrava region, due to its composition of sources, especially metallurgy, steel, coke ovens and power generation facilities, is the most polluted region. Significantly important factors included outdoor pollution and residence in Ostrava or Karvina, in addition to other

known factors such as ETS, type of heating, cooking, travelling and some other personal activities connecting with VOC exposure. A more detailed study would be beneficial to simultaneously measure indoor/outdoor/personal concentrations to reveal the main indoor sources and the strength of their effects in comparison with outdoor sources such as the impact of industrial plants, traffic and local heating. In such cities as Ostrava, there is a great need to monitor, regulate and decrease the prolonged and unsustainably high levels of pollutants to protect public health, including the most susceptible group – children.

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Table 1. Time distribution of activities in hours during the 24-h monitoring period for individual cohorts in both seasons according to time-location-activity diary (TLAD).

	Winter						Summer							
Participant information	Prague		Ostrava		Karvina		Prague		Ostrava		Karvina		Havirov	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
At home	8.6	2.9	**11.7**	**3.3**	8.8	3.0	8.6	2.9	**10.4**	**4.3**	9.7	2.0	10.4	3.8
Travelling	1.9	1.0	**1.5**	**0.8**	**1.3**	**0.8**	1.9	1.7	1.3	0.8	1.7	2.9	1.0	0.7
At work	11.9	2.4	**8.8**	**3.4**	11.2	3.5	11.5	3.1	**8.4**	**3.1**	10.5	4.1	**7.4**	5
Restaurant	0.1	0.3	0.2	0.9	**0.3**	**0.5**	0.3	0.6	0.2	0.6	0.6	1.0	0.7	1.1
Elsewhere	0.7	2.0	0.4	0.8	1.0	2.4	0.3	1.2	**0.9**	**1.9**	0.8	2.0	**2.8**	3.1
Outdoors	8.2	4.9	**0.3**	**0.6**	**5.1**	**5.5**	6.6	5.2	**1.1**	**1.7**	6.5	5.7	5.0	4.8
Indoors	11.1	4.3	**20.8**	**5.0**	**14.3**	**4.6**	11.8	5.5	**18.1**	**6.9**	11.8	5.3	11.8	6.8
Ventilation by windows	2.4	4.9	**3.6**	**4.8**	3.1	4.7	4.8	6.6	**8.7**	**8.0**	4.2	4.6	**8.2**	5.2
Car	2.6	4.6	0.8	1.0	**3.5**	**4.6**	2.4	4.4	0.7	1.0	1.5	2.9	1.9	3.4
Public transport	0.5	0.5	0.5	0.5	**0.1**	**0.3**	0.5	0.5	0.5	0.5	**0.1**	**0.3**	**0.1**	0.3
Passive smoking	0.0	0.1	0.1	0.6	**0.9**	**2.5**	0.3	1.4	0.0	0.2	**0.8**	**1.6**	0.2	0.6

Significant differences are highlighted in bold. Comparisons done using the Mann-Whitney U-test. The reference group is Prague. Unit-hour.

Table 2. Personal BTEX (µg/m^3) exposures at all locations by season; p values were obtained from the Mann-Whitney U-test.

	Period			**2-3/2009**				**5-6/2009**	
	Region	N	Mean ± SD	Median (Range)	P	N	Mean ± SD	Median (Range)	P
Benzene	Prague	65	5.28 ± 9.80	3.39 (2.14-76.20)	-	60	3.36 ± 1.90	2.97 (1.21-15.90)	-
	Havirov	0	-	-	-	12	3.80 ± 1.07	3.62 (2.54-5.90)	0.055
	Karvina	23	8.54 ± 5.33	7.00 (3.89-28.40)	**<0.001**	23	16.39 ± 28.62	5.29 (2.67-111.00)	**<0.001**
	Ostrava	72	7.17 ± 2.92	6.77 (2.48-19.40)	**<0.001**	64	5.93 ± 3.24	5.30 (1.52-20.80)	**<0.001**
Toluene	Prague	65	36.93 ± 95.21	13.30 (3.54-490.00)	-	60	19.52 ± 21.74	10.80 (3.76-97.60)	-
	Havirov	0	-	-	-	12	12.25 ± 8.74	8.97 (4.01-36.60)	0.75
	Karvina	23	115.13 ± 423.32	18.40 (4.91-2050.00)	0.25	23	28.44 ± 46.19	14.70 (7.05-226.00)	0.09
	Ostrava	72	16.87 ± 17.99	11.25 (3.72-128.00)	0.37	64	11.03 ± 8.96	8.28 (2.60-54.30)	0.06
Ethylbenzene	Prague	65	3.69 ± 2.61	2.82 (0.84-12.20)	-	60	2.90 ± 3.54	1.90 (0.83-21.00)	-
	Havirov	0	-	-	-	12	3.26 ± 4.11	1.80 (1.00-16.00)	0.80
	Karvina	23	6.11 ± 8.01	2.80 (1.32-31.30)	0.86	23	5.80 ± 7.42	3.13 (1.40-33.60)	**<0.001**
	Ostrava	72	3.56 ± 3.82	2.21 (0.83-24.90)	0.14	64	2.40 ± 1.62	1.70 (0.35-8.50)	0.31
m,p-Xylene	Prague	65	12.58 ± 8.72	10.60 (2.54-41.40)	-	60	9.14 ± 7.95	7.00 (2.73-55.60)	-
	Havirov	0	-	-	-	12	9.50 ± 11.33	5.97 (3.39-44.60)	0.19
	Karvina	23	19.74 ± 28.58	7.41 (4.43-105.00)	0.38	23	16.69 ± 20.12	8.66 (4.48-83.00)	0.07
	Ostrava	72	10.35 ± 13.06	6.45 (2.06-81.10)	**<0.001**	64	7.44 ± 5.11	5.77 (0.78-30.90)	**<0.01**
o-Xylene	Prague	65	4.43 ± 2.98	3.84 (0.89-15.40)	-	60	3.00 ± 2.73	2.28 (0.93-16.90)	-
	Havirov	0	-	-	-	12	2.76 ± 2.57	1.87 (1.17-10.50)	0.21
	Karvina	23	6.12 ± 8.00	2.80 (1.43-33.80)	0.47	23	5.27 ± 6.93	2.98 (1.12-34.70)	**<0.01**
	Ostrava	72	3.08 ± 2.10	2.31 (1.10-11.10)	**<0.001**	64	2.52 ± 1.67	1.97 (0.30-8.39)	0.069

P-values refer to comparisons between other localities and Prague.

Supplemental Material

PERSONAL EXPOSURE TO VOLATILE ORGANIC COMPOUNDS IN THE CZECH REPUBLIC

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Supplemental Material, Figure 1. The map of the Czech Republic with highlight of measured localities.

Supplemental Tables

Supplemental Material, Table 1. Personal exposures to BTEX (μg/m^3) according to time-activity questionaires in all groups by season; p values were obtained from the Mann-Whitney U-test.

	Variable		2-3/2009				5-6/2009			
			N	Mean ± SD	Median (Range)	P	N	Mean ± SD	Median (Range)	P
Benzene	Public transport	BM	93	8.28 ± 12.87	5.70 (0.52-99.70)	-	98	7.62 ± 14.76	4.34 (1.82-111.00)	-
		AM	72	5.53 ± 3.28	4.57 (2.14-19.40)	**<0.05**	62	4.44 ± 2.79	3.58 (1.21-15.90)	**<0.05**
	Car	BM	81	5.33 ± 3.07	4.32 (0.52-19.40)	-	85	4.87 ± 4.09	3.48 (1.21-31.00)	-
		AM	84	8.76 ± 13.47	5.82 (2.48-99.70)	**<0.01**	75	8.11 ± 16.51	4.49 (1.82-111.00)	**<0.05**
	At home	BM	62	6.40 ± 9.86	4.14 (0.52-76.20)	-	68	7.18 ± 16.82	3.19 (1.21-111.00)	-
		AM	103	7.49 ± 10.06	5.93 (2.36-99.70)	**<0.01**	92	5.81 ± 5.70	4.62 (1.52-44.70)	**<0.01**
	Passive smoking	0	152	7.05 ± 10.37	5.16 (0.52-99.70)	-	146	6.58 ± 12.28	4.26 (1.21-111.00)	-
		Above 0	13	7.34 ± 2.00	7.12 (4.20-12.20)	**<0.01**	14	4.34 ± 2.12	3.46 (2.11-8.78)	0.52
	Travelling	BM	74	7.69 ± 11.50	6.05 (0.52-99.70)	-	25	9.27 ± 17.54	3.62 (2.18-90.90)	-
		AM	91	6.58 ± 8.56	4.85 (2.14-76.20)	**<0.05**	135	5.85 ± 10.35	4.24 (1.21-111.00)	0.47
	Ventilation by window	BM	78	6.19 ± 5.18	4.80 (0.52-34.70)	-	72	6.68 ± 11.52	4.18 (1.21-90.90)	-
		AM	87	7.87 ± 12.81	5.68 (2.36-99.70)	0.21	88	6.15 ± 12.01	4.20 (1.52-111.00)	0.5
	Indoors	BM	80	5.79 ± 8.82	3.90 (0.52-76.20)	-	76	7.29 ± 16.14	3.59 (1.71-111.00)	-
		AM	85	8.29 ± 10.86	6.47 (2.48-99.70)	**<0.001**	84	5.57 ± 5.28	4.59 (1.21-44.70)	0.0759
	Outdoors	BM	78	7.65 ± 11.01	6.12 (0.52-99.70)	-	69	6.31 ± 11.62	4.11 (1.52-90.90)	-
		AM	87	6.56 ± 8.98	4.11 (2.14-76.20)	**<0.01**	91	6.44 ± 11.93	4.35 (1.21-111.00)	0.9
	At work	BM	73	8.04 ± 11.39	6.26 (0.52-99.70)	-	74	7.31 ± 13.02	4.99 (1.52-111.00)	-
		AM	92	6.31 ± 8.67	4.24 (2.14-76.20)	**<0.001**	86	5.59 ± 10.57	3.36 (1.21-90.90)	**<0.001**
	Restaurant	0	145	6.52 ± 7.24	5.20 (0.52-76.20)	-	127	5.58 ± 8.47	4.11 (1.21-90.90)	-
		Above 0	20	11.08 ± 21.02	6.50 (2.62-99.70)	0.12	33	9.49 ± 19.80	4.50 (2.13-111.00)	0.66
Toluene	Public transport	BM	93	39.09 ± 211.74	13.00 (0.39-2050.00)	-	98	20.59 ± 27.71	11.70 (3.24-226.00)	-
		AM	72	37.10 ± 91.50	11.85 (3.54-490.00)	0.95	62	11.50 ± 13.00	8.28 (2.60-97.60)	**<0.01**
	Passive smoking	0	152	37.05 ± 173.08	12.15 (0.39-2050.00)	-	146	15.33 ± 16.25	8.92 (2.60-97.60)	-
		Above 0	13	51.99 ± 126.80	14.30 (6.99-473.00)	0.34	14	35.22 ± 58.54	13.85 (5.22-226.00)	**<0.05**
	Ventilation by window	BM	78	54.51 ± 239.88	11.80 (0.39-2050.00)	-	72	23.33 ± 32.53	11.30 (2.60-226.00)	-

		AM	87	23.62 ± 53.83	13.30 (3.54-490.00)	0.83	88	11.94 ± 9.37	8.34 (3.07-51.20)	<**0.01**
	Outdoors	BM	78	15.65 ± 17.35	10.90 (0.39-128.00)	-	69	15.11 ± 17.65	8.31 (2.60-82.90)	-
		AM	87	58.46 ± 231.79	13.80 (3.54-2050.00)	<**0.01**	91	18.55 ± 27.13	11.50 (3.24-226.00)	0.065
	Restaurant	0	145	37.36 ± 176.82	12.20 (0.39-2050.00)	-	127	16.60 ± 25.25	8.41 (2.60-226.00)	-
		Above 0	20	44.49 ± 106.67	15.10 (3.54-490.00)	0.27	33	18.85 ± 15.27	14.30 (3.89-70.60)	<**0.01**
Ethylbenzene	Public transport	BM	93	4.28 ± 5.27	2.75 (0.10-31.30)	-	98	3.75 ± 4.62	2.30 (0.89-33.60)	-
		AM	72	3.42 ± 2.52	2.56 (1.02-12.20)	0.74	62	2.30 ± 2.56	1.73 (0.35-20.20)	<**0.001**
	Ventilation by window	BM	78	3.99 ± 4.79	2.52 (0.10-31.30)	-	72	3.46 ± 3.43	2.35 (0.35-20.40)	-
		AM	87	3.83 ± 3.85	2.69 (0.83-26.40)	0.94	88	2.96 ± 4.43	1.84 (0.80-33.60)	<**0.01**
	Car	BM	81	3.42 ± 3.41	2.45 (0.10-24.90)	-	85	2.83 ± 4.15	1.86 (0.35-33.60)	-
		AM	84	4.37 ± 5.00	2.85 (1.02-31.30)	0.15	75	3.59 ± 3.83	2.23 (0.92-21.00)	<**0.05**
	Outdoors	BM	78	3.20 ± 2.85	2.21 (0.10-16.80)	-	69	2.47 ± 2.53	1.78 (0.35-20.40)	-
		AM	87	4.54 ± 5.22	2.80 (0.84-31.30)	<**0.05**	91	3.73 ± 4.78	2.07 (0.83-33.60)	<**0.05**
m.p-Xylene	Public transport	BM	93	13.55 ± 18.59	7.41 (0.28-105.00)	-	98	11.16 ± 12.05	7.09 (2.73-83.00)	-
		AM	72	10.87 ± 8.19	8.19 (3.20-41.40)	0.94	62	7.40 ± 6.96	5.96 (0.78-55.60)	<**0.01**
	Car	BM	81	10.75 ± 10.85	7.56 (0.28-78.20)	-	85	8.63 ± 10.63	6.37 (0.78-83.00)	-
		AM	84	13.94 ± 18.04	8.32 (3.27-105.00)	0.29	75	10.91 ± 10.32	7.22 (2.98-55.20)	<**0.05**
	Ventilation by window	BM	78	12.79 ± 15.42	7.95 (0.28-102.00)	-	72	11.19 ± 9.90	7.66 (0.78-55.60)	-
		AM	87	12.01 ± 14.67	7.63 (2.06-105.00)	0.65	88	8.49 ± 10.90	6.21 (2.73-83.00)	<**0.001**
	Indoors	BM	80	14.50 ± 17.13	9.67 (0.28-105.00)	-	76	10.96 ± 12.58	7.04 (2.73-83.00)	-
		AM	85	10.38 ± 12.43	6.49 (2.06-81.10)	<**0.01**	84	8.57 ± 8.13	6.07 (0.78-54.40)	<**0.01**
	Outdoors	BM	78	9.63 ± 10.33	6.44 (0.28-81.10)	-	69	7.97 ± 6.98	5.94 (0.78-54.40)	-
		AM	87	14.84 ± 17.89	8.83 (2.54-105.00)	<**0.01**	91	11.02 ± 12.43	6.92 (2.73-83.00)	<**0.05**
	At work	BM	73	11.70 ± 14.56	6.84 (0.28-81.10)	-	74	9.73 ± 11.41	6.48 (0.78-83.00)	-
		AM	92	12.92 ± 15.38	8.61 (2.54-105.00)	<**0.05**	86	9.67 ± 9.75	6.87 (2.73-55.60)	0.32
o-Xylene	Public transport	BM	93	4.26 ± 4.67	2.73 (0.28-33.80)	-	98	3.66 ± 4.10	2.38 (0.93-34.70)	-
		AM	72	3.68 ± 2.58	2.85 (1.07-12.00)	0.92	62	2.43 ± 2.15	1.97 (0.30-16.90)	<**0.01**
	Car	BM	81	3.47 ± 2.54	2.73 (0.28-12.00)	-	85	2.93 ± 4.06	2.05 (0.30-34.70)	-
		AM	84	4.53 ± 4.83	2.88 (1.07-33.80)	0.23	75	3.46 ± 2.79	2.36 (1.02-14.10)	<**0.05**
	Ventilation by window	BM	78	4.20 ± 4.56	2.82 (0.28-33.80)	-	72	3.67 ± 3.14	2.62 (0.30-16.90)	-
		AM	87	3.83 ± 3.22	2.59 (0.89-22.70)	0.72	88	2.78 ± 3.78	2.00 (0.90-34.70)	<**0.001**
	Indoors	BM	80	4.85 ± 4.96	3.72 (0.28-33.80)	-	76	3.66 ± 4.54	2.38 (0.93-34.70)	-

	AM	85	3.21 ± 2.29	2.33 (1.10-11.10)	**<0.01**	84	2.75 ± 2.19	1.97 (0.30-13.10)	**<0.01**
Outdoors	BM	78	3.13 ± 2.24	2.20 (0.28-11.10)	-	69	2.59 ± 1.92	2.00 (0.30-13.10)	-
	AM	87	4.79 ± 4.82	3.24 (0.89-33.80)	**<0.01**	91	3.62 ± 4.32	2.30 (0.93-34.70)	**<0.05**

BM-below median; AM-above median.

Indoors-hours spent indoors; outdoors-hours spent outdoors.

Supplemental Material, Table 2. BTEX (µg/m^3), PM2.5 or PM10 (µg/m^3) and temperature (°C) from stationary monitoring in Prague and Ostrava during the period of personal monitoring.

Locality	Period	Benzene		Toluene		Ethylbenzene		m,p-Xylene		o-Xylene		PM2.5 or PM10[*)]		t (°C)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Prague-Smichov	08.02.-20.02.2009	1.4	0.4	1.7	0.7	0.2	0.1	0.8	0.6	0.2	0.2	19.2	5.5	-0.8	2.1
Ostrava-Privoz	02.03.-13.03.2009	5.4	5.7	1.5	1.3	0.2	0.2	0.8	0.5	0.1	0.1	48.0	33.5	4.4	2.1
Ostrava-Fifejdy	02.03.-13.03.2009	3.8	2.1	2.3	1.5	0.5	0.6	1.8	2.2	0.3	0.4	39.7 *)	26.2		
Ostrava-Ceskobratrska	02.03.-13.03.2009	3.3	1.6	2.1	1.2	0.4	0.3	0.3	0.2	0.5	0.3	46.0 *)	28.3		
Prague-Smichov	17.05.-27.05.2009	0.8	0.2	3.0	1.0	0.4	0.2	3.6	1.5	0.6	0.3	14.8	3.8	18.2	2.7
Ostrava-Privoz	10.06.-19.06.2009	5.7	3.7	1.9	1.6	0.3	0.2	0.4	0.6	0.2	0.3	18.9	5.0	17.6	3.7
Ostrava-Fifejdy	10.06.-19.06.2009	2.8	2.4	2.0	2.3	0.3	0.2	0.7	0.5	0.2	0.1	19.1 *)	5.1		
Ostrava-Ceskobratrska	10.06.-19.06.2009	2.3	0.6	3.2	1.8	0.1	0.0	0.1	0.1	0.3	0.2	21.5 *)	3.8		

Source: Czech Hydrometeorological Institute. *) PM10

PM2.5 were measured by Environnement SA, MP 101M analyzed by gravimetry in Prague and Thermo ESM Andersen, FH 62 I-R analyzed by radiometry in Ostrava.
PM10 were measured by Environnement SA, MP101M analyzed by radiometry in Prague, Thermo ESM Andersen, FH 62 I-R measured in Ostrava Fifejdy and Privoz analyzed by radiometry and Digitel Elektronik, DHA 80 measured in Ostrava-Ceskobratrska analyzed by gravimetry.

Supplemental Material, Table 3. Bivariate associations between personal levels (μg/m^3) and concentrations of BTEX (μg/m^3) measured using stationary monitors by the Czech Hydrometeorological Institute in Prague and Ostrava during the day of personal monitoring.

		2-3/2009				**5-6/2009**			
	Region	N	Intercept	B	P	N	Intercept	B	P
Benzene	All	137	3.55	1.13	**<0.01**	123	2.74	1.26	**<0.001**
	Prague	65	5.50	-0.17	0.96	60	2.89	0.54	0.62
	Ostrava	72	1.53	1.66	**<0.001**	63	4.12	0.83	**<0.05**
Toluene	All	137	24.65	0.94	0.87	123	4.59	1.43	**<0.01**
	Prague	65	16.75	12.53	0.52	60	-0.97	2.65	0.08
	Ostrava	72	14.03	1.36	0.43	63	7.22	1.11	**<0.05**
Ethylbenzene	All	137	3.43	0.64	0.38	123	1.12	10.31	**<0.001**
	Prague	65	4.96	-8.38	**<0.05**	60	4.36	5.64	0.30
	Ostrava	72	3.12	1.05	0.25	63	1.59	3.90	0.07
m,p-Xylene	All	137	11.22	0.19	0.85	123	7.23	-0.45	**<0.05**
	Prague	65	14.48	-2.64	0.19	60	4.15	0.18	0.59
	Ostrava	72	8.97	1.16	0.39	63	4.68	4.30	**<0.05**
o-Xylene	All	137	3.69	0.10	0.92	123	0.53	18.88	**<0.001**
	Prague	65	5.46	-4.03	0.09	60	9.03	8.37	0.45
	Ostrava	72	2.59	1.62	0.06	63	0.81	10.34	**<0.05**

B-row regression coefficient.