HODNOCENÍ EXPOZICE CHEMICKÝM LÁTKÁM A JEJICH RIZIK V LIDSKÉ POPULACI

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CENTRUM PRO VÝZKUM TOXICKÝCH LÁTEK V PROSTŘEDÍ

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Habilitační práce

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Habilitační práce

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Poděkování

Na tomto místě bych chtěl poděkovat své rodině, přátelům a také především svým kolegům za jejich podporu, pomoc a inspiraci.

OBSAH

Seznam použitých zkratek

ADME	Adsorpce, Distribuce, Metabolismus, Eliminace (*Absorption, Distribution, Metabolism, Elimination*)
C	Koncentrace dané látky v expoziční matrici (*Concentration*)
CA	Kometový test (*Comet Assay*)
CDI	Chronický denní příjem (*Chronic Daily Intake*)
D	Difuzní koeficient (*Diffuse coefficient*)
DAD	Celková dermální absorbovaná dávka (*Dermally Absorbed Dose*)
DA_{event}	Absorbovaná dávka při jednom případu dermální expozice
DDD	1,1-dichloro-2,2-bis-(4-chlorfenyl)-ethan
DDD	1,1-dichloro-2,2-bis-(4-chlorfenyl)-ethan
DDE	1,1-dichlor-2,2,-bis(4-chlorfenyl)-ethylen
DDT	1,1,1-trichlor-2,2,-bis(4-chlorfenyl)-ethan
DDX	Označení pro sumu DDT a jejich metabolitů ve vzorku
δC	Rozdíl koncentrací na různých stranách membrány
ED	Trvání expozice (*Exposure Duration*)
EDC	Látky způsobující endokrinní disrupci (*Endocrine Disrupting Chemicals*)
EF	Frekvence expozice (*Exposure Frequency*)
EHBMI	Evropská iniciativa lidského biomonitoringu *(European Human Biomonitoring Initiative*)
EHMC	Ethylhexyl methoxycinnamát
EMEP	Evropský monitoring a hodnotící program (*European Monitoring and Evaluation Programme*)
ET	Doba expozice (*Exposure duration*)
FR	Zhášeče hoření (*Flame retadrants*)
GAČR	Grantová agentura České republiky
GENASIS	Globální informační systém pro hodnocení životního prostředí (*Global Environmental Assessment and Information System online database*)
GIS	Geografický informační systém (*Geographic Information System*)
GIT	Gastrointestinální trakt (*Gastrointestinal Tract*)
GWAS	Srovnávací asociační studie genomů (*Genome-Wide Association Studies*)
HBM	Lidský biomonitoring (*Human Biological Monitoring*)
HCB	Hexachlorobenzen
HCH	Hexachlorcyklohexan (α-HCH, β-HCH, γ-HCH)
HEAR	Odborná skupina "Hodnocení humánní expozice a zdravotních rizik" (*Human Exposure Assessment and Risks*)
HHRA	Hodnocení zdravotních rizik (*Human Health Risk Assessment*)
IDW	Metoda interpolace v GIS (*Inverse Distance Weighting*)
IR	Příjmová rychlost (*Intake Rate*)
log K_{ow}	Logaritmus rozdělovacího koeficientu v systému *n*-oktanol/voda
J	Difůzní tok (*Flux*)
Kp	Koeficient permeability
NUTS	Označení normalizované klasifikace územních celků v ČR
MDE	Monitoring dietární expozice SZÚ

MZSO	Systém monitorování zdravotního stavu obyvatelstva České republiky ve vztahu k životnímu prostředí
OCP	Organochlorové pesticidy
PAS	Pasivní vzorkovače
PBPK	Fyziologicky založené Farmakokinetické Modely (*Physiogically based Pharmacokinetic Models*)
PBTK	Fyziologicky založené toxikokinetické modely (*Physiogically based Toxicokinetic Models*)
PCDDs	polychlorované dibenzo-p-dioxiny (furany - PCDFs)
PCA	Analýza hlavních komponent (*Principal Component Analysis)*
PCB	Polychlorované bifenyly (*Polychlorinated Biphenyls*)
PFC	Perfluorované sloučeniny (*Perfluorinated Compounds*)
PK	Farmakokinetika (*Pharmacokinetics*)
PM	Pevné - prachové částice s informací o velikosti v µm (*Particulate Matter – PM*)
POPs	Persistentní organické polutanty (*Persistent Organic Pollutants*)
POPRC	Výbor pro hodnocení POPs (*Persistent Organic Pollutants Review Committee of the Stockholm Convention*)
PUF	Polyuretanová pěna - materiál pro pasivní vzorkovače (*Polyuretane foam*)
QIVIVE	Metoda Kvantitativní extrapolace dat in vitro - in vivo (*Quantitative in vitro-to-in vivo extrapolation*)
REZZO	Registr emisí a zdrojů znečištění ovzduší
RfD	Referenční dávka (*Reference Dose*)
SA	Povrch kůže dostupný expozici (*Surface Area*)
SCOPUS	*Database ELSEVIER Scopus©,*
SCGE	Metoda metodu buněčné gelové elektroforézy (*Single-Cell Gel Electrophoresis*)
SVOC	Semivolatilní organické sloučeniny (*Semi-volatile organic compounds*)
SÚ	Stockholmská úmluva (*Stockholm Convention*)
SZÚ	Státní zdravotní ústav
$t_{1/2}$	Biologický poločas eliminace (*Biological Half-time*)
TDI	Tolerovaná denní dávka (*Tolerable Daily Intake*)
TK	Toxikokinetika (*ToxicoKinetics*)
UNEP	Program organizace spojených národů pro životní prostředí (*United Nations Environment Programme*)
WHO	Světová zdravotnická organizace (*World Health Organization*)
WOS	*Web of Science™*

1. ABSTRAKT

Problematika výskytu chemických látek v okolním prostředí je především spojována s potenciální možností přestupu do lidského organismu. Jedná se o expozici člověka, která přichází v úvahu pouze v případě, že je člověk vystaven kontaktu s chemickými látkami. Tento expoziční transport má však mnoho přirozených a aktivně fungujících barier. Pokud však dojde k tomuto přestupu, může se u některých látek projevit negativní biologický efekt. K pochopení principu transportu do lidského těla je nutné celý proces expozice detailně studovat. Důležitý je exaktní popis osudu toxikologicky významných chemických látek v prostředí s důrazem na prostorovou a časovou variabilitu výskytu a na identifikaci hlavních parametrů, které mají na výskyt v prostředí dominantní vliv. Zásadní je také charakteristika podmínek samotné expozice a hodnocení následné toxikokinetiky. Expoziční dávka, následná distribuce a osud chemické látky pak totiž v lidském těle významně ovlivňuje možný výsledný biologický efekt. Z detailních dat o expozici konkrétní chemické látky a s využitím současných znalostí toxikodynamiky lze pak provést predikci potenciálních zdravotních rizik (Calabrese, 2014). Hodnocení zdravotních rizik je tedy zároveň metodou interpretace dat o expozici.

Tyto přístupy hodnocení a interpretace dat o expozici toxikologicky významným chemickým látkám a z nich plynoucích potenciálních zdravotních rizik v lidské populaci jsou v odborné skupině autora habilitační práce často využívány v mnoha vědeckých studiích.

Hlavní výzkumné aktivity odborné skupiny "Human Exposure Assessment and Risks" (HEAR) pod vedením autora habilitační práce jsou uvedeny v následujícím seznamu:

- Nové metody hodnocení a interpretace výskytu a osudu nebezpečných látek v prostředí (v půdě, sedimentech, ve vodách, ve volném ovzduší a v potravinách).
- Hodnocení významu podílu expozičních cest, scénářů a expozičních parametrů na celkových přijatých dávkách (dietární, inhalační a dermální expozice).
- Retrospektivní hodnocení expozice – využití biomonitorovacích studií pro zpětnou rekonstrukci expozice (pomocí biomonitoringu mateřského mléka v lidské populaci – v ČR).
- Vývoj nových metod hodnocení expozice.
- Pravděpodobnostní predikce rizik v lidské populaci.
- Cílené studie pro zpřesňování kinetiky expozice vybraným chemickým látkám – SkinRISK projekt (GAČR) a využití těchto parametrů pro toxikokinetické (PBTK) modely.

Tyto nejvýznamnější vědecké aktivity jsou více popsány v následujících kapitolách této habilitační práce. Je zde stručně shrnut současný stav poznání s důrazem na aktuální nové směry vývoje v této oblasti výzkumu a v jeho kontextu jsou prezentovány vlastní výsledky autora v podobě přiložených publikací.

1. ABSTRACT

The presence of chemical substances in the environment is associated with a potential for their transfer to the human body**.** Human exposure comes into consideration only if the person is exposed to chemicals. This exposure transport has many naturally and actively functioning barriers. However, if exposure occurs, the transfer of certain substances may cause negative biological effects. To understand the principle of transport into the human body, it is necessary to study the whole process of exposure in detail. The exact description of the fate of toxicologically-relevant chemical substances in the environment is very important, with an emphasis on the spatial and temporal variability of their occurrence and identification of main parameters that have a dominant influence on the environmental presence of chemicals. Other crucial influences are also the characteristics of the conditions of exposure and subsequent evaluation of toxicokinetics. Thus, the exposure dose, its distribution and the fate of chemicals in the human body significantly affects the possible final biological effect. The prediction of potential health risks can be realised from detailed data about exposure to an exact chemical substance and by use of present knowledge about toxicodynamics (Calabrese, 2014). Therefore, evaluation of health risks is simultaneously a method for interpretation of data about exposure.

These main approaches of evaluation, interpretation of exposure data for toxicologically-important chemicals and the resulting potential health risk in the human population are used in many studies in this author´s habilitation thesis. A list of research activities of the group "**Human Exposure Assessment and Risks**" (**HEAR**) under the supervision of author is in the following part:

- New methods of evaluation and interpretation of the occurrence and fate of hazardous substances in the environment (soil, sediment, water, ambient air and foodstuffs).
- Evaluation of the proportion of exposure pathways and parameters on the total received doses (dietary, inhalation and dermal exposure).
- Retrospective exposure assessment – the use of biomonitoring studies for retrospective reconstruction of exposure (biomonitoring of breast milk in the human population in CR).
- The development of new methods for exposure assessment.
- Probabilistic risk prediction in human population.
- Targeted studies for refinement of exposure of selected chemical substances – SkinRISK project (GAČR) and use of these parameters for toxicokinetic (PBTK) models.

The most important scientific activities are further described in the following chapters in this habilitation thesis. Here is summarised the current state of knowledge, with an emphasis on current new developments in this field of research. The author´s own results are presented in the attached publications, also in the context of the above-described research activities.

2. Úvod

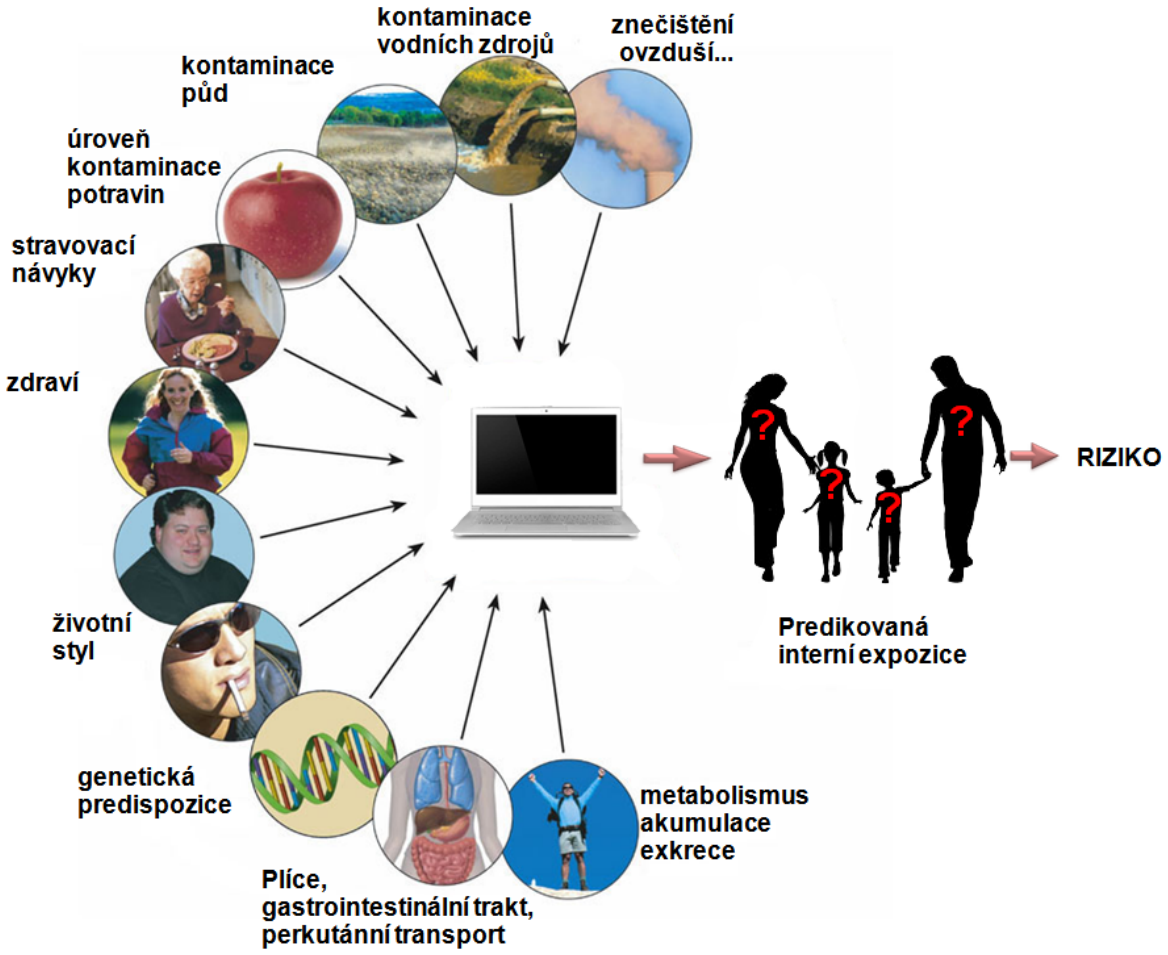
V minulosti bylo uvedeno na trh mnoho nových, mnohostranně využitelných průmyslových látek či pesticidů (Holoubek et al., 2006), u kterých byly následně identifikovány významné negativní efekty a to jak na lidském zdraví, tak i na životním prostředí (Jones and De Voogt, 1999). Mezi tyto látky patří především perzistentní organické polutanty (POPs). Mnoho z nich však již podléhá globální Stockholmské úmluvě (SÚ), která vstoupila v platnost roku 2004 (www.pops.int). Cílem SÚ je zákaz, omezení a případně regulace produkce, distribuce či nakládání 26 chemických látek, jejich metabolitů, isomerů, či solí. Například v posledním roce 2015 byla na seznam zařazena skupina chlorovaných naftalenů, hexachlorbudadien a pentachlorfenol. I když je tato úmluva v platnosti již několik let (od roku 2004), stále lze nalézt tyto látky v matricích životního prostředí a také i ve vzorcích v lidské populaci. Česká republika má například ve srovnání s jinými evropskými zeměmi významně vyšší hladiny PCB v mateřském mléce i přesto, že byla jejich produkce zakázána již v roce 1984 (Fång et al., 2015; Holoubek et al., 2009; Malisch and Van Leeuwen, 2003).

Významnou aktivitou SÚ je také kontinuální hodnocení účinnosti výše zmíněných opatření na eliminaci výskytu těchto látek v životním prostředí (Klánová and Harner, 2013). Z této aktivity plyne jeden z hlavních cílů SÚ, což je snižovat u těchto látek celkovou chronickou expozici v lidské populaci. Co nejpřesnější retrospektivní a prospektivní hodnocení expozice je tedy zcela zásadním a klíčovým nástrojem. V současné době je hodnocení účinnosti opatření realizováno formou monitoringu identifikovaných klíčových matric: mateřského mléka v lidské populaci (Mikeš et al., 2012) a volného ovzduší (Fiedler et al., 2013). Monitoring výskytu toxikologicky významných chemických látek v hlavních matricích poskytuje užitečné podklady pro hodnocení dlouhodobých trendů potenciální expozice a z nich plynoucích rizik (Přibylová et al., 2012; Roots et al., 2015, 2010).

Je nutné zdůraznit, že při aplikaci přístupu dvou klíčových matric je hodnocena a interpretována screeningově jen část možných expozičních cest (z hlediska SÚ jde o ovzduší a mateřské mléko). U volného ovzduší se jedná o hodnocení nabídnuté, externí expoziční dávky při inhalačním scénáři (Čupr et al., 2013). V případě mateřského mléka se jedná o hodnocení interní expoziční hladiny kojících matek, což je výsledek dlouhodobé celoživotní komplexní expozice (Ritter et al., 2009), ale bez primární znalosti hlavních expozičních cest (Gyalpo et al., 2012). Proto je velmi důležité směřovat výzkumné aktivity i k ostatním typům expozice a vyvíjet takové metody, které budou schopny tyto predikce zpřesňovat a to jak u prospektivních, tak i retrospektivních metod. Zpřesňování odhadů dílčích vstupů expozičních cest přispěje k identifikaci hlavních zdrojů těchto toxických látek (například - textil při dermální expozici, dominantně při sportovní aktivitě; či konkrétní potravinová komodita při dietární expozici) a tím pádem k možnosti účinně omezovat zdravotní rizika pro lidskou populaci (Bányiová et al., 2015).

Zdravotní rizika pro člověka v podobě reálného výskytu chronických poruch a onemocnění je však třeba dávat do souvislosti nejen s environmentálními faktory (patří sem zde popisovaná celoživotní expozice chemickým látkám - toxickým polutantům), ale také do souvislosti s genetickými dispozicemi ve sledované populaci, sociálními a ekonomickými faktory, se stárnutím a s celou řadou dalších faktorů (Obrázek 1).

Srovnávací studie genomu "genome-wide association studies" (GWAS) mohou k vysvětlení příčin některých chorob významnou měrou přispívat, ale k vysvětlení incidence a jejich trendů jako jediný zdroj informací rozhodně také nestačí (Bogdanos et al., 2013).

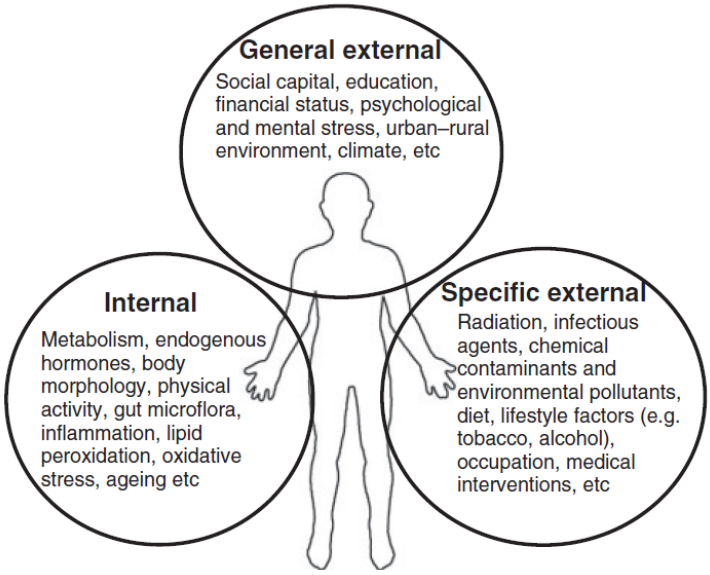


Obrázek 1 Přehled základních faktorů, které mohou ovlivňovat celkovou interní expozici a výsledná rizika.

V posledních letech se tedy pozornost vědeckých studií přesouvá od detailního studia jednotlivých faktorů ke studiu tzv. exposomu jako záznamu celoživotní expozice člověka od jeho početí po současnost. Velmi důležitou složkou jsou tedy vlivy jednotlivých faktorů, a také časový vývoj síly jejich vlivu v průběhu expoziční doby (Wild et al., 2013). Pojem exposom ("exposome") poprvé použil Christopher Wild v roce 2005 (Wild, 2005). Tento koncept je dále diskutován a rozvíjen v předních vědeckých časopisech (Mao and Wang, 2015; Slama and Vrijheid, 2015; Wild, 2011). Exposom lze chápat jako komplexní vliv řady faktorů, jako je znečištění vnitřního a vnějšího prostředí nebezpečnými chemickými látkami,

ale i sociální prostředí, faktory životního stylu jedince, jeho metabolismus a stav střevní mikroflóry, výživa a fyzická aktivita, odezva vlastního těla na různé druhy záření, hluk, infekci, stres, nadměrné požívání alkoholu nebo kouření (Vrijheid, 2014; Wild, 2011) (Obrázek 2). Případná nerovnováha nebo významná expozice některým z těchto faktorů může přispívat ke zvýšené zátěži jedince a v dlouhodobém horizontu tak způsobovat celou řadu chronických nemocí a poruch, zvláště u jedinců se zvýšenou vnímavostí k určitým stresorům (Wild, 2011). Integrující pohled na významnost jednotlivých faktorů podílejících se potenciálně na vzniku chorob a zhodnocení jejich váhy v rozvoji neurologických, behaviorálních či imunologických poruch mohou poskytnout dlouhodobé prospektivní studie.

V seznamu všech faktorů, které se jistou mírou podílejí na celkovém zdravotním stavu lidské populace, lze identifikovat dvě hlavní skupiny: faktory externího a interního prostředí (viz Obrázek 2). Wild i Vrijheid ve svých konceptech ještě odlišují v rámci externího prostředí specifickou skupinu (Specific External Environment), kam patří právě expozice chemických látkám (Vrijheid, 2014; Wild, 2011). Do skupiny základních externích faktorů pak patří například socio-ekonomické faktory, stres, klimatické vlivy, a podobně.



Obrázek 2 Základní schéma konceptu exposomu. Zdroj: (Wild, 2012).

Na tomto místě je tedy nutné zdůraznit, že vliv environmentálních faktorů v podobě expozice chemických látkám je jedním z mnoha významných faktorů celkového exposomu, které je potřebné do budoucích vědeckých studií zahrnout.

Tato habilitační práce se zaměřuje na hodnocení a interpretaci expozice chemickým látkám (především perzistentním organickým polutantům) a z nich plynoucích potenciálních zdravotních rizik v lidské populaci.

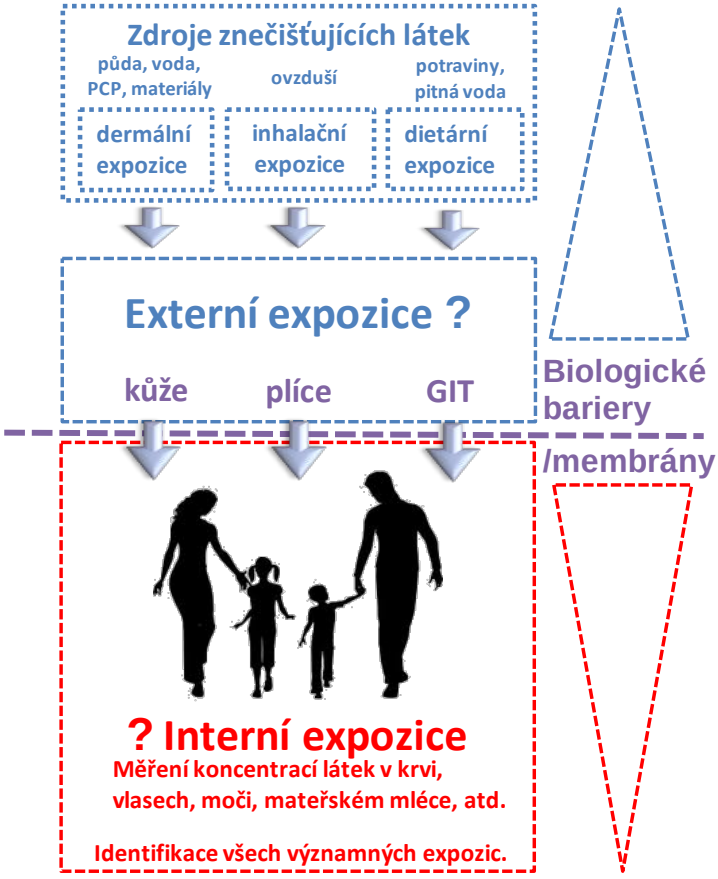
3. Metody hodnocení expozice chemickým látkám

Nutnost detailnějšího hodnocení expozice vychází z výsledků dlouhodobých biomonitorovacích studií, které často identifikují vysoké hladiny toxikologicky významných chemických látek ve vzorcích biomatric (Mikeš et al., 2012) (Příloha 15).

Expozice je dle WHO definována jako kontakt hodnocené chemické látky s vnějšími hranicemi organismu takovým způsobem, že může dojít k jejímu vstupu do organismu a tím

pádem se mohou projevit její škodlivé účinky (Knudsen et al., 2011). Klíčovým slovem v této definici je kontakt. Expoziční cestu tvoří 4 hlavní prvky. V první řadě je to zdroj a mechanizmus úniku hodnocené chemické látky. Dále pak příjmové a transportní médium či média. Důležitým prvkem je také místo možného kontaktu člověka s kontaminovaným médiem a v neposlední řadě i způsob průniku do organismu včetně jeho časové složky (délka expoziční doby) (Phillips and Moya, 2014).

Parametr, který nejvíce charakterizuje expozici je pak výsledná dávka, která vyjadřuje množství chemické látky, které skutečně vstupuje do organismu inhalací, ingescí nebo je v kontaktu s kůží. Pokud ještě nepřekročí hranice organismu, je definována jako nabídnutá dávka (také v některých publikacích označovaná jako expoziční dávka). Po překročení biologických membránových barier pak označujeme toto množství také jako vstřebanou dávku. Vstřebaná dávka je tedy množství chemické látky vstřebané jednotlivcem v definovaném čase (například v jednotkách $ng.kg^{-1}.den^{-1}$). Tyto dávky jsou pak důležitým vstupním parametrem při hodnocení možných zdravotních rizik (viz kapitola 4).



Obrázek 3 Metody hodnocení externí a interní expozice (modifikováno z původního schématu Top-down a Buttom-up Exposomic - (Rappaport, 2011).

Samotný výzkum v hodnocení expozice chemickým látkám lze provádět v zásadě dvěma metodickými postupy (Frederiksen et al., 2009). První metodou je **hodnocení expozice formou analýz matric a materiálů, které jsou v kontaktu s lidským tělem**. Tyto metody jsou také nazývány jako hodnocení **externí expozice.** Jde o takzvanou nabídnutou expozici. Druhým postupem je metoda **retrospektivního hodnocení expozice formou analýz míry kontaminace biologických vzorků,** jako je krev, mateřské mléko, moč, vlasy a podobně (Černá et al., 2015; Choi et al., 2015b). Jsou tedy vyhodnocovány jako **interní expozice**.

Obě metody cíleně zpřesňují odhady příspěvků k celkové expozici za účelem vyhodnocení možných zdravotních rizik (Lu et al., 2014). Proto je ve výzkumu velmi vhodné oba přístupy vzájemně kombinovat (Sharma et al., 2014) (Příloha 23).

Tyto dva přístupy také popsal další zakladatel konceptu exposomu Rappaport (Rappaport, 2011). První z těchto strategických metod označuje jako "bottom–up", při které jsou analyzovány nejdůležitější matrice: ovzduší, voda, potraviny. Jedná se tedy o externí expozici. Druhá z nich - "top–down" je adekvátní k metodě hodnocení interní expozice. V současných vědeckých studiích jsou používány oba dva typy označení (Gyalpo et al., 2015).

Pro hodnocení externí expozice ("buttom–up") je nutná znalost transportního mechanizmu od zdroje až k biologické membráně přestupu (kůže, gastro–intestinální trakt, plicní epitel). Čím přesněji je tato cesta popsána a charakterizována, tím je výstup z této predikce expozice shodnější s realitou. Transport je také závislý na mnoha faktorech, které je nutné co nejpřesněji definovat pro zvolené expoziční scénáře. Dalším používaným konceptem při analýze externí expozice je přístup nasazení *in vitro* testů toxicity. Při tomto přístupu jsou vzorky expoziční matrice aplikovány do testů toxicity *in vitro*. Výhodou je, že výsledek testů prezentuje komplexní biologickou odpověď na všechny přítomné chemické látky a stresory. Testy jsou schopny reagovat na výsledek případných synergických, či antagonických biologických účinků chemických směsí. Je ale nutné zdůraznit, že je zde testována kompletní nabídnutá dávka, nikoli vstřebaná - biodostupná dávka. S tímto omezením je výsledek také nutné interpretovat.

V případě hodnocení interní expozice ("top–down") formou analýz míry kontaminace biologických vzorků je výstupem výsledná koncentrace hodnocené chemické látky (či jejího metabolitu) v konkrétní tělní tekutině, či tkáni. Ta ale představuje aktuální stav interní expozice. Aby byl tento parametr použitelný pro správné vyhodnocení expozice, je zapotřebí dostatečně charakterizovat všechny děje, které mají na tuto výslednou koncentraci vliv. Jedná se primárně o procesy ADME: absorpce, distribuce, metabolismu a eliminace (Roberts and Renwick, 2014). Na základě rekonstrukce těchto procesů lze retrospektivně predikovat původní chronickou denní expoziční dávku (reverzní dozimetrie) (Abass et al., 2013; Clewell and Clewell III, 2008; Gyalpo et al., 2015; Ulaszewska et al., 2012). Čím přesnější parametrizaci těchto procesů lze aplikovat, tím je predikovaná expoziční vstřebaná dávka bližší realitě. Problematikou procesů ADME se zabývá toxikokinetika (viz kapitola 3.2.1).

V případě interní expozice by měla být také hodnocena dávka v cílovém orgánu. Jedná se o integrované množství stresoru - chemické látky v cílovém orgánu v průběhu určitého času (nutné odlišovat dávku od koncentrace v cílové matrici). Navíc biodostupná – biologicky účinná dávka tvoří pouze část celkové dávky v cílovém orgánu (Loccisano et al., 2013). U retrospektivního posouzení biodostupné dávky v cílovém orgánu jsou nezbytně nutné znalosti procesů ADME včetně důležité kinetiky transportu této látky.

Z výčtu všech hlavních přístupů je nutné také zmínit metodu hodnocení pomocí biomarkerů toxického efektu (či jinak nazývané biomarkery biologicky účinné dávky - například chromozomální aberace periferních lymfocytů, či bukálních buněk), které ale principem spadají do skupiny metod hodnocení interní expozice (Rossner et al., 2013).

3.1. Hodnocení externí expozice

Hodnocení expozice bývá velmi často prováděno formou analýz matric a materiálů, u kterých lze předpokládat kontakt s lidským tělem. Hodnoceným parametrem externí expozice ("buttom–up approach") je pak nabídnutá dávka chemické látky. Výběr matric a materiálů je podmíněn fyzikálně-chemickým parametrům chemické látky a jejím schopnostem transportu od zdroje až po místo kontaktu.

V rámci procesu hodnocení se provádějí screeningové predikční výpočty expozice, tj. přijatých dávek a to podle základních expozičních rovnic specifických pro jednotlivé expoziční scénáře (Bartoš et al., 2009; Čupr et al., 2013; Hayes and Kruger, 2014).

Základní rovnice pro výpočet chronického denního příjmu (CDI – chronic daily intake):

CDI = C x IR x ET x EF x ED / (BW x AT)

CDI – chronický denní příjem (obecně příjem) [např. v $ng.kg^{-1}.den^{-1}$]

C – koncentrace dané látky v expoziční matrici

IR – příjmová rychlost (Intake Rate; například inhalace vzduchu v $m^3.hod^{-1}$ dle převládající fyzické aktivity participantů studie) (EPA, 2011)

ET – doba expozice (Exposure Time)

EF – frekvence expozice (Exposure Frequency)

ED – trvání expozice (Exposure duration) [rok]

BW – váha těla [kg]

AT – doba průměrování [den]

(Fjeld et al., 2007; López-Roldán et al., 2015; Phillips and Moya, 2014)

Cílem predikce by měl být odhad chronického příjmu, tedy příjmu, který podle dostupných informací lze dlouhodobě předpokládat (CDI).

V následujících kapitolách jsou uvedeny příklady hodnocení externí expozice chemickým látkám s využitím znalosti jejich osudu v prostředí.

3.1.1. Osud chemických látek v prostředí

Chemické látky označované jako perzistentní organické polutanty (POPs) jsou stále v centru pozornosti, protože představují rizika jak pro lidské zdraví, tak i pro ekosystémy (Gevao et al., 2010; Jones and De Voogt, 1999; Konkel, 2014; Lohmann et al., 2001; Taylor et al., 2013). Mezi nejdůležitější kritéria kategorizace látek do skupiny perzistentních organických polutantů jsou: perzistence v prostředí, schopnost významné bioakumulace, potenciál "long–range" transportu (transport na dlouhé vzdálenosti) a navíc mají tyto látky toxikologicky významné negativní efekty. Patří mezi ně nejen organochlorové pesticidy (OCPs: například p,p‘-DDT, hexachlorcyklohexan, hexachlorbenzen), polychlorované bifenyly

(PCBs), či polycyklické aromatické uhlovodíky (PAHs), ale jsou to také přehodnocené látky, jejichž evaluace byla provedena až na základě novějších vědeckých studií.

Chemické látky, které jsou z různých zdrojů emitovány do prostředí, totiž dále podléhají nejrůznějším transportním a transformačním procesům (chemická či fotochemická degradace, biotransformace, finální biodostupnost atd.) (Klán et al., 2003). POPs se mohou díky svým vlastnostem také kumulovat jak v abiotických složkách prostředí, tak především v živých organismech (v lidských tkáních; jsou rozpustné v tucích). Navíc u nich často dochází k dálkovému transportu přes ovzduší do oblastí, kde se ani nepoužívaly ani nevyráběly. Proto je znalost těchto jevů důležitá pro korektní hodnocení externí expozice v lidské populaci.

Proces evaluace nových kandidátských POPs v současné době provádí tým mezinárodní odborníků v rámci Výboru pro hodnocení POPs – POPRC: Persistent Organic Pollutants Review Committee of the Stockholm Convention (Stockholm Convention, 2015). Za Českou republiku je nyní členem výboru právě autor této habilitační práce.

3.1.2. Inhalační expozice

Pro hodnocení externí expozice inhalační cestou je velmi důležitá znalost koncentrací toxikologicky významných chemických látek v ovzduší. Koncentrace v ovzduší jsou ovlivňovány mnoha faktory prostředí. Za důležité procesy kontrolující hladiny semivolatilních látek v atmosféře jsou také považovány procesy výměny na rozhraní půdy a volného ovzduší (Růžičková et al., 2008). Intenzita těkání je ovlivněna fyzikálně–chemickými vlastnostmi sledovaných látek (tlak nasycené páry, rozpustnost), množstvím a kvalitou organického materiálu, písku a jílu v půdě, a také klimatickými podmínkami (Kobližková et al., 2009) (publikace – jejímž výstupem je experimentální stanovení hodnot volatilizačních toků pro jednotlivé polutanty).

V ovzduší pak dochází k rozdělování SVOC mezi jednotlivé složky atmosféry v závislosti na teplotě, dostupnosti prachové frakce a fyzikálně-chemických vlastnostech jednotlivých látek (Landlová et al., 2014; Radonic et al., 2009) (Příloha 19). Právě tato distribuce POPs mezi jednotlivé frakce volného ovzduší je rozhodující pro další osud těchto látek, protože na ní závisí doba jejich života v atmosféře (různá degradační rychlost či účinnost suché a mokré depozice) a tím pádem i schopnost dálkového transportu.

3.1.2.1. Význam atmosférických částic pro inhalační expozici

Při inhalaci dochází ke vdechování nejen plynné složky, ale také i prachových částic. Do intenzivního výzkumu distribuce chemických látek na různé velikostní frakce, který probíhá v posledních letech, přispěl také autor této habilitace (Čupr et al., 2013; Degrendele et al., 2016, 2014; Landlová et al., 2014; Novák et al., 2014; Okonski et al., 2014). V následujícím textu jsou popsány cíle a hlavní výstupy našich studií, které přispěly k lepšímu poznání významu atmosférických částic pro hodnocení inhalačních rizik.

Pro zjištění koncentrace látky v ovzduší za účelem vyhodnocení inhalační externí expozice lze využít jak aktivní, tak i pasivní typy vzorkovačů. Při vzorkování by měly být odebírány obě složky – plynná frakce i prachové částice (Bartoš et al., 2009) (Příloha 9). Navíc při vhodné volbě odběrového zařízení lze prachové částice při odběru separovat na dílčí velikostní frakce. To umožnilo v případové studii (Čupr et al., 2013) provést separátní analýzy až 6 velikostních frakcí: <0.49 µm, 0.49-0.95 µm, 0.95-1.5 µm, 1.5-3.0 µm, 3.0-7.2 µm a 7.2-10 µm (Příloha 18). Cílem této studie byla detailní analýza atmosférických prachových částic s aerodynamickým průměrem menším nebo rovným 10 µm (PM10) a to pomocí nového kombinovaného přístupu gravimetrické, chemické, mineralogicko-morfologické a toxikologické charakterizace různých velikostních frakcí typově odlišných atmosférických částic. Do plicních alveol se totiž při inhalaci dostávají především jemné až ultrajemné částice (menší než PM2,5) (Oberdörster and Utell, 2002). Proto byly všechny vyjmenované analýzy provedeny shodně na všech velikostních frakcích. Záměrem analýz bylo přispět ke komplexnímu poznání polétavého prachu, který se značně podílí na znečištění atmosféry a představuje potenciální zdravotní rizika při inhalaci (Kampa and Castanas, 2008; Rossner et al., 2013). Součástí naší studie byla také predikce celkových přijatých dávek z inhalační expozice, vztažené k výsledkům expozičních koncentrací (metodika popsána v článku Čupr et al., 2013). Finálně pak byla vypočítána pravděpodobnostní zdravotní rizika. Zvlášť byly hodnoceny dílčí frakce a jejich podíly k celkovým zdravotním rizikům. Výsledek jasně potvrdil vstupní hypotézu, že největší příspěvky do celkově přijaté dávky a tím i zdravotních rizik byly právě u nejjemnějších frakcí PM0.45 – PM0.95. Bylo to také potvrzeno i nejvyšším přímým i nepřímým genotoxickým potenciálem *in vitro* v této frakci (potvrzeny přítomné genotoxické a pregenotoxické látky). Výsledek je ve shodě i s porovnávanými hodnotami celkového aktivního povrchu frakcí částic, kde největší hodnoty povrchů jsou právě u ultrajemných frakcí. Významná zdravotní rizika byla aplikovaným modelem potvrzena ve třech nejmenších frakcích: tedy v <0.49 µm, 0.49-0.95 µm, 0.95-1.5 µm (PM1.5). Dalším přínosem publikace je také podrobně popsaná metodika, která je doporučená jako vhodný nástroj pro přesnější charakterizaci možných zdravotních rizik z inhalační expozice v případě atmosferických částic PM (viz kapitola 4).

V navazující studii (Landlová et al., 2014) (Příloha 19) byly naše výsledky ještě více potvrzeny a to pomocí stejného plánu analýz, ale na 6 výrazně odlišných lokalitách. Z výsledků studie jasně vyplynulo, že kromě analýz koncentrací polétavého prachu PM, jsou velmi zásadní chemické analýzy látek, které jsou na něm sorbovány. Důkazem byly hodnocené lokality s prokazatelně vyššími hodnotami prašnosti PM10, ale s velmi nízkými hladinami chemických látek. Ze všech hodnocených lokalit byla nejvíce zatížená malá obec s převládajícím vlivem lokálních topenišť na tuhá paliva (bez plynofikace), což opět výrazně poukázalo na největší problém venkovských regionů podobného typu. Tento výsledek byl potvrzen i navazující toxikologickou analýzou všech vzorků i dílčích frakcí prachových částic (jejich extrakty): testy aktivity dioxinového typu (*dioxin-like aktivity test*), test

antiestrogenicity a test genotoxicity SOS chromotest with Escherichia coli PQ 37 (Novák et al., 2014) (Příloha 20). Základní popis výhod tohoto přístupu je v kapitole níže (kapitola 3.1.2.2.).

Provedené studie byly dosud zaměřeny hlavně na aktuální popis rozdílů mezi frakcemi částic, případně mezi lokalitami. Proto byla provedena studie s cílem vyhodnocení sezónních vlivů na distribuci chemických látek na dílčích frakcích atmosférických částic (Degrendele et al., 2014; Okonski et al., 2014) (Příloha 24). Byly hodnoceny vzorky ze dvou lokalit (Brno - Kotlářská a Telnice), kde odběry probíhaly aktivními kaskádovými impaktory kontinuálně celý rok. Byla zjištěna velmi výrazná sezonalita v distribuci PAHs, polychlorovaných dibenzo-p-dioxinů a -furanů (PCDDs/PCDFs) a dioxinům podobných PCBs (Degrendele et al., 2014). Výrazná část těchto látek byla sorbována na částicích PM0.95 (až 73%). Zdravotní inhalační rizika byla nejvyšší v zimním období se zvýšenými emisemi z lokálních topenišť a především v inverzních meteorologických situacích (až 40 x vyšší rizika než v letním období) (Degrendele et al., 2014). Studie potvrdila zásadní přínos aplikace kaskádových impaktorů pro odběry, analýzy a následnou interpretaci potenciálních zdravotních rizik z inhalační expozice v oblastech, které jsou zatížené atmosférickými částicemi.

3.1.2.2. Využití pasivních vzorkovačů

Pasivní vzorkování je další alternativní možností pro dlouhodobý monitoring vývoje expozičních hladin polutantů v ovzduší. Tato konstrukčně jednoduchá zařízení mohou být použita na celé řadě lokalit současně v kontinuálním designu studií. Pasivní vzorkovače ovzduší (PAS) poskytují informaci o dlouhodobé kontaminaci vybraného místa a mohou být použity jako screeningová metoda pro semikvantitativní srovnání různých lokalit s výhodou malé citlivosti ke krátkodobým náhodným změnám koncentrace polutantů (Klánová and Harner, 2013). Velkou výhodou je možnost využití těchto vzorkovačů i ve vzdálených a nepřístupných oblastech, protože nejsou závislé na zdroji energie ani nevyžadují přítomnost obsluhy. Jsou také vhodné v dlouhodobých monitorovacích programech, které jsme realizovali v rámci Evropy (Přibylová et al., 2012; Roots et al., 2010) (Příloha 17, 14) a ve vybraných zemích v Africe (Klánová et al., 2009b) (Příloha 10), kde mohou posloužit k identifikaci / charakterizaci primárních a sekundárních zdrojů a transportu kontaminantů v jednotlivých regionech a mezi nimi (Čupr et al., 2015a, 2015b).

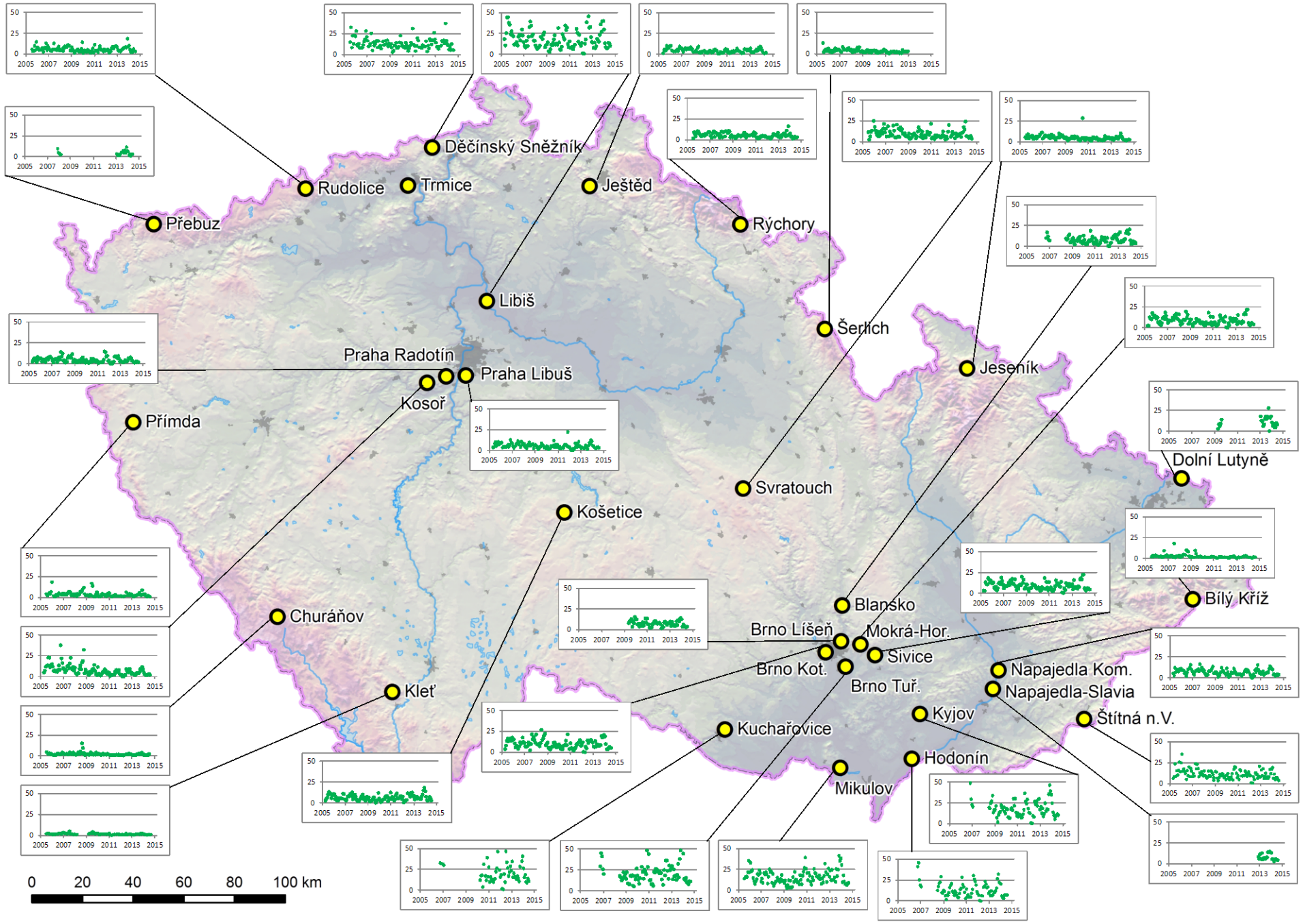
Jiné studie s PAS jsou zaměřeny na hledání příčin výskytu expozičních koncentrací. Slouží k tomu například kombinace metody hodnocení hlavních komponent (PCA- *principal component analysis*) nebo shlukové analýzy (*cluster analysis*), které jsou aplikované na matice koncentrací. Porovnávají se s dostatečně charakterizovaným referenčním otiskem typických zdrojů (Stafilov et al., 2011). Studie přináší praktické popisy postupů a nástrojů, jak tyto zdroje identifikovat, či alespoň kategorizovat, včetně podrobného výčtu jejich nejistot a omezení (Dvorská et al., 2012a).

Problematika přepočtů výsledků pasivního vzorkování získaných ve formě hmotnosti polutantu na vzorkovacím médiu za časové období (ng/PUFdisk za expoziční čas) na výsledky v jednotkách v $ng.m^{-3}$ a jejich závislosti na konkrétních parametrech je řešena v několika vědeckých studiích (Klánová et al., 2008; Markovic et al., 2015) (Příloha 8). V současné době je finalizována publikace, která právě hodnotí všechny detaily postupu přepočtů včetně jejich nejistot (v přípravě). Tento popis je i součástí certifikované metodiky pasivního typu vzorkování volného ovzduší (Čupr et al., 2015a).

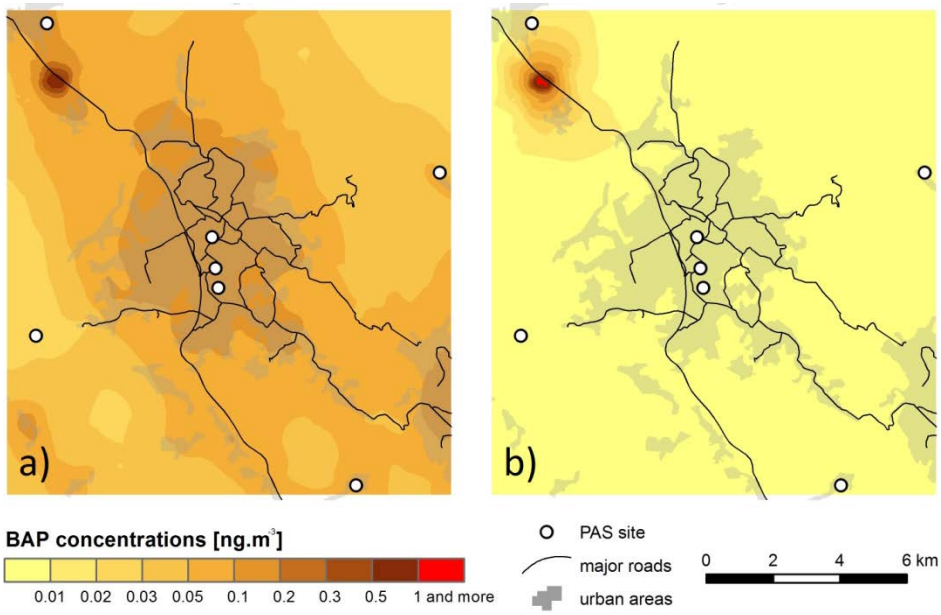
Pasivní vzorkování lze také využít ke screeningovému hodnocení inhalační expozice a z ní plynoucích zdravotních rizik, což jsme aplikovali v několika našich studiích (Bartoš et al., 2009; Klánová et al., 2007) (Příloha 9 a Příloha 6). Zásadní výhodou PAS je právě možnost dlouhodobého monitoringu vývoje expozice a predikovaných rizik. Tento typ vzorkovače na bázi polyuretanové pěny (PUF - z bílé, nebarvené polyuretanové pěny o hustotě 0,030 $g.cm^{-3}$, zpěňované bez přídavku mletého vápence - typ T 3037, výrobce Molitan, a.s., ČR; kruhový tvar, tloušťky 15 mm a průměru 150 mm.) lze využít nejen pro venkovní, ale také i pro vnitřní prostředí (Bohlin et al., 2014) - viz následující kapitola 3.1.2.3.

Náš tým v posledních letech realizoval projekt vybudování národní monitorovací sítě pro dlouhodobé kontinuální sledování perzistentních organických polutantů (POPs) ve volném ovzduší České republiky metodou pasivního vzorkování (Čupr et al., 2015b). Tato monitorovací síť je realizovaná s využitím těchto vzorkovačů tak, aby poskytovala data a výstupy vhodné pro hodnocení prostorových a časových trendů výskytu POPs v ovzduší ČR a poskytovala podklady pro plnění závazků mezinárodních smluv k chemickým látkám (Stockholmská úmluva o POPs či Úmluva o dálkovém přenosu znečišťování ovzduší překračujícím hranice států). Monitorovací síť nyní zahrnuje 32 lokalit. Odběry jsou realizovány dle nově publikované certifikované metodiky (Čupr et al., 2015a). Na Obrázku 4 je ukázka ze souboru map časových a prostorových trendů koncentrací hodnocených chemických látek ve volném ovzduší v ČR (Čupr et al., 2015b). Všechny mapy jsou dostupné na webových stránkách této monitorovací sítě (monet.recetox.muni.cz) včetně certifikované metodiky (Čupr et al., 2015a) a data jsou veřejně přístupná na našem portálu GENASIS (www.genasis.cz) přímo přes mapovou interaktivní web aplikaci.

Obrázek 4 Časové a prostorové hodnocení koncentrací sumy DDXs (DDT a jejich metabolitů; ng/PUF disk) ve volném ovzduší, měřené pomocí pasivního vzorkování kontinuálně v období 1/2006 - 6/2015 (bod v grafu odpovídá jedné odběrové kampani v délce 28 dní) (Čupr et al., 2015b).



PAS vzorkovače byly také úspěšně použity v kombinaci s rozptylovým modelováním (Sáňka et al., 2014) (Příloha 21). Cílem studie byl návrh a ověření nového nástroje pro hodnocení zátěže volného ovzduší v situaci, kdy jsou v hodnoceném regionu dostatečně známé zdroje znečištění. Na vybranou oblast města Liberce a okolí byl aplikován rozptylový Gaussovský model. S využitím databáze znečišťujících zdrojů REZZO (Registr emisí a zdrojů znečištění ovzduší) a meteorologických charakteristik oblasti byly namodelovány predikované koncentrace vybraných PAHs (viz Obrázek 5).



Obrázek 5 Koncentrace BaP (benzo(a)pyren) v Liberci a okolí, vypočítané rozptylovým modelem SYMOS'97 (a) pro zimní období a (b) pro letní období (Sáňka et al., 2014). Na lokalitách (bílé terče) bylo pomocí dlouhodobého kontinuálního vzorkování provedeno porovnání s modelem.

Časový vývoj modelových predikcí pak byl porovnán s vývojem skutečně vzorkovaných lokalit pomocí techniky PAS. Odběrové lokality byly totiž současně v bodech referenční sítě rozptylového modelu. Výstupem publikace je kromě srovnání a jeho zdůvodnění také podrobný návod jak tento nový nástroj použít (Sáňka et al., 2014). Velmi často se totiž v reálu používají pro zhodnocení imisních koncentrací polutantů jen rozptylové modely. Což je pro správný odhad expozice nedostatečné. Proto je pro takovéto situace velmi vhodné současné použití i pasivních vzorkovačů a nástrojů GIS (Geografický informační systém).

Další metodou pro zhodnocení vývoje expozičních hladin POPs v ovzduší je využití jehličí jako pasivní vzorkovače (Ratola et al., 2011). POPs jako skupina látek s nízkou rozpustností ve vodě a vysokým rozdělovacím koeficientem *n*-oktanol-voda má tendence se akumulovat v lipidech, což zahrnuje i voskovou vrstvu na povrchu jehlic. Ta pasivně sorbuje POPs z atmosféry po dlouhou dobu a lze tedy jehličí využít jako pasivní vzorkovač ovzduší. V naší studii (Klánová et al., 2009a) jsme se zaměřili na porovnání výsledků koncentrací z povrchu jehlic borovice a paralelně prováděných odběrů ovzduší aktivními vzorkovači na lokalitě Košetice (pozaďová stanice EMEP). Výsledky potvrdili použitelnost metody PAS pro

dlouhodobé hodnocení časových trendů vývoje kontaminace ovzduší (Klánová et al., 2009a) (Příloha 11).

Metodu pasivního vzorkování jsme dále aplikovali i ve vysokohorských oblastech Velké a Malé Fatry, Vysokých a Nízkých Tater v rámci našeho projektu Needle-Net. Jako indikační druh jsme zvolili kosodřevinu a jejich jehlice (*Pinus mugo* Turra.). Výhodou tohoto druhu je, že u něho lze odebírat 1. až 5. ročník jehlic (jehlice opadávají až koncem pátého roku). V případě borovice lesní to jsou pro porovnání většinou jen maximálně tři roky expozice. Publikace naší nové studie byla přijata v časopisu Ecological Indicators (Chropeňová et al., 2016). Velmi zajímavým výstupem studie byly zjištěné významně vyšší hladiny perfluorovaných sloučenin (PFC) v oblasti, kde jsou lyžařská střediska. Tyto látky byly totiž dříve používány do lyžařských vosků či sportovních textilií (typu GORE-TEX®) z důvodu velmi vhodných vlastností odpuzovat vodu a případnou špínu (Freberg et al., 2010). Na základě výsledků projektu Needle-Net byla v roce 2015 provedena naše navazující studie v Norsku, která nová zjištění měla potvrdit i v jiných oblastech. Odběry a analýzy jehlic tento výskyt PFC látek v okolí lyžařských center potvrdil. Ale poměry dílčích perfluorovaných látek v analyzovaných vzorcích v Norsku byly výrazně odlišné od vzorků ze Slovenska. Tyto překvapivé výsledky jsou nyní zpracovávány do publikace.

3.1.2.3. Inhalační expozice ve vnitřním prostředí

Ve vnitřním prostředí lidé tráví až 90 % času (Ma and Harrad, 2015; Schweizer et al., 2007). Proto je hodnocení výskytu koncentrací nebezpečných chemických látek ve vzduchu ve vnitřním prostředí zásadní v hodnocení celkové expozice člověka. I když byly již mnohé látky legislativně zakázané (výroba, aplikace do výrobků), stále se mohou vyskytovat v původních materiálech právě ve vnitřním prostředí (například FR – *flame retardants* - zhášeče hoření). Z nich se pak mohou uvolňovat do prostředí. Studium těchto emisních potenciálů patří k velkým prioritám výzkumu (Ma and Harrad, 2015).

Ve vnitřním prostředí jsou navíc sorpční materiály, které mohou fungovat jako rezervoáry chemických látek a mohou tedy způsobovat sekundární emisi. Vnitřní prostředí navíc zvyšuje perzistenci některých látek z důvodu velmi limitovaného přímého slunečního svitu (bez UV degradace). Navíc zateplení budov a omezená ventilace může ve vnitřním prostředí významně zvyšovat koncentrace nebezpečných látek. Koncentrace mnoha semivolatilních látek ve vnitřním prostředí mohou být i několikanásobně vyšší než ve venkovním prostředí (Bohlin et al., 2014; Rudel et al., 2010; Zhang et al., 2011).

Pro studie hodnocení výskytu vybraných SVOC látek ve vnitřním prostředí jsme použili pasivní vzorkovače PAS. Výstupem je publikovaný detailní popis metodiky PAS pro vnitřní prostředí včetně správného výběru expoziční doby PUF filtru, výsledných vzorkovacích rychlostí a to vždy pro každou chemickou látku (Bohlin et al., 2014) (Příloha 22). Jsou zde

také detailně kvantifikovány nejistoty s predikcí zdravotních rizik, které jsou s touto metodou spojeny.

Kromě pasivních vzorkovačů lze pro zjištění koncentrací ve vzduchu vnitřního prostředí samozřejmě použít aktivní typy vzorkovačů. Tento přístup jsme zvolili ve studii hodnocení koncentrací DDT a jejich metabolitů ve vnitřním prostředí v Ománu. DDT tam bylo totiž velmi intenzivně použito pro postřik vnitřního prostředí proti malárii a to v letech 1976 až 1992. Z analýz odebraných vzorků (v roce 2005) jsme identifikovali, že i po více než 15 letech od poslední aplikace DDT jsou koncentrace ve vnitřním prostředí stále významně vysoké – představující potenciální zdravotní rizika (Booij et al., 2016) (Příloha 28). Vzhledem k poměrně dobré evidenci použití postřiků s DDT a s využitím cíleného designu studie s výběrem různého stáří aplikace (12 domácností ve třech regionech), bylo možné provést zpětný odhad region-specifických poločasů života ve vnitřním prostředí (t1/2; *half-lives*). Model využívající region-specifických poločasů jsme pak využili k predikci koncentrací ve vzduchu vnitřních prostředí hodnocených lokalit v současné době (tedy pro rok 2015). Zdravotní rizika plynoucí z prospektivně predikovaných koncentrací byla již na akceptovatelných hladinách. Prezentovaný model/přístup lze metodicky doporučit a aplikovat i v jiných regionech, ve kterých se DDT používalo.

Velmi specifickou skupinou je také hodnocení expozice na palubě letadel (Allen et al., 2013) - viz Obrázek 6. Vnitřní vybavení letadel musí vyhovovat striktním požadavkům bezpečnosti v případě požárů. Proto mnoho materiálů obsahuje velmi vysoké hladiny zhášečů hoření. Lze tedy předpokládat, že expozice v případě delšího pobytu na palubě letadla může být významná. Tato vstupní hypotéza byla potvrzena pro případ bromovaných difenyletherů (BDEs). Hlavní hodnocenou matricí byly prachové částice.

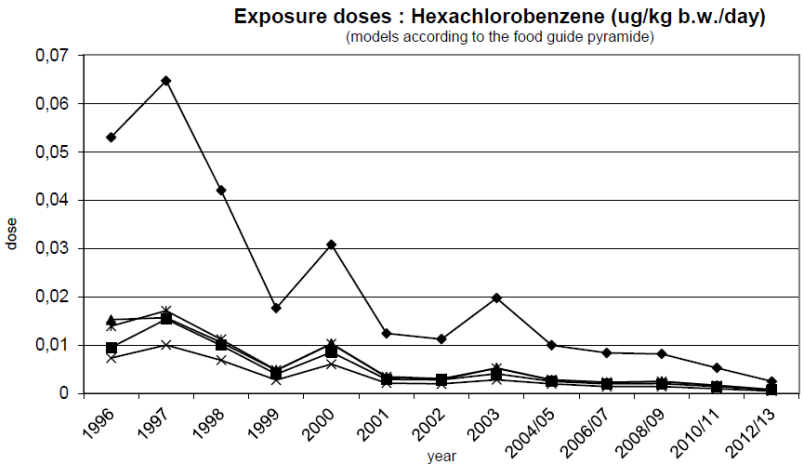
Obrázek 6 Paluba letadla může představovat významný zdroj pro expozici FR (flame retardants) – zdroj: (Allen et al., 2013).

Na celkových přijatých dávkách se v případě vnitřního prostředí ovšem podílí nejen inhalační cesta, ale také příjem prachu orální cestou (kontaminace potravin a vody, *"hand to mouth behavior"*), a dermální kontakt s prachovými částicemi, či s materiálem, který může představovat zdroj uvolňovaných chemických látek (Booij et al., 2016). A právě identifikace významu a kvantifikace těchto dílčích expozičních cest ve srovnání s inhalací a také celkovým expozičním příjmům patří k doporučovaným směrům posledních vědeckých studií (Ma and Harrad, 2015).

3.1.3. Dietární expozice

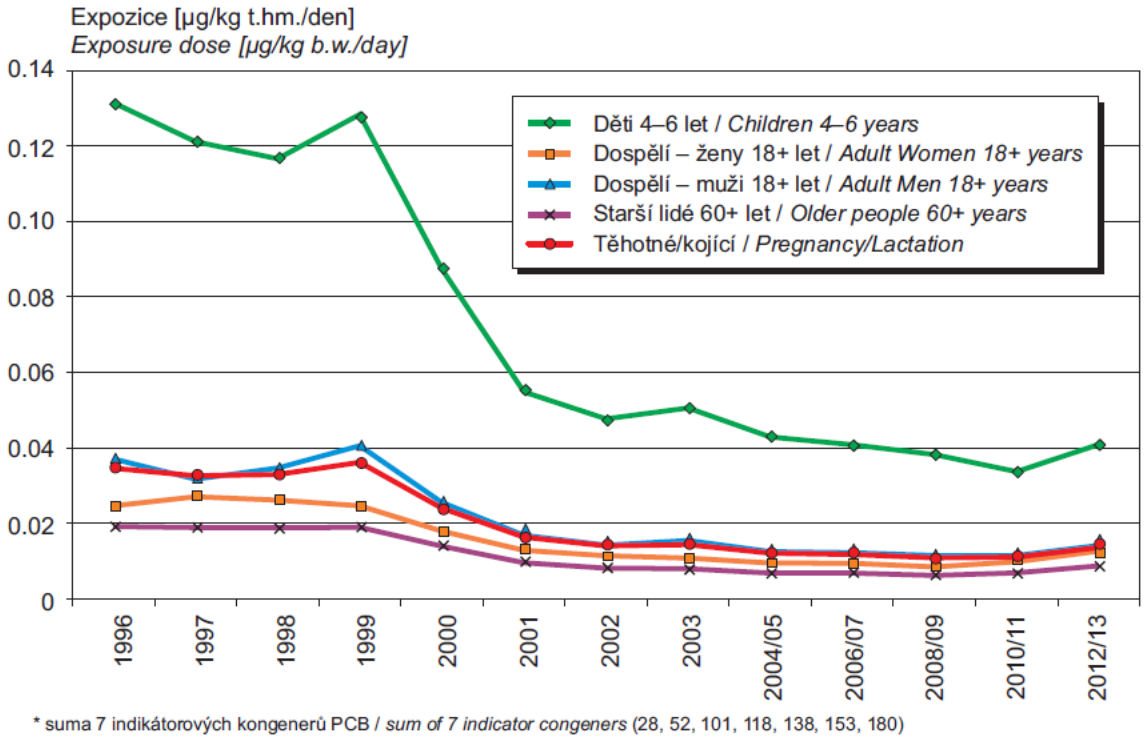
Dietární expozice hraje v celkovém chronickém denním příjmu mnoha látek velmi významnou roli (Choi et al., 2015a). Pro co nejpřesnější predikci dietárního expozičního příjmu je kromě koncentrací v potravinách také důležitý vstupní parametr spotřeby potravinových komodit (Dvorská et al., 2012b) (Příloha 16 – popis parametrů, rovnice

příjmu). Monitoring potravních řetězců je v tomto ohledu nenahraditelný zvláště u látek, které nemají legislativně kontrolovaný limit, ale jejich expozice a následné toxické efekty mohou být významné (Choi et al., 2015b).



Obrázek 7 Vývoj celkových chronických denních příjmů z dietární expozice v lidské populaci v ČR pro hexachlorobenzen z dietárního monitoringu. Zdroj: převzato z (SZÚ, 2014).

I v rámci ČR je dlouhodobě prováděn monitoring dietární expozice (MDE) a to ve dvouletých intervalech od roku 1994. Kromě cíleného monitoringu vybraných chemických látek v potravinových komoditách, jsou také prováděny bakteriologické a mykologické analýzy. Cílem MDE je odhad průměrné expozice populace ČR. K tomuto odhadu jsou využity právě zjištěné průměrné hodnoty chemických látek v potravinách a jejich dílčích komoditách, a také jejich doporučené denní dávky (takzvaný model potravní pyramidy, který vychází z doporučených dávek potravin); (Ruprich et al., 2011; SZÚ, 2012). Výsledky jsou pravidelně publikovány ve formě grafů dlouhodobých trendů pro hodnocené látky (viz ukázka: Obrázek 7 a 8).



Obrázek 8 Časový trend vývoje celkových chronických denních příjmů z dietární expozice v lidské populaci v ČR pro sumu 7 indikátorových PCBs z dietárního monitoringu. Zdroj: převzato z (SZÚ, 2014).

Pro specifické případy lze tento přístup také doplnit modelováním přestupů z kontaminovaných půd do rostlin a jejich plodů (Mikeš et al., 2009) (například při dobré znalosti kontaminace půd). Z naší studie plyne, že pro transport z kontaminované zeminy do plodin hrají také velkou roli resuspendované půdní částice, které ulpívají na listech plodin (ve studii byly použity salát a ředkvička).

K velkým nejistotám metod modelových výpočtů dietární expozice patří nedostatek exaktně stanovených přestupů látek z přijatých potravin do krevního oběhu. Výzkumná skupina autora habilitační práce aktuálně vyvíjí nové, zpřesňující experimentální metodiky přestupu přes gastrointestinální trakt - GIT (Partyková, 2015). Cílem je využít inovované postupy k experimentálnímu stanovení toxikokinetických koeficientů pro GIT.

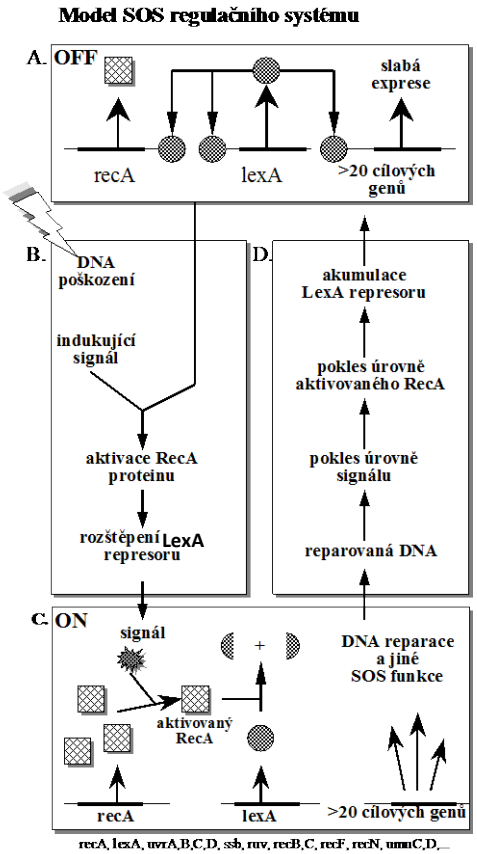
3.1.4. Využití přístupu testování toxických efektů

Vzhledem k tomu, že v nabídnuté dávce při expozici je velmi komplexní směs mnoha chemických látek, není v současné době možné chemickou analýzou determinovat všechny tyto stresory. Jsou postupně identifikovány nové a nové skupiny látek, které by měly být dlouhodobě monitorovány pro svoje významné toxikologické efekty (Lammel, 2015). Proto je v tomto směru velmi vhodné doplňovat chemické analýzy i biologickými testy *in vitro* na odebraných vzorcích, které představují velmi komplexní směs chemických látek (Bartoš et al., 2006, 2005; Čupr et al., 2013, 2005; Flegrová et al., 2008, 2007; Novák et al., 2014; Škarek et al., 2007a, 2007b).

Mezi jedny z významných typů efektů patří schopnost některých látek interagovat s buněčnou DNA. Výsledkem expozice, může být poškození DNA v buňce. To lze predikovat s využitím progresivních metod hodnocení genotoxického potenciálu. Při interpretační syntéze však platí striktní zásada akceptování významných vlivů predikčních nejistot. Metody využívají experimentální toxikologii pro odhad účinků adekvátních reálných vzorků expozičních matric na testované biologické modelové systémy *in vitro* (prokaryotické či eukaryotické buněčné linie). Test genotoxicity lze definovat jako detekční systém, který umožňuje na základě interakce studovaného faktoru a biologického systému provést kvalitativní i kvantitativní hodnocení jeho genotoxického potenciálu.

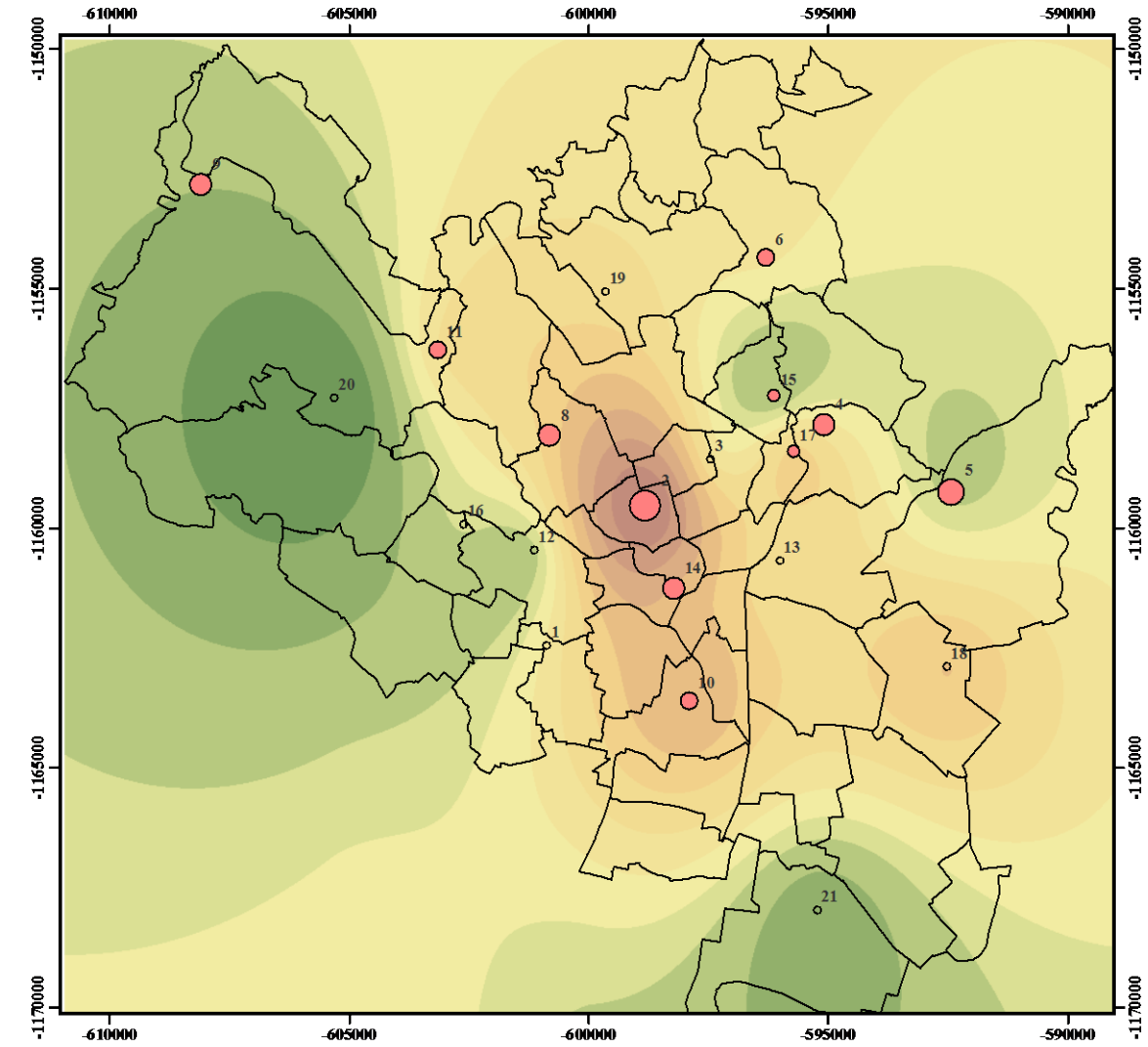
V současné době existuje velké množství dostupných testů genotoxicity. Důležitý požadavek je, aby bylo možné provést testy velmi rychle po úpravě vzorku a pokud možno s co nejmenšími náklady a nejmenší spotřebou primárního vzorku. Velmi progresivním je test na principu indukce SOS reparace v důsledku interakce mezi genotoxickými faktory a DNA (Anjum and Krakat, 2015; Kováts et al., 2013). Indukce SOS reparace je detekována s využitím specifických reportérových genů jako je například gen pro ß-galaktosidázu (*lac-Z*). Podléhají stejné kontrole a jsou společně přepisovány s *din* geny SOS systému (př. recA, sfiA, sulA, umuC) v podobě fúzního genu, promotor SOS genu::reportérový gen (M. Abdel-Massih et al., 2013) - Obrázek 9. Fúzní gen sulA::lacZ je umístěn v plazmidu společně s genem zajišťujícím rezistenci k ampicilínu. Pokud tedy u buňky náhodou dojde ke ztrátě toho

plazmidu, buňka v přítomnosti antibiotika nepřežije. Tento antibiotikový tlak udržuje kulturu v původní kvalitě a navíc eliminuje možnost nechtěné kontaminace při nedokonalé aseptické práci při testu. Podstatu indukce pozitivní odpovědi detekčního systému lze shrnout do sledu několik kroků. V důsledku genotoxického zásahu (poškození DNA) je spouštěn SOS reparační systém (Biran et al., 2010). Hlavní kontrolní úlohu v aktivaci systému hrají produkty SOS genů lexA a recA. LexA gen kóduje protein s represorovou aktivitou. Protein je vázán ve specifických místech promotorové části některých SOS genů, a tak blokuje celý SOS reparační systém. Avšak genotoxickým efektem je odstartován přepis recA genu, který umožňuje aktivaci specifické proteázy, která štěpí represor lexA a umožňuje přepis jednotlivých SOS genů a tedy spuštění celého SOS reparačního systému. Extracelulární produkovaná ß-galaktosidáza pak kvantifikuje míru genotoxického efektu. Výsledkem je aktivace dějů, které následně vedou k částečné či úplné opravě poškozené DNA. Případný cytotoxický efekt se musí sledovat paralelně pomocí detekce extracelulární alkalické fosfatázy.



Obrázek 9 Model SOS reparačního systému při poškození DNA buňky.

Výše uvedené testy genotoxicity jsou z hlediska svých vlastností velmi vhodné pro screeningové hodnocení genotoxického potenciálu vzorků nabídnuté expozice. A to nejen pro testování čistých chemických látek (viz naše realizovaná studie na skupině azaarenů (Bartoš et al., 2006) (Příloha 3), či na potenciální genotoxicitu toxafenu (Bartoš et al., 2005) (Příloha 1). Autor této habilitační práce aplikoval tyto testy genotoxicity v mnoha studiích s cílem screeningově identifikovat vzorky, u kterých lze předpokládat významnější přítomnost genotoxicky aktivních látek v celkové směsi. Poprvé s využitím extraktů vzorků PAS byly tyto testy použity v naší studii kvality ovzduší na 20 lokalitách městské aglomerace (Čupr et al., 2006) (Příloha 4). Paralelně k chemické analýze hladin POPs v jednotlivých vzorcích volného ovzduší byla využita *Escherichia coli* (fúzní gen *sulA::lacZ*) v testu genotoxicity a podrobně studován vztah dávka - odpověď. V řadě vzorků byla detekována přímá mutagenita a statistická analýza ukázala významnou korelaci mezi pozorovanými biologickými efekty a mírou kontaminace formou koncentrací sumy PAHs (viz Obrázek 10).



negenotoxický

RGTU

0,01 - 5,90

5,91 - 7,86

7,87 - 8,90

8,91 - 9,50

9,51 - 11,67

Hranice katastru Brna

Koncentrace 28 PAH (ng/PUF)

2 344 - 6 000
6 001 - 8 200
8 201 - 10 000
10 001 - 11 200
11 201 - 12 600
12 601 - 14 100
14 101 - 16 000
16 001 - 18 000
18 001 - 20 500
20 501 - 23 500
23 501 - 27 000
27 001 - 32 000

Hranice katastru Brna

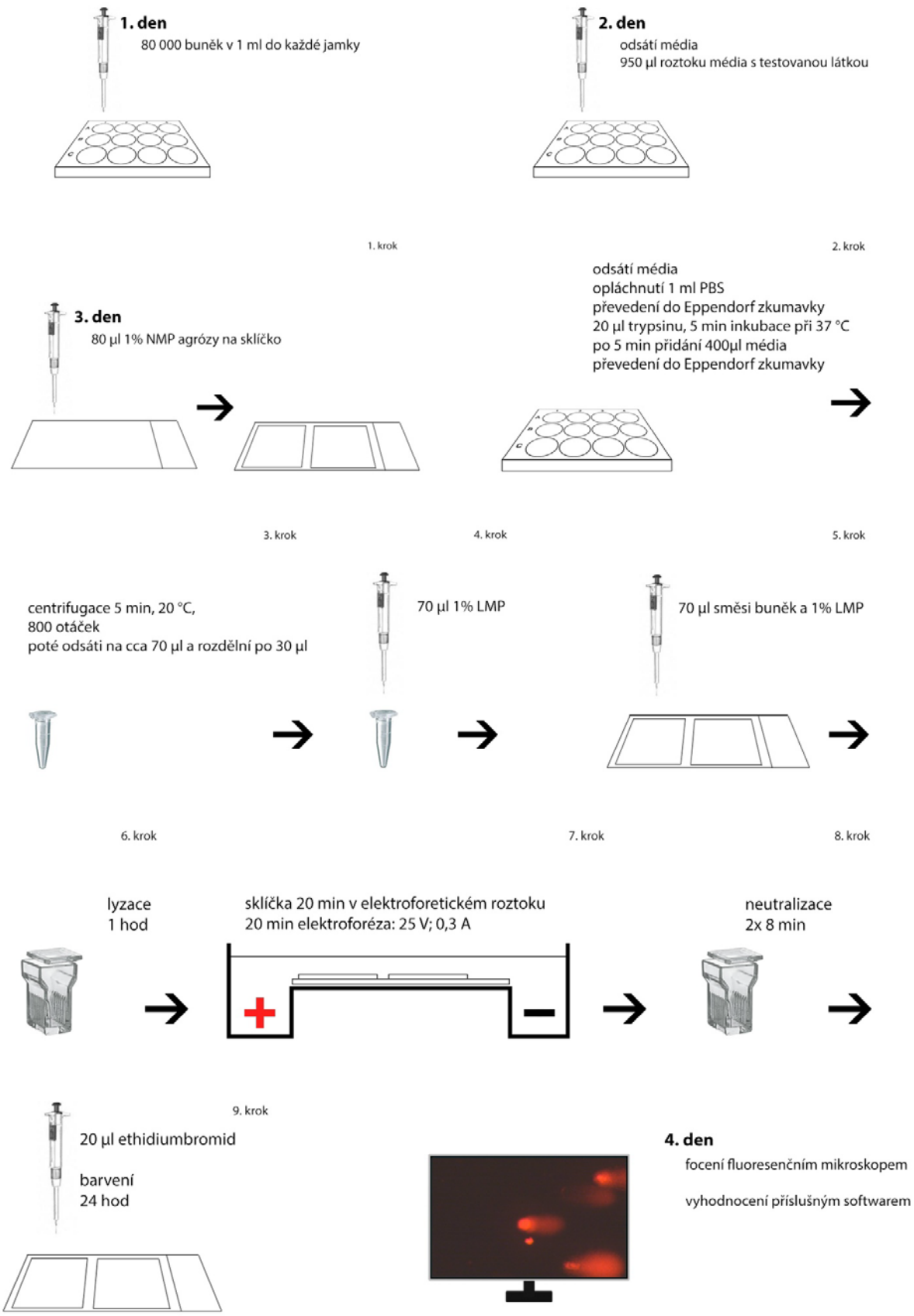
Code	Locality
1	Brno - Bohunice
2	Brno - Kotlářská
3	Brno - Černá Pole
4	Brno - Maloměřice
5	Brno - Líšeň
6	Brno - Soběšice
8	Brno - Žabovřesky
9	Brno - Hrad Veveří
10	Brno - Komárov
11	Brno - Kníničky
12	Brno - Pisárky
13	Brno - Židenice
14	Brno - Střed (Petrov)
15	Brno - Lesná
16	Brno - Kohoutovice
17	Brno - Husovice
18	Brno - Slatina
19	Brno - Řečkovice
20	Brno - Bystrc
21	Brno - Chrlice

Obrázek 10: Prostorová prezentace relativního genotoxického potenciálu RGTU (RGTU = [1/MGC]*100); 11 vzorků z 20 vykazovalo statisticky významný genotoxický potenciál) (Čupr et al., 2006).

Důležitým směrem vývoje hodnocení možného poškození DNA v exponovaných buňkách představuje další soubor testů na eukaryotických buňkách - tzv. Comet assay (dále již CA). CA nebo také Single-Cell Gel Electrophoresis (SCGE) je metoda používaná ke stanovení poškození DNA na úrovni jednotlivých eukaryotických buněk. Je velmi rozšířená především pro biomonitoring a genotoxické studie (Forchhammer et al., 2010). Název Comet assay souvisí se vzhledem buňky s poškozenou DNA, která při elektroforéze migruje směrem k anodě a tím vzniká „ocas" komety (Obrázek 11). Princip této metody spočívá v tom, že genotoxické látky způsobují poškození a zlomy na DNA. Taková DNA se zlomy je potom nabitá. Když jsou buňky imobilizovány a poté lyzovány, tak při elektroforéze DNA se zlomem migruje z buněčného obalu směrem k anodě, což se po obarvení příslušným barvivem zobrazí jako „kometa". DNA buněk bez zlomů nenese žádný náboj, a proto zůstává v buněčném obalu, který se poté zobrazí jako nepoškozená, celistvá buňka (Azqueta and Collins, 2013).

Největšími výhodami CA oproti ostatním metodám stanovování genotoxicity chemických látek jsou rychlost, stanovení na úrovni jedné buňky a možnost používat velké spektrum i lidských buněk (Kang et al., 2013), což přináší výrazně lepší interpretaci výsledků. Lze aplikovat přístup *in vitro* – použití extraktů vzorků externí expozice (např. extrakty vzorků ovzduší na bronchiální buňky) a nebo přístup *in vivo* – izolaci buněk ze vzorků interní expozice přímo od hodnocených participantů studie (například izolované periferní lymfocyty nebo neinvazivně získané buňky z bukálních stěrů) (Bolognesi and Fenech, 2013). Pro hodnocení poškození DNA *in vivo* lze použít i test frekvence mikrojader (*micronucleus test*), test chromosomálních aberací či výměn sesterských chromatid v provedení *high-throughput* biomonitoringu (vysoce výkonné screeningové metody) (Balamuralikrishnan et al., 2014; Rossner et al., 2013; Rossnerova et al., 2011; Schunck et al., 2004). Právě i k těmto přístupům výzkumu interní expozice *in vivo* směřuje tým autora této práce (viz další detail v kapitole 3.2.2.).

V posledních letech je také výzkum intenzivně směřován k využití *in vitro* dat pro *in vivo* extrapolaci QIVIVE (quantitative in vitro to in vivo extrapolation) (McNally and Loizou, 2015; Meek and Lipscomb, 2015; Wilk-Zasadna et al., 2014; Yoon et al., 2015, 2012). Zásadní roli v extrapolaci hraje důkladná znalost všech čtyř procesů ADME pro dílčí hodnocené chemické látky. Pomocí toxikokinetiky lze pak predikovat koncentrační hladiny látky v cílovém orgánu (zde se již ale jedná o hodnocení interní expozice). Pokud však nejsou k dispozici experimentálně stanovené toxikokinetické parametry, lze je pro účely QIVIVE extrapolace odvodit například pomocí QSAR přístupu (Quantitative structure–activity relationship). Pro vstupy do QIVIVE se však oprávněně preferují experimentálně ověřené hodnoty.



Obrázek 11: Schéma optimalizovaného pracovního postupu Comet assay (Hložková, 2015).

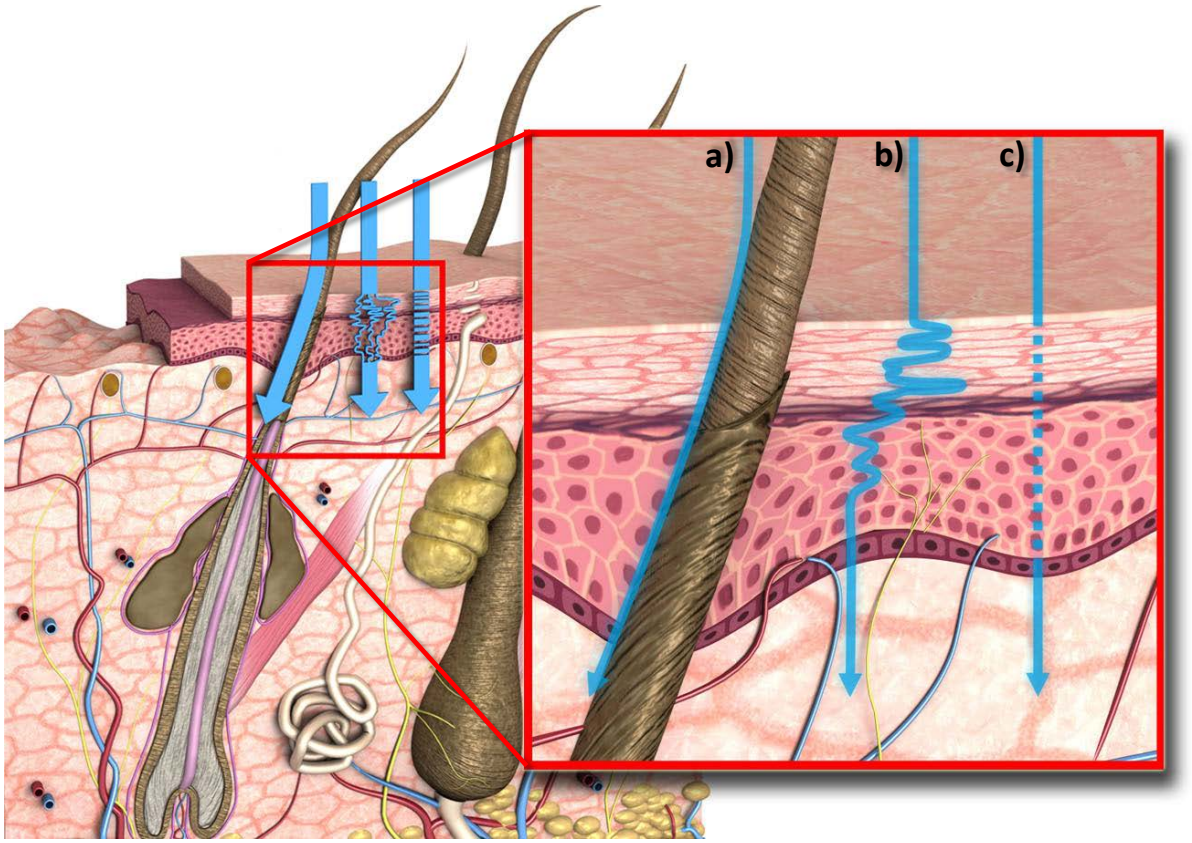
3.1.5. Dermální expozice

Dermální expozice představuje další možnou transportní cestu chemických látek do lidského organismu. V mnoha studiích je její význam nesprávně zanedbáván (Kalantzi and Siskos, 2011). Toto podhodnocování plyne z nedostatečných znalostí možné kinetiky transportu a to především ve specifických scénářích. V posledních vědeckých studiích je zpřesňování významu dermální expozice zdůrazňováno a označováno jako jeden z prioritních směrů výzkumu (Bányiová et al., 2016, 2015; Rostami and Juhasz, 2011).

3.1.5.1. Základní principy dermální absorpce

Dermální / perkutánní absorpce je termín, vyjadřující komplexní proces přestupu chemických látek přes kůži (Huong et al., 2009; WHO, 2006). Skládá se z několika fází, které jsou vzájemně propojené. Penetrace představuje vstup látky do dané kožní vrstvy, či struktury. Permeace je přestup z jedné vrstvy do druhé, která je strukturálně a funkčně odlišná od té první. Resorpcí je pak nejčastěji označován proces vstupu látky do vaskulárního systému lidského organismu (Huong et al., 2009).

Přestup látky kůží může probíhat třemi základními cestami (Chilcott and Price, 2008): a) folikulární cestou (do tohoto typu transportní cesty řadíme i cestu potními žlázami), b) intracelulární cestou (mezibuněčnou) a c) intercelulární cestou (transcelulární) – viz následující schéma (Obrázek 12).



Obrázek 12: Schéma znázorňující strukturu kůže s vyznačením třech základních cest transportu chemických látek po dermální expozici: a) folikulární (včetně cesty potními žlázami), b) intercelulární cesty vstupu (tento mezibuněčný transport patří mezi nejvýznamnější) a c) intracelulární přestup (Chilcott and Price, 2008). Část základního obrázku kožního řezu byla převzata (BASF, 2013). Detailní popis lidské kůže je v práci Kataríny Bányiové (Bányiová, 2012) a (Atkinson et al., 2015; Mathes et al., 2014).

Pokud látka dokáže pronikat z lipofilního prostředí kožní mazové žlázy (folikulární mazové žlázy) do hydrofilního prostředí pokožky, lze u látky předpokládat vstup do vaskulárního systému (Chilcott and Price, 2008).

3.1.5.2. Expoziční parametry při dermálním transportu

V této kapitole jsou stručně uvedeny možné postupy hodnocení dermální expozice. Klíčová je především co největší znalost nabídnuté externí expoziční koncentrace v matrici, se kterou je lidské tělo v kontaktu. Dalším důležitým expozičním parametrem, vstupujícím do výpočtu chronického denního příjmu při přestupu přes kůži je celkový exponovaný povrch kůže a koeficient permeability Kp průniku kůží ($cm.hod^{-1}$) – tedy konstanta specifická pro každou hodnocenou chemickou látku.

Přestup chemických látek kůží se pokládá obecně za pasivní difúzní proces, který se řídí dle Fickova zákona: tok látky membránou, která je limitujícím faktorem pro rychlost přestupu, je přímo úměrný rozdílu koncentrací na různých stranách membrány:

$$J = -D \times (\delta C/\delta x),$$

kde J je tok (Flux, $g.cm^{-2}.h^{-1}$), D je difuzní koeficient, δC je rozdíl koncentrací na různých stranách membrány ($g.cm^{-3}$) a δx je tloušťka membrány. Platí tedy i následující rovnice, která je v problematice dermální expozice často používána:

$$Jss = Kp \times C_0,$$

kde Jss je tok látky membránou v ustáleném stavu ($g.cm^{-2}.h^{-1}$), Kp je permeační koeficient pro danou látku v dané koncentraci v daném nosiči ($cm*h^{-1}$)a C_0 je koncentrace látky v nosiči ($g*cm^{-3}$). Permeační koeficient může být použit jen pro predikci absorpce látky v tom stejném nosiči (Chilcott and Price, 2008). Pro přestup látek hraje samozřejmě velkou roli i fyzikálně chemické vlastnosti samotných látek – jako je rozpustnost, molekulová hmotnost, rozdělovací koeficient log K_{ow}.

Pro zpřesněný výpočet celkové dermální absorbované dávky (DAD) je vhodné použít následující postup a rovnici (Bányiová et al., 2015) (Příloha 25):

$$DAD = \frac{DA_{event}\ x\ EV\ x\ ED\ x\ EF}{AT} \times \frac{SA}{BW}$$

kde DA_{event} ($mg.cm^{-2}.případ^{-1}$) se odvozuje zvlášť pro krátkodobé a dlouhodobé expozice:

- krátkodobé působení ($t_{event} \leq t^*$), kde t* je čas potřebný k dosažení rovnovážného stavu (*steady state*) a platí $t^*=2.4\ \tau$, kde τ je doba zpoždění (lag-time; v hodinách za případ):

$$DA_{event} = 2\ FA\ x\ K_p\ x\ C_w \sqrt{\frac{6\tau\ x\ t_{event}}{\pi}}$$

- pro případ dlouhodobého působení (t_{event} > t*) pak:

$$DA_{event} = FA\ x\ K_p\ x\ C_w\ \left[\frac{t_{event}}{1+B} + \ 2\tau\ \left(\frac{1+3B+3B^2}{(1+B)^2}\right)\right]$$

kde:

DAD – dermální absorbovaná dávka [mg.kg^{-1}.den^{-1}]

DA_{event} – absorbovaná dávka při jednom případu [mg.cm^{-2}.případ^{-1}]

EV – počet případů za den [případ.den^{-1}]

EF – frekvence expozice [den.rok^{-1}]

ED – trvání expozice [rok]

SA – povrch kůže [cm^2]

BW – váha těla [kg]

AT – doba průměrování [dny]

FA – absorbovaná frakce vody s kontaminantem [0 až 1, bezrozměrný]

Kp – koeficient permeability průniku kůží [cm.hod^{-1}]

CW – koncentrace kontaminantu ve vodě [mg.l^{-1}]

CF – konverzní faktor [0,001 l.cm^{-3}]

t_{event} – trvání případu [hod.případ^{-1}];

t* – čas potřebný k dosažení rovnovážného stavu [hod]; t* = 2,4τ

τ - je doba zpoždění [lag-time; v hodinách za případ]

B – poměr Kp pro průchod zrohovatělou částí a živými buňkami pokožky [bezrozměrný];

pro stanovení tohoto koeficientu je doporučováno použít aproximační vztah (EPA, 2004):

$$B = \frac{K_p}{K_{p,ve}} \cong K_p \frac{\sqrt{MW}}{2.6}\ (aproximační\ vztah)$$

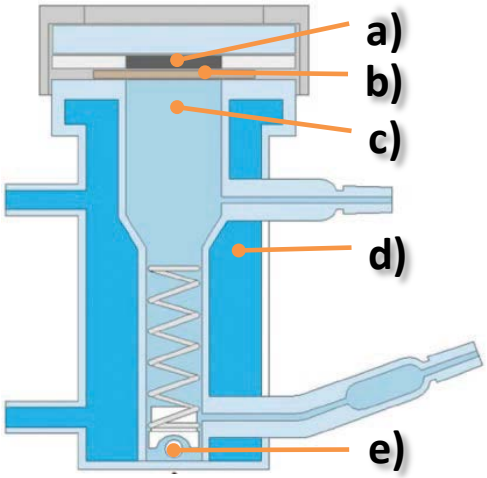
kde MW je molekulová hmotnost [g.mol^{-1}].

Detailní popis využití tohoto modelu k predikci celkové dermální absorbované dávky pro zvolené expoziční scénáře jsou součástí našich publikací (Bányiová et al., 2016, 2015).

3.1.5.3. Experimentální hodnocení kinetiky perkutánního transportu

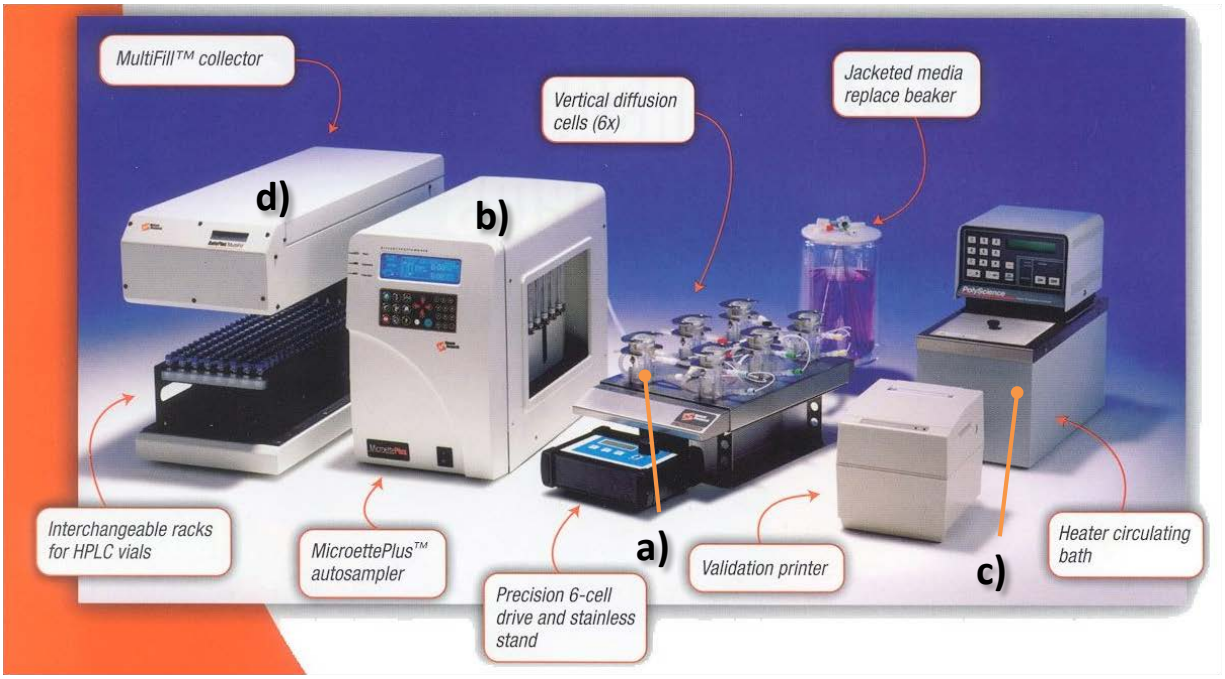
Jak již bylo výše zdůrazněno, kinetika přestupu toxikologicky významných chemických látek do lidského organizmu přes kůži patří mezi důležité expoziční parametry metodiky hodnocení zdravotních rizik. Nenahraditelnou roli v získávání těchto parametrů představují experimentální studie založené na metodice Franzových cel. Jedná se o experimentální přístup *ex vivo* (v některých publikacích ji označují *in vitro*), využívající reálné vzorky lidské kůže. Základní princip metody spočívá v tom, že mezi dvě komory (donor / receptor), je umístěná membrána – tedy například štěp lidské kůže (případně jiné biologické membrány –

GIT, a podobně). V receptorové části systému je kapalina, která je v neustálém přímém kontaktu s kůží, je promíchávána a temperována na konstantní teplotu (Obrázek 13 a 14). Na membránu v donorové části je aplikována testovaná látka či vzorek. V pravidelných časových intervalech jsou odebírány vzorky receptorové kapaliny, které jsou pak následně analyzovány vhodnou analytickou metodou (Chilcott and Price, 2008).



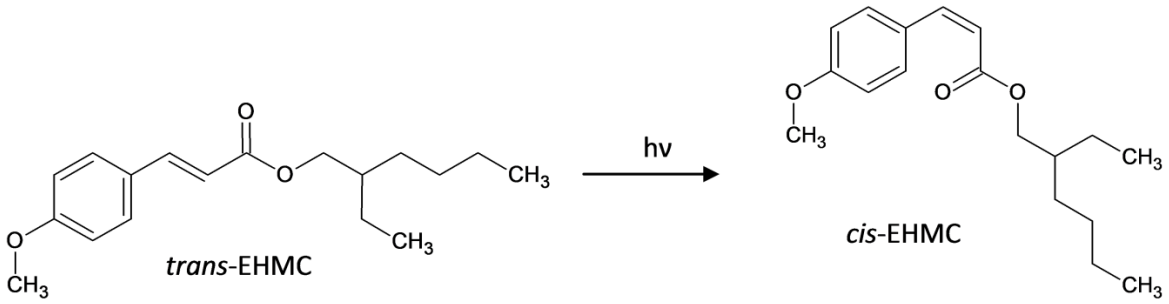
Obrázek 14: Schéma Franzovy cely pro experimentální stanovení kinetiky dermální absorpce (Hanson Research, USA); a) donorová část; b) štěp lidské kůže; c) receptorová část (horním otvorem se provádí vzorkování receptoru a spodním otvorem se přivádí nová receptorová kapalina); d) temperační plášť cely pro zajištění konstantní teploty; e) magnetické míchadlo.

Pro experimentální stanovení parametrů kinetiky transportu polutantů je zapotřebí postupovat dle přísných standardů (Bányiová et al., 2016; OECD, 2004a, 2004b), které jsou striktně závazné i pro farmaceutické aplikace (včetně dodržování etických principů při aplikaci lidských kožních štěpů). V našich experimentech využíváme plně automatizovaný systém MiroettePlus™ (Hanson Research, USA). Systém funguje zcela automaticky a disponuje celkem 6 celami, které jsou temperované (32 °C). Pod každou celou je pomocí magnetických míchadel zajištěna homogenizace receptorové kapaliny. Vzorky jsou průběžně v předem nastaveném časovém harmonogramu vzorkovány propojeným systémem až do minivialek. Čerstvá receptorová kapalina je paralelně doplňovaná do cel při každém provedeném vzorkování.



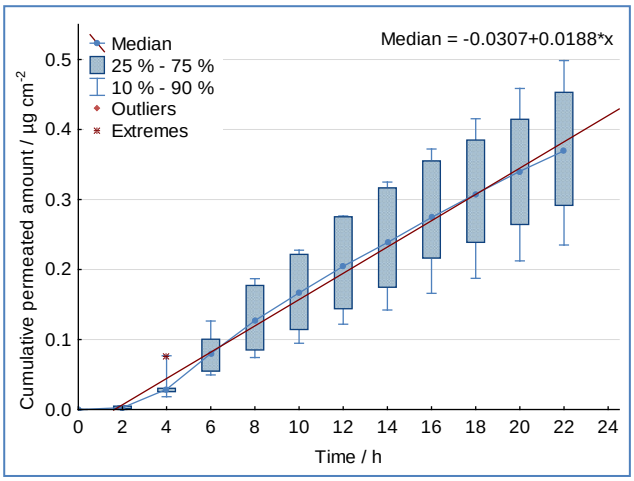
Obrázek 13: Plně automatizovaný systém na experimentální stanovení kinetiky dermálního transportu MicroettePlus (Hanson Research, USA) a) stojan s magnetickými míchadly pro 6 Franzových cel, b) řídící a programovací procesor se vzorkovací pumpou, c) termostat s cirkulací d) a automatický sběrač vzorků.

V rámci aktuálně řešeného projektu GAČR (hlavní řešitel Pavel Čupr: „*Kinetika perkutánní penetrace optických izomerů filtrů ultrafialového záření, bromovaných retardérů hoření a polárních pesticidů při dermální expozici*“) je využíván tento systém pro výzkum a zpřesnění hlavních kinetických parametrů dermálního transportu u vybraných chemických látek, u kterých tato data dosud chybí. Jsou to optické izomery Ethylhexyl methoxycinnamátu (EHMC - je aktivní látka používaná ve většině opalovacích krémů jako UV-filtr – sunscreen – Obrázek 15), vybrané bromované zhášeče hoření a také polární pesticidy (CUP – *currently used pesticides*).



Obrázek 15: Fototransformace trans izomeru EHMC na cis izomer (Ethylhexyl methoxycinnamát), který vykazuje v našich provedených experimentech odlišné toxikologické vlastnosti (Nečasová et al., 2016).

Výstupem experimentů jsou například série ověřených toxikokinetických parametrů (Obrázek 16), které jsou důležité pro predikci kinetiky transportu (Bányiová et al., 2016, 2015), (Příloha 27 a 25).



	Carbendazim		
	J_{ss} (µg cm^{-2} h^{-1})	K_p (cm h^{-1})	τ (h)
Mean	0.0188	0.0034	1.6
Standard Deviation	0.0004		0.3
Coefficient of Variation		0.06	0.2

Obrázek 16: Ukázka výsledků experimentů stanovení perkutánní penetrace carbendazimu vyjádřené jako kumulativní množství přestupu přes lidskou kůži za daný čas. Výsledné toxikokinetické parametry: Jss (tok látky lidskou kůží v ustáleném stavu - *Steady-state flux*), Kp (koeficient permeability průniku kůží - *permeability coefficient*) a τ (doba zpoždění - *lag-time*). Převzato z naší publikace: (Bányiová et al., 2016).

Tyto informace lze pak využít pro modelování expozičních scénářů s cílem zpětnovazebně eliminovat výsledná rizika plynoucí z dermální expozice.

3.1.5.4. Studie dermální expozice využívající monitoringu koncentrací v environmentálních matricích

V roce 2009 jsme publikovali detailní screeningovou databázi výsledků analýz koncentrací POPs v půdách V ČR, jako typické země centrální Evropy (Holoubek et al., 2009) (Příloha 12). Byly sledovány možné vlivy různých parametrů půdy ve vztahu k využití půdy (*land use*) a k nadmořské výšce lokalit. Koncentrace silně korelovaly s obsahem organického uhlíku. Zatímco HCHs a HCB byly detekovány s významnějšími koncentracemi spíše v orných půdách, vyšší koncentrace PCDDs/Fs, PCBs, PAHs and DDTs byly analyzovány ve výše položených oblastech lesních půd. Na této databázi s celkem 471 půdních vzorků odebraných v průběhu let 1996 – 2006 ve spojení s databází starých ekologických zátěží (SESEZ - Systém evidence starých ekologických zátěží – databáze 3061 lokalit), byly pak následně provedeny predikce expozice a následných zdravotních rizik (Čupr et al., 2010) (Příloha 13). Kromě dermální expozice byla hodnocena také inhalační a orální expozice prachových částic (sekundární emise jemných částic z půdy prašností). Prostorová distribuce expozice byla modelována pomocí metod IDW s pomocí GIS (IDW - Inverse Distance Weighting). Výstupem je publikovaná celoplošná mapa ČR predikcí koncentrací vybraných POPs a zdravotních rizik s odhady celkových zásob včetně kvantifikace nejistot.

Popsaná problematika se vztahuje i k možné dermální expozici při zemědělské práci v záplavových oblastech. Sedimenty říčních toků mohou totiž představovat významný zdroj kontaminantů pro zemědělský půdní fond (Hilscherová et al., 2007) a zároveň zdroj potenciálních toxikologicky významných efektů (Hilscherová et al., 2010).

3.2. Hodnocení interní expozice

U hodnocení interní expozice se jedná o retrospektivní interpretaci expozice a to formou analýz kontaminací biologických matric, jako je krev, mateřské mléko, vlasy, moč a podobně v rámci cíleného biomonitoringu (tedy "Top-down Exposomic" přístup – Obrázek 3). Zjištěné koncentrace ale představují aktuální stav interní expozice. Pro správnou rekonstrukci expozice (tedy predikce původní chronické denní dávky CDI) je však zapotřebí dostatečně charakterizovat všechny důležité procesy toxikokinetiky (ADME procesy) (Roberts and Renwick, 2014). Právě ty výrazně ovlivňují poločasy života v těle organismu, jejich biodostupnost a také expoziční čas působení jejich možných biologických efektů v cílených orgánech.

3.2.1. Toxikokinetika

V současné době probíhá výrazný vývoj fyziologicky založených farmako/toxiko-kinetických modelů (PBTK) - viz detailní rešerše v diplomové práci Jany Václavíkové - vedoucí Pavel Čupr (Václavíková, 2015). Hlavním cílem toxikokinetiky je časově dependentní predikce tkáňových koncentrací toxikantů ze známé přijaté expoziční dávky (externí expozice) nebo

naopak retrospektivní rekonstrukce celkových chronických dávek ze známé interní koncentrace.

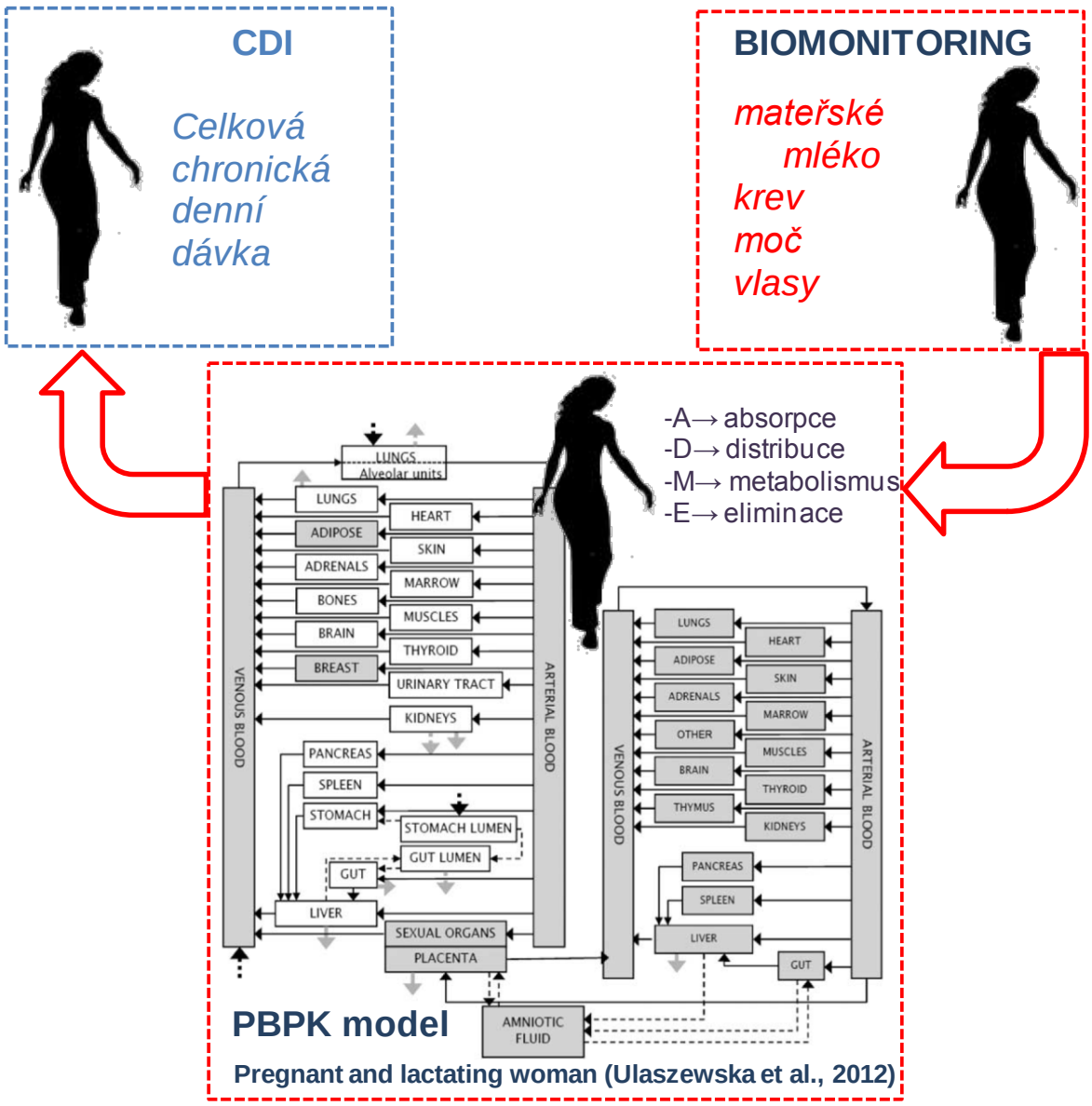
Toxikokinetika tedy představuje kvantitativní způsob studia osudu chemických látek a jejich metabolitů v organismu od momentu vstupu do těla, distribuce do orgánů a tkání prostřednictvím krevního oběhu včetně finálních biotransformačních metabolických procesů a vylučování z těla ven (ADME).

Po přestupu látky přes biologické bariery do organismu na ni totiž dále působí mnoho faktorů, které ovlivňují její potenciál pro projev negativního efektu v cílovém orgánu nebo tkáni. Rychlost transportu do těchto cílových míst účinku v organismu je určena hlavně vstupní dávkou toxické látky a poločasem života (persistencí). Sledovaná látka může mít několik cílových míst účinku. Případně několik látek může mít naprosto stejný cíl účinku. Nicméně i vysoká interní koncentrace nemusí nutně vést k projevu toxicity. Typickým příkladem jsou některé lipofilní látky, které se kumulují primárně v tukových tkáních (depotní tuk), které však pro tyto látky nejsou cílovými místy účinku (Lehman-McKeeman, 2013; Nadal et al., 2013).

Využití toxikokinetických parametrů a jejich modelů pro reverzní dozimetrii právě patří k progresivně se vyvíjejícím novým přístupům v této oblasti (Obrázek 17). Cílem reverzní dozimetrie je predikce chronické denní dávky (CDI) z naměřených tkáňových koncentrací (Abass et al., 2013; Gyalpo et al., 2015; Ulaszewska et al., 2012). Pomocí CDI a dobré znalosti ADME procesů hodnocených látek lze pak parametricky křížově porovnávat vstupní data o koncentracích v různých biologických matricích (krev, moč, mateřské mléko atd.) a to nejen v rámci jedné studie, ale také i referenčně mezi různými kohortami. Tento přístup tedy má velmi silný potenciál pro propojování výsledků z různých monitorovacích programů.

Aplikace dostupných, experimentálně stanovených toxikokinetických dat v modelech umožní definovat (Roberts and Renwick, 2014):

- vnitřní expozici (interní expozici) na základě koncentrací toxikantu v přijímané matrici (např. nabídnutá dávka v potravinách) – prediktivní toxikokinetické modelování,
- vztah mezi koncentrací v plazmě, krvi či moči a cílovým místem toxicity,
- retrospektivní rekonstrukci expozice na základě koncentrací toxikantu v biologických matricích,
- porovnání studií zaměřených na hodnocení koncentrací látek v různých matricích (predikované CDI lze pak přímo porovnávat mezi studiemi/kohortami),
- potenciálně rizikové skupiny exponované populace včetně jejich porovnání.



Obrázek 17: Ukázka využití toxikokinetického modelu v reverzní dozimetrii pro zpětnou rekonstrukci expozice (schéma toxikokinetického PBPK modelu převzato z: (Ulaszewska et al., 2012).

Následnou potenciální biologickou interakcí chemických látek v organismech se zabývá toxiko-dynamika. Její výstupy jsou pak využívány pro modely predikcí možných zdravotních rizik (viz kapitola 4).

3.2.2. Biomonitoring v lidské populaci

Pro hodnocení interní expozice v lidské populaci je nejčastěji využíván biomonitoring konkrétních biotických matric (Human Biomonitoring – HBM). Samotná data pak lze pomocí dlouhodobých časových trendů vyhodnotit. Interpretace těchto dat formou časových trendů koncentrací je však omezena jen na jejich směr a významnost (vzestupný, klesající, stagnující trend). V současné době je výzkum v této oblasti směřován právě k vývoji vhodnějších metod interpretace těchto dat pomocí použití nejnovějších toxikokinetických modelů pro zpětnou

rekonstrukci expozic s cílem vyhodnotit vývoj celkových chronických denních dávek (Gyalpo et al., 2015).

Definice HBM je souhrn metod, zabývající se systematickým, opakovaným, dlouhodobým a standardizovaným odhadem expozice člověka potenciálně nebezpečným chemickým látkám z okolního prostředí, které jsou analyzovány v lidských tekutinách a tkáních (Knudsen et al., 2011). Je to nástroj nejen k ověření expozice populace chemickým látkám z prostředí, ale slouží také ke sledování časových trendů zátěže, posouzení možných zdravotních rizik a ověření účinnosti nápravných opatření.

HBM je prováděn například formou longitudinálních dlouhodobých studií, kde jsou odběry lidských matric opakovány vždy u stejných participantů. Nebo jde případně o „cross-sectional“ studie, kdy se v daný časový interval hodnotí odebrané vzorky u respondentů v různých věkových kategoriích. Ve státech EU jsou HBM studie prováděny v různých formách a se zaměřením na různé chemické látky. Možnost přímého porovnání výsledků je pak v mnoha případech velmi omezená. Proto v loňském roce (2015) vznikla iniciativa EHBMI (European Human Biomonitoring Initiative), jejíž hlavním cílem je co nejvíce propojit aktivity HBM v dílčích státech EU za účelem hodnocení expozice prioritním chemickým látkám v lidské populaci v Evropě. Náš tým je aktuálně do přípravy tohoto projektu aktivně zapojen.

Biomonitoring je realizován nejčastěji pomocí sledování biomarkerů expozice případně i pomocí biomarkerů efektu. Biomarker expozice je v nejnovějších publikacích této problematiky používán ve smyslu hodnot zjištěných koncentrací sledované látky či jejich metabolických produktů v lidských matricích (Brown et al., 2015; Knudsen et al., 2011), což odpovídá přístupu interní expozice. Biomarker efektu pak představuje paralelní hodnocení expozice biologickou specifickou analýzou. Příklad využití přístupu expozičních biomarkerů toxicity *in vivo* byl již popsán v kapitole 3.1.4 (biomarkery poškození DNA *in vivo*). Dalším typem biomarkeru je takzvaný diagnostický biomarker, který již slouží ke stanovení skutečné diagnózy onemocnění. Doplňující hodnotu mají v některých případech biomarkery vnímavosti (susceptibility biomarkers). Některé biologicky významné enzymy se totiž mohou u různých osob vyskytovat v různých formách s odlišnou biologickou aktivitou. Jestliže má tato aktivita pro organismus zásadní ochrannou funkci a u daného jedince se vyskytuje forma enzymu s nízkou aktivitou, je tento jedinec citlivější / vnímavější vůči danému stresoru (např. detekce málo aktivní formy enzymu zodpovědného za odbourávání určitého typu chemické látky indikuje zvýšené riziko z expozice těmto látkám) (Knudsen et al., 2011).

Velký důraz je nyní také kladen na neinvazivní metody biomarkerů expozice: odběry a analýza vlasů, nehtů, zubů, moči nebo vzorků slin z bukálních stěrů (Alves et al., 2014).

V neposlední řadě jsou také v rámci HBM prováděny i analýzy externí expozice (ovzduší, voda, potraviny). Tyto hodnoty jsou velmi cenné právě v případě, kdy lze pomocí toxikokinetiky a vstupních dat interní expozice predikovat retrospektivně chronické denní dávky. Ty jsou pak porovnávány s údaji nabídnuté dávky (externí expozice - například

v dílčích potravinových komoditách), což ve výsledku může napomoci k identifikaci hlavní expoziční cesty.

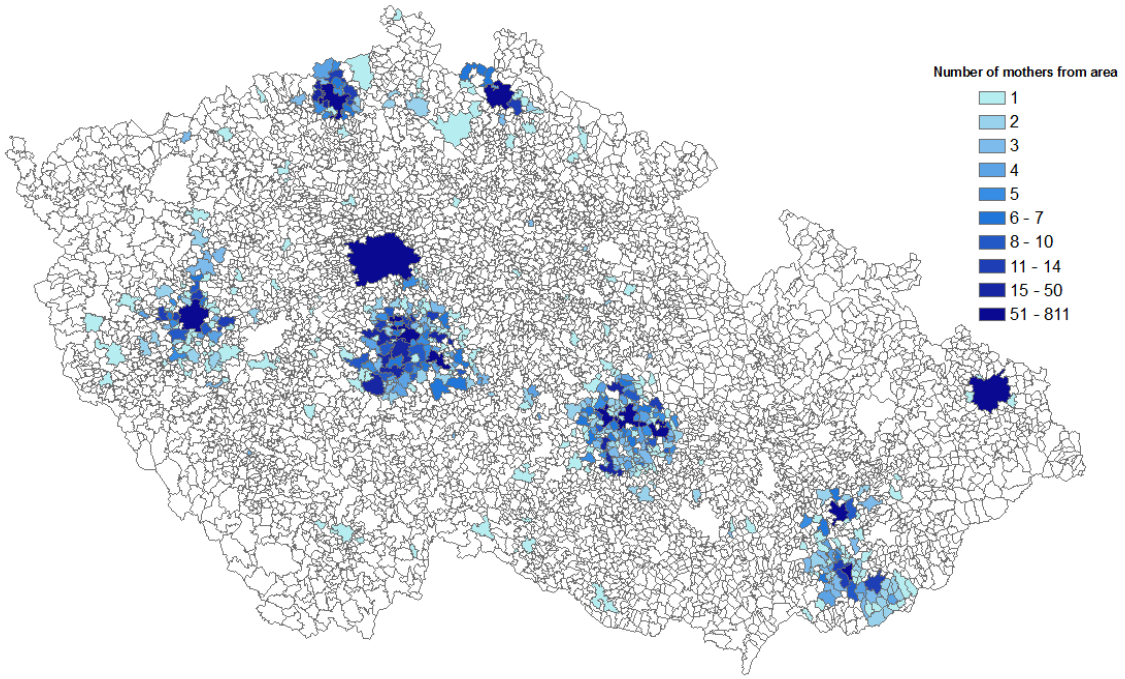
Velmi důležitým přístupem v oblasti lidského biomonitoringu je také odběr vzorků za účelem budování infrastrukturních biobank. Tento přístup funguje na principu bezpečného ukládání odebraných vzorků lidských matric pro budoucí možné využití. Pokud dojde v budoucnosti ke zpětnému přehodnocení nebezpečných vlastností některých již používaných chemických látek, bude možné i zpětně vyhodnotit prostorový i časový vývoj expozice těmto látkám. Právě k těmto zpětným opakovaným hodnocením dochází v případě POPs poměrně často. Dokazuje to výčet chemických látek, které jsou na seznamu Stockholmské úmluvy, nebo případně listu kandidátských látek hodnotícího výboru POPRC.

Proto vývoj nových analytických metod, které umožní flexibilně reagovat na zařazení nových emergentních látek na seznam mezinárodních dohod, je výzkumnou prioritou rozvoje HBM. Do tohoto směru vývoje patří nejen přístup biobank, ale také progresivní metody necílového screeningu (non-target screening) (Baduel et al., 2015; Ganna et al., 2016)**.**

Biomonitoring v ČR je realizován každoročně již od roku 1994. Hlavní matricí je mateřské mléko a v některých fázích projektu jsou hodnoceny i vzorky krve, moči a vzorky vlasů (Černá et al., 2012, 2010, 2007). Realizaci projektu zajišťuje Státní zdravotní ústav v Praze (SZÚ). Název tohoto projektu je „Systém monitorování zdravotního stavu obyvatelstva České republiky ve vztahu k životnímu prostředí“. Tým autora této habilitační práce úzce spolupracuje se SZÚ právě v oblasti dílčí interpretace výsledků biomonitoringu mateřského mléka v ČR (Mikeš et al., 2012).

Odběry biologického materiálu jsou průběžně zajišťovány pracovníky SZÚ nebo příslušnými zdravotními ústavy ve sledovaných regionech. Postup při odběrech vzorků biologického materiálu byl definován Standardním operačním postupem (SOP – Protokol odběru a manipulace se vzorky). Odběru biologického materiálu vždy předcházel informovaný souhlas – po vysvětlení účelu studie každá osoba (respondentka) vyjádřila písemně souhlas s odběrem materiálu a jeho použitím pro biologický monitoring (v rámci protokolu schváleného etickou komisí).

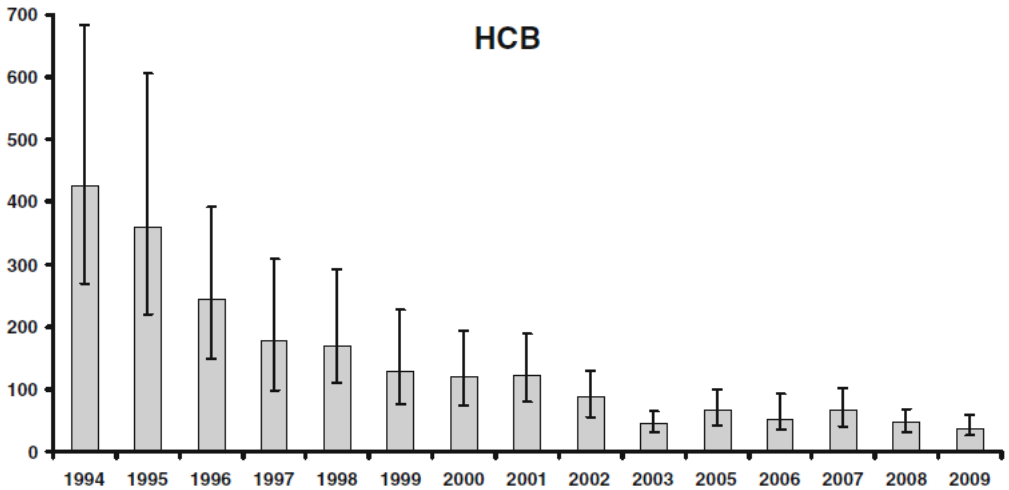
Na následující mapě (Obrázek 18) je prostorové hodnocení účasti respondentek za hodnocené období 1994-2009 na 9 monitorovacích oblastí/měst: Benešov, Žďár n/S, Plzeň, Ústí n/L, Ostrava, Praha, Liberec, Kroměříž a Uherské Hradiště (NUTS 5; 4754 respondentek) (Mikeš et al., 2012) (Příloha 15). Podrobný popis detailů studie je k dispozici také v práci Jany Václavíkové (Václavíková, 2015). Studie byla zaměřena na vybrané POPs: konkrétně na 7 polychlorovaných bifenylů – PCB (28, 52, 38, 118, 138, 153 a 180) a vybrané organochlorové pesticidy – OCP (DDT, DDE, DDD, α-HCH, β-HCH, γ-HCH a HCB).



Obrázek 18: Biomonitoring mateřského mléka v ČR – počty respondentek v hodnocených oblastech (geograficky v NUTS 5) z celkového počtu 4754 respondentek. Převzato z (Mikeš et al., 2012).

Byly vyhodnoceny dlouhodobé trendy výskytu vybraných POPs v mateřském mléce včetně odhadu populačních poločasů života dílčích chemických látek (Mikeš et al., 2012). Na Obrázku 19 je ukázka výsledků hexachlorbenzenu v mateřském mléce, kde je jasně patrný sestupný trend.

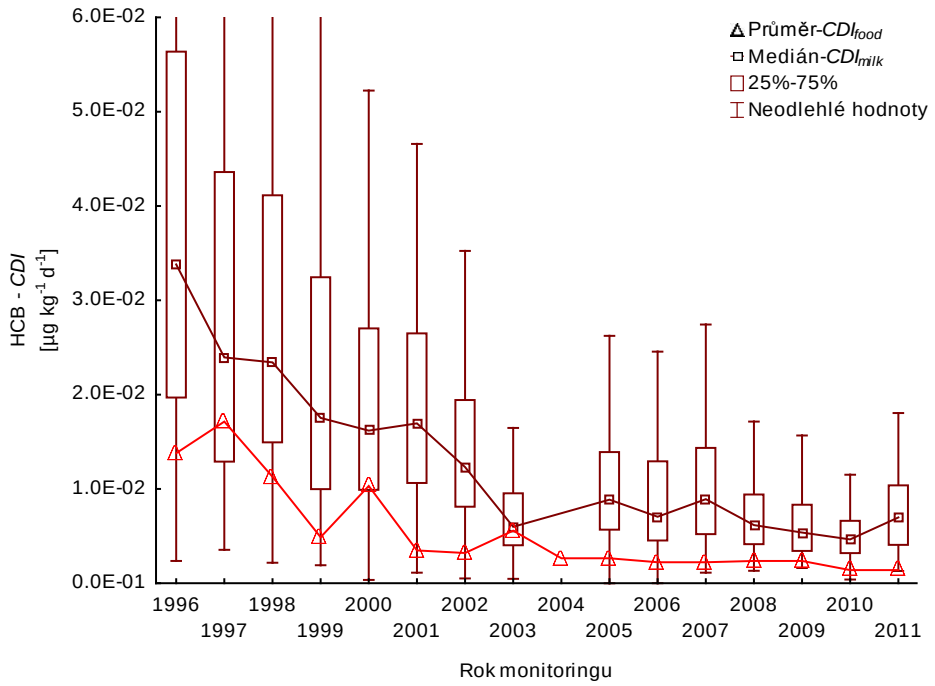
Mateřské mléko bylo vybráno jako matrice z několika důvodů. V prvé řadě pro vysoký obsah lipidů, pro poskytnutí relevantní informace o expozici matky a následně kojence, ale také i z důvodu charakteru neinvazivního typu odběru vzorků. Dlouhodobá data indikují pokračování klesajícího trendu POPs koncentrací v této indikátorové matrici v ČR.



Obrázek 19: Časový trend výskytu hexachlorbenzenu (HCB) v mateřském mléku v ČR. Publikováno 2012: (Mikeš et al., 2012).

Studie na rozdíl od některých literárních zdrojů nepotvrdila kojení jako faktor důležitý pro outflux. Bylo také zjištěno, že hodnoty BMI (body mass index) byly v přímé asociaci s vyšším množstvím HCB a s nižším množstvím výše-chlorovaných PCBs. Celkově tato dlouhodobá studie jasně potvrzuje efektivnost restrikcí používání POPs v ČR. Jedná se o velmi užitečný nástroj pro parametrické hodnocení efektivnosti Stockholmské úmluvy a jejich opatření.

Zjištěné koncentrace POPs za dílčí roky byly následně použity pro inovovaný přístup reverzní dozimetrie s využitím PBPK modelu vyvinutého týmem dr. Trappa (Trapp et al., 2008). Výsledky toho zpětného výpočtu CDI pomocí PBPK na základě koncentrací v mateřském mléce (označované jako CDI_{milk}) byly pak porovnány s hodnotami chronických denních dávek CDI (více na Obrázku 7) z monitoringu SZÚ dietární expozice (MDE) za celé období (CDI_{food}) (Václavíková et al., 2016). V současné době jsou nejaktuálnější výsledky této studie finalizovány do publikace (viz ukázka pro vývoj situace v interní expozici hexachlorbenzenu HCB v ČR; Obrázek 20).



Obrázek 20: Časový trend chronických denních dávek hexachlorbenzenu (HCB) predikovaných pomocí PBPK z koncentrací v mateřském mléku (CDImilk) v ČR a jejich porovnání s CDIfood z dietárního biomonitoringu kontaminace potravinových komodit (Václavíková et al. 2016).

Hodnoty CDImilk sledovaných látek v mateřském mléce vykazují u některých POPs sestupný trend. Pro správné vyhodnocení tohoto trendu je velmi vhodné pokračovat v biomonitoringu i v následujících letech. Zpětně predikované CDI_{milk} pro většinu hodnocených látek byly vůči měření SZÚ na základě kontaminace potravin CDI_{food} významně vyšší, ale trendově se velmi podobaly výsledkům CDI_{food}. Může to indikovat přítomnost jiné

(dosud neznámé) expoziční cesty, kterou bude nutné v dalších studiích případně identifikovat. Výjimkou byly hodnoty γ-HCH, které se blížily se svým trendem a výsledky CDI_{food} nejvíce ze všech sledovaných látek.

I přesto, že byly tyto látky antropogenního původu zakázány v ČR v rozmezí let 1970 – 1980, stále se jejich vysoké hladiny nachází v prostředí a také v lidských matricích.

Dalším aktuálním směrem výzkumu, který s využitím dat realizujeme, je zpřesnění hodnot poločasů života jednotlivých látek v lidské populaci (publikace v přípravě). Tyto lze pak použít do modelů zpětné rekonstrukce expozice (Gyalpo et al., 2015).

4. Interpretace expozičních dat v analýze zdravotních rizik

Data o míře kontaminace dílčích matric životního prostředí (externí expozice) nebo údaje o míře kontaminace biologických matric (interní expozice - viz předcházející kapitola) lze využít pro možné vyhodnocení potenciálních zdravotních rizik s důrazem na karcinogenní a nekarcinogenní efekty polutantů (Čupr et al., 2007) (Příloha 5). Jedná se o screeningovou parametrickou metodu interpretace údajů o expozici v lidské populaci. Predikce zdravotních rizik je prováděna na základě dlouhodobých epidemiologických studií a také s využitím experimentů se zvířaty (Calabrese, 2014). Je však nutné zdůraznit, že se jedná o vyhodnocení jen potenciální vazby mezi expozicí chemickým látkám a možnými zdravotními efekty. Tato metoda interpretace tedy nezahrnuje vlivy dalších významných faktorů celkového exposomu. Výsledek hodnocení zdravotních rizik je tedy nutné správně interpretovat a to jako potenciální příspěvek k celkovým rizikům včetně detailního popisu nejistot (Meek et al., 2011).

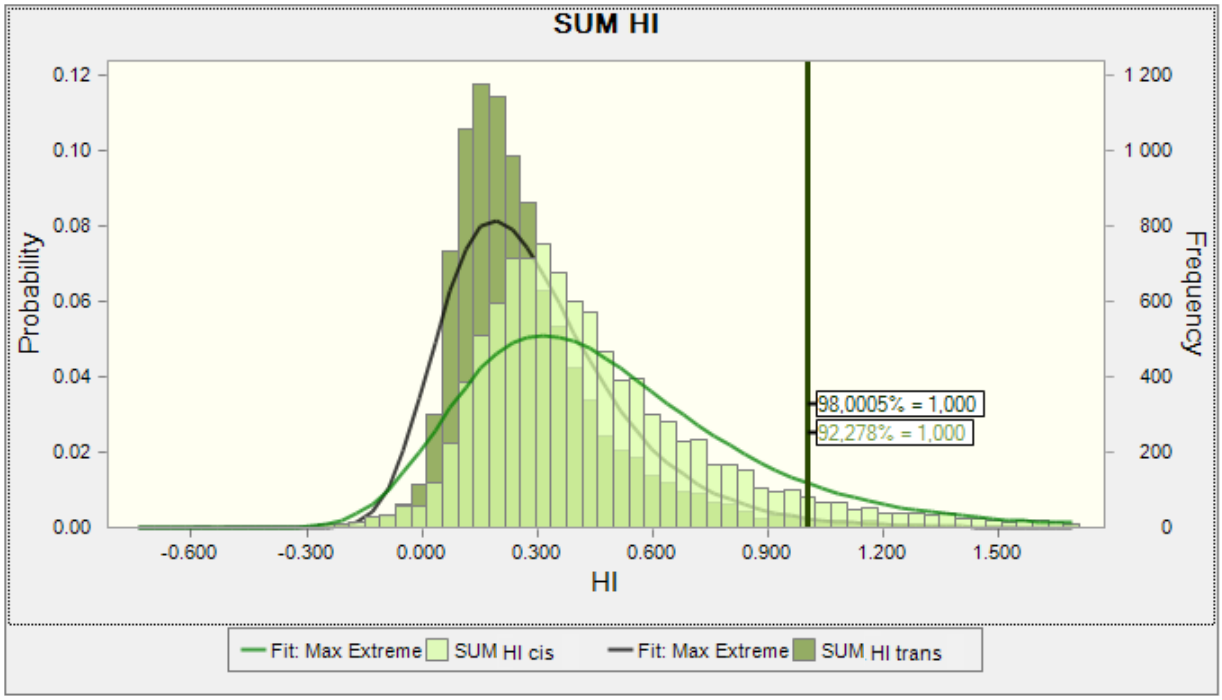
Hodnocení zdravotních rizik představuje metodický postup, který umožňuje systematickým vyhodnocováním epidemiologických studií, experimentů *in vitro* a *in vivo* odhadnout a kvantifikovat potenciální vliv chemického znečištění prostředí na lidské zdraví (Fjeld et al., 2007). Metodika umožňuje na základě souboru toxikologických informací o působících chemických látkách (či obecně stresorech) a jejich vlivu na zdraví modelovat pravděpodobné dopady na zdravotní stav populace (EPA, 2016, 1989). Ovšem jen při důkladné znalosti těchto faktorů a při znalosti hodnocené populace. Výhodou je také možnost prospektivního modelování vlivu ještě neexistujících situací, které je v současné době V ČR v praxi ze zákona povinně aplikováno například při plánování nových technologií, aktivit či staveb.

Hodnocení zdravotních rizik má celkem 4 základní části (EPA, 2005, 1989):

- identifikace nebezpečnosti (*hazard identification*)
- určení vztahu dávka – účinek (*evaluation of dose – response relationship*)
- hodnocení expozice (*exposure assessment*)
- charakterizace rizika (*risk characterisation*)

Po důkladné identifikaci nebezpečnosti hodnocených stresorů je nutné pomocí co nejpřesnějších metod a přístupů predikovat skutečnou expoziční absorbovanou dávku (Čupr, 2016). Ta je pak následně vyhodnocena buď metodou referenční dávky RfD pro případ nekarcinogenní chemické látky, nebo formou pravděpodobnostní charakterizace karcinogenního rizika metodou CSF (*Cancer Slope Factor approach*) pro zvolené populační skupiny (Čupr et al., 2013, 2007; Fjeld et al., 2007).

V současné době je výzkum v oblasti interpretace expozice chemickým látkám, pomocí analýzy zdravotních rizik, směřován k využití pravděpodobnostního modelování (Bányiová et al., 2016; McNally and Loizou, 2015). V postupu pravděpodobnostních výpočtů celkových chronických denních dávek a z nich plynoucích zdravotních rizik vstupují celé distribuční statistické hodnoty/charakteristiky parametrů a nikoli tedy jen jedno číslo za danou proměnnou (Bányiová et al., 2016) – Příloha 27. Výsledek je pak také ve formě pravděpodobnosti (ukázka výsledku pravděpodobnostního hodnocení - Obrázek 21).



Obrázek 21: Výsledky analýzy nekarcinogenních rizik ve formě celkové sumy HI za všechny hodnocené expoziční cesty (HI - hazard index): optický izomer trans-EHMC (tmavě zelená distribuční křivka) a cis-EHMC (světlezelená). EHMC je ethylhexyl methoxycinnamát – je to aktivní látka používaná ve většině opalovacích krémů jako UV-filtr. Detail výzkumu je v kapitole 3.1.5.3. Graf je převzat z manuskriptu (Nečasová et al., 2016).

Pravděpodobnostní modelování zdravotních rizik se stává součástí komplexních modelů hodnocení expozice (další směr vývoje a výzkumu). Příkladem je systém Merli-Expo (Suciu et al., 2014) (http://merlin-expo.eu/), který kombinuje v současné době devět modelů s predikcí externí expozice (osud látek v akvatickém a terestrickém prostředí s modely transportu do expozičních médií: potraviny, ovzduší) s PBPK modelem expozice a hodnocení následných zdravotních rizik v lidské populaci.

5. Závěr

Předložená práce shrnuje aktuální problematiku hodnocení expozice chemickým látkám a jejich možných zdravotních rizik v lidské populaci. Jsou zde popsány významné směry výzkumu v této oblasti. Vybrané metody hodnocení jsou doplněné souborem publikací autora předložené habilitační práce.

V minulosti bylo uvedeno na trh mnoho nových chemických látek využitelných v průmyslových či zemědělských aplikacích. U řady z nich byly až následně identifikovány významné negativní biologické efekty. Pro správné vyhodnocování jejich zdravotních rizik je kromě informací o toxikodynamice (účincích) důležité důkladně znát celý proces expozice včetně parametrů, které na ni mají největší vliv. Nutnost detailnějšího hodnocení expozice vychází i z výsledků dlouhodobých biomonitorovacích studií, které často identifikují vysoké hladiny toxikologicky významných chemických látek ve vzorcích biotických matric (krev, mateřské mléko, moč, atd.). Výzkum je proto zaměřen na dílčí fáze expozice chemickým látkám od identifikace zdroje po místo jejich kontaktu, následného transportu a také včetně dalšího studia osudu uvnitř organismu až po možný biologický efekt.

V předložené práci byly popsány metody pro zpřesňování hodnocení expozice chemickým látkám, což je zásadně důležité pro identifikaci a parametrizaci nejvýznamnějších cest transportu těchto látek do lidského těla. Vhodným propojením dostupných dat a modelů externí a interní expozice lze pak přesněji popsat celý proces a jeho klíčové parametry a efektivněji eliminovat nebo alespoň redukovat potenciální zdravotní rizika. Práce kombinuje toxikologické a chemické metody a propojuje výsledky terénních studií zaměřených na kontaminaci složek prostředí a lidských matric s výsledky laboratorních experimentů. Tento výzkum je klíčový pro další strategické směřování centra RECETOX, které usiluje o pochopení potenciálních vztahů mezi expozicí populace a rozvojem některých onemocnění. To však vyžaduje neustálý rozvoj interdisciplinární expertízy a vazeb, které by nebyly možné bez úzké spolupráce s dalšími týmy centra RECETOX. Kolegům i studentům centra proto patří závěrečné poděkování autora.

6. Zkrácený životopis

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2002 - dosud, Centrum pro výzkum toxických látek v prostředí - RECETOX, Přírodovědecká fakulta, Masarykova univerzita. (odborný asistent, vědecký pracovník).

Absolvovaná vědecká stáž:

2009 National Research Centre for Environmental Toxicology (EnTox) University of Queensland, Brisbane, Australia (3,5 měsíce).

Odborná činnost:

Výzkum v oblasti hodnocení expozice chemickým látkám v lidské populaci; charakterizace potenciálních zdravotních rizik z expozice toxickým a karcinogenním látkám (POPs a další xenobiotika); interní a externí expozice; perkutánní transport - experimentální *ex vivo* metody pomocí Franzových cel s kožními štěpy lidské kůže; toxikokinetické PBTK modely a jejich zpřesňování; interpretace dat biomonitoringu; výskyt POPs v mateřském mléce; hodnocení dlouhodobých trendů výskytu POPs v lidské populaci; dietární, inhalační a dermální expozice; pravděpodobnostní hodnocení zdravotních rizik; aplikace nových progresivních testů genotoxicity; biomarkery expozice; indoor/outdoor expozice; vývoj nových vzorkovacích technik; publikační činnost; řízení projektů vědy a výzkumu, koordinace a řízení aktivit dalších pracovníků; oponování projektů vědy a výzkumu; výuka – analýza rizik; ekotoxikologické biotesty (genotoxicita); tvorba výukových materiálů; vedení diplomantů a doktorandů.

Členství ve společnostech:

- SETAC - Society of Environmental Toxicology and Chemistry
- ISEE - International Society for Environmental Epidemiology
- Člen mezinárodního panelu odborníků POPRC - Persistent Organic Pollutants Review Committee (při Stockholmské Úmluvě SC) - http://chm.pops.int/;
- Člen Rady Národního centra pro toxické látky (od 2006) http://www.recetox.muni.cz/index.php?pg=narodni-pops-centrum--rada-narodniho-centra

7. Seznam publikací autora habilitační práce (dle databází SCOPUS a WOS)

V habilitační práci je v textu citována většina publikací autora. V následujícím seznamu jsou uvedeny tyto publikace (z WOS a SCOPUS) s indikací těch, které jsou v plném znění v označené příloze (Příloha XX; C - *corresponding author* je Pavel Čupr; IF - WOS *impact factor* dostupný při přijetí článku v tisku; Q1 - *Quartile in Environmental Sciences* - kvartil hodnocení časopisu při akceptaci článku; SS - *Soil Science*). Úplný seznam všech publikací autora a jejich kompletní kopie jsou součástí dokumentace k habilitaci. Jejich seznam i s odkazy na plné verze je též v databázích RESEARCH GATE, REASERCHERID WOS, SCHOLAR GOOGLE nebo ORCID ID. Viz adresy na konci seznamu.

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9. Přílohy

Příloha 1

Bartoš, T., Škarek, M., **Čupr, P.**, Kosubová, P., Holoubek, I., 2005. Genotoxic activity of a technical toxaphene mixture and its photodegradation products in SOS genotoxicity tests. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 565, 113–120.

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Genotoxic activity of a technical toxaphene mixture and its photodegradation products in SOS genotoxicity tests

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Abstract

Toxaphene (CAS No. 800-35-2) is a complex mixture of several hundred components that was used worldwide primarily as an agricultural pesticide with insecticide effects in the second half of the 20th century. In vitro investigations of the genotoxicity and mutagenicity of toxaphene were generally described in the literature, but they provided somewhat equivocal results. We re-evaluated the genotoxicity of technical toxaphene in two prokaryotic systems. The SOS Chromotest showed high sensitivity to toxaphene: three concentrations (40, 20 and 10 mg/l) were clearly positive and the dose–response effect was evident. In the *umu*C assay, a dose-dependent increase in genotoxic activity was observed at toxaphene concentrations from 2.5 to 40.0 mg/l, but these results were found to be not significant. The genotoxicity of toxaphene and its photodegradation products after UV-irradiation (3–6–9 h) at concentrations ranging from 7.5 to 60.0 mg/l was also examined in this study. An irradiated solution of technical toxaphene after 3 h showed no significant evidence of bacterial growth inhibition. However, exposure of *Salmonella* to 6 h UV-irradiated toxaphene showed a toxic effect compared with the negative control. After 9 h irradiation, a decrease of bacterial growth was observed. Activity of β-galactosidase in the presence of a toxaphene solution was significantly increased after 6 and 9 h irradiation, reaching values that were 2.4- and 3.1-fold higher, respectively, than the control, which exceeded the criteria of significant genotoxicity. These results show that while technical toxaphene is a weak, direct-acting mutagen in some bacterial tests, a dose-dependent toxicity and genotoxicity of its photoproducts could be conclusively demonstrated by the *umu*C test.

Keywords: Toxaphene; Genotoxicity assays; SOS response; *umu*C test; Photodegradation; UV-irradiation

1. Introduction

Toxaphene (CAS No. 800-35-2) is a complex mixture of several hundred components that results from the chlorination of technical camphene. The theoreti-

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cal number of different chlorinated congeners is 32,768 [1], but only a few hundreds of them have an environmental significance [2]. Toxaphene has been in use worldwide, primarily as an agricultural pesticide with insecticide effects in the second half of the 20th century [3]. Although the use of toxaphene has been banned during the 1980s, some countries have no restrictions on application of this pesticide and are still suspected of current use of toxaphene or toxaphene-like products [4]. Due to the persistent volatile and lipophilic nature of some toxaphene compounds in the mixture, these compounds have a tendency to bioaccumulate in animals. More than 10 years after its major use, toxaphene was found to be one of the most abundant pesticides detected in polar aquatic organisms [5]. The widespread distribution of toxaphene indicates that this compound may be transported over long distances. The atmosphere is its main environmental transport medium. It may enter surface water and soil due to wet and dry deposition processes.

Because of its high persistence and bioaccumulative potency the toxic effects of toxaphene are of great interest. The complexity of toxaphene complicates its evaluation from the toxicological point of view. Up to now, the toxic and genotoxic effects of individual congeners of toxaphene have not been evaluated adequately. Some toxaphene congeners may be more toxic than the technical toxaphene mixture itself [6]. Although in vivo studies showed no evidence for mechanisms of a genotoxic interaction of toxaphene, in vitro studies did show that toxaphene is genotoxic in mammalian cell systems and mutagenic in the Ames test [3]. These in vitro investigations of genotoxicity and mutagenicity of toxaphene were generally described in the literature, but they provided somewhat equivocal results. Studies by Hooper et al. [7] showed that the toxaphene mixture is mutagenic in *Salmonella typhimurium* tester strains TA98 and TA100 in the presence or absence of liver S9 fraction. S9 added for metabolic activation caused a decrease in mutagenicity. The genotoxic response of the technical mixture disappeared in the presence of rat S9 liver homogenate, indicating that the parent compounds causing direct genotoxicity are easily metabolised to non-genotoxic metabolites. A dose-dependent increase in *his* revertants was also observed in tester strains TA97, TA98, TA100, TA102 and TA104 in the absence of S9 metabolic activation [8]. In contrast to these conclusions, a study by Mortelmans et al. [9] described somewhat inconsistent results. Using several different strains, toxaphene was mutagenic in *Salmonella* tester strains TA98 and TA100, but no evidence of mutagenicity was observed with strain TA1537 at toxaphene concentrations ranging from 0 to 10 mg/ml. Negative results were obtained also in tester strain TA1535 without S9 fraction. The genotoxicity of technical toxaphene, as well as toxaphene congener B[30012]-(1 1 1), but not B[12012]-(2 0 2), B[12012]-(2 1 2) and B[30030]-(1 2 2), was also demonstrated by Boon et al. [10]. Toxaphene and four its congeners B[12012]-(2 0 2), B[12012]-(2 1 2), B[30030]-(1 2 2) and B[30012]-(1 1 1) were tested for mutagenic activity in *Salmonella* strains TA98 and TA100 using the micro-suspension procedure. Toxaphene was mutagenic only in the TA100 strain at concentrations of 2.5, 5.0 and 10.0 mg/ml. None of the four tested toxaphene congeners was mutagenic in strain TA100 [11]. In vitro evidence for genotoxicity was found in studies examining SCE induction [12]. Small but significant increases in SCE have been observed in human lymphoblasts. In contrast, toxaphene failed to induce mutation in the Chinese hamster V79/HGPRT mutation assay. Small increases in SCE relative to untreated control cultures were found in V79 cells exposed to 5.6 and 10 μg/ml, but these results were found to be statistically non-significant [8].

The transformation of toxaphene in hexane under UV-light conditions is comparable with its degradation in anaerobic soil [13] and sediment [14]. Exposure of toxaphene to UV-irradiation results in dechlorination and dehydrochlorination, mainly of the highly chlorinated congeners. Toxaphene congeners can be divided in three groups: the first group (e.g. Parlar 26, 40, 50) is very stable, whereas representatives of the second group (e.g. Parlar 39, 58) degrade very easily via photoelimination of a chlorine atom. This reaction leads to the formation of chlorobornanes that also degrade in the presence of UV-radiation or by reaction with reactive oxygen species. The third group is also relatively stable except for congeners with a second dichloro group in the C10 position [15]. The degradation of chlorobornanes primarily depends on the individual substitution pattern at the six-membered ring. The presence of a geminal dichloro group at the ring in the C2 position is responsible for the high instability of certain chlorobornanes, whereas the alternating chlorine substitution (2-*endo*, 3-*exo*, 5-*endo*, 6-*exo*) leads to an extremely high

stability [13]. Although chlorobornanes cannot react with ozone, these compounds are readily attacked by the OH radical. UV-radiation is the only abiotic process that terminates the reaction induced by the OH radical. In the troposphere, UV-radiation contributes to toxaphene photolysis much more than expected [15].

In this study, the genotoxic effects of a toxaphene mixture are investigated by means of the SOS Chromotest and the *umu*C test. Both are based on the induction of the SOS repair system as a result of interaction of the test compound with DNA of a genetically modified tester strain. In addition, the degradation pathways for toxaphene components and their influence on the genotoxic activity of the possible UV-induced degradation products are discussed.

2. Materials and methods

2.1. Chemicals

Toxaphene (technical mixture, LA84599) was purchased from Supelco, Bellefonte, PA, USA. The concentration of the stock solution in methanol was 5 mg/ml. All other chemical reagents were of the highest available quality.

2.2. UV-irradiation of toxaphene and chemical analysis

An aliquot of the stock solution of toxaphene in methanol was diluted in water of milliQ quality and irradiated in quartz tubes [16]. Irradiation was conducted at 5 cm distance from the light source (medium pressure Hg lamp, 125 W, Teslamp Co., Praha, Czech Republic) and lasted for 3, 6 or 9 h. The same irradiation time was used in a previous study of toxaphene photoproducts [17]. The irradiated samples were then divided into portions for chemical analysis and for toxicity and genotoxicity tests.

For the chemical analysis, the water samples were extracted with dichloromethane and concentrated in hexane. Analyses were performed using a Finnigan GCQ gas chromatograph coupled with an external ionization ion-trap mass spectrometer. The ion trap was operated in the MS/MS mode, with electron ionization at 70 eV, ion source temperature 200 °C and transfer line temperature 275 °C [18]. Before the toxicity and genotoxicity tests, the irradiated water samples were adjusted to pH = 7.0 ± 0.5.

2.3. SOS genotoxicity tests

The SOS Chromotest was performed according to a slightly modified method of Xu et al. [19]. The tester strain *Escherichia coli* PQ 37, described in detail by Quillardet et al. [20], was grown overnight at 37 °C in LB medium containing ampicillin (20 μg/ml). After the incubation period, the culture was diluted 50-fold into a fresh LB medium with ampicillin and incubated for another 2 h. The optical density (600 nm) of the incubated culture was adjusted to 0.04 and mixed (3:1) with phosphate buffer (pH 7.4). The stock solution of the test substance was diluted with methanol. Eight microliters of each of the concentrations was mixed with 992-μl portions of the bacterial dilution in tubes (1.5 ml) to reach final concentrations of 40.0, 20.0, 10.0, 5.0 and 2.5 mg/l. Eight microliters of methanol were taken as negative control, and 4-nitroquinoline *N*-oxide (30, 15 mg/l) was used as positive control. The mixtures were incubated for 2 h at 37 °C. Meanwhile, two microplates were prepared for measurements of enzyme activities. β-Galactosidase activity (genotoxicity assay) was determined after addition of 25 μl of the content of the incubated tubes into 100 μl of B-buffer solution (pH 7.0) with *o*-nitrophenyl-β-D-galactopyranoside (2 mg/ml). Alkaline phosphatase activity (toxicity assay) was determined after the addition of 25 μl of the content of the incubated tubes into 100 μl of P-buffer solution (pH 8.8) with *p*-nitrophenyl-phosphate (2 mg/ml). The microplates were incubated for 45 min at 37 °C and the enzyme activity was determined by spectrophotometric measurement at 420 nm. Toxic effects were quantified as a percentage of the alkaline phosphatase activity in comparison with the negative control. The concentrations that showed more than 50% inhibition were excluded. The SOS induction factor (IF) was then calculated for each of the test concentrations. When the induction factor for any of the test concentrations reached 1.5, the test substance was scored as a significant genotoxin. This approach is equivalent with using statistical methods and makes evaluating easier [21].

The *umu*C test was performed according to a slightly modified ISO method [22]. The tester strain

S. typhimurium TA1535/pSK1002 was grown in TGA medium (containing 20 μg of ampicillin per ml) overnight at 37 °C. After the incubation period, the optical density (600 nm) was adjusted to 0.08 by addition of fresh TGA medium. Complete reaction mixtures were prepared in 1.5-ml tubes and consisted of 75 μl 10 × TGA medium, 260 μl of the bacterial suspension and 665 μl of each of test dilutions. Methanol was taken as a negative control and a solution of 4-nitroquinolin *N*-oxide (0.078, 0.156 mg/l) was used as a positive control. In the case of UV-irradiated samples, final concentrations (7.5, 15, 30 and 60 mg/l) were prepared by mixing the water sample with methanol, after which 250 μl was transferred (in three replicates) into a microplate. The microplate was incubated for 4 h at 37 °C. Before and after the incubation, the optical density (600 nm) of each microplate well was measured to quantify growth inhibition during the exposition (toxicity assay). Twenty-five microliters of the content of the wells were then transferred into wells of a second microplate with 100 μl of B-buffer (pH 7.0). Then 25 μl of phosphate buffer with *o*-nitrophenol-β-D-galactopyranoside (4.5 mg/ml) was added into the wells. The second microplate was incubated for 30 min at 37 °C. The optical density (420 nm) was measured before and after the incubation to determine β-galactosidase activity. Toxic and genotoxic effects were quantified and evaluated in the same way as in the case of the SOS Chromotest.

3. Results

3.1. Genotoxic activity of technical toxaphene mixture

The genotoxic effect of the technical toxaphene mixture in the absence of S9 fraction was investigated in *E. coli* tester strain PQ 37 with the SOS Chromotest. The results are shown in Fig. 1A. No evidence of a significant cytotoxic effect was observed at any test concentration (Fig. 1B). According to the criteria of Quillardet, a substance is unambiguously genotoxic when the induction factor IF is at least 1.5 times that of the negative control. With methanol as a solvent, the highest three concentrations (40, 20 and 10 mg/l) were clearly genotoxic according to these criteria and the dose–response effect was evident.

The technical mixture of toxaphene was also tested in the *umu*C test system with *S. typhimurium* TA1535/pSK1002 in the absence of metabolic activation. A dose–response dependence of β-galactosidase activity was seen with test concentrations ranging from 2.5 to 40 mg/l toxaphene (Fig. 2A). No toxic effects detected on the basis of reduced bacterial growth were observed, as shown in Fig. 2B. In contrast to the SOS Chromotest, results from the *umu*C test were found to be statistically non-significant.

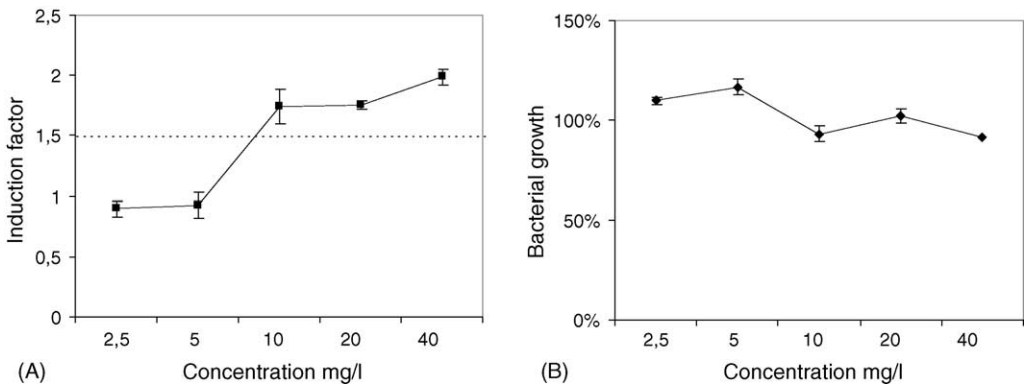

Fig. 1. Genotoxic (A) and cytotoxic effects (B) of a technical toxaphene mixture the in SOS Chromotest. Results are presented as means ± S.D. for triplicate determinations. The genotoxicity criterium of IF = 1.5 is marked by the dashed line.

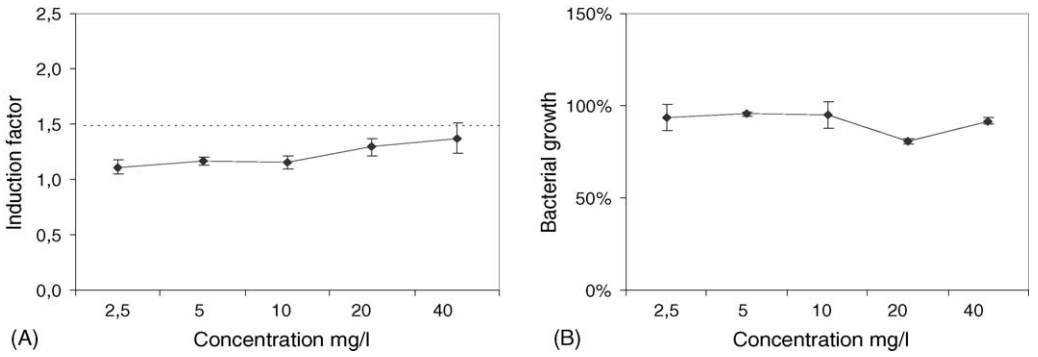

Fig. 2. Genotoxic (A) and cytotoxic effects (B) of a technical toxaphene mixture in the *umu*C test. Results are presented as means ± S.D. for triplicate determinations. The genotoxicity criterium of IF = 1.5 is marked by the dashed line.

3.2. Genotoxic activity of UV-irradiated technical toxaphene

Unirradiated and 3 h irradiated solutions of the technical toxaphene mixture did not give rise to significant inhibition of bacterial growth, but the growth of *Salmonella* exposed to toxaphene at a concentration of 60 mg/l after 6 h irradiation was only 22.2% compared with the negative control. After 9 h irradiation, the bacterial growth at toxaphene concentrations of 30 and 60 mg/l was reduced to 14.8 and 0%, respectively. The results of all test concentrations after 0, 3, 6 and 9 h of UV-exposure are presented in Fig. 3. The rapid growth inhibition did not allow the calculation of an induction factor from the activity of β-galactosidase.

Results at a toxaphene concentration of 7.5 mg/l showed a dose–response dependence of β-galactosidase activity, but this relation was found to be statistically non-significant. The same conclusion was reached at an eight-fold higher concentration of technical toxaphene, but this was caused by the impossibility to calculate a relevant induction factor after the 6 and 9 h irradiations, due to a strong growth inhibitory effect. The activity of β-galactosidase was significantly increased at a toxaphene concentration of 15 mg/l after 9 h irradiation, reaching even 3.2-fold higher values in comparison with the negative control (Fig. 4—open circles). The same effect was observed at 30 mg/l, where toxaphene after 6 h irradiation induced a 2.4-fold stronger genotoxic effect compared with the control and exceeded the criteria for significant genotoxicity (Fig. 4—closed circles). The genotoxic data for a 9 h irradiation were excluded due to growth inhibition of *Salmonella*.

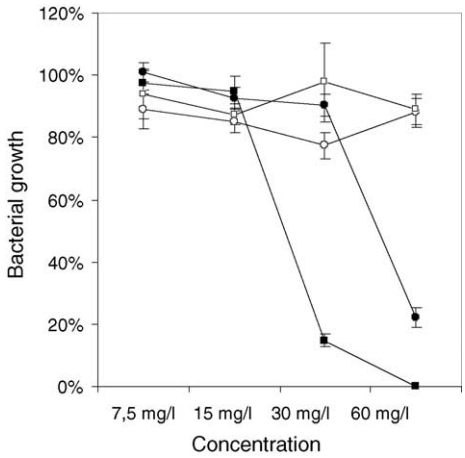

Fig. 3. Cytotoxic effect of a toxaphene mixture before (t = 0 h—open circles) and after UV-irradiation at three times (t = 3 h—open squares, t = 6 h—closed circles, t = 9 h—closed squares). Results are presented as means ± S.D. for triplicate determinations.

The chemical analysis of irradiated toxaphene showed the decrease of all selected congeners in relation to toxaphene unexposed to UV-light. The decrease of 22 congeners was determined at three irradiation times (Fig. 5, Table 1). The significant degradation after 6 and 9 h of irradiation was recorded. Examples are shown in Fig. 6.

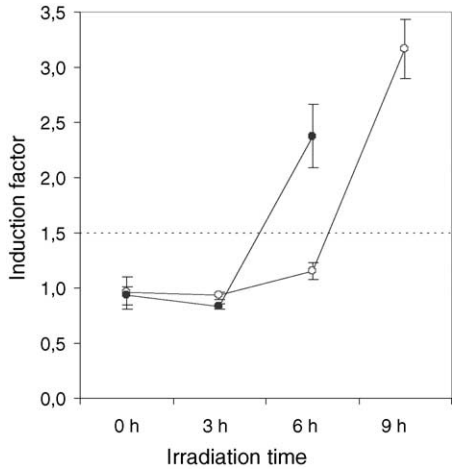

Fig. 4. Relationship of activity of β-galactosidase presented as induction factor at two concentrations (15 mg/l—open circles and 30 mg/l—closed circles) of a toxaphene mixture to the UV-irradiation time. Results are presented as means ± S.D. for triplicate determinations. The genotoxic criterium of IF = 1.5 is marked with a dashed line.

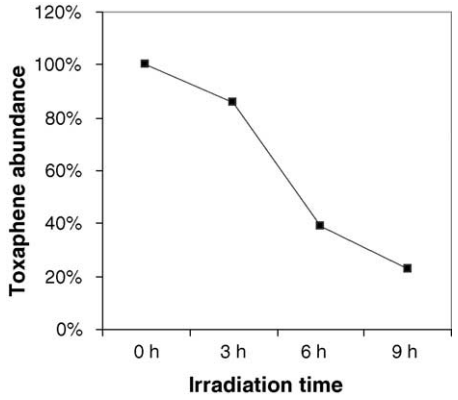

Fig. 5. Average abundance of 22 toxaphene congeners in relation to irradiation time (compared to time 0 h).

4. Discussion

Many studies over the past years have focused on the mutagenicity and genotoxicity of toxaphene. Several human studies have shown that the incidence of cancer could be associated with inhalation exposure to a number of pesticides, including toxaphene. However, these studies were inconclusive due to lack of information on exposure levels and concurrent exposure to other pesticides [6]. A study by the National Toxicology Program (NTP) reported an increase in liver tumours in male and female mice and an increase in thyroid tumours in male and female rats when toxaphene was fed in the diet [23], but toxaphene has proven negative in the mouse dominant lethal assay [24]. The International Agency for Research on Cancer (IARC) has concluded that while there is inadequate evidence for the carcinogenicity of toxaphene in humans, there is sufficient evidence in some experimental animals. Therefore, IARC has classified toxaphene as a possible human carcinogen—Group 2B [25]. The present study was undertaken to fill some data gaps on the genotoxicity of technical toxaphene in prokaryotic genotoxicity assays and to resolve the inconsistencies between published genotoxicity effects.

In this study, toxaphene induced the SOS repair system of *E. coli* PQ 37. The review by Quillardet and Hofnung [26] stated that the concordance between the SOS Chromotest and the *Salmonella*/microsome assay is very high (over 82%). The induction of β-galactosidase synthesis in this test reflects the level of expression of SOS functions involved in inhibition of cell division

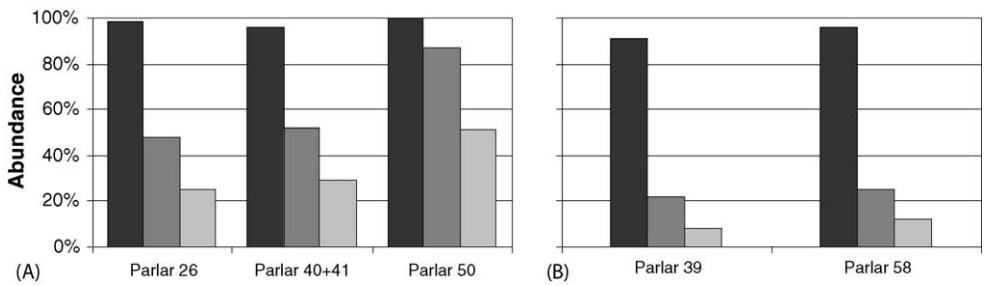

Fig. 6. Abundance of (A) a relatively stable and (B) an easily eliminated group of toxaphene congeners after 3 h (black bars), 6 h (dark grey bars) and 9 h (grey bars) of UV-irradiation compared with time 0 h.

Table 1
Abundance (%) of 22 toxaphene congeners in relation to irradiation time (compared to time 0 h)

Congener name	3 h	6 h	9 h
Parlar 11	54	23	12
Parlar 12	97	67	41
Parlar 15	100	95	82
Tox metab 1	90	32	33
Parlar 21	77	30	17
Parlar 25	30	14	9
Parlar 26	98	48	25
Parlar 31	30	14	10
Parlar 32	88	22	10
Parlar 38	94	27	12
Parlar 39	91	22	8
Parlar 40 + 41	96	52	29
Parlar 42	95	32	15
Parlar 44	99	45	28
Parlar 50	100	87	51
Parlar 51	91	30	17
Parlar 56	88	16	8
Parlar 58	96	25	12
Parlar 59	93	39	18
Parlar 62	100	42	20
Parlar 63	95	48	23

controlled by lexA, which also controls the indirect mutagenesis caused by DNA-damaging agents as detected in the *Salmonella*/microsome assay. Our results confirm the concordance between the two test systems and are in agreement with recently published reports [8] in which toxaphene is mutagenic in all tester strains of *S. typhimurium* TA97, TA98, TA100, TA102 and TA104. The dose–response curve of β-galactosidase activity measured at toxaphene concentrations ranging from 2.5 to 40 mg/l was found also in the *umu*C test with *S. typhimurium* TA1535/pSK1002. In contrast to the SOS Chromotest, the induction factor was lower than 1.5 relative to the control variant and the results were found to be statistically non-significant. Growth inhibition of the test bacteria due to cytotoxicity is often an interfering factor that may affect the observed genotoxic response and invalidate the test results. Both in the SOS Chromotest and the *umu*C test there was no evidence of significant growth inhibition at any of the test concentrations ranging from 2.5 to 60 mg/l of technical toxaphene mixture. The complex nature of toxaphene with possibly a heterogeneous composition may cause some of the differences between genotoxicity observed in this study and other reports.

Besides technical toxaphene, we also studied the genotoxic properties of UV-irradiated toxaphene, because of lack of data about genotoxic activation. In parallel chemical analyses of the transformed toxaphene photoproducts, it was observed that the concentration of 22 congeners of technical toxaphene decreased with increasing irradiation time. Table 1 shows the photostability of the toxaphene congeners, analogous to other studies [13,15]. The main reaction has been dechlorination. Because of the decrease in concentration of 22 detectable congeners and the simultaneous increase in genotoxic potency of irradiated water samples, we suppose also that formation of possible oxygenous toxaphene products could have occurred [27] (DNA binding species), whose effect is caused through interaction with oxygen radicals [8]. These compounds may have strong, genotoxic properties compared with technical toxaphene.

Toxaphene may also contribute to genotoxicity and mutagenesis through a number of indirect mechanisms not examined in the present report. For example, toxaphene might act as a co-mutagen by interfering with the repair of DNA damage caused by another agent [8]. These possibilities are currently under investigation in our laboratory.

Acknowledgements

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Příloha 2

Čupr, P., Škarek, M., Bartoš, T., Cigánek, M., Holoubek, I., 2005. Assessment of human health risk due to inhalation exposure in cattle and pig farms in South Moravia. *Acta Veterinaria Brno* 74, 305–312.

Assessment of Human Health Risk due to Inhalation Exposure in Cattle and Pig Farms in South Moravia

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Abstract

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The main topic of this study was human health risk assessment of defined inhalation exposure scenario in selected cattle and pig farms in south Moravia (Czech Republic). This exceptional evaluation of potential risks for farms manipulators was the main contribution of this study. Possible both human health risks, non-carcinogenic and carcinogenic (according to US EPA Human health risk assessment methodology), for feeders and other workers exposed to polluted indoor air in the farm stables were quantified in the selected pig and cattle farms with significantly increased concentrations mainly of carcinogenic PAHs and PCBs in the indoor air. No non-carcinogenic risks were determined in any of the localities, but also increased carcinogenic risks were observed. The highest carcinogenic health risk was found in the cattle stable ($MAX_{IECR} = 8.08 \cdot 10^{-6}$), the lowest one in the pig stable ($MIN_{IECR} = 2.57 \cdot 10^{-6}$). Carcinogenic risk values in the farms under study were not extremely high, but those were approximately twice higher than a median value of the risk determined for research workers from Košetice $IECR = 5.96 \cdot 10^{-7}$ (years 1996 – 1999), Central European background monitoring station of EMEP.

Carcinogenic and non-carcinogenic risk, PAHs, PCBs, farm stable

Air pollution is one of the most serious environmental problems. Due to various anthropogenic activities a broad spectrum of pollutants are emitted in huge amounts in the air. Nowadays a major concern is focused on organic pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs). They are generally called persistent organic pollutants (POPs). These compounds are ubiquitous air pollutants and their presence in the air results from emissions from diverse sources (Buehler et al. 2001; Breivik et al. 2004). They are able to show serious toxic effects on humans as well as wildlife in very low concentrations (Holoubek et al. 1999). Moreover they persist for a long time in the environment and tend to bio-concentrate in animal tissues. Increased exposure to these chemicals may be associated with increased health and ecological risks (Eljarrat and Barcelo 2003).

While quite a lot of information about outdoor air concentrations of POPs and human exposure exist (Halsall et al. 1995; Buehler et al. 2001; Kim et al. 2004), not too much is known about their levels indoor and their health risks. Due to indispensable emissions of indoor sources and insufficient aeration, higher concentrations of air pollutants, including POPs, may be achieved indoor. Indoor inhalation exposure to air pollutants is one of significant factors that may increase health risks (Jones 1999). An emphasis is placed mainly on the occupational exposure, where there are efforts to recognize, monitor and eliminate high exposures that may cause serious damage of human health as described in numerous studies (Tucek et al. 1998; Sweeney et al. 2000; Palus et al. 2003; Turci et al. 2003).

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For this purpose human health risks may be assessed. One of the approaches is based on assessment via determination of concentrations of priority pollutants. And then either the concentrations of the pollutants may be simply considered according to established limits or a complete health risk assessment may be performed. While the former approach is based on simple comparison of each of pollutants concentrations with safe levels, the latter enables to integrate exposures to several pollutants under exactly defined exposure conditions. The significance of the second approach is mainly emphasized by the fact that the limits are not very often available and persons are exposed usually to more than one pollutant. These assessments are done predominantly in risk workplaces such as hospitals and laboratories, chemical industry, steelworks, gas plants etc.

However there are many other workplaces that are not monitored and under any control from the point of exposure to such pollutants as POPs, because their high concentrations are unexpected there. This study presents one of the examples. Higher concentrations of PAHs (mainly carcinogenic PAHs), PCBs and OCPs in indoor air were detected in pig and cattle farms in the Hodonín District (see Table 1 and 2). Concentrations of individual PAH were 3–8 times higher than concentrations in the outdoor air. In the case of PCB congeners the indoor concentrations were 2–15 times higher. These data were obtained during the study focused on ecotoxicological assessment of carcinogenic PAHs in pig and cattle farms (Cigánek et al. 2000).

Table 1. Concentration of PAHs in indoor and outdoor air of pig and cattle farms

	Compounds	pig farm		cattle farm	
		indoor air[1]	outdoor air[1]	indoor air[1]	outdoor air[1]
1	Naphthalene	0.91 ± 0.36	0.90 ± 0.85	2.15 ± 0.57	1.69 ± 1.08
2	Acenaphthylene	0.26 ± 0.10	0.25 ± 0.27	2.97 ± 2.11	1.59 ± 1.40
3	Acenaphthene	0.19 ± 0.03	0.17 ± 0.11	1.18 ± 0.75	0.52 ± 0.35
4	Fluorene	1.44 ± 0.10	2.09 ± 1.13	10.7 ± 3.27	4.77 ± 3.25
5	Phenanthrene	9.48 ± 3.00	7.50 ± 2.75	26.9 ± 4.61	10.8 ± 5.17
6	Anthracene	0.59 ± 0.48	0.15 ± 0.01	1.65 ± 0.52	0.42 ± 0.19
7	Fluoranthene	4.07 ± 2.51	2.35 ± 0.50	7.79 ± 1.12	3.61 ± 1.22
8	Pyrene	2.64 ± 1.63	1.40 ± 0.22	6.80 ± 1.18	2.50 ± 0.89
9	Benz[a]anthracene	0.27 ± 0.15	0.11 ± 0.04	0.70 ± 0.10	0.34 ± 0.21
10	Chrysene	0.41 ± 0.21	0.24 ± 0.06	1.09 ± 0.17	0.59 ± 0.39
11	Benzo[b]fluoranthene	0.36 ± 0.22	0.25 ± 0.01	0.96 ± 0.14	0.52 ± 0.35
12	Benzo[k]fluoranthene	0.18 ± 0.12	0.14 ± 0.07	0.51 ± 0.07	0.27 ± 0.19
13	Benzo[a]pyrene	0.24 ± 0.15	0.09 ± 0.02	0.55 ± 0.06	0.26 ± 0.17
14	Indeno[1,2,3-cd]pyrene	0.28 ± 0.18	0.14 ± 0.08	0.79 ± 0.21	0.34 ± 0.24
15	Dibenz[a,h]anthracene	0.03 ± 0.03	0.01 ± 0.01	0.07 ± 0.01	0.04 ± 0.03
16	Benzo[g,h,i]perylene	0.25 ± 0.15	0.15 ± 0.08	0.98 ± 0.25	0.31 ± 0.22
	Σ of PAHs (Nos. 1-16)	**21.6 ± 8.5**	**15.9 ± 4.6**	**65.9 ± 7.23**	**28.5 ± 14.5**
	Σ of carc. PAHs (Nos. 9-15)	**1.76 ± 1.01**	**0.98 ± 0.36**	**4.66 ± 0.44**	**2.36 ± 1.58**

[1] concentration in $ng \cdot m^{-3}$, mean value from three analysis ± S.D. (standard deviation)

Determination of significantly increased concentrations mainly of carcinogenic PAHs and PCBs in the indoor air led us to attempt to quantify possible health risks for feeders and other workers exposed to polluted indoor air in the farm stables. This decission was reasonable due to the fact that the workplace has never been monitored from the point of health risks, even if high concentrations of dangerous pollutants are there and people spend long working hours there.

The mostly used approach for the risk assessment is an US EPA method of health risk assessment (EPA 1989). This method enables an effective quantification of both non-

Table 2. Concentration of PCB and chlorinated pesticides in indoor and outdoor air of pig and cattle farms

	Compounds	pig farm		cattle farm	
		indoor air[1]	outdoor air[1]	indoor air[1]	outdoor air[1]
1	PCB No. 28	75.0 ± 24.8	52.0 ± 23.3	57.0 ± 15.3	36,7 ± 20.1
2	PCB No. 52	63.3 ± 27.6	49.7 ± 26.4	54.3 ± 18.0	44.7 ± 25.1
3	PCB No. 101	59.0 ± 5.0	36.0 ± 17.3	42.3 ± 4.9	33.7 ± 13.2
4	PCB No. 118	13.7 ± 1.7	10.0 ± 4.9	9.7 ± 2.9	8.0 ± 2.2
5	PCB No. 153	61.0 ± 12.2	38.7 ± 15.6	41.7 ± 4.9	34.7 ± 5.0
6	PCB No. 138	45.7 ± 18.9	29.0 ± 17.7	28.0 ± 9.2	24.7 ± 7.3
7	PCB No. 180	18.7 ± 9.2	11.0 ± 5.7	11.3 ± 2.9	9.3 ± 2.1
	Σ of PCB (Nos. 1-7)	**336 ± 47.3**	**226 ± 97.1**	**244 ± 41.3**	**191 ± 65.4**
8	alfa-HCH	53.0 ± 38.7	28.0 ± 9.6	51.7 ± 23.6	25.0 ± 8.5
9	beta-HCH	6.3 ± 5.3	9.0 ± 7.5	4.3 ± 2.1	4.0 ± 2.2
10	gama-HCH=Lindane	1473 ± 1943	57.0 ± 26.5	493 ± 656	42.7 ± 13.8
11	delta-HCH	< 1.0	1.3 ± 1.9	< 1.0	< 1.0
	Σ of HCHs (Nos. 1-4)	**1533 ± 1981**	**95.3 ± 40.1**	**549 ± 679**	**71.7 ± 15.2**
12	p,p'-DDE	83.7 ± 12.6	94.3 ± 29.5	59.0 ± 6.4	56.7 ± 20.1
13	p,p'-DDD	4.7 ± 1.2	3.3 ± 2.1	2.7 ± 2.5	1.3 ± 1.9
14	p,p'-DDT	15.0 ± 5.7	14.3 ± 6.9	8.7 ± 2.5	12.0 ± 6.5
	Σ of DDT (Nos. 1-3)	**103 ± 17.9**	**112 ± 24.2**	**70.3 ± 8.7**	**70.0 ± 28.4**
	Hexachlorobenzene	57.7 ± 16.9	69.7 ± 42.1	91.7 ± 37.8	85.0 ± 53.2

[1] concentration in $pg \cdot m^{-3}$, mean value from three analysis ± S.D. (standard deviation)

carcinogenic and carcinogenic health risks. Moreover, the final risk is a result of an integration exposure to several pollutants. It consists of four basic steps: (i) hazard identification, (ii) dose-response assessment, (iii) exposure assessment, and (iv) risk characterization. Hazard identification is the qualitative assessment dealing with the inherent toxicity of an agent/stressor. This qualitative assessment addresses the question of whether there is any potential for human toxicity. Dose-response assessment serves for identification of the relationship between the dose of an agent/stressor and the induction of an adverse effect. Exposure assessment enables the determination of the extent of human exposure to an agent/stressor. In the end risk characterization describes the nature and likelihood of health risk to humans, including attendant uncertainties. If significant human health risks are identified, a risk management, suggesting actions for decrease of risks, must follow. This method was successfully used for risk assessment of different pollution exposure including outdoor and indoor air (Sweeney et al. 2000; Wcislo et al. 2002).

Materials and Methods

Site description

For the study 3 swine and 2 cattle farms were selected. They were located in the Hodonín District (eastern part of the Czech Republic) that belongs among agricultural regions of the Czech Republic. Indoor air samples were collected on places inside the buildings that fulfilled requirements for representative sampling. The samples were collected (1999 – 2000) in three campaigns (June 199; February 2000; November 2000) to find out indoor concentrations of selected persistent organic pollutants in warm and cold part of the year.

1. L1 (locality 1) was located in the pig farm in Milotice (Agropodnik Hodonín). Air samplers were placed in the middle of a pig fattening hall No. 7 (100·15·2.5 meters). The hall, where 1300 pigs were housed, was aerated with 32 air blowers and open small windows. During a cold period of the year the hall was heated with a gas bunner and the windows were closed.

2. L2 (locality 2) was located in the pig farm in Dubňany (Gigant Dubňany). Air samples were collected in the hall No. 16 (96·18·3 meters). The hall was divided into two halves and in each of parts there were 750 pigs. The collectors were placed in the middle of one part. 12 air blowers and 12 windows were used for aeration of each of the parts. During a cold period of the year the windows were closed.

3. L3 (locality 3) was located in the cattle farm in Nesyt (ZD Mikulčice) situated in a distance of 1.5 km from

a power-station Hodonín. Indoor air was sampled in the calving house (60·10·4 meters). The house was aerated with 7 vacuum ventilation blowers, 14 windows, 4 run gates and 3 entrance gates. In winter only vacuum ventilation blowers were used for the aeration. Every day a tractor operated for about an hour in the house (littering, cleaning, feed distribution).

4. L4 (locality 4) was located in a cattle farm in Násedlovice (ZEMAS Čejč). Air samplers were placed in the corner of cow house No. 1 (50·30·3.5 meters). About 160 heads of cattle were housed there. For the aeration only roof ventilation flaps and 4 run gates and 4 entrance gates. In a cold period all gates were closed. Every day a tractor operated for about an hour in the house (littering, cleaning, feed distribution).

5. L5 (locality 5) was located in a pig farm in Terezín (ZEMAS Čejč). Air samplers were placed in the middle of the hall No. 1 (70·8·2.8 meters). In the hall there were 500 pigs. The hall was aerated with 6 air blowers, roof ventilation flaps, small windows and entrance gates. In winter only air blowers were used for the aeration.

Stock feeders and other workers spend inside 6 hours per day and 6 days per week in average.

Besides indoor air samples collected inside farm buildings, reference outdoor samples were collected in parallel on two localities. L2b was located outdoor at the farm in Dubňany and L3b was located in the farm in Nesyt. Both of localities were affected by a traffic (tractors, lorries and other vehicles) at the farms.

Sample collection

The indoor and outdoor air samples were collected three times in the localities during 1999 – 2000. For a 24-hour sampling (350 – 450 m^3 per day) high-volume samplers PS-1 (Graseby-Anderson U.S.A.) were used. These samplers, with a tandem of filters, enable sampling of both gas-phase and particle phase semi-volatile organic compounds. The absorbed pollutants on particulate matter were collected on a quartz filter and pollutants in a vapour phase were collected on a PUF filter. All sample collections were done according to U.S. EPA recommendations. The exposed filters were extracted with DCM in the Soxhlet extractor. The extracts were then fractioned with different polarity solvents on a silicagel column for the chemical analysis of PAHs and their nitro- and oxy-derivates. For the analysis of OCPs and PCBs were organic extracts purified on H_2SO_4 modified silicagel column. PAHs (16 compounds according to U.S. EPA) and its derivates, OCPs (HCB, α-HCH, β-HCH, γ-HCH, δ-HCH, p,p'- DDT, p,p'-DDD and p,p'-DDE) and PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138 and PCB 180) were analyzed with GC/MS (Finnigan MAT, Austin USA). All steps including sample collection, extraction and chemical analyses were done under QA/QC (Cigánek et al. 2000).

Risk assessment method

The risks were quantified under the present environmental conditions for the selected exposure scenario (Table 3). Indicator chemicals, also termed COPCs (*chemical of potential concern*), are typically selected as an initial step in a site-specific risk assessment in order to characterize the site and to focus assessment activities on those POPs compounds that may pose the most significant potential risks to humans. The risk characterization was considered separately for carcinogenic and non-carcinogenic effects, and includes a discussion on factors that may result in either an overestimation or an underestimation of the risks.

Table 3. Selected exposure scenario

Exposure parameter	Value	Unit	Notice
Body weight [BW]	70	kg	
Exposure time [ET]	6	hours per day	
Exposure frequency [EF]	200	days per year	
Inhalation rate [IR]	20	m^3 per day	
Exposure duration [ED]	30	years	
Lifetime expectancy [LA]	70	years	
Averaging time – non-cancer [AT-N]	10 950	days	(ED · 365 days)
Averaging time – cancer [AT-C]	25 550	days	(LA · 365 days)

Human health risks, both non-carcinogenic (*HI – hazard index*) and carcinogenic (*IECR – incremental probability of an individual developing cancer over a lifetime*) were computed according to US EPA methodology (EPA 1989) - upgraded for new reference values.

Potential non-cancer risks for exposure to COPCs were evaluated by comparison of the estimated contaminant intakes from inhalation exposure with the *RfD* to produce the *HQ*, defined as follows (EPA 1989):

$$HQ = \frac{CDI}{RfD}$$

where *HQ* is hazard quotient (unitless); *CDI*, chronic daily intake (mg/kg/day); *RfD*, reference dose (mg/kg/day).

The *HQ* assumes that there is a level of exposure (i.e., *RfD*) below which it is unlikely for even sensitive populations to expect any adverse health effects. If the HQ exceeds unity (a value of 1), there may be a concern for potential non-carcinogenic effects. To assess the overall potential for non-carcinogenic effects posed by more than

one chemical, the *HQ* calculated for each chemical are summed (assuming additivity of effects) and expressed as *HI* (hazard index) (EPA 1989):

$$HI = \Sigma HQ_i$$

In cases where the non-cancer *HI* does not exceed unity (*HI* < 1), it is assumed that no chronic risks are likely to occur at the site (EPA 1989). If the *HI* is greater than unity as a consequence of summing several *HQs* it would be appropriate to segregate (separate) the compounds by effect and by mechanism of action and to derive specific *HIs* for each of target organ groups.

The health risk assessment has been carried on with the determination of the individual excess cancer risk index (*IECR*) (EPA 1996a, 1996b).

Cancer risks *IECR* were estimated as the incremental probability of an individual developing cancer over a lifetime as a result of exposure to a potential carcinogen; the following linear low-dose carcinogenic risk equation was used for each of exposure routes (EPA 1989):

$$IECR = 1 - e^{(-LAIC \cdot IUR)}$$

where *LAIC* is livetime average inhalation concentration ($\mu g \cdot m^{-3}$); *IUR*, inhalation unit risk ($1/\mu g \cdot m^{-3}$) – values used for calculation are summarized in Table 4. If a site has multiple carcinogenic contaminants, cancer risks for each carcinogens (assuming additivity of effects) and compared with the acceptable risk.

$$IERC = \Sigma IERC_i$$

Risks in the range of 1E-06 to 1E-04 typically have been judged to be acceptable by EU and US EPA (EPA 1991a, 1991b).

Table 4. RfDs and IURs for the selected COPCs

COPCs	RfD [$mg \cdot kg^{-1} \cdot day^{-1}$]	Ref.	IUR [$1/\mu g \cdot m^{-3}$]	Ref.
Naphthalene	9.00E-04	NCEA		
Acenaphthene	6.00E-02	IRIS		
Fluorene	4.00E-02	IRIS		
Anthracene	3.00E-01	IRIS		
Fluoranthene	4.00E-02	IRIS	8.70E-05	WHO
Pyrene	3.00E-02	IRIS		
Benz[a]anthracene			1.20E-04	WHO
Chrysene			8.70E-05	WHO
Benzo[b]fluoranthene			8.70E-03	WHO
Benzo[k]fluoranthene			8.70E-04	WHO
Benzo[a]pyrene			8.70E-02	WHO
Indeno[1,2,3-cd]pyrene			5.80E-03	WHO
Dibenz[a,h]anthracene			7.70E-02	WHO
PCB 28			1.00E-04	IRIS
PCB 52			1.00E-04	IRIS
PCB 101			1.00E-04	IRIS
PCB 118			1.00E-04	IRIS
PCB 153			1.00E-04	IRIS
PCB 138			1.00E-04	IRIS
PCB 180			1.00E-04	IRIS
alpha-HCH			1.80E-03	IRIS
beta-HCH			5.40E-04	IRIS
gamma-HCH	3.00E-04	IRIS		
p,p'-DDT	5.00E-04	NCEA	9.71E-05	NCEA
HCB	8.00E-04	IRIS	4.60E-04	IRIS

IRIS - Integrated Risk Information Systém (U.S.EPA, *http://www.epa.gov*)
WHO – World Health Organization (*http://www.who.int*)
HEAST - Health Effects Summary Tables (U.S.EPA, *http://www.epa.gov*)
NCEA - National Center for Environmental Assessment (U.S.EPA, *http://www.epa.gov*)

Results and Discussion

Analysis of indoor air in the stables of cattle and pig farms revealed increased concentrations of POPs in comparison to outdoor levels (Cigánek et al. 2000). Even if available data included only three one-day samplings for each of five analysed localities, non-carcinogenic and carcinogenic risks for feeders and other workers were assessed.

The values of *HI* and *IECR* for all samplings are in Fig. 1, Fig. 2 and Table 5. While no non-carcinogenic risks were determined in any of the localities, increased carcinogenic risks were observed. In same cases level of $IECR = 1 \cdot 10^{-6}$ was passed over. The highest *IECR* was found in locality L4 in February 2000. The lowest IECR was found also in February 2000 and it was in locality L2.

Table 5. Summary of risk values for inhalation scenario (indoor and outdoor air)

Indoor air				Outdoor air			
Locality	Campaigns	*HI*	*IECR*	Locality	Campaigns	*HI*	*IECR*
L1	VI-99	0.00015	**1.153E-06**				
	II-00	0.00013	**3.611E-06**				
	XI-00	0.00018	7.189E-07				
L2	VI-99	0.00063	**2.646E-06**	L2b	VI-99	0.00003	9.303E-07
	II-00	0.00007	2.565E-07			0.00006	6.187E-07
	XI-00	0.00015	**2.179E-06**			0.00019	5.296E-07
L3	VI-99	0.00028	**4.248E-06**	L3b	VI-99	0.00004	3.271E-07
	II-00	0.00032	**4.849E-06**			0.00032	3.676E-06
	XI-00	0.00024	**4.025E-06**			0.00024	2.348E-06
L4	VI-99	0.00022	9.127E-07				
	II-00	0.00023	**8.08E-06**				
	II-00	0.00025	**2.372E-06**				
L5	VI-99	0.00042	3.93E-07				
	II-00	0.00008	3.031E-07				
	XI-00	0.00031	9.519E-07				

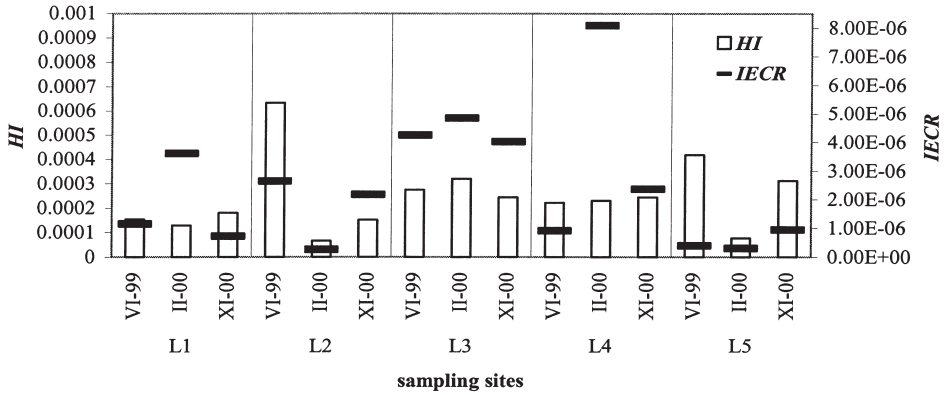

Fig. 1. Summary of risk values for inhalation scenario (indoor)

The average *IECR* levels for the investigated locality are graphically compared in Figs 1 and 2. The highest average carcinogenic risk was detected in locality L3. On the other hand the lowest average level of *IERC* was in locality L5.

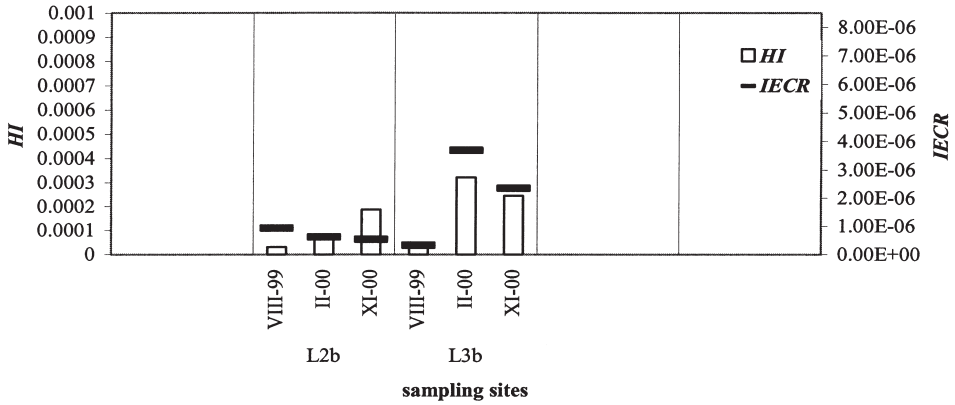

Fig. 2. Summary of risk values for inhalation scenario (outdoor)

In the carcinogenic risks, the most important role was played by PAHs. The influence of other measured POPs was only marginal.

Any clear difference between the samplings in warm and cold part of the year was not observed. Neither an open-fire heating nor decreased aeration in the cold periods increased the health risks. The pig farms did not differ significantly from the cattle farms.

Even if outdoor air was sampled only on two farms, health risk assessment showed that non-carcinogenic as well as carcinogenic risks for indoor air were only slightly higher or comparable with the outdoor risks.

In case of pesticide risks, any demands to reduce contamination are not necessary because of their low indoor concentrations. The main contributors to human health risk are PAHs. Therefore ventilation while tractors, lorries and other vehicles are running should be the main purpose how to decrease estimated human health risk, mainly in winter season.

In comparison with a median value of *IECR* for research workers from Košetice (years 1996 – 1999), Central European background monitoring station of EMEP, that achieves $5.96 \cdot 10^{-7}$ (Holoubek et al. 2003), the carcinogenic risks in farms are approximately twice higher.

As a specific result of the risk assessment for the case-study area, obtained in line with the applied principle of reasonable maximum exposure scenarios, it has been shown that used indoor inhalation exposure pathway in the farms may pose increased carcinogenic health risk ($MAX_{IECR} = 8.08 \cdot 10^{-6}$).

Hodnocení zdravotních rizik z inhalační expozice ve stájích prasat a skotu na jižní Moravě

Hlavním tématem této studie bylo hodnocení zdravotních rizik z inhalačního expozičního scénáře na vybraných vepřínech a kravínech na jižní Moravě (Česká republika). Toto výjimečné hodnocení potenciálních rizik pracovníků farem bylo hlavním přínosem této studie. Ve vybraných vepřínech a kravínech tedy byly hodnoceny možné nekarcinogenní i karcinogenní zdravotní rizika (podle metodiky Hodnocení zdravotních rizik US EPA) pro profese krmiče a ostatní zaměstnance těchto farem s významně vyššími koncentracemi především karcinogenních PAHs a PCBs v ovzduší vnitřního pracovního prostředí. Nekarcinogenní rizika nebyla zjištěna ani na jedné z hodnocených lokalit, ale zvýšená karcinogenní rizika byla pozorována. Nejvyšší karcinogenní rizika byla nalezena v prostorech stájí krav ($MAX_{IECR} = 8.08 \cdot 10^{-6}$), nejnižší ve vepřínech ($MIN_{IECR} = 2.57 \cdot 10^{-6}$).

Hodnoty karcinogenních rizik z vnitřních prostor hodnocených stájí této studie však nebyly extrémně vysoké, ale přibližně dvakrát vyšší než je hodnota mediánu rizika, determinovaného pro výzkumné pracovníky Středoevropské pozaďové monitorovací stanice sítě EMEP v Košeticích *IECR*=5.96·10^{-7} (1996 - 1999).

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Příloha 3

Bartoš, T., Letzsch, S., Škarek, M., Flegrová, Z., **Čupr, P.**, Holoubek, I., 2006. GFP assay as a sensitive eukaryotic screening model to detect toxic and genotoxic activity of azaarenes. Environmental Toxicology 21, 343–348.

GFP Assay as a Sensitive Eukaryotic Screening Model to Detect Toxic and Genotoxic Activity of Azaarenes

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ABSTRACT: Azaarenes are nitrogen-containing polyaromatic heterocyclic compounds (NPAHs). The majority of the azaarenes found in the environment originate from anthropogenic sources. Concentrations of NPAHs found in the environment are reported to be one to two orders of magnitude lower than polycyclic aromatic hydrocarbons (PAHs) concentrations, yet their biological effects can be of similar magnitude. Very few studies on the genotoxicity of azaarenes are available in the literature. In the present study, a preliminary profile of both the toxic and genotoxic potential of 5 PAHs and their 20 aza-analogues were investigated. To assess the toxic and genotoxic activity, a green fluorescent protein (GFP) assay based on the yeast *Saccharomyces cerevisiae* was selected. To compare the sensitivity of this eukaryotic short-term assay with bacterial screening tests, the Toxi-Chromotest for toxicity and SOS-Chromotest for genotoxicity assessment were also performed. This comparison indicates that in most cases, the yeast GFP assay is apparently of comparable specificity to the bacterial toxicity or genotoxicity tests with respect to the correlation of positive/negative responses, but much more sensitive with respect to the effective concentration values. In the cases of phenazine, phenanthridine, 1,10-phenanthroline, or 4,7-phenanthroline, one to two orders of magnitude lower IC20 and minimum genotoxic concentration values in the yeast GFP assay were observed. In this study, the authors present evidence that genotoxicity assessment using the yeast GFP assay can provide a simple system to monitor the activity of these environmental pollutants that could possess mutagenic potential at low concentrations. © 2006 Wiley Periodicals, Inc. Environ Toxicol 21: 343–348, 2006.

Keywords: azaarenes; GFP yeast bioassay; genotoxicity; toxicity

INTRODUCTION

Nitrogen heterocyclic aromatic hydrocarbons or azaarenes contain one or more nitrogen atom(s) in place of a carbon atom. This makes them more soluble in water than their homocyclic analogues (Pearlman et al., 1984), and consequently perhaps also more bioavailable. Although partly of natural origin (e.g., as alkaloids; Kaiser et al., 1996), the majority of the azaarenes found in the environment originate from anthropogenic sources, and are formed and released into the environment by incomplete combustion of fossil fuels, in spills or effluents of a variety of industrial activities, oil drilling, refining and storage (Kochany and Maguire, 1994), and coal tar distillation (Pereira et al., 1983). N-heterocycles are also associated with wood pres-

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ervation (Pereira et al., 1983) and pesticide use (Kuhn and Suflita, 1989). Their presence has been shown in air (Santodonato and Howard, 1981), groundwater (Pereira et al., 1987), and in both marine and freshwater environments (Blumer et al., 1977; Kozin et al., 1997; Van Genderen et al., 1994). Concentrations of nitrogen-containing polyaromatic heterocyclic compounds (NPAHs) found in the environment are reported to be one to two orders of magnitude lower than polycyclic aromatic hydrocarbons (PAH) concentrations (Blumer et al., 1977), yet their biological effects can be of similar magnitude. Azaarenes are known to exhibit mutagenic and carcinogenic activity, just as do neutral polyaromatic hydrocarbons. Because of their planar fused-ring structures, azaarenes are capable of intercalating between the bases of DNA. Highly electrophilic intermediates formed during biotransformation (e.g., diol epoxides) can easily bind strongly (but reversibly) with nucleophilic carboxyl, amino, and sulfhydryl groups on nucleic acids or proteins. When a covalent bond between a carbonium ion intermediate and the DNA helix are formed, these DNA adducts lead to a disruption of the DNA configuration and possibly to mutagenesis and cancer.

To assess mutagenic and carcinogenic hazards, several biological test systems have been developed. At present, standardized prokaryotic genotoxicity procedures include the Ames test (Gee et al., 1994; Maron and Ames, 1983) and the umuC test (Oda et al., 1985) based on genetically engineered *Salmonella typhimurium* strains or the SOS Chromotest (Quillardet and Hofnung, 1985) using *E. coli* strain. Although these bacterial assays have proved their effectiveness, they have some disadvantages, principally because they use noneukaryotic cells and hence will not detect genotoxins that interact with eukaryote-specific targets. More recently, *Saccharomyces cerevisiae* tester strains have been developed for detection of genotoxic potential with yeast-optimized green fluorescent protein (GFP) fused to the RAD54 promoter (Afanassiev et al., 2000; Walmsley et al., 1997). The DNA damage inducible promoter of the RAD54 gene was fused to yeast codon-optimized gene for the jellyfish GFP and responds to all agents known to a broad spectrum of genotoxins, suggesting that yeast DNA damage sensing pathways activate the homologous recombinational repair pathway as a default for failure or saturation of the other repair pathways (Cahill et al., 2004). The amount of GFP accumulated reflects the genotoxic effect of the substance, i.e., the more damage caused, the greater the activation of the DNA repair system, the greater the amount of GFP produced. This GFP can then be noninvasively quantified when illuminated by blue light.

In this study, we present evidence that the yeast GFP assay can be successfully used for studying toxicity and genotoxicity of these environmental pollutants. Their structures and nomenclature are given in Figure 1.

MATERIALS AND METHODS

Chemicals

Quinoline (CAS No. 91-22-5, purity 98%), benzo[h]quinoline (CAS No. 230-27-3, purity 97%), acridine (CAS No. 260-94-6, purity 97%), quinazoline (CAS No. 253-82-7, purity 99%), isoquinoline (CAS No. 119-65-3, purity 97%), phenanthridine (CAS No. 229-87-8, purity 98%), 4,7-phenanthroline (CAS No. 230-07-9, purity 98%), 1,10-phenanthroline (CAS No. 66-71-7, purity 99%), carbazole (CAS No. 86-74-8, purity 96%), 6-methylquinoline (CAS No. 91-62-3, purity 98%), 1,7-phenanthroline (CAS No. 230-46-6, purity 99%), phenazine (CAS No. 92-82-0, purity 98%), phthalazine (CAS No. 253-52-1, purity 98%), naphthalene (CAS No. 91-20-3, purity 98%), anthracene (CAS No. 120-12-7, purity 97%), benz[a]anthracene (CAS No. 56-55-3, purity 99%), dibenzo[a,h]anthracene (CAS No. 53-70-3, purity 97%), fluorene (CAS No. 86-73-7), phenanthrene (CAS No. 85-01-8) were purchased from Sigma-Aldrich. Benz[a]acridine (CAS No. 225-11-6, purity 99,5%), benzo[c] acridine (CAS No. 225-51-4, purity 99,8%), dibenz[a,i] acridine (CAS No. 226-92-6, purity 99,7%), dibenz[a,j]acridine (CAS No. 224-42-0, purity 99%), dibenz[a,h]acridine (CAS No. 226-36-8, purity 99,86), dibenz[c,h]acridine (CAS No. 224-53-3, purity 99,3%), and 7-H-dibenz[c,g]carbazole (CAS No. 194-59-2, purity 99,7%) were obtained from Dr. Ehrenstorfer GmbH. All other used chemical reagents were of the highest available quality.

Strains and Growth Condition

The *Saccharomyces cerevisiae* strain used in this study was FF18984 (MATa, leu2-3,112 ura3-52 lys2-1 his7-1). The reporter strain (GenT01) contains a multiple copy plasmid bearing the entire upstream noncoding DNA sequence of the S. cerevisiae *RAD54* gene fused to a yeast codon-optimized derivative of the Aequorea Victoria (jellyfish) *GFP* gene (Cormack et al., 1997). The control strain (GenC01) contains the identical plasmid except that 2 bp have been removed at the start of the *GFP* gene, such that no GFP is made. Yeast cells were grown using a well-defined minimal media which exhibits low autofluorescence (F1) (Cahill et al., 2004). Single colonies of yeast strains, grown on selective SD medium, were used to inoculate F1 medium (10 mL) and grown to stationary phase.

GFP Assay

This assay, accurately described (Cahill et al., 2004), was carried out in 96-well microtitre plates (black, clear bottom) obtained from Greiner (catalogue no. 655097). 1.2 mM stock of the test chemical was prepared in 4% (v/v) aqueous dimethyl sulfoxide (DMSO) and used to make two identical

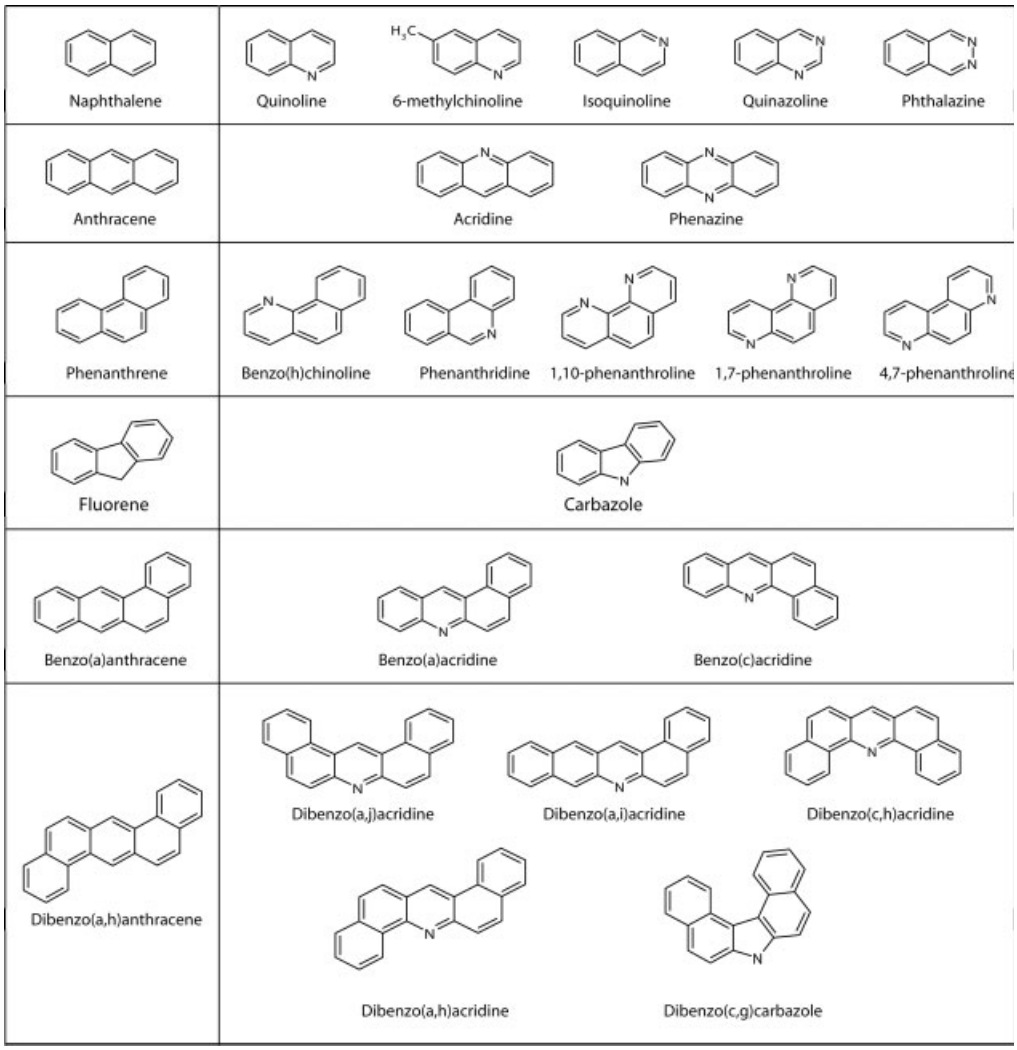

Fig. 1. Structures and nomenclature of PAHs and their nitrogenic analogues (azaarenes).

dilution series across the microplate. To achieve this, 150 mL of the test chemical solution were put into two microplate wells. Each sample was serially diluted by transferring 75 mL into 75 mL of 4% DMSO, mixing and then taking 75 mL out and into the next well. This produced nine serial dilutions of 75 mL each.

Controls were added as follows: (i) Compound alone, to provide information on compound absorbance/fluorescence; (ii) Yeast cultures diluted with 4% DMSO alone, to give a measure of maximum proliferative potential; (iii) methyl methanesulfonate as a genotoxicity control: ''high'' = 0.00125% (v/v), ''low'' = 0.0001875% (v/v); (iv) Methanol as a cytotoxicity control: ''high'' = 3.5% (v/v), ''low'' = 1.5% (v/v); and (v) Growth medium alone, to confirm sterility/lack of contamination.

Stationary phase cultures of GenT01 and GenC01 were diluted to an optical density (OD600 nm) = 0.2 in double strength F1 medium (Billinton et al., 1998). An aliquot of 75 mL of the yeast suspension was added to each well of the diluted chemical: GenT01 to one series and GenC01 to the second series of each compound, and to appropriate standards and controls (i.e., GenT01 to methyl methanesulfonate-containing wells and GenC01 to methanol-containing wells). After the plates were filled, they were sealed using a gas-permeable membrane (Breath-Easy; Diversified Biotech, USA) and then incubated, without shaking, overnight (16–20 h) at 30°C. Following overnight incubation, GFP reporter fluorescence and yeast culture absorbance data were collected from the microplates. The microplate reader used was a Wallac 1420 Victor (Perkin Elmer), which combines fluorescence and absorbance functionality. Filter set: excitation 485 nm, emission 535 nm, absorbance 550 nm.

To compare the sensitivity of GFP yeast assay and bacterial screening tests, unpublished data of the Toxi-Chromotest (toxicity data) (Kwan, 1993) and SOS-Chromotest

TABLE I. Summary of toxicity and genotoxicity assessment

		Toxicity—IC20 (μM)		Genotoxicity—MGC (μM)	
Group	Compound	GFP Assay	Toxi-Chromotest	GFP Assay	SOS-Chromotest
PAH	Fluorene	187.6	123.0	–	150.0
	Naphthalene	–	–	–	–
	Phenanthrene	35.1	53.0	–	–
	Benzo(a)anthracene	–	–	–	–
	Dibenzo(a, h)anthracene	–	–	–	–
	Anthracene	–	–	–	–
Analogues to naphthalene	quinoline	–	–	–	–
	6-methylchinoline	–	–	–	–
	Isoquinoline	147.8	–	304.0	134.0
	quinazoline	–	–	–	–
	phthalazine	–	–	–	206.0
Analogues to anthracene	acridine	87.9	54.0	81.7	30.0
	phenazine	9.4	264.0	2.1	132.0
Analogues to phenanthrene	benzo(h)chinoline	329.7	103.0	119.4	78.0
	phenanthridine	39.6	114.0	30.3	–
	1, 10-phenanthroline	5.1	10.0	3.9	–
	1, 7-phenanthroline	131.5	–	51.2	139.0
	4, 7-phenanthroline	191.5	218.0	7.4	209.0
Analogues to fluorene	carbazole	66.2	50.0	–	–
Analogues to benzo(a)anthracene	Benzo(a)acridine	54.7	5.0	43.3	30.0
	Benzo(c)acridine	–	9.0	–	–
Analogues to dibenzo(a, h)anthracene	Dibenzo(c, g)carbazole	2.1	1.0	–	–
	Dibenzo(a, j)acridine	–	–	–	–
	Dibenzo(a, h)acridine	–	–	–	–
	Dibenzo(a, i)acridine	2.7	10.0	1.4	3.0
	Dibenzo(c, h)acridine	–	–	–	–

–, not detected.

(genotoxicity data) (Quillardet and Hofnung, 1985) from a parallel study are included. IC20 and minimum genotoxic concentration values were calculated using linear regression model.

RESULTS

To assess toxic and genotoxic activity, 5 polyaromatic hydrocarbons and 20 of their nitrogen-containing heterocyclic analogues were selected. The response from each substance tested at the concentrations of 0, 2.34, 4.69, 9.38, 18.8, 37.5, 75, 150, 300, and 600 μM were examined. However, some compounds were tested only at lower concentrations because of their low solubility (especially 4- and 5-ring analogues).

To assess toxicity and genotoxicity, the IC20 and minimum genotoxic concentration values of each substance are listed in Table I.

Taking these results as whole, both toxicity and genotoxicity values indicate azaarenes have higher toxic and genotoxic potencies than their homocyclic analogues. These results are obvious especially in case of genotoxicity evaluation, where none of the PAHs showed any adverse effect in GFP test, however, 10 their aza- homologues were scored as potential genotoxins. These findings for most potent group of phenanthrene analogues are shown in Figure 2.

Taking these results test by test, it appears that they have different sensitivity. It can be seen that GFP test system for toxicity assessment responded to the same substances as bacterial short-term assay Toxi-Chromotest. Even in case of isoquinoline and 1,7-phenanthroline, the adverse impact on cell growth was observed only in the GFP assay.

Also the results using the same genotoxicity endpoints using two different bioassays are in good agreement, even if in some cases (phenazine or 4,7-phenanthroline) the GFP assay detect genotoxic potencies two orders of magnitude lower than bacterial SOS Chromotest.

DISCUSSION

Very few studies on the genotoxicity of azaarenes are available in the literature. This study established both the toxic

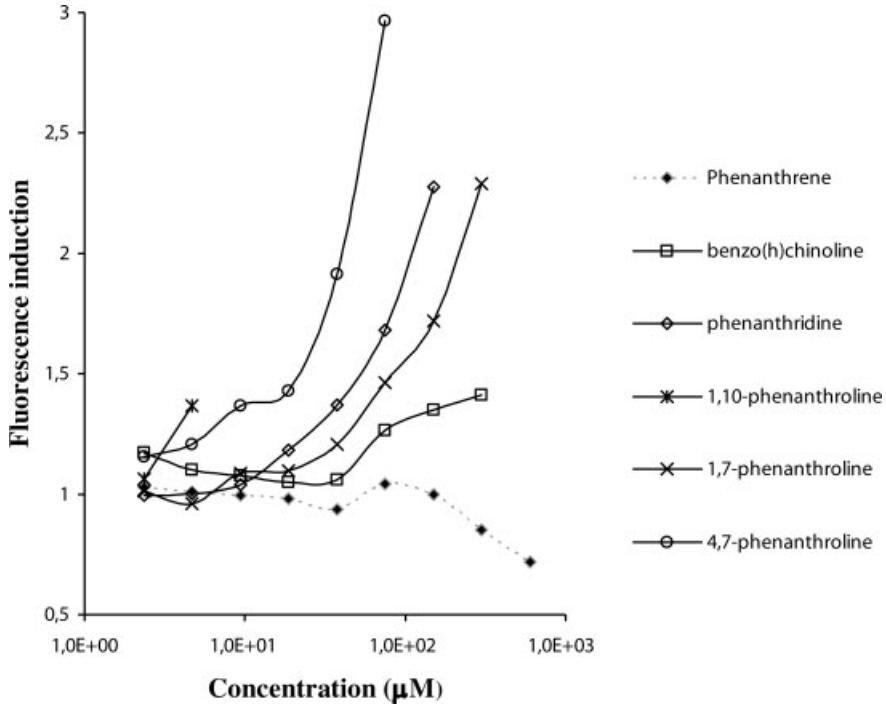

Fig. 2. Genotoxicity results of most potent group—phenanthrene and its analogues.

and genotoxic potential of 20 azaarenes using a eukaryotic short-term assay system.

The simultaneous use of GFP assay (Afanassiev et al., 2000) and Toxi-Chromotest (Kwan, 1993) for toxicity assessment and GFP assay (Afanassiev et al., 2000) and SOS-Chromotest (Quillardet and Hofnung, 1985) for genotoxicity assessment allows us to compare the sensitivity of bacterial and eukaryotic bioassays. The principle of both toxicity and genotoxicity assays is very similar. For toxicity evaluation, IC20 (growth inhibition) is determined by absorbance measurement, genotoxicity assays are based on expression of reporter gene (β-galactosidase or GFP synthesis). This comparison indicates that in most cases the GFP assay is apparently of comparable sensitivity to the bacterial toxicity or genotoxicity tests with respect to the correlation of positive/negative responses, but much more sensitive with respect to the effective concentration values.

It is important to note that in contrast to the Ames and the SOS tests (Ames et al., 1973; Quillardet et al., 1982), which are bacterially based, GFP assay system provides all the typical features of eukaryotic cell architecture and metabolism. Although lacking many metabolic pathways found in animals and humans, basic repair mechanisms as a response to genetic damage are more similar between yeast and mammals (Crichlow and Jackson, 1998; Kim and Weinert, 1997).

The monitoring of the DNA-damage potential of chemical substances is a main goal in understanding the risk that can be caused by the presence of dangerous substances in the environment. The toxicity and genotoxicity assessment using yeast strain *Saccharomyces cerevisiae* can provide a simple system to monitor the activity of these substances that could possess mutagenic potential at low concentrations not only due to its eukaryotic advances, but especially due to its high sensitivity.

Presence of azaarenes has been shown in air (Santodonato and Howard, 1981), groundwater (Pereira et al., 1987), and in both marine and freshwater environments (Blumer et al., 1977; Kozin et al., 1997; Van Genderen et al., 1994). Apart from their natural origin, NPAHs enter the environment as spills or waste materials generated by mining industry, coal tar- and oil shale processing operations, wood preserving facilities, and chemical manufacturing plants (Kaiser et al., 1996). NPAHs are present in the environment in amounts up to 1–10% of those of their homocyclic analogue PAHs (Wild and Jones, 1995), but their biological effects can be of similar magnitude. Information about the genotoxicity of each specific group of pollutants in environmental samples are lacking in the literature and that is why GFP assay can help to define the toxic or genotoxic potential of a variety of these compounds and can be very useful tool to determine environmental risks of these compounds in mixtures in environmental monitoring.

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Příloha 4

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Passive air sampler as a tool for long-term air pollution monitoring: Part 2. Air genotoxic potency screening assessment

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Extracts from passive air samples can be used to assess genotoxic potency.

Abstract

The capability of passive air sampling to be employed in the evaluation of direct genotoxicity of ambient air samples was assessed. Genotoxic effects of the total extracts from the polyurethane foam filters exposed for 28 days during a regional passive air sampling campaign were investigated. Twenty sampling sites were selected in Brno city on the area of approximately 20 × 20 km in October and November 2004. Brno is the second largest city of the Czech Republic, highly industrialized with approximately 370,000 of permanent inhabitants. The levels of PAHs, PCBs, and chlorinated pesticides were determined in all samples. Fraction of each extract was also assayed in the bacterial genotoxicity test using *Escherichia coli sulA::lacZ*. Complete dose–response relationships of the air extracts were determined. The statistical analysis showed significant correlation between observed biological effects and PAHs concentrations in samples.

Keywords: Passive air sampling; *Escherichia coli*; SOS reparation system; Air genotoxic potency

1. Introduction

Residents of industrialized and densely populated regions are exposed to the ambient air pollution arising primarily from the industrial activities, heavy traffic and combustion sources. Epidemiological studies carried out to investigate the health risks related to the air pollution suggest that ambient air pollution may be responsible for increased rates of diseases like lung cancer (Kappos et al., 2004; Tam and Neumann, 2004; Parodi et al., 2005; Pauk et al., 2005). Estimation of the human inhalation exposure to the mutagenic and genotoxic compounds in the air is imperative for the public health risk evaluation. However, relatively few investigations have been published for the typical urban areas and information required for conducting human risk assessments (especially exposure assessment) is still very limited. Moreover, the complexity and potential synergic effects of the airborne toxic compounds cannot be adequately ascertained by the chemical analysis itself (Buschini et al., 2001; Zhao et al., 2002) and for the purpose of potential public health risk assessment, the biomonitoring of ambient air in addition to chemical monitoring is receiving increasing attention (Isidori et al., 2003; Brits et al., 2004; Claxton et al., 2004). These demands require specific and sensitive methods capable of indicating the presence of genotoxic compounds in the environment. Several biological test systems have been developed recently to assess the mutagenic and carcinogenic hazards. Among them, the genotoxicity assessment bioassays are valuable bacterial assays based on the response to DNA damage induced by the genotoxic compounds in cells (Hamers et al., 2000). The assays based on the transcriptional fusions between DNA-damage inducible promoters and reporter systems have been used to detect a variety of environmental genotoxins. These sensitive tests utilize the gene promoters involved in the SOS response to the DNA damage such as sulA (McDaniels et al., 1990).

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High-volume air sampling has been a standard method for monitoring of semi volatile organic compounds in the air for a number of years, providing samples for both chemical and toxicological analyses. However, difficulties and high costs associated with this conventional air sampling are partly responsible for the general lack of air quality measurements. Various passive air samplers were developed recently as technically feasible and cost-effective alternative for investigating chemical signatures in air, capable of semi-quantitative measurements of the air pollution as well as relative comparisons of the individual sampling sites (Ockenden et al., 1998; Shoeib and Harner, 2002; Wania et al., 2003; Harner et al., 2004; Jaward et al., 2004a,b). Besides the low cost and versatility, an integrative character eliminating the impact of accidental short-term contamination is the main advantage of these methods (Petty et al., 1993; Isidori et al., 2003; Claxton et al., 2004).

Assessment of genotoxic potency of the passive sampling obtained air samples using the screening genotoxicity test (SOS chromotest), was performed in this study. This method is based on the induction of the SOS repair system as a result of the sample interaction with DNA of a genetically modified tester strain. Our objectives were: (1) to perform regional scale urban air sampling campaign (area 20 × 20 km) using passive samplers and consecutive spatial GIS analysis; (2) to evaluate the feasibility of screening genotoxicity tests on the air samples obtained from the polyurethane foam-based passive samplers; (3) to detect potential significant induction of the SOS genes in this bioassay; and (4) to compare this information with the results of chemical analysis. The uncertainty factors of this approach are discussed as well.

2. Materials and methods

2.1. Air sampling

Passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5 cm thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in the protective chambers were employed in this study. Theory of passive sampling using similar devices was described elsewhere (Shoeib and Harner, 2002; Harner et al., 2004). Sampling chambers were pre-washed and solvent-rinsed with acetone prior to installation. All filters were pre-washed, cleaned (8 h extraction in acetone and 8 h in dichloromethane), wrapped in two layers of aluminum foil, placed into zip-lock polyethylene bags and kept in freezer prior deployment. Exposed filters were wrapped in two layers of aluminum foil, labeled, placed into zip-lock polyethylene bags and transported in cooling box at 5 °C to the laboratory where they were kept in freezer at −18 °C until the analysis. Field blanks were obtained by installing and removing the PUF disks at all sampling sites.

2.2. Sample analysis

All samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. The crude extracts were divided in two parts. One half was evaporated under a gentle stream of nitrogen and re-dissolved in dimethyl sulfoxide (DMSO) at the concentration scale appropriate for biological experiments. The other half was used for the chemical analysis. Volume was reduced under a gentle nitrogen stream at ambient temperature, and fractionation achieved on silica gel column; sulfuric acid modified silica gel column was used for PCB/OCP samples. One laboratory blank and one reference material were analyzed with each set of ten samples. Terfenyl and PCB 121 were used as the internal standards for PAHs and PCBs analyses, respectively. Samples were analyzed using a GC-ECD (HP 5890) supplied with a Quadrex fused silica column 5% Ph for PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, and OCPs (α-HCH, β-HCH, γ-HCH, δ-HCH, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT). Twenty-eight polycyclic aromatic hydrocarbons (naphthalene, biphenyl, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzonaphtho-thiophene, benzo[*b*]fluorene, benzo[*g,h,i*]fluoranthene, cyclopenta[*c,d*]pyrene, benz[*a*]anthracene, triphenylene, chrysene, benzo[*b*]fluoranthene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, perylene, indeno[1,2,3-*c,d*] pyrene, dibenz[*a,h*]anthracene, dibenz[*a,c*]anthracene, benzo[*g,h,i*]perylene, athanthrene, coronene) were determined in all samples using a GC-MS instrument (HP 6890–HP 5972) supplied with a J&W Scientific fused silica column DB-5MS.

2.3. Quality assurance/quality control

Recoveries were determined by spiking the surrogate standards (D8-naphthalene, D10-fenantrene, D12-perylene for PAHs analysis, PCB 30 and PCB 185 for PCBs analysis) on the pre-cleaned filter prior to extraction. The amounts were similar to the detected quantities of analytes in the samples. Recoveries were higher than 75% and 72% for all samples for PCBs and PAHs, respectively. Recovery factors were not applied to any of the data. Recoveries of the native analytes measured for the reference material varied from 88% to 103% for PCBs, from 75% to 98% for OCPs, from 72% to 102% for PAHs. Laboratory blanks were very low. Field blanks consisting of pre-extracted PUF disks were taken on each sampling site. They were extracted and analyzed in the same way as the samples, and the levels in field blanks never exceeded 5% of quantities detected in samples for PCBs, 1% for OCPs, 3% for PAHs, indicating minimal contamination during transport, storage and analysis. Previous air sampling studies performed in our laboratory showed a good agreement between samples from duplicate passive air sampling, in which variability ranged from 5% to 20% for all analytes.

2.4. Genotoxicity test

Genetically modified bacteria cells (tester strain *Escherichia coli* PQ 65 harboring a sulA::lacZ fusion) were employed in the study (Quillardet et al., 1982, 1997; Quillardet and Hofnung, 1985). DNA is a molecular target and the reporter responds directly to the DNA damage. Cytotoxicity as a result of more general macro-molecular damage can be detected in this test as well. The 96-well microtiter plate format was used for slightly modified SOS chromotest (Xu et al., 1989; Bartos et al., 2005). The tester strain was grown overnight in LB medium containing ampicillin (20 μg ml^{-1}) at 37 °C. After the incubation period, the culture was diluted 50-fold into a fresh LB medium with ampicillin and it was incubated for another 2 h. The optical density (600 nm) of the incubated culture was adjusted to 0.04 and prepared culture was mixed (3:1) with a phosphate buffer (pH 7.4). The stock solution of the sample (20% of PUF filter aliquot in DMSO) was diluted 3:1, 1:1 and 1:3 with DMSO.

Eight microliters of each of the dilutions was mixed with 392 μl of the bacterial inoculum in tubes (1,5 ml) to reach the final concentrations: 20%, 15%, 10% and 5% of the original PUF filter in 1 ml of the reaction mixture (% PUF ml^{-1}). Eight microliters of DMSO was taken as a negative control; a solution of 4-nitroquinoline-*N*-oxide was used as a positive control. The mixtures were incubated for 2 h at 37 °C. Two microplates were prepared for measurements of enzymatic activities. β-Galactosidase activity (genotoxicity assay) was determined after the addition 25 μl of the contain of the incubated tubes into 100 μl of a B-buffer solution (pH 7.0) with *o*-nitrophenyl-β-D-galactopyranoside (2 mg ml^{-1}). Alkaline phosphatase activity (toxicity assay) was determined after the addition of 25 μl of the contain of the incubated tubes into 100 μl of a P-buffer solution (pH 8.8) with *p*-nitrophenylphosphate (2 mg ml^{-1}). The microplates were incubated for 45 min at 37 °C and enzymatic activity was determined spectrophotometrically at 420 nm. Toxic effects were quantified as a percentage of the alkaline phosphatase activity in comparison with the negative control. The concentrations showing more than 50% inhibition were excluded. The SOS induction factor (IF) was calculated for every tested concentration. The samples with the induction factor higher than 1.5 for any tested concentration were marked as a significant genotoxins.

2.5. Statistics and correlation analyses

Non-parametric test was selected for the data analyses of environmental samples because it does not require a normal distribution and a homogeneous variance. Correlations were tested using the non-parametric Spearman test (Statistica for Windows 6).

2.6. Spatial GIS analyses

Selected data were processed using the geographic information system (ArcView 3.2, ESRI). A simple terrain based-model (Hoef et al., 2004) was applied for the spatial analysis of POPs concentrations (Surfer 8.0, Golden Software). A geostatistical gridding method (Kriging) was used to produce maps from the irregularly spaced PAHs air level data (Cressie, 1990).

3. Results

Brno, with its 370,000 of permanent residents and 240,000 of the working opportunities, is the second largest city of the Czech Republic. It is a natural center of a territory with 2 million inhabitants. It is located between the highlands and lowlands in the territory of southern Moravia at 49°12′ N, 16°34′ E, 190–479 m above sea level. The territory of the city is well ventilated; no climatic calamities have been recorded in Brno over the recent years. Average air temperature is +9.4 °C, average yearly rain precipitation 505 mm, prevailing wind direction from northwest. A variety of industries is located mostly in the southern parts of the city with the northern part having a residential and recreational character.

Twenty sampling sites (described in Table 1) were selected in the Brno city including industrial, agricultural, residential and background areas. The sampling campaign lasting 28 days was carried out in October and November 2004, and the average temperature was +8.1 °C. Samplers were placed 1.5–2.0 m above the ground in the man breathing zone, in the open terrain sites without significant obstacles for a free air stream around the sampler. To gain information on the spatial distribution of pollutants, all samples were analyzed for polychlorinated biphenyls, organochlorinated pesticides, and polyaromatic hydrocarbons; and at the same time their toxicity and genotoxicity were evaluated. The experimental design of this parallel assessment as a new tool for the air pollution monitoring is presented in Fig. 1. The results of chemical analysis of the samples from passive sampling are given in Table 2. A sum of 16 EPA PAHs collected on individual filters varied from 15 μg to 30 μg in the center of the city, industrial areas to the south and in the places with the heavy traffic, and from 6 μg to 15 μg in the residential areas and peripheries of the city. A sum for 7 indicator PCBs remained between 8 and 47 ng except for the southern industrial zone where it reached 404 ng. At the same site, the sum of *p,p′*-DDT, DDE, DDD reached 101 ng, while in all other places it stayed between 9 and 46 ng.

An estimated spatial distribution of PAHs based on the passive sampling is demonstrated in Fig. 2. A three-dimensional shaded model was created rendering from a grid database (Kriging geostatistical method). The elevation of the surface corresponds to the amount of PAHs.

Results of the mutagenicity assay on the PUF filter extracts are shown in Table 2 and Fig. 3. They are expressed as an induction factors (a genotoxic potency of direct mutagens in the samples from filters after 28 days of exposure). Biological answers of the samples from 11 sites were scored as significantly mutagenic (IF >1.5; Xu et al., 1989).

The correlation between the genotoxicity of extracted filter and the amount of pollutants sequestered by the filter was significant in the case of induction factors for the sample concentration of 10% PUF ml^{-1}. A very good correlation was observed especially for the sum of polycyclic aromatic hydrocarbons (induction factors versus the total amounts of 28 PAHs determined in all filter extracts are shown in Fig. 4) and also

Table 1
List of the sampling sites for the passive sampling campaign in the region of Brno city

No.	Sampling site	Description
1	Brno—Bohunice	Service gate of the hospital, residential area, large urban settlements, apartment buildings, SW part of the city
2	Brno—Kotlarska	Crossroad on the inner traffic ring, heavily polluted by transport emissions, close to the city center
3	Brno—Cerna Pole	Administration building of the arboretum, woody area, close to the city center
4	Brno—Malomerice	Gate of the power station, gas combustion, industrial area, NE part of the city
5	Brno—Lisen	Urban residential area, large suburb of apartment buildings, E part of the city
6	Brno—Sobesice	Backyard of the village family house, N periphery of the city
8	Brno—Zabovresky	Czech hydro meteorological institute, residential area, family houses, close to the city center
9	Brno—Hrad Veveri	Upper courtyard of the Veveri castle, currently under reconstruction, background area, N of the city
10	Brno—Komarov	Backyard of the service station, industrial part of the city, S of the center
11	Brno—Kninicky	Administration building of zoo, woody area, N part of the city
12	Brno—Pisarky	Grassy area of water treatment plant, close to the crossroad on the outer traffic ring
13	Brno—Zidenice	Garden of the family house, residential area, E part of the city
14	Brno—Stred (Petrov)	Garden of the vicarage near the Petrov cathedral, top of the hill in the center of the city
15	Brno—Lesna	Backyard of the apartment house, large suburb of apartment houses, NE part of the city
16	Brno—Kohoutovice	Apartment house, suburban settlement, close to the woods, W part of the city
17	Brno—Husovice	Apartment house, construction site of the new houses, E part of the city
18	Brno—Slatina	Apartment house, urban settlement, close to the highway and industrial area, SE part of the city
19	Brno—Reckovice	Backyard of the family house, residential area, N part of the city
20	Brno—Bystrc	Apartment house, urban settlement, NW part of the city
21	Brno—Chrlice	Private orchard in the village, agricultural area, S periphery of the city

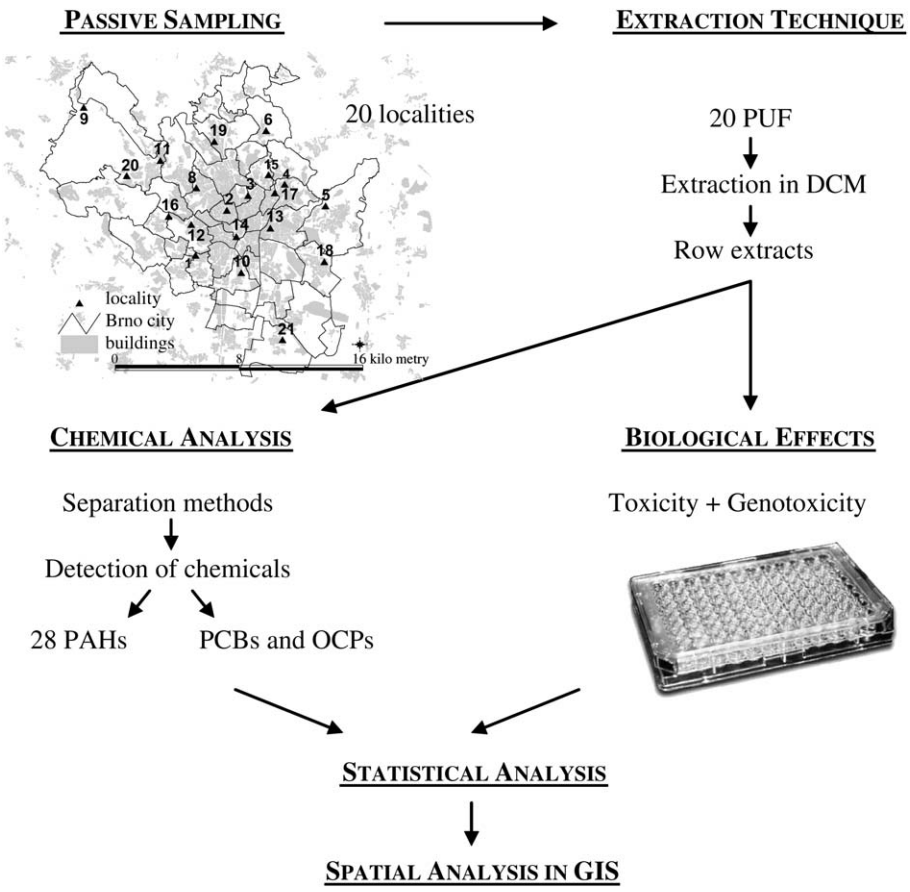

Fig. 1. Experimental design of the air genotoxic potency screening assessment.

for the sum of *p,p′*-DDT, DDE, DDD. All Spearman rank correlation values (Rho) are presented in Table 2. The fact that correlation that is significant for lower concentration has not been observed for the higher ones can probably be explained by a masking effect of the cell growth inhibition caused by the high concentrations of pollutants. The most significant statistical Rho was found for acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene.

4. Discussion

When the PUF disks are deployed in the sampling chambers, they were estimated to give the typical sampling rates of 3–4 m^3 of air per day (Shoeib and Harner, 2002). The ambient concentrations represented by the measured amounts of POPs per sample values can therefore be derived. Previous works have also shown that characterization of the uptake profiles of various POPs based on their octanol–air partition coefficients gives satisfactory results and equivalent air sample volumes can be calculated (Harner et al., 2004). However, since one of the goals of this study was to compare the results of chemical analysis of the polyurethane foam disk extracts with the results of genotoxicity assay, the total amounts of POPs sequestered by the PUF filters were used for comparison rather than estimated air concentrations. Since it is difficult to identify fully and to quantify the complex mixture of organic compounds to which we are exposed through the air pollution, the chemical analyses in this study was limited to 7 indicator PCBs, selected OCPs, and 28 PAHs, most of which are biologically active and some even carcinogenic (Du Four et al., 2004).

To compare the results with the background site not influenced by this region, the sampling site No. 22 (Kosetice), EMEP observatory (Holoubek et al., 2001) where the parallel passive air sampling was carried out, was included in Table 2. It can be seen from the table that organochlorinated pesticides (HCH and DDT) and PCBs are distributed quite evenly and the amounts of OCPs measured in most sites in Brno are similar or lower than the amounts in Kosetice. On the contrary, amounts of PCBs determined in most sites in Brno are slightly elevated when compared with Kosetice, which is in agreement with the anthropogenic origin of these compounds. However, there is an exceptional site No. 10 (Komarov) where the levels of PCBs and DDTs more than one order of magnitude higher were detected. They can originate from the former application or disposal of products or wastes containing these compounds or from unauthorized storage in surrounding buildings of the old industrial zone.

In the case of PAHs, we can see a different situation where the levels measured in Kosetice are significantly lower than those in Brno. The highest amounts were found in the sites affected by the traffic (No. 2—Kotlarska, No. 8—Zabovresky,

Table 2
Summary of genotoxicity data expressed as the induction factors for all four tested concentrations and the results of the statistical analysis (non-parametric Spearman test) expressed as Spearman rank correlation (Rho) between the chemical data and the genotoxicity for concentration 10% PUF ml^{-1}

Sampling site	Genotoxic potency[b]				Amount of chemicals (ng PUF^{-1})						
	IF 5	IF 10	IF 15	IF 20	Sum 16 EPA PAHs	Sum 28 PAHs	Sum PCB	Sum HCH	Sum DDT	HCB	PeCB
1	1.00	0.90	**1.44**	1.05	12,725.20	12,914.00	20.10	17.64	21.27	55.95	7.09
2	1.13	1.80	**1.81**	1.65	30,678.80	31,306.40	47.32	24.23	22.12	48.98	7.05
3	0.94	1.34	1.41	**1.45**	13,909.20	14,075.60	46.26	18.10	17.65	32.83	7.31
4	1.11	1.32	**1.86**	1.72	12,802.40	12,934.00	33.30	14.59	15.88	27.41	5.69
5	0.95	1.21	1.41	**1.66**	9473.20	9617.60	8.81	11.38	10.91	28.99	4.24
6	1.01	1.33	**1.54**	1.38	12,718.00	12,855.20	9.59	10.82	16.67	30.80	7.61
8	1.19	1.31	**2.24**	1.51	16,798.40	17,002.40	12.08	10.92	11.85	25.80	6.31
9	1.07	0.98	2.35	**2.53**	8196.00	8277.60	11.51	15.20	11.43	48.03	6.70
10	1.26	1.36	1.65	**2.02**	20,092.00	20,385.60	404.22	18.90	101.87	51.18	7.70
11	1.48	1.14	1.75	**1.99**	13,874.80	14,058.40	13.05	13.16	16.14	39.05	5.81
12	1.35	0.99	1.15	**1.28**	9707.60	9818.00	11.68	11.61	12.16	38.26	7.24
13	1.13	1.02	1.46	**1.48**	14,265.60	14,416.40	15.18	11.88	17.34	36.98	5.36
14	1.41	1.42	1.77	**1.78**	19,863.20	20,131.20	25.30	21.79	26.13	53.78	6.44
15	1.28	0.97	1.49	**1.52**	8634.00	8749.20	11.36	14.24	16.06	41.10	6.31
16	0.43	0.66	0.81	**1.02**	10,074.00	10,187.20	12.19	13.73	14.67	47.17	5.53
17	0.59	1.00	1.23	**1.96**	16,170.80	16,495.60	16.54	20.38	46.09	47.07	4.94
18	0.54	1.01	1.18	**1.36**	17,746.80	18,028.40	33.02	22.52	32.70	63.66	7.07
19	0.47	0.88	0.98	**1.05**	14,902.40	15,028.40	17.50	10.73	10.51	35.16	4.35
20	0.41	1.12	**1.32**	1.20	7140.40	7593.60	10.21	5.68	24.25	15.26	4.38
21	0.79	0.90	1.07	**1.30**	6397.60	6450.80	13.12	10.04	9.24	32.97	4.11
22[a]					5482.20		13.93	19.43	18.31	80.88	6.94
Rho[c]					**0.52**[d]	**0.52**[d]	0.34	0.34	**0.45**[d]	−0.12	0.38

[a] RECETOX background site: observatory of EMEP (Cooperative Program for Monitoring and Evaluation of Air Pollutants in Europe).
[b] Induction factors for each sample dilution.
[c] Spearman rank correlation.
[d] Statistically significant Spearman rank correlation ($p < 0.05$).

No. 14—Petrov) or close to the industrial areas (No. 10—Komarov, No. 18—Slatina) (see Table 1) in accordance with the previous studies (Pospisil et al., 2004). Several elevated levels in the southeastern sites can be explained by a prevailing wind direction from the northwest.

Two important aspects of the induction factor IF need to be evaluated: maximum IF (Fig. 3) and minimum genotoxic concentration (MGC) (Table 3). From the map in the Fig. 3 it is evident that sampling site No. 2 (Kotlarska) does not have as high induction factor as could be expected from the results

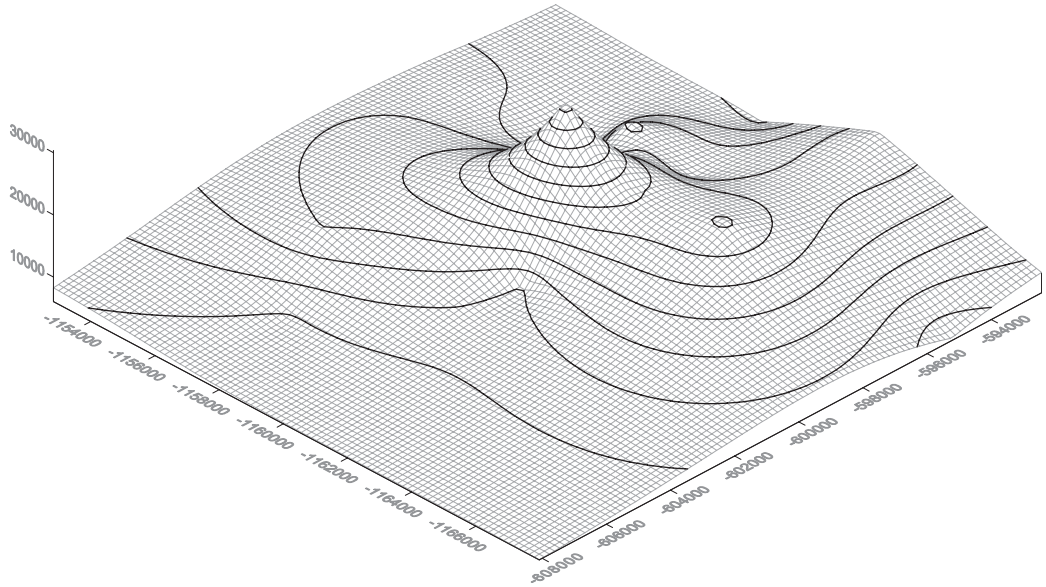

Fig. 2. Wire frames: the three-dimensional presentation of the relative PAHs contamination (Surfer 8.0, Golden Software—Kriging geostatistical method).

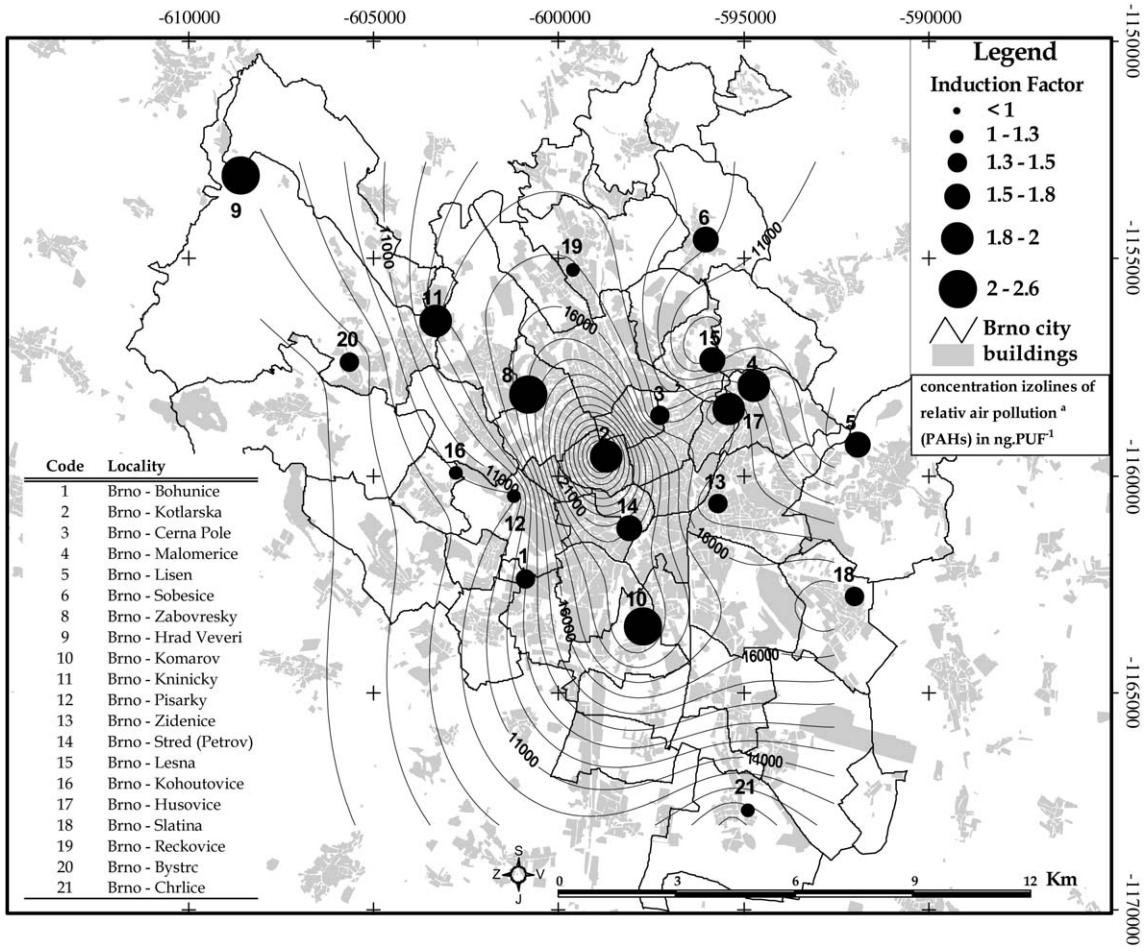

Fig. 3. Spatial presentation of the relationship between the relative air contamination and the direct genotoxic potency (significant genotoxicity factors in the air samples with IF $\geq$1.5). Izolines correspond with the amounts of PAHs sequestered by the filter (in ng PUF^{-1}). (Surfer 8.0, Golden Software).

of chemical analysis and from the literature (Pospisil et al., 2004). Nevertheless, the sample from this site was scored as genotoxic at very low concentrations as can be seen in Table 3 and in Fig. 5. On the contrary, relatively high IF-value was observed at the background sampling site No. 9 (Veveri Castle). It can probably be explained by the intensive reconstruction of the castle during which significant amounts of direct genotoxic chemicals other than those analyzed in the polyurethane foam disks in this study can be released to the ambient air.

It is obvious that selected bioassay only covers a specific part of all chemicals present in the air samples. The significant correlations between the direct genotoxicity of the samples and the amounts of indirect-acting PAHs indicate that the amount of PAHs in the air is probably linked to the amount of direct-acting mutagens like oxy- and nitro-PAHs

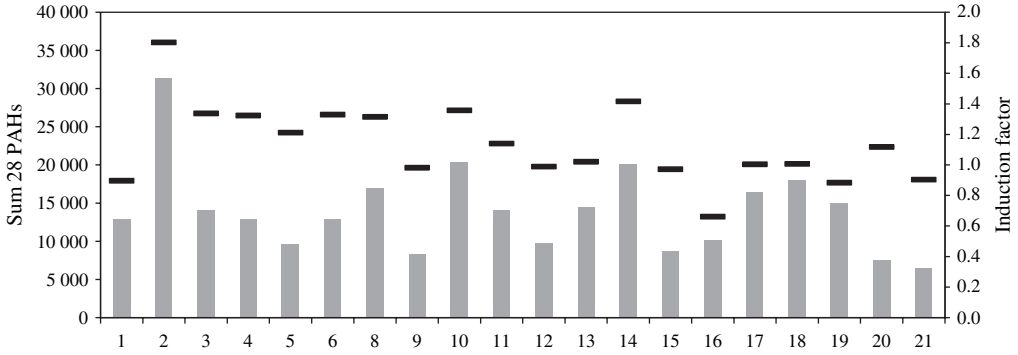

Fig. 4. Correlation of the sum of PAHs (bars) and induction factors for concentration 10% PUF ml^{-1}.

Table 3
Minimum genotoxic concentration for the sampling sites with the significant adverse genotoxic effect

Locality	MGC		RGTU[a]
	% PUF ml^{-1}	SD	
2	8.57	2.07	11.67
4	11.65	0.23	8.58
5	10.53	6.74	9.50
6	14.09	3.96	7.10
8	11.33	0.98	8.82
9	11.92	0.43	8.39
10	12.84	5.53	7.79
11	12.72	1.33	7.86
14	11.24	1.69	8.90
15	21.55	3.94	4.64
17	16.96	1.81	5.90

SD, standard deviation.
[a] Relative genotoxic units (RGTU = [1/MGC] × 100).

(Wada et al., 2001; Du Four et al., 2004). Dinitropyrenes, for instance, are the known products of diesel combustion and 1-nitropyrene and 1,8-dinitropyrene are expected to be fully responsible for 20% and 80%, respectively, of the direct genotoxicity of a diesel exhausts (Hamers et al., 2000). It can be expected from their physicochemical properties that these compounds are sequestered by polyurethane foam filter of the passive sampler and they can be responsible for the genotoxicity of the sample. Detection of the wider spectra of compounds adsorbed on the filter and verification of this hypothesis should be the focus of future research.

5. Conclusions

Results of the study indicate not only a very good capability of the passive air samplers to reflect the spatial fluctuation in concentrations of persistent organic pollutants in the ambient air, but the feasibility of using this method for the direct genotoxic potential assessment. The integration of the passive air sampling technique with the genotoxicological analysis may provide an effective tool for the air monitoring on various scales and for the screening of the genotoxic potential of ambient air samples, which can be used for exposure assessment as a part of human health risk assessment. The geographical information system (GIS) analysis facilitated the spatial results interpretation of this combination of the chemical analysis with the genotoxicity assay, and provided an attractive tool for displaying the levels of air contamination and their genotoxic risks.

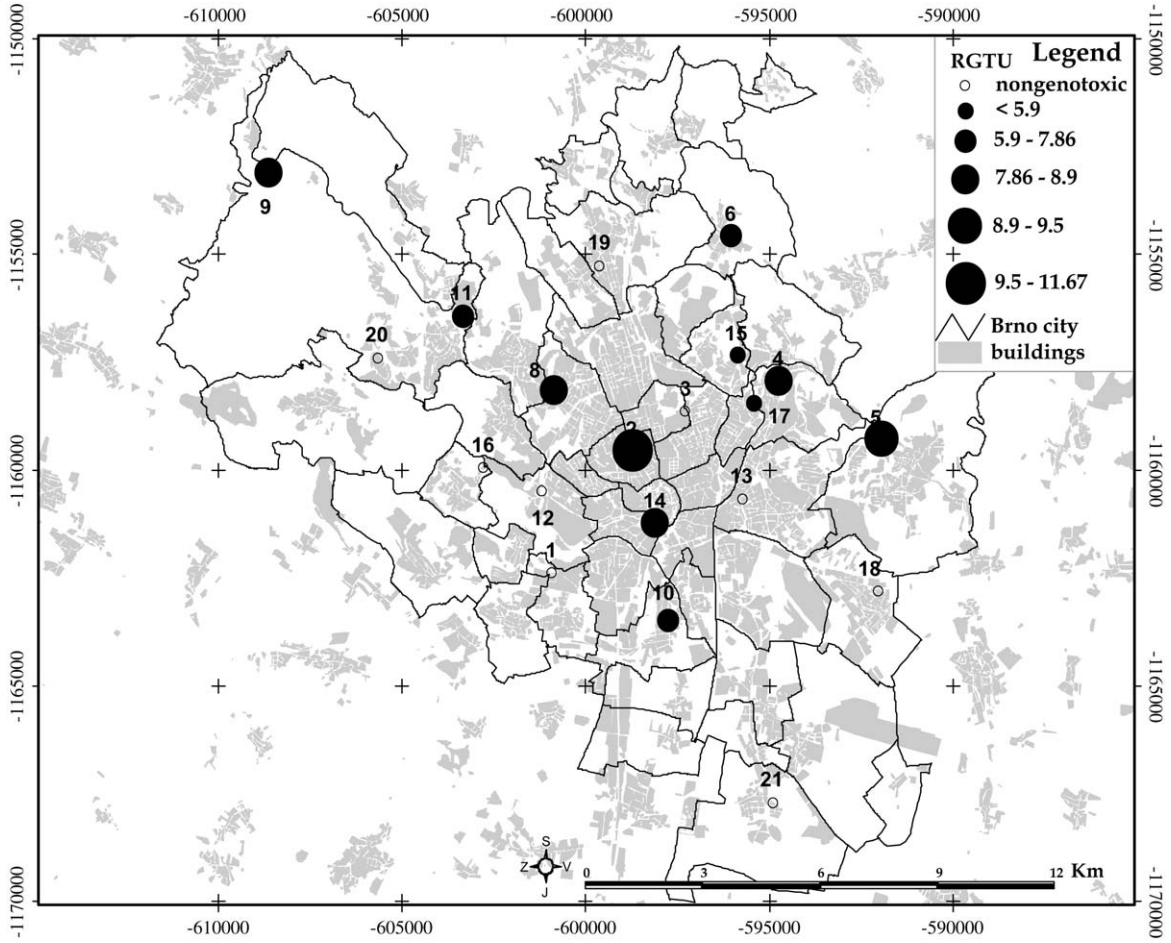

Fig. 5. Spatial presentation of relative genotoxic units RGTU (11 out of 20 sampling sites were marked as significantly mutagenic).

Acknowledgments

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METODICKÉ MOŽNOSTI HODNOCENÍ ZDRAVOTNÍCH RIZIK NA POPULAČNÍ ÚROVNI S VYUŽITÍM DOSTUPNÝCH ENVIRONMENTÁLNÍCH DAT A EPIDEMIOLOGIE ZHOUBNÝCH NÁDORŮ V ČR

METHODICAL ASPECTS OF POPULATION–BASED AND ENVIRONEMNTALLY RELATED CANCER RISK ASSESSMENT IN THE CZECH REPUBLIC

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Souhrn
Článek se zabývá problematikou hodnocení karcinogenních rizik na populační úrovni a komentuje dostupnost potřebných dat v České republice. Rozbor mezinárodně používané metodiky hodnocení vlivu environmentálních polutantů prokázal, že Česká republika je dostatečně datově vybavena pro tento typ analýz. Práce s environmentálními databázemi byla dokumentována na příkladu informačních systémů sledujících kvalitu ovzduší. Reprezentativní epidemiologická data o zhoubných nádorech jsou dostupná v podobě online software na portálu NOR: www.svod.cz. Již pilotní studie s arsenem, kadmiem, benzo(a)pyrenem a benzenem odhalila velké rozdíly v rizikovosti jednotlivých látek a také rozdíly mezi regiony ČR. Databáze Národního onkologického registru v tomto systému hraje nepostradatelnou roli jako zdroj populačních referenčních standardů pro hodnocení rizika. Environmentální studie mohou naopak významně přispět k vizualizaci dat epidemiologických dat implementací geografických informačních systémů. Práce je pilotní metodickou studií pro rozsáhlejší zhodnocení zdravotních rizik české populace ve vztahu k zhoubným nádorům.

Klíčová slova: hodnocení rizik, onkologický registr, GIS.

Summary
Paper is focused on cancer risk assessment and its methodical and information background in the Czech Republic. The study proved that there are sufficient data sources, both environmental and epidemiologic. Representative epidemiologic cancer data are available in web portal of National Cancer Registry (NCR): www.svod.cz. Even pilot study with arsene, cadmium, benzene and benzo(a)pyrene indicated remarkable differences not only among model pollutants but also among regions of the country. Database of NCR with more than 1,3 million cancer cases reported since 1977 forms an indispensable base for definition of reference standards in risk assessment studies. And on the other hand, environmental projects can enrich NCR with implementation of geographic information systems. Paper is a pilot methodical introduction to more representative evaluation of health status of Czech population as related to cancer risks.

Key words: risk assessment, cancer registry, GIS.

Úvod

Iniciace nádorového bujení je podmíněna řadou různých, navzájem souvisejících faktorů s možnými synergistickými či právě naopak antagonistickými účinky. Dědičné mutace nebo polymorfismus vybraných genů, environmentální agens, které ovlivňují incidenci somatických genetických změn, a některé další systémové a lokální faktory se ve vzájemné kombinaci zcela jistě podílejí na procesech vedoucích ke vzniku rakoviny [1,2]. V důsledku této komplikované podstaty vlivů je hodnocení populačních rizik ve vztahu k nádorovým onemocněním velmi složitý a komplexní problém. Dnes již známe řadu možných faktorů a hledisek, pomocí nichž je můžeme analyzovat obraz možných expozic či rizikových vlivů. Postupně vzniká ucelenější obraz poznání, který zcela jistě umožní i předcházet nepříznivým následkům [3,4].

Znečištění ovzduší je jedním z faktorů, které se spolupodílejí na ovlivnění lidského zdraví. Za posledních několik desítek let byla nashromážděna řada důkazů o negativním působení znečištěného ovzduší na lidské zdraví [6,7]. V exponované populaci můžeme pozorovat zvýšený výskyt onemocnění dýchacího ústrojí, alergických onemocnění, nemocnost a úmrtnost osob s chronickým onemocněním dýchacího a kardiovaskulárního ústrojí, snížení reprodukčních schopností, ale také výskyt nádorových onemocnění, na kterých se může určitou měrou podílet expozice

karcinogenním látkám z ovzduší [5]. Tyto skutečnosti samozřejmě platí i pro českou populaci. Na národním portálu sloužícím pro analýzy populačních dat epidemiologie nádorů v ČR (www.svod.cz) lze nalézt jednoznačně zvýšenou incidenci i mortalitu karcinomu plic v regionech se zhoršenou kvalitou ovzduší.
Snižování koncentrací znečišťujících látek v ovzduší vytváří podmínky pro zlepšení ukazatelů zdravotního stavu, které jsou v souvislosti s expozicí populace těmto látkám uváděny [5]. Otázkou je, nakolik a jakými přístupy a metodami lze tento vliv teoreticky a prakticky hodnotit v běžné praxi. Pro odpověď na tuto otázku jsme analyzovali využitelnost dostupných populačních dat o zdravotním stavu obyvatel a environmentálních dat v metodice hodnocení zdravotních rizik [3–6, 8–18].

Výběr parametrů zdravotního stavu obyvatel
Výběr ukazatelů o incidenci nádorových onemocnění byl proveden v úvodní části této pilotní studie tak, aby odrážel nejvíce pravděpodobné vlivy faktorů životního prostředí. Přestože je známa řada možných příčin vzniku ZN, komplexní hodnocení možných rizik a jasné vymezení preventabilních složek je stále nemožné. Iniciace většiny nádorových onemocnění je podmíněna vzájemnými interakcemi řady faktorů, které vznikají následkem několika recesivních genetických událostí nebo interakcí environmentálních faktorů s pacientovou vlastní DNA. Jen velmi malé procento onkologických onemocnění je podmíněno čistě genetickými příčinami. I samotná interpretace interakce genetických a environmentálních faktorů stále zůstává značně komplikovanou záležitostí [19].
Jednotlivé typy onkologických onemocnění se ve své incidenci a prevalenci liší více než řádově, a to jak vzhledem k různým typům populací, tak i geografické poloze dané populace. Konvergence cizí populace k epidemiologickým hodnotám v dané lokalitě, známé na příkladu imigrantů, vylučuje vysvětlení čistě jen pomocí genetických faktorů. Na základě tohoto zjištění byly již v šedesátých letech učiněny závěry, z nichž vyplývalo, že většina onkologických onemocnění je ve svém principu preventabilní, přičemž vhodnou volbou či změnou životního stylu a stavem životního prostředí, lze značnému množství případů onemocnění předejít.
Dnes je již známo, a na řadě nejenom epidemiologických studií prokázáno, poměrně velké množství faktorů iniciujících rakovinu. Mezi nejvýznamnější patří zcela jistě kouření, obezita a několik onkogenních virů, dále též profesní rizika ve vztahu k určitým typům onkologických onemocnění [8–10, 20–23]. Poměrně velký podíl celosvětové variability v epidemiologických parametrech, a to nejenom u častějších typů nádorů, jakými jsou rakovina prsu, prostaty, tlustého střeva a konečníku, zůstává i přesto nadále nevysvětlen [24]. V rámci řešení tohoto projektu byla zařazena do výše zmíněných prostorových a časových analýz data o incidencích následujících diagnostických skupin zhoubných novotvarů:

- incidence ZN tlustého střeva a konečníku (diagnózy C18, C19, C20, C21),
- incidence ZN plic (diagnóza C34),
- incidence ZN prsu (diagnóza C50 – pouze ženy),
- incidence ZN prostaty (diagnóza C61 – pouze muži),
- incidence ZN varlete (diagnóza C62 – pouze muži).

Je ovšem na místě zdůraznit, že hodnocení onkologických rizik na populační úrovni rozhodně nelze provádět prostou korelací sumárních incidenčních a mortalitních dat s environmentálními charakteristikami daného území v daném čase. Řada zhoubných nádorů se před svým zachycením zdravotnickým systémem dlouho vyvíjí a jejich hostitel (budoucí pacient) může migrovat a také i v tomto prediagnostickém období procházet řadou vlivů. V působení externích vlivů i vlivů souvisejících s životním stylem existuje tedy časové okno, perioda, která musí být v analýzách respektována, i když ji na populační úrovni nelze přesně určit. Jedinou cestou, jak zabránit zkreslení, je sledovat nejen sumární incidenci a mortalitu pro nádory jako takové, ale respektovat co nejpodrobnější charakteristiky pacientů a diagnostickou identifikaci nově zachycených případů. Nádor zachycený v časném stadiu u mladého člověka indikuje externí nebo genetický vliv jistě silněji (pravděpodobněji) než velmi pokročilé stadium onemocnění zachycené u člověka ve vyšším věku. Z parametrů, které musí být při hodnocení rizik respektovány na straně epidemiologických onkologických databází, lze jmenovat především:

- **diagnostickou skupinu zhoubných nádorů**
  - Onkologie je diagnosticky velmi heterogenní terén a bez rozlišování jednotlivých diagnostických skupin nelze sumární čísla interpretovat. Zcela jinou vazbu k vlivu externích faktorů budou mít nádory mozku, hematoonkologické malignity a např. nádory plic.
- **klinické stadium onemocnění v době diagnózy**
  - Pokročilost onemocnění určuje dobu, po kterou byl nádor v těle před vlastní diagnózou. U pomalu rostoucích nádorů se tak velmi snižují možnosti přímé korelace s možnými externími vlivy v daném místě a čase.
- **pohlaví pacienta**
  - Některé malignity samozřejmě může mít pouze jedno z pohlaví, např. nádory pohlavních orgánů. U jiných je ale při hodnocení rizik užitečné pohlaví sledovat, neboť se mohou lišit např. životním stylem.
- **věk pacienta v době diagnózy**
  - Velmi podstatný parametr, neboť incidence i mortalita má u jednotlivých diagnostických skupin daný typický věkově specifický průběh. Záchyt nádorů mimo tuto typickou křivku může indikovat vliv externích faktorů.

Jelikož jde o parametry, které jsou zároveň běžným a minimálním záznamem onkologických epidemiologických registrů, neměl by být problém využít je i pro hodnocení populačních rizik. Dojde tím sice ke zkomplikování interpretace všech nalezených korelačních vztahů, nicméně je to nutná cena, kterou platíme za korektní interpretaci výsledků. Situaci přitom umíme ještě více zkomplikovat, neboť už samotný záchyt malignity a také doba přežití diagnostikovaného pacienta souvisí se stavem, možnostmi a výkonností zdravotnictví v dané oblasti. Tím bychom ale výrazně překročili záměr a možnosti tohoto úvodního sdělení, proto se dále primárně zaměříme na popis dostupných environmentálních dat a metodických postupů.

Využitelnost dostupných zdrojů environmentálních dat s ohledem na zdraví populace
Jako hlavní zdrojovou databázi pro charakterizaci nejvýznamnějších environmentálních faktorů jsme v této pilotní studii využili databázi ČHMU o míře imisního

zatížení látkami s potenciálním karcinogenním efektem ve volném ovzduší. Do analýzy byly zařazeny látky s bezprahovým typem účinku: benzo(a)pyren, benzen, arzen a kadmium. Dílčí databáze obsahují průměrné roční imisní koncentrace těchto látek gridové síti 2x2 km pro celou plochu ČR. Všechna tato vstupní data byla implementována do analytického GIS prostředí (geografické informační systémy) jednotlivých podkladových vrstev. ČHMU vytvořil tyto vrstvy v rámci řešení projektu VaV/740/3/02 jako výstup statistických analýz s využitím výsledků reálných monitorovacích měření z více než 150 měřicích stanic ČR v kombinaci s aplikací rozptylových modelů (aplikace Bayesovské asimilace – [25], interpolace s využitím metody kriging, IDW, Radial Basic Function).

Metodika hodnocení zdravotních rizik

Data o míře kontaminace volného ovzduší pro daný rok byla využita pro retrospektivní vyhodnocení potenciálních zdravotních rizik s důrazem na karcinogenní efekty přítomných polutantů. Hodnocení zdravotních rizik je metodický postup, který umožňuje systematickým vyhodnocováním škodlivých faktorů odhadnout a kvantifikovat vliv faktorů prostředí na zdraví [26–29]. Metodika umožňuje na základě souboru informací o působících látkách a jejich vlivu na zdraví modelovat pravděpodobné dopady na zdravotní stav populace, ovšem při znalosti těchto faktorů a při znalosti stavu dané populace. Výhodou je možnost prospektivního modelování vlivu ještě neexistujících situací. Metodika zahrnuje následující základní kroky:

- identifikace nebezpečnosti,
- určení vztahu dávka – odpověď,
- hodnocení expozice charakterizace rizika,
- řízení a komunikace rizika.

Identifikace nebezpečnosti

Účelem tzv. identifikace nebezpečnosti je posoudit závažnost důkazů o nežádoucích účincích studovaného faktoru na člověka. Provádí se na základě hodnocení dat získaných z pozorování u lidí, z experimentálních studií na zvířatech, na izolovaných orgánech, tkáních, buněčných systémech nebo z dat získaných ze studií vztahů mezi chemickou strukturou a biologickou účinností látek (QSAR). Standardní návody pro toto hodnocení mnoho světových organizací (WHO, OECD, EU, US EPA, US ATSDR, IPCS, FDA). Údaje o nebezpečnosti látek lze vyhledat v toxikologických databázích.

Určení vztahu „dávka – účinek"

Pro posouzení zdravotních rizik se využívá několika možných přístupů a postupů. Existují dva základní vstupy do procesu odhadu zdravotních rizik. První vychází z epidemiologických studií, které vyhledají vztah mezi dávkou (expozicí) a účinkem u člověka. Druhým vstupem jsou experimentálně získané toxikologické charakteristiky látek aproximované do hodnot blížících se reálné expozici člověka. Hlavním cílem tohoto hodnocení je stanovení maximální úrovně stresoru (koncentrace chemické látky), která škodlivě neovlivní hodnocený „endpoint". Lze hovořit o stanovení ještě bezpečné = limitní expozice. Na základě experimentálních a epidemiologických dat US EPA stanovuje v rámci informačního systému IRIS tyto koncentrační úrovně (CSF, IUR,…), jejichž podrobnější definice jsou uvedeny v následujícím textu.

Princip stanovení konstanty karcinogenního potenciálu v praxi vychází z hypotézy, že vztah mezi velmi nízkými dávkami studované látky a vyvolaným efektem (vznikem nádoru) bude lineární. To umožňuje stanovit směrnici závislosti takového lineárního vztahu a na základě znalosti expozice odhadovat pravděpodobnost vzniku nádorových procesů (cancer slope factor – CSF). Pokud se použije pro tento vztah nelineární závislost, je odhad efektu často o 3–4 řády nižší. Pro použití lineárního modelu však hovoří větší „míra ochrany" zdraví. V posledních letech však US EPA v IRIS systému (a také WHO) preferuje pro stanovení karcinogenního efektu využití hodnot inhalačních jednotek rizika rakoviny (dále IUR). Na základě nových poznatků o farmakokinetice a metabolismu celé řady karcinogenních látek tak byly přehodnoceny dříve používané hodnoty CSFi na IUR dle metodiky EPA [26].

Hodnocení expozice

Expozice je zjednodušeně definována jako „kontakt" člověka s chemickým, biologickým nebo fyzikálním faktorem. Její posouzení je klíčovou a současně nejobtížnější fází hodnocení rizika. V tomto kroku je odhadována velikost, četnost a doba trvání expozice sledovanými látkami a také velikost, povaha a typ populace, která je daným látkám vystavena. Výstupem hodnocení je numerický odhad přijaté dávky, který je dále použit v charakterizaci rizika. Základním předpokladem pro správné hodnocení expozice je určení všech pravděpodobných expozičních cest (vstupů) a jejich charakteristika s ohledem na zájmovou populaci.

Kvantitativní odhad expozice sledovanými látkami je spolu s hodnocením dávka – odpověď určující pro charakterizaci rizika. K tomuto účelu jsou sestavovány expoziční scénáře, které umožňují odhadovat velikost expozice. Obsahem scénáře je soubor vybraných expozičních parametrů, které umožňují charakterizovat a specifikovat expozici dané populace.

Inhalační expozice - pro výpočet chronického denního příjmu inhalační cestou *CDI* platí následující rovnice:

$$CDI = CA \cdot IF \qquad r1$$

kde je CA koncentrace v ovzduší
IF faktor příjmu

Pro samotný faktor příjmu pak platí

$$r2$$

kde je
- IR inhalované množství
- ET doba expozice
- EF frekvence expozice
- BW váha těla
- AT čas průměrování
- ED trvání expozice

Charakterizace karcinogenních rizik

Výpočet karcinogenního rizika se provádí podle následujícího vztahu:

$$CVRK = LAIC \times IUR \qquad r3$$

- kde $LAIC$ je průměrná celoživotní koncentrace.

Hodnoty IUR jsou ve většině případů odvozeny od dříve používaných hodnot CSF a je možné je použít v případě standardního expozičního scénáře, kdy uvažujeme následující parametry expozičního scénáře:

IR	inhalované množství	20 $m3.den^{-1}$
ET	doba expozice	24 $hod.den^{-1}$
EF	frekvence expozice	365 $dní.rok^{-1}$
BW	váha těla	70 kg
AT	čas průměrování	ED x 365 $dnů.rok^{-1}$
ED	trvání expozice	70 let

V případě odchylky v některém parametru je nutné zařadit do výpočtu další korekce v přímé či nepřímé úměře k výslednému riziku (vztah mezi jednotlivými parametry definuje rovnice r2).

Takto vypočítané riziko (*CVRK)* se považuje za celoživotní vzestup pravděpodobnosti incidence nádorových onemocnění nad průměr pro populaci i jednotlivce, vždy v důsledku definované expozice daným faktorem. V případě odhadu karcinogenního rizika, kde se předpokládá bezprahový typ účinku, je tedy hodnocenou informací navýšení incidence onkologických onemocnění v populaci exponovaných lidí (CVRK). V oficiální literatuře je za hranici akceptovatelného rizika považován interval 1–100 případů zhoubného novotvaru v milionové populaci (1.10^{-6} až 1.10^{-4}) [29]. Hraniční úroveň rizika je ovšem nutné stanovit vždy pro každou danou regionálně specifickou studii [30]. Pravděpodobnostní riziko slouží především k relativnímu porovnání s referenčními oblastmi. Hodnocení absolutních hodnot predikovaných na základě aplikace konkrétního expozičního scénáře není možné.

Aplikace GIS v pilotní studii na datech Národního onkologického registru

Pro tuto pilotní studii byl vybrán jako analytický nástroj GIS (geografický informační systém, ArcGIS 8, ESRI). Výhody jeho použití spočívají především v možnosti paralelního provedení prostorových a časových analýz v systému i několika zdrojových databází. V případě incidencí zhoubných novotvarů jsou mapy prezentovány s dvěma informačními GIS vrstvami. Plochy představují barevné odlišení regionů – okresů, a to na základě průměrných hodnot incidencí za celé hodnocené období (1995–2002). Na ploše každého okresu je pak informační sloupcový graf časového vývoje hodnoceného parametru pro daný rok.

Pro hodnocení inhalační expozice byl použit expoziční scénář, který předpokládá, že lidé jsou vystaveni hodnoceným koncentracím celých 24 hodin. Tento přístup nadhodnocuje význam koncentrací látek ve venkovním ovzduší, ale celkovou expozici nadhodnocuje pouze v případě, že jsou koncentrace ve venkovním ovzduší vyšší než uvnitř budov.

Bylo hodnoceno karcinogenní riziko expozice arsenu, kadmia, benzenu a benzo(a)pyrenu pro roky 2003 a 2004, zvlášť pro jednotlivé látky dostupné v databázi celého území ČR. Výsledné úrovně rizik jsou prezentovány jednotlivě a jako suma rizik odpovídající jejich součtu (viz Obr. 1 – rok 2004). Z výsledků pro oba roky je patrné, že ke karcinogennímu riziku nejvýznamněji přispívá expozice benzo(a)pyrenu v menší míře benzenu a arsenu. Naopak kadmium představuje v celkovém riziku naprosto minoritní podíl.

Z rozložení odhadů rizika je možné usoudit na jeho eskalaci v aglomeracích větších měst (zejména Praha a Ostrava) a také v blízkosti významných dopravních komunikací. Lze tedy konstatovat, že příspěvek dopravy k těmto rizikům je významný (Praha + přilehlé dálniční spojení). Nejvyšších rizikových hodnot je však dosahováno v průmyslových oblastech Ostravska a severních Čech, kdy hodnoty pravděpodobnostního rizika vzniku nádorových onemocnění překračují hranici 1:10000. Doplňující analýzou pak bylo vyhodnocení území, kde jsou překračovány imisní limity dané naší legislativou pro dané látky přispívající ke zdravotním rizikům (Obr. 2.). Jednalo se o hlavně o benzo(a)pyren.

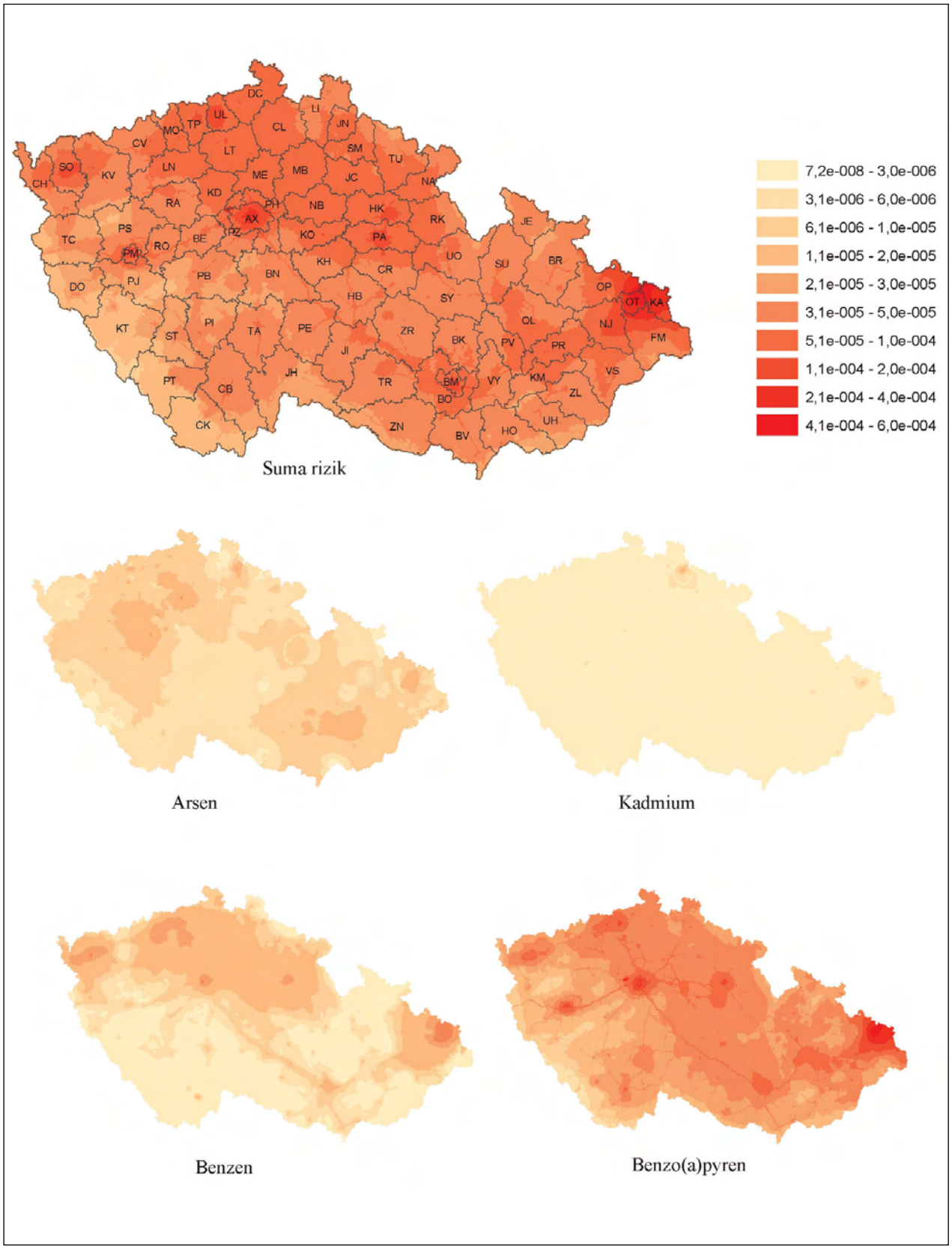
Podrobnější analýza dat skutečného výskytu ZN byla provedena paralelně. Samotné srovnání aktuálních koncentrací a z nich vypočítaných odhadů zdravotních rizik se skutečnou incidencí různých novotvarů je ovšem problematické. Problémem pro Českou republiku také je, že věrohodné a kvantifikovatelné údaje o stavu životního prostředí máme za období, kdy ještě nemáme k dispozici plně validovaná epidemiologická data. Tato skutečnost se ale ve velmi krátké době změní a díky připravené metodice hodnocení a podchyceným environmentálním datům bude možné plošné a plně kvantifikovatelné hodnocení rizik.

Závěr

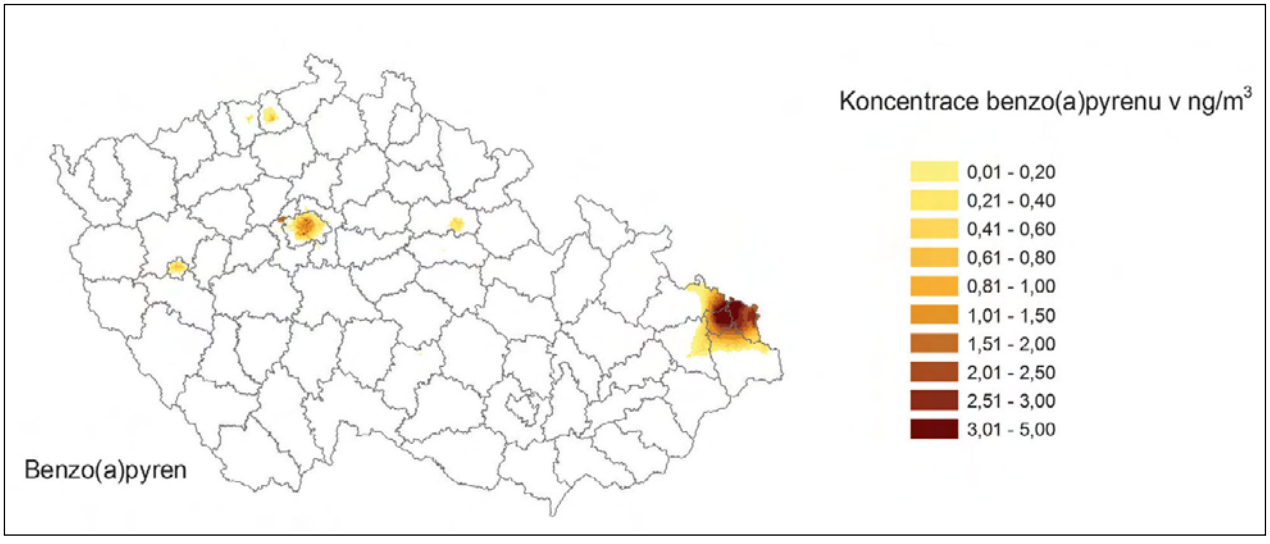
Na příkladu dat o kvalitě ovzduší práce dokumentuje dostupnost environmentálních dat, aktuálních pro území České republiky. Rovněž jsou takto k dispozici reprezentativní data o epidemiologii zhoubných nádorů (www.svod.cz) a mezinárodně používaná, validovaná metodika hodnocení karcinogenních rizik na populační úrovni. Lze tedy konstatovat, že v České republice je možné data Národního onkologického registru (NOR) využít jako velmi cenný zdroj informací pro hodnocení zdravotních rizik. Tato data představují velmi cenný zdroj informací pro definici referenčních hodnot karcinogenního rizika, ať již pro celou populaci nebo na regionální úrovni. A naopak zde dokumentovaná implementace geografických informačních systémů by velmi prospěla vizualizaci dat NOR.

Poděkování

Projekt byl realizován za podpory MŽP ČR, MŠMT MSM 0021622412 INCHEMBIOL a ČHMÚ.



Obr. 1: Výsledky hodnocení potenciálních zdravotních rizik plynoucích z inhalační expozice vybraným karcinogenním látkám (pro definovaný modelový expoziční scénář; *CVRK* pro rok 2004, zdroj vstupních dat ČHMÚ).



Obr. 2: Prostorové vymezení území, na kterém je nutné snížení koncentrací benzo(a)pyrenu s využitím metody srovnání s platným limitem pro volné ovzduší (polygony identifikující, o kolik je nutné snížit koncentraci; data pro rok 2004).

Tab. 1: Dostupná environmentální data v ČR vhodná pro hodnocení humánních rizik v kombinaci s databází populační onkologie.

Data	Charakter dat	Monitoring
Radonová mapa ČR	polygonální mapy radonového rizika	ČGS – Česká geologická služba
Imisní koncentrace nejvýznamnějších polutantů v ovzduší (databáze ISKO)	Oxid dusičitý, Oxid siřičitý PM10 Arsen, Kadmium Benzen, Benzo(a)pyren (gridový model 2 x 2 km)	od 1995. (ČHMÚ – Český hydrometeorologický ústav, ISKO – Informačního systému kvality ovzduší)
Znečištění pitné vody	veřejné vodovody; veřejné a soukromé studny	1996–2004 (SZÚ– Státní zdravotní ústav)
Imisní zátěž volného ovzduší POPs	kontinuální monitoring pasivního vzorkování 50 lokalit v ČR metodou	od 1995 (RECETOX – Výzkumné centrum pro chemii životního prostředí, MU, Brno)
Koncentrace polutantů v půdě	bazální monitoring půd, registr kontaminovaných ploch, databáze RECETOX	od 1990 (ÚKZÚZ – Ústřední kontrolní a zkušební ústav zemědělský; RECETOX)
Obsahy látek a dalších stresových faktorů v potravinách na trhu v ČR	koncentrace polutantů; analýzy spotřebního koše (1997)	od 1994 (SZÚ)
Kontaminace povrchových a podzemních vod	koncentrace polutantů	od 1994 (ČHMÚ)

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Příloha 6

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Are the residents of former Yugoslavia still exposed to elevated PCB levels due to the Balkan wars? Part 2: Passive air sampling network

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Abstract

Many Eastern European countries suffer the lack of data on concentrations of persistent organic pollutants in the environmental matrices. This absence of information is preventing the local authorities from taking the adequate actions to protect the people and environment. This is even more alarming in the countries recently affected by the wars where the chemicals released to the environment during the military operations can cause a significant ecological damage and health effects on the population. A potential of passive air sampling technique as a tool capable of providing seasonally and spatially resolved information about the local sources and levels of contamination was explored in this study as a first step to the establishment of a cost-effective long-term monitoring in this area. The passive air samplers proved to be a powerful technique capable of detecting the concentrations ranging over four orders of magnitude providing the information very comparable with the conventional techniques.

Keywords: Persistent organic pollutants; Polychlorinated biphenyls; Atmosphere; Soil; High volume sampling; Passive air sampling; Balkan war; Yugoslavia

1. Introduction

There is a general lack of information on the levels of persistent organic pollutants (POPs) in the countries of Central and Eastern Europe (CEE) (UNEP, 2002a). A better situation is in the Czech Republic, Slovakia, Poland, and Slovenia. Satisfactory information about the pesticides exists also in some others, like Hungary, Bulgaria, and Croatia. In the rest of CEE countries, available data on the POP sources and levels are very limited, and there is no systematic monitoring of POPs in the countries of former Yugoslavia (UNEP, 2002b). Considering the fact that this region has very specific environmental problems resulting from the recent wars, an improvement of this situation is obviously needed.

Epidemiological studies focused on the evaluation of health risks related to the air pollution suggest that the ambient air pollution may be responsible for increasing rates of diseases like a lung cancer (Kappos et al., 2004; Pavuk et al., 2004; Tam and Neumann, 2004; Parodi et al., 2005). From this point of view, frequent measurements of the air concentrations in affected areas as well as an establishment of the monitoring program with the priority in the identification and evaluation of the sources of POPs are the matter of a great importance.

Although the high volume air sampling campaigns performed in Croatia, Serbia, Bosnia and Herzegovina in 2003 and 2004 (Klanova et al., 2007) offered first systematic data on the atmospheric levels of POPs, more coordinated effort is required for determination of the seasonal fluctuations and long-term trends, for the assessment of impacts and potential risks, and for the effectiveness evaluation of applied measures. This is, however, complicated by the lack of both sampling equipment and laboratory capacities in the region.

Addressing these needs, the additional atmospheric campaign was designed for the APOPSBAL project using the passive air sampling (PAS) technique as an alternative method of a sample collection. Various passive air samplers were developed recently as cost-effective tools enabling a semi-quantitative measurement of the atmospheric pollution and a relative comparison of the POPs concentrations on the individual sampling sites (Ockenden et al., 1998; Shoeib and Harner, 2002; Wania et al., 2003; Harner et al., 2004; Jaward et

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al., 2004a, 2004b). They have been currently employed in many large scale projects (Pozo et al., 2006) as well as in the assessments of the regional trends (Gouin et al., 2005; Motelay-Massei et al., 2005; Harner et al., 2006a; Harner et al., 2006b). The capability of those devices to reflect the spatial and temporal fluctuation in the POP concentrations was investigated (Klanova et al., 2006b) as well as their ability to serve the purpose of toxicological studies (Cupr et al., 2006).

The feasibility of the long-term application of passive air samplers for the evaluation of persisting influence of the war damages on the atmospheric contamination of the Western Balkan region was assessed in this study. Results of this project were compared to those of previous high volume sampling campaigns.

2. Materials and methods

2.1. Sampling sites

In order to collect an extended number of parallel air samples, to put the data from the short-term high volume sampling events into the right perspective, to gain more information about the spatial and temporal distribution of POPs, and to collect the samples from remote places, a passive air sampling (PAS) campaign was organized in Croatia, Serbia, Bosnia, Herzegovina, and Kosovo in 2004. PUF based passive samplers were employed at 34 sampling sites for 5 consecutive periods of 28 days between July and December of 2004. The sampling design of previous high volume campaigns (Klanova et al., 2007) was extended to cover a central part of Croatia (Zagreb) and Western Slavonia, the industrial, residential, and rural areas were included. Two sites in Kosovo were added to the network where an active air sampling proved to be difficult to organize as well as new background site in Serbia to learn more about the transport (Fruska Gora). Additional eighteen sites in the Czech Republic – including the background monitoring station in Kosetice serving as an EMEP observatory (Holoubek et al., 2001) – were sampled accordingly and they served as a reference region. The map of the sampling sites in the Balkan region as well as in the Czech Republic is provided in Fig. 1.

2.2. Air sampling

Passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5 cm thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in the protective chambers were employed in this study. The theory of the passive sampling using similar devices was described elsewhere (Shoeib and Harner, 2002; Harner et al., 2004). Sampling chambers were prewashed and solvent-rinsed with acetone prior to installation. All filters were prewashed, cleaned (8 h extraction in acetone and 8 h in dichloromethane), wrapped in two layers of aluminum foil, placed into zip-lock polyethylene bags and kept in the freezer prior to deployment. The exposed filters were wrapped in two layers of aluminum foil, labeled, placed into zip-lock polyethylene bags and transported in cooling box at 5 °C to the laboratory where they were kept in the freezer at −18 °C until the analysis. Field blanks were obtained by installing and removing the PUF disks at all sampling sites.

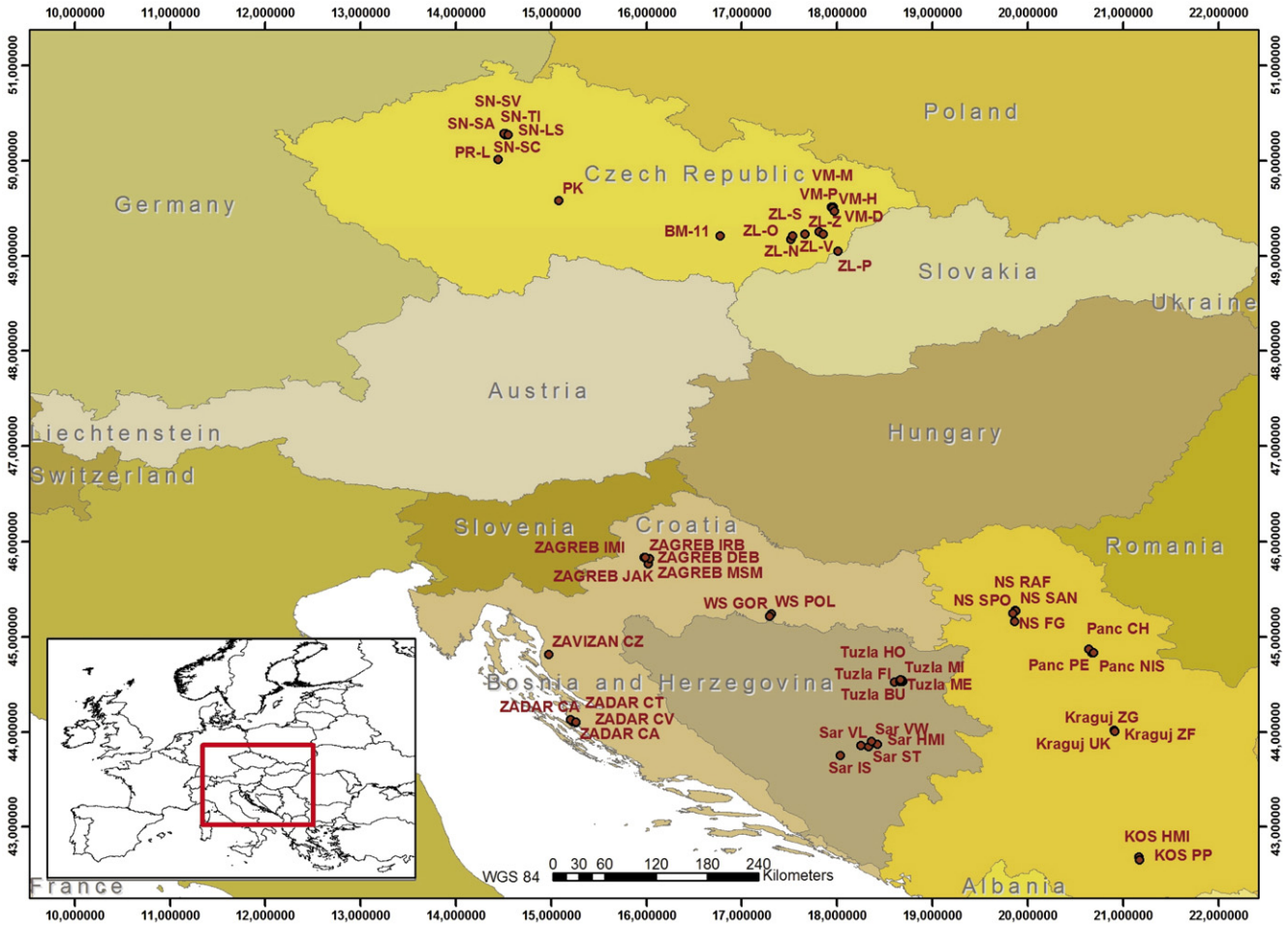

Fig. 1. The map of the sampling sites.

Table 1
Amounts of PCBs sequestered in the polyurethane foam filters in Croatia and Western Slavonia (ng filter^{-1})

Country	Croatia											
Sample	PAS [a]											
Sampling site	Zavizan CZ	Zadar CT	Zadar CA	Zadar CV	Zadar CM	Zagreb IMI	Zagreb IRB	Zagreb MSM	Zagreb JAK	Zagreb DEP	WS POL	WS GOR
PCB 28	2.2	274.1	4.3	4.5	1.7	4.0	3.0	4.7	11.8	29.8	3.1	2.2
PCB 52	2.7	77.9	3.7	3.1	2.0	4.5	4.3	4.4	12.1	17.9	3.6	2.2
PCB 101	1.6	9.7	4.0	1.1	1.3	1.8	2.4	2.8	5.4	12.1	0.5	0.4
PCB 118	0.5	5.6	0.9	0.5	0.4	0.9	0.6	2.0	2.9	3.7	0.5	0.7
PCB 153	2.4	6.9	4.5	1.7	1.6	2.2	3.0	3.6	3.6	4.4	1.2	1.6
PCB 138	1.8	5.4	3.7	1.3	1.2	1.3	1.6	1.8	2.5	4.2	0.7	0.9
PCB 180	1.9	2.4	1.1	0.8	0.6	0.5	0.8	0.6	0.7	1.2	0.3	0.5
Total PCB	13.1	382.0	22.2	13.0	8.8	15.2	15.8	19.9	39.0	73.3	9.9	8.5

[a] Passive air sample.

2.3. Sample analysis

All samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. One laboratory blank and one reference material were analyzed with each set of ten samples. Surrogate recovery standards (D_8-naphthalene, D_{10}-phenanthrene, D_{12}-perylene for PAHs analysis, PCB 30 and PCB 185 for PCBs analysis) were spiked on each filter prior to extraction. Terfenyl and PCB 121 were used as internal standards for polyaromatic hydrocarbon (PAH) and polychlorinated biphenyl (PCB)/organochlorine pesticide (OCP) analyses, respectively. Volume was reduced after extraction under a gentle nitrogen stream at ambient temperature, and fractionation achieved on a silica gel column; a sulphuric acid modified silica gel column was used for PCB/OCP samples. Samples were analyzed using GC-ECD (HP 5890) supplied with a Quadrex fused silica column 5% Ph for PCBs: PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, and OCPs: α-hexachlorocyclohexane (HCH), β-HCH, γ-HCH, δ-HCH, 1,1-dichloro-2,2-bis (*p*-chlorfenyl) ethylene (*p,p′*-DDE), 1,1-dichloro-2,2-bis (*p*-chlorfenyl) ethan (*p,p′*-DDD), 1,1,1-trichloro-2,2-bis (*p*-chlorfenyl) ethan (*p,p′*-DDT). 16 US EPA polycyclic aromatic hydrocarbons were determined in all samples using GC-MS instrument (HP 6890-HP 5972) supplied with a J&W Scientific fused silica column DB-5MS.

2.4. Quality assurance/quality control

Recoveries were determined for all samples by spiking with the surrogate standards prior to extraction. The amounts were similar to detected quantities of analytes in the samples. Recoveries were always higher than 76% and 71% for PCBs and PAHs, respectively. Recovery factors were not applied to any of the data. The recovery of native analytes measured for the reference material varied from 88 to 103% for PCBs, from 75 to 98% for OCPs, from 72 to 102% for PAHs. Laboratory blanks were very low. Field blanks consisted of pre-extracted PUF disks which were taken on each sampling site. They were extracted and analyzed in the same way as the samples; the levels in field blanks never exceeded 3% of quantities detected in samples for PCBs, 1% for OCPs, 3% for PAHs, indicating a minimal contamination during the transport, storage, and analysis.

3. Results

A sampling rate of the passive sampler of similar design was estimated to be 3–4 m^3/day based on the laboratory calibration experiments co-employing passive and active samplers (Shoeib and Harner, 2002). Our own calibration experiments performed in the field under various conditions are showing that the sampling rates range between 3 and 8 m^3 in the field conditions (Klanova et al., 2006c) probably due to the fluctuations of the wind speed, temperature, irradiation or humidity. This means that using a flat sampling rate to derive the atmospheric concentrations from the amounts of POPs sequestered in the filter can cause a significant error. Since no performance reference compounds indicating the sampling rates on the individual sites were applied, the amounts of POPs found in polyurethane foam filters on various sampling sites after 28-day exposure are reported bellow. Data given in the following tables represent the amounts of PCBs determined in the samples from the first exposure period (between 7/14/2004 and 8/11/2004).

The highest amount of PCBs captured in the PUF filter in 28 days was 6 μg for the sum of 7 indicator congeners (2 μg for the individual congeners) in Zastava factory in Kragujevac, Serbia, while the same sum only reached hundreds of nanograms in other PCB contaminated sites (Zadar, Tuzla), and stayed in the range of tens of nanograms in the residential areas (Tables 1–3). This corresponds to the results of the

Table 2
Amounts of PCBs sequestered in the polyurethane foam filters in Bosnia and Herzegovina (ng filter-1)

Country	Bosnia & Herzegovina									
Sample	PAS [a]									
Sampling site	Sarajevo ST	Sarajevo HMI	Sarajevo VW	Sarajevo VL	Sarajevo IS	Tuzla HO	Tuzla FI	TuzlaMI	TuzlaME	Tuzla BU
PCB 28	10.9	9.4	6.7	2.9	2.3	57.6	13.8	2.9	2.1	10.2
PCB 52	5.4	7.4	4.5	4.9	3.4	26.4	14.4	5.2	5.7	7.0
PCB 101	2.0	2.5	3.2	3.3	1.5	10.7	16.0	1.5	1.8	2.3
PCB 118	0.7	0.8	0.8	1.2	0.6	2.7	3.4	0.3	0.3	0.3
PCB 153	2.7	1.6	3.8	8.3	2.1	2.3	9.9	1.3	1.6	2.5
PCB 138	2.0	1.1	2.6	5.0	1.3	1.6	6.9	0.8	1.4	1.0
PCB 180	0.6	0.4	0.8	1.9	0.6	0.3	1.4	0.3	0.5	0.4
Total PCB	24.3	23.0	22.4	27.5	11.8	101.6	65.8	12.3	13.4	23.7

[a] Passive air sample.

Table 3
Amounts of PCBs sequestered in the polyurethane foam filters in Serbia and Kosovo (ng filter^{-1})

Country	Serbia											
Sample	PAS [a]											
Sampling site	Kragujevac ZG	Kragujevac ZF	Kragujevac UK	Pancevo NIS	Pancevo PE	Pancevo CH	Novi Sad RAF	Novi Sad SAN	Novi Sad SPO	Novi Sad FG	Kosovo HMI	Kosovo PP
PCB 28	175.2	87.2	3.9	8.4	2.0	5.9	6.4	6.8	6.5	3.4	6.7	7.2
PCB 52	1931.0	43.6	13.5	8.5	10.2	6.0	7.3	6.6	6.7	3.3	5.7	7.6
PCB 101	2290.4	58.6	22.3	11.9	18.2	1.0	6.6	3.9	4.1	2.0	2.7	15.3
PCB 118	789.6	36.9	11.7	5.6	7.8	2.4	4.0	4.1	0.8	1.0	1.1	3.0
PCB 153	517.2	44.2	7.9	9.5	5.3	3.4	3.1	3.2	1.7	1.9	1.4	16.9
PCB 138	548.6	50.4	9.3	9.0	5.6	3.9	4.2	3.9	1.4	1.8	1.4	11.3
PCB 180	84.0	38.1	1.5	1.9	1.1	0.7	0.6	0.8	0.6	1.1	0.4	6.6
Total PCB	6336.0	359.0	70.1	54.8	50.2	23.3	32.2	29.3	21.8	14.5	19.4	67.9

[a] Passive air sample.

active air sampling when the air concentrations in Zastava were as high as 40 ng m^{-3}, but the levels in other industrial objects and the storage places were bellow 10 ng m^{-3}, and the concentrations in residential and background areas never exceeded 200 pg m^{-3} (Klanova et al., 2007).

A generally decreasing trend in the levels of PCBs in the atmosphere corresponding with a decrease of the average daily temperatures in this region between July and December was observed in all sampling sites indicating enhanced evaporation of chlorinated compounds from the secondary sources during the warm season (Fig. 2).

The top layer soil samples were collected near all passive air sampling sites and the range of PCB concentrations (7 congeners) between 1 ng g^{-1} and 3 μg g^{-1} was determined. A correlation between both the PCB levels in the atmosphere and in the soil surface (Fig. 3), and between the congener distribution in the air and soil (Fig. 4), is obvious. Correlations between the passive air samples and the corresponding soil levels of PCBs were tested using the non-parametric Spearman test (Statistica for Windows 6). A strong correlation was found between the ambient air and soil data for the sum of indicator congeners (0.83). Majority of the sites with the sum of 7 PCBs in the PAS filter bellow or around 30 ng had the soil concentration between 1 ng g^{-1} and 10 ng g^{-1} indicating that soil is not a main source of PCBs in the atmosphere. In the sites where the amount of PCBs in the filter reached several hundreds of ng, concentrations in hundreds of ng g^{-1} were also found in the soil showing that evaporation from contaminated soils adds to the total atmospheric pollution (Trafostation Zadar: 382 ng filter^{-1}, 242 ng g^{-1} in soil; FNP Kragujevac: 359 ng filter^{-1}, 197 ng g^{-1} in soil). However there were two places with a high PCB content in the soil (Zastava Kragujevac: 1292 ng g^{-1}, Gorica: 3085 ng g^{-1}) and very different result of the air sampling (Zastava Kragujevac: 6336 ng filter^{-1}, Gorica: 8 ng filter^{-1}) (Fig. 5).

OCP levels were determined in all air and soil samples as well. The concentrations in soils were bellow 1 ng g^{-1} for HCHs and between 1 and 60 ng g^{-1} for DDTs with maxima in Zastava Kragujevac and Elektrodistribucia Tuzla. The amounts sequestered in the PAS filters remained bellow 20 ng per filter for both HCHs and DDTs in all places except for Zastava Kragujevac, where the levels reached 80 ng for HCHs and 100 ng for DDTs. It means the atmospheric concentrations in Zastava factory were almost an order of the magnitude higher than on the other sites.

The PAH concentrations in the soils varied between 35 ng g^{-1} and 8 μg g^{-1} with the maxima in industrial centers — in Zastava Kragujevac, Zadar, and Sarajevo the levels were above 2 μg g^{-1}, in

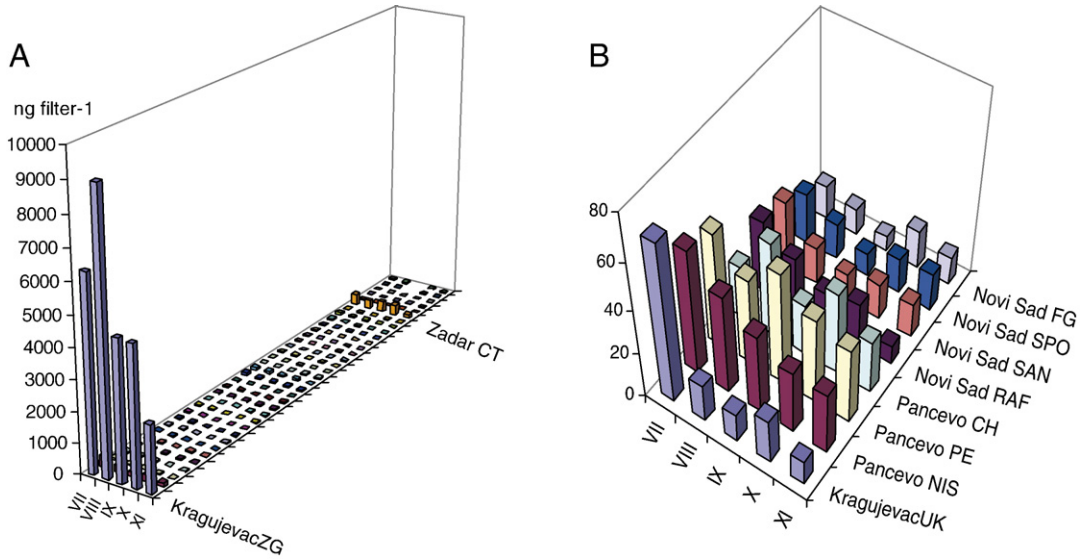

Fig. 2. Decreasing trend in the amount of PCBs in the atmosphere (ng filter^{-1}) on various sampling sites from July to December. (A) all sampling sites, (B) sampling sites in Serbia excluding the most contaminated sites in Zastava Kragujevac.

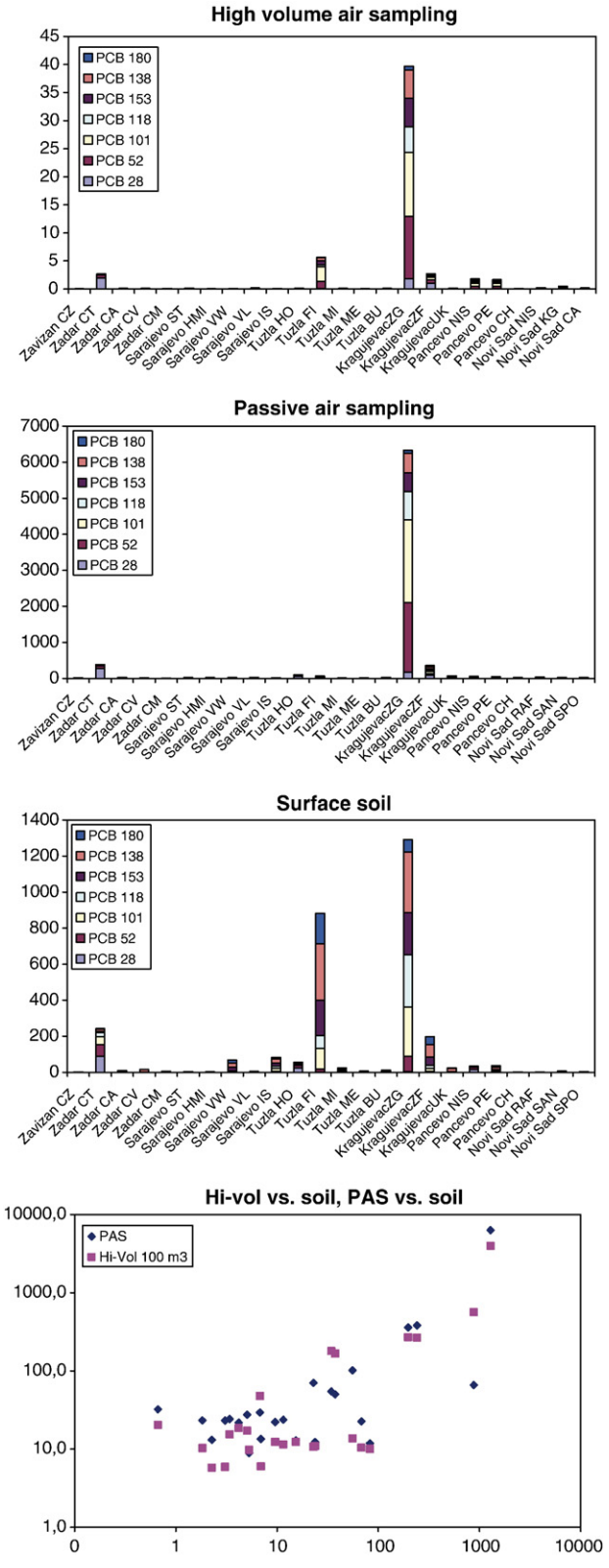

Fig. 3. Corresponding PCB concentrations in the ambient air (ng m^{-3}), PAS (ng $filter^{-1}$), and the soils (ng g^{-1}) for all sampling sites. PAS (ng $filter^{-1}$) vs. soil (ng g^{-1}) and hi-vol (ng 100 m^{-3}), vs. soil (ng g^{-1}) correlations are presented bellow.

Kragujevac University or Zagreb IMI above 6 μg g^{-1}. Summer air maxima never exceeded 10 μg per filter even in the industrial cities (Zagreb, Poljana, Tuzla, Pancevo, Kragujevac) while the winter maxima went as high as 140 μg per filter in Tuzla. The winter levels doubled in Kragujevac or Pancevo when compared with those of summer but they increased more than twenty-fold between summer and winter in Tuzla. Whole Tuzla region has the PAH levels about one order of the magnitude higher than all the other sampling sites and the pollution sources as well as toxicological risks were assessed in independent studies and published separately (Skarek et al., in press).

4. Discussion

Development of passive air sampling devices capable of being deployed in many locations at the same time opens new possibilities not only for the large scale but also for regional monitoring projects (Klanova et al., 2006b). Since this technique offers information about a long-term contamination of selected sites, it also becomes a very suitable tool for the evaluation of the spatial and temporal variations and trends of the atmospheric concentrations of POPs. Here the passive samplers were successfully applied on the regional level as a screening method for the comparison of the atmospheric contamination of various sites in the Western Balkan affected by war accidents.

The study revealed a very good agreement between the results obtained from the initial high volume air sampling campaigns performed in Croatia, Serbia, and Bosnia and Herzegovina in 2003–2004 and from the passive air sampling campaign. Both the range of concentrations and the congener distribution derived from two techniques corresponded very well. While the air concentrations in Serbia determined with a high volume sampler varied from 100 pg m^{-3} to 40 ng m^{-3} for the sum of 7 indicator PCBs, the amount of PCBs in a PAS filter ranged between 20 and 6000 ng per filter which indicates the average sampling rate around 7 m^3/day.

Screening of the surface soils drew our attention to several heavily polluted sites and it confirmed that the evaporation from contaminated surfaces is still an important source of elevated PCB concentrations in the atmosphere. However, there are sites where the contaminated soil is not fully responsible for the high levels of PCBs in the atmosphere. The soil concentrations of PCBs in Zastava factory are similar to the other sites with damaged capacitors (about 1 μg g^{-1}) but the air concentrations are an order of the magnitude higher. This is probably due to the evaporation from the primary source — a Pyralene filled transformer still operational in this facility. Several serious hotspots deserving further investigation were found during the soil survey. In destroyed factories and storage facilities in Banja Luka, Bosnia, the concentrations as high as 3800 μg g^{-1} were found in the soils, for instance, threatening to contaminate the aquatic environment. A detailed soil and sediment survey as well as the application of PAS for the air monitoring were suggested and both are currently in progress.

A concentration range of PCBs found in the Balkan region was compared to 18 sampling sites in the Czech Republic as a

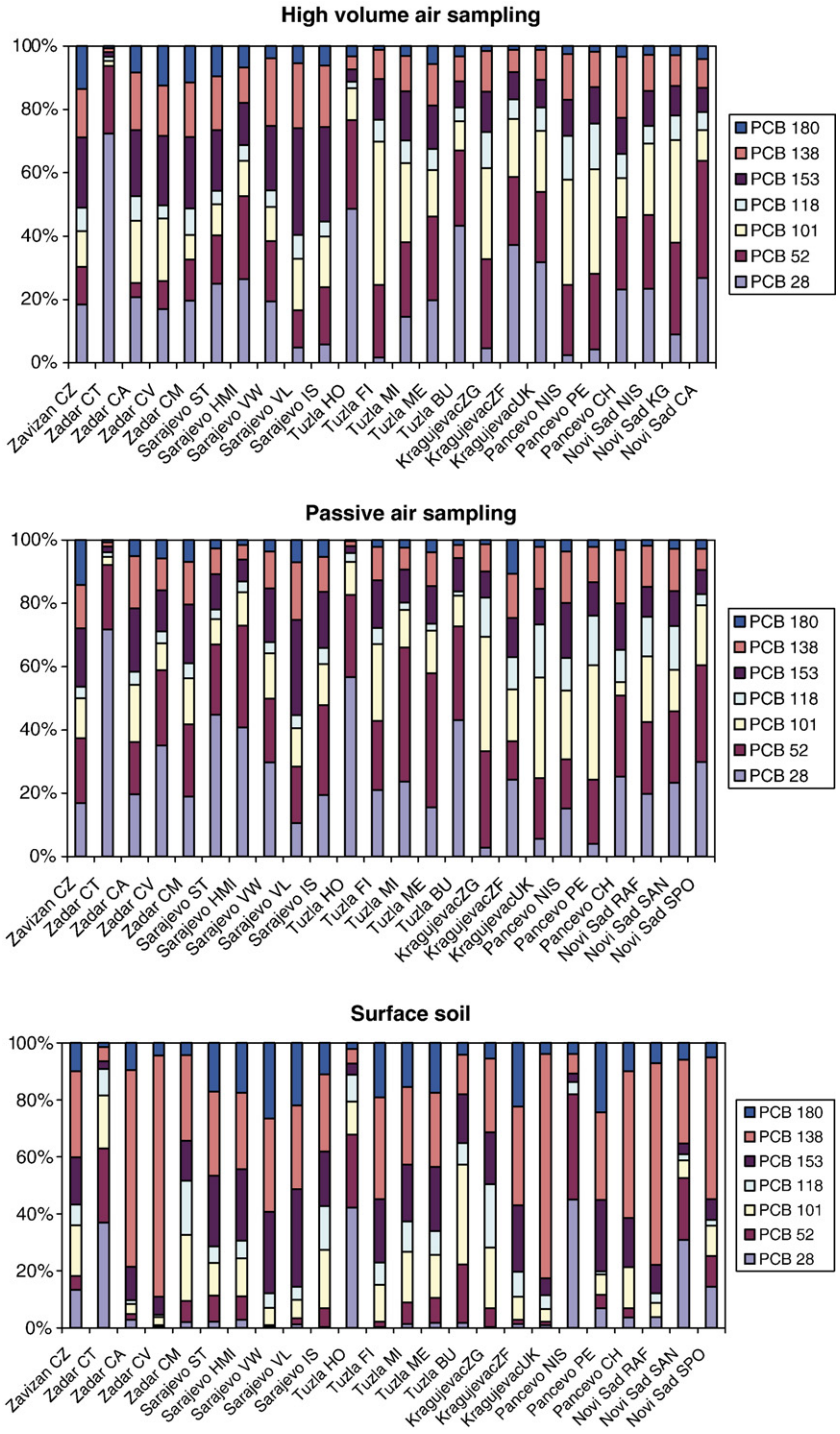

Fig. 4. PCB congener pattern in the ambient air, PAS, and the soil for all sampling sites.

country of reference. In the Czech Republic, the average amount of PCBs in a PUF filter of the passive sampler was 50–130 ng for the sum of 7 indicator congeners for the industrial sites, 10–40 ng for the residential and rural sites, and 7 ng for the background observatory which means the PCB levels in residential and background areas were comparable in both countries while the concentrations in the areas with a war damage were about two orders of the magnitude higher than in any industrial area of the reference region.

The human health risks were quantified for the basic exposure scenario (Fig. 6). The incremental probability of an individual developing a cancer over a lifetime (*IECR)* was computed according to the US EPA methodology (EPA, 1989) upgraded for the new reference values (Cupr et al., 2005). For an inhalation of evaporated congeners, the ambient air PCB concentrations can be associated with the specified risk levels (upper-bound inhalation unit risk: 1×10^{-4} [ug m^{-3}]$^{-1}$). The risks in the range of *IECR* from 1E−06 to 1E−04 have been

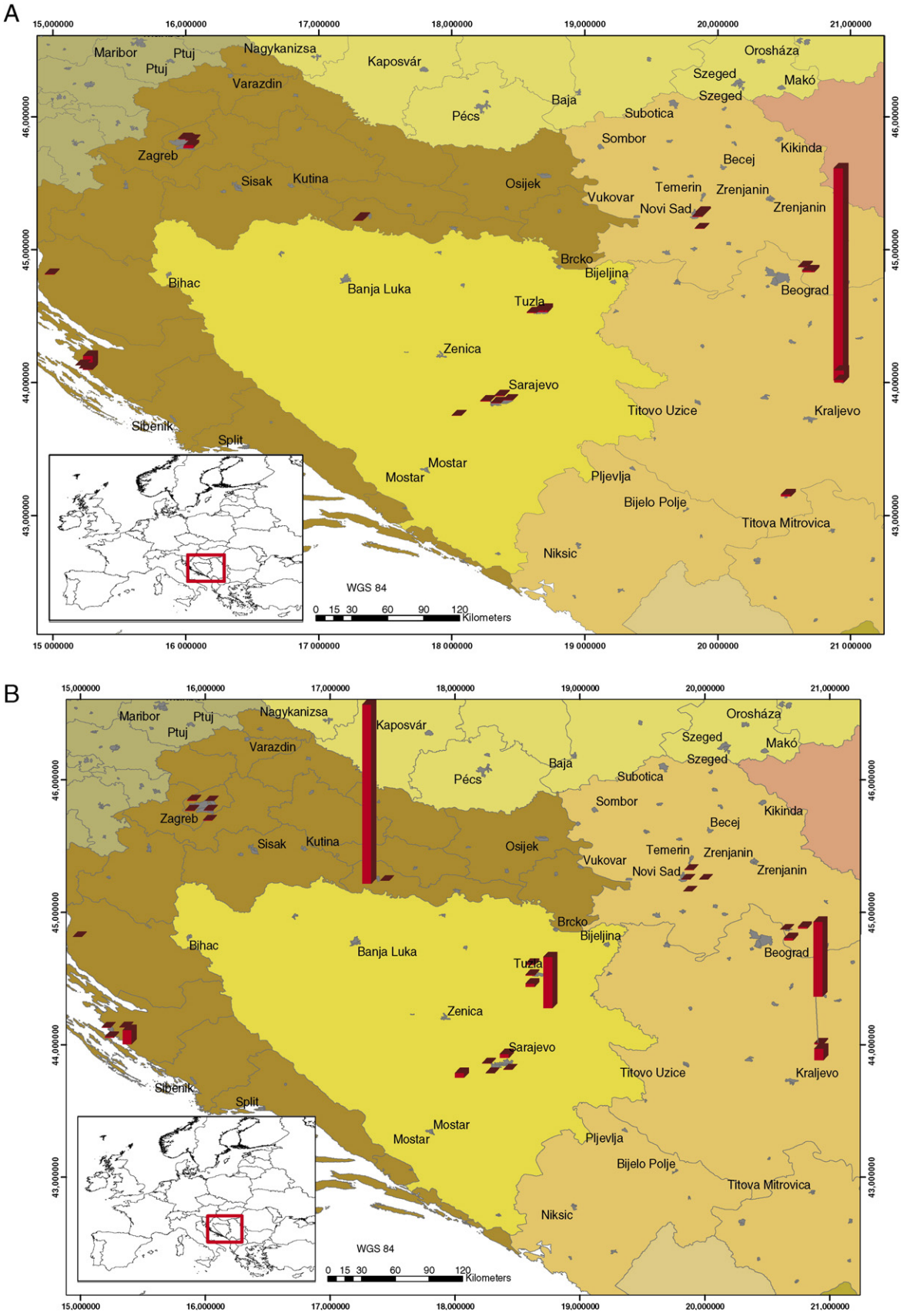

Fig. 5. Atmospheric (A) and soil (B) contamination of the Western Balkan countries with PCBs. Highest bar represents the amount of 6336 ng filter^{-1} (A) or the concentration of 3085 ng g^{-1}(B).

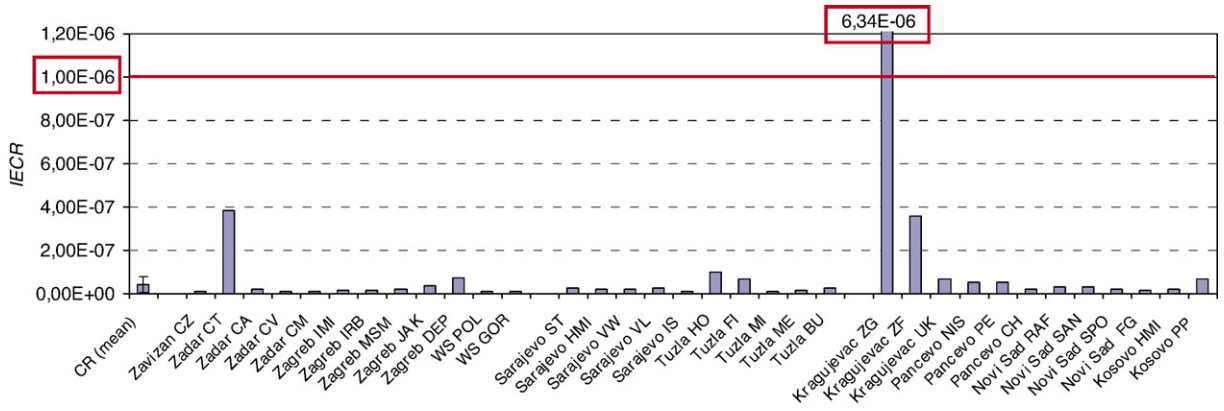

Fig. 6. Summary of risk values *IECR* (incremental probability of an individual developing a cancer over a lifetime) for basic inhalation scenario (exposure duration ED = 70 years, exposure frequency EF = 365 days per year, inhalation rate IR = 20 m^3 day^{-1}, exposure time ET = 24 h per day) based on the estimated concentrations of PCBs determined in the PAS samples from the first exposure period (7/14/2004–8/11/2004) (sampling rate 100 m^3/28 days). Red line — acceptable IECR = 1.00E−06.

typically judged to be acceptable by EU and US EPA. This level (IECR = 1E−06) was exceeded only in the Zastava factory in Kragujevac.

There were no hot spots of OCP contamination detected in the investigated areas. With the exception of Zastava in Kragujevac where the levels of HCHs and DDTs in the atmosphere were significantly higher, the soil and air concentrations on other sampling sites were similar to the Central Europe.

A contamination of the atmosphere with PAHs is apparently not connected with the war but with the existing industrial activities and especially with the local heating and incineration processes. Very pronounced seasonal trend was observed. The range of concentrations on most sampling sites resembled the one in the reference region; the PAH levels in the Tuzla area were however significantly higher and they are the subject of further investigation.

The passive air samplers proved to be a powerful technique capable of detecting the POP concentrations ranging over four orders of the magnitude providing the information very comparable to the conventional techniques for the fraction of the price. Beside the costs, an integrative character of the sample and the feasibility of obtaining the temporally resolved data are the main advantages of this method. It is necessary to consider a possible uncertainty caused by the variations in the sampling rates in the field conditions when interpreting the results. However it is encouraging that even in the simplest design without the application of performance reference compounds the error is limited to the factor of 3. This is very acceptable for a preliminary screening of the regions with no monitoring data.

The Stockholm Convention on Persistent Organic Pollutants defines the problems of the effectiveness evaluation of the Convention measures and parties to the Convention are required to demonstrate how the obligations of the Convention will be implemented. Therefore they need to establish arrangements to provide themselves with comparable monitoring data on the presence of the chemicals listed in the Annexes and their regional and global environmental transport. Several countries involved in this study are currently working on development of such a monitoring system however it is understood that it will take several years before it is fully operational. The APOPS-BAL project not only provided a suitable set of information for ongoing POPs inventories in these countries; it also evaluated the potential of the passive air sampling technique in the current effort to establish an appropriate monitoring capacity in the area.

Acknowledgments

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Příloha 7

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A combined approach to the evaluation of organic air pollution — A case study of urban air in Sarajevo and Tuzla (Bosnia and Herzegovina)

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Abstract

Organic pollution is a complex mixture where besides usually discussed polycyclic aromatic hydrocarbons (PAHs) a lot of other toxic or potentially toxic compounds occur. In this case, the organic air pollution in two important industrial cities, Sarajevo and Tuzla, in Bosnia and Herzegovina (part of former Yugoslavia) was assessed with the emphasis placed on genotoxic risks using both chemical (PAHs analyses) and biological approaches (genotoxicity testing with a screening bacterial genotoxicity test — SOS chromotest). The study was performed as a part of the APOPSBAL project (ICA2-CT2002-10007). So far there has not been any information either about the PAHs pollution or the genotoxic activity of the organic air pollution for the localities under the study. Therefore, the presented information is considered absolutely unique. Both used approaches made possible to identify the localities with the highest pollution level and genotoxic risks in both cities. Generally, higher levels of both parameters were determined in Tuzla, which is much more industrialized than Sarajevo, and especially at localities close to city centers and affected by traffic emissions, but also at localities polluted by emissions from industry and household heating. Even if benzo(a)pyrene concentrations exceeded the maximum permitted levels for this pollutant at some localities in Tuzla, the PAHs concentrations were fully comparable with the levels determined in other industrial European cities. Significant genotoxicity of the organic extracts was detected for almost all of the urban localities in the test both without (−S9; direct genotoxicity) and with the addition of metabolic activation (+S9; indirect genotoxicity). The observed direct genotoxic activities were discussed in relation to a potential presence of PAHs derivatives in the air. The indirect genotoxic activities were apparently higher at the localities with higher contents of carcinogenic PAHs. The significant relationship between the determined genotoxic activities and the PAHs pollution was also confirmed by a regression analysis. However, the correlations were not absolute because the observed genotoxic activity was also dependent on the presence of other organic pollutants than the PAHs. It concerns predominantly direct genotoxicity which is not related with the PAHs, but with their nitro-, oxi-, and hydroxy-derivatives and also other unknown polar organic pollutants. However, the concentrations of the direct genotoxins apparently correlated with the PAHs contents in the air. The study showed that screening genotoxicity tests, such as the SOS chromotest, could be effectively used for the identification of localities with increased genotoxic risks. In comparison with the health risk assessment which is usually based on the chemical analyses of only a small part of the pollution mixture, the bioassays enable us to evaluate the risks of all the mixture. The localities with the highest detected human health risks according to the screening bioassays may then be analyzed in detail with specific chemical methods to identify their causes.

Keywords: Air pollution; Urban air; PAHs; Genotoxicity; Bioassay

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1. Introduction

Adverse effects of air pollution on human health and a close relationship between the levels of air pollution and increased frequencies of certain diseases (e.g. acute respiratory infections, chronic respiratory diseases, asthma, bronchitis, cardiovascular diseases and cancer) have been proved by numerous epidemiological studies (Dockery et al., 1993; Pope et al., 1995; Abbey et al., 1999; Hoek et al., 2002). The increased risks were observed mainly for the population exposed to urban air which is affected predominantly by traffic emissions, emissions from household heating and industries.

Air pollution is a very complex mixture consisting of hundreds of different inorganic and organic compounds. Regarding health effects importance is attached to the organic part of the mixture and mainly to polycyclic aromatic hydrocarbons (PAHs) and their derivatives. Some of them and their environmental mixtures show strong mutagenicity and carcinogenicity (Møller et al., 1985; Nardini and Clonfero, 1992; Černá et al., 2000). According to IARC (1983) the PAHs are the main cause of the genotoxic activity of the urban air.

The PAHs, emitted into the air especially as a result of combustion of fossil fuels, occur in the air both in gas and particulate phases (Kamens et al., 1995). The adverse health effects are related mainly to PAHs of high molecular weight (benzo(a)fluoranthene, benzo(a)pyrene, dibenz(a,h)anthracene etc.) absorbed on particles, but some toxic effects may be also shown by PAHs of low molecular weight (naphthalene, anthracene, phenanthrene etc.) present in the gas phase. Moreover, as proved, these low molecular weight compounds have a potency to increase the genotoxic activity of the PAHs of high molecular weight (Hass et al., 1981). Therefore, it is necessary to analyze joined samples of both the particulate matter and gas phase. Besides the parental PAHs, growing attention is also pointed to their derivatives (i.e. nitro-, hydroxy-, oxi-PAHs). They may come from the same sources as the parental PAHs, but a large amount of them may also result from reactions of the PAHs with other pollutants in fumes and in the ambient air (NO_x, O_3, OH), or from transformation reactions under irradiation (Nielsen, 1984; Nielsen et al., 1984; Finlayson-Pitts and Pitts, 2000). While the PAHs require metabolic activation to show their genotoxic effects (Legator and Au, 1994), the PAHs derivatives may show direct genotoxicity in bacterial genotoxicity tests. In addition, some of these reaction products show even higher toxicity than the starting materials (Rosenkranz and Mermelstein, 1983; Nardini and Clonfero, 1992; Finlayson-Pitts and Pitts, 2000; Feilberg et al., 2002).

The level of organic air pollution is generally evaluated based on results of chemical analyses that enable us a qualitative and quantitative evaluation of the pollution based on only a limited number of compounds. Detailed analyses of a larger spectrum of pollutants are very expensive and time-demanding. However, human health may also be affected by other pollutants than the ones generally monitored. Alternatively, air pollution may be evaluated by the quantification of various biological effects (toxic effects) of the pollution using screening bioassays. One advantage of this approach is the possibility to evaluate air pollution as the complex mixture that is or at least close to its original state and, especially, in relation to the identification of human health risks. Even if the bioassays do not make it possible to determine the exact composition of the pollution mixture, they are able to provide quite exact information about its toxic effects. The samples or localities with the highest found toxic potencies may consequently be analyzed using chemical methods to identify possible causes of the found toxicity. Therefore, there are efforts to find suitable screening tools based on bioassays that are simple, cheap, quick and sensitive to an objective group of pollutants. Genotoxicity dominates the concern about toxic effects of air pollution. For the analyses of air pollution the well-known bacterial screening test on *Salmonella typhimurium* (Ames test) is used exclusively (Černá et al., 1999, 2000; Feilberg et al., 2002; Cigánek et al., 2004 etc.). On the contrary, other screening genotoxicity tests like the SOS chromotest are seldom used for this purpose (Courtois et al., 1988; Schleibinger et al., 1989).

In this paper, the usage of both approaches, chemical analyses and toxicity testing, for the evaluation of organic air pollution and its genotoxic activity is demonstrated. The analyses of PAHs contents in organic extracts from air samples collected in two important industrial cities of Bosnia and Herzegovina (part of former Yugoslavia), Sarajevo and Tuzla, and analyses of the genotoxic activity, using the SOS chromotest, were performed. Besides the evaluation of air pollution at the urban localities, both approaches were compared to confirm the practicability of the SOS chromotest for the evaluation of air pollution and identification of localities with increased health risks.

2. Materials and methods

2.1. Air sampling

Sarajevo and Tuzla (which lies approximately 75 km north of Sarajevo) are important industrial cities of

Bosnia and Herzegovina. While in Sarajevo there are mainly light industries, Tuzla is characterized by a high concentration of heavy industries (heavy engineering, chemical industry) and mining. In both cities dispersion conditions for air pollution are affected by the fact that they are situated in basins with west-eastern orientation. Ten sampling sites covering both urban and reference background localities were chosen for the study. The localities are described in Table 1a and b, and their positions are visualized in maps in Fig. 1a and b. Five 24-hour samples of total suspended particles (TSP) and organic compounds in the gas phase were collected during 5 consecutive days at the localities. The samples were collected using a high volume sampler PS-1 (Graseby-Andersen, USA) equipped with a tandem of a glass fiber filter (GF), where TSP was collected, and polyurethane filter (PUF), where organic compounds in the gas phase were collected. Sampling volumes were 200–400 m^3 of air per day. All the samplings were performed during May 2004 as a part of the EU project

Table 1
Description of the sampling localities in Sarajevo area (a) and Tuzla area (b)

Identification	Position (WGS 84)	Characteristics
a. Sarajevo area		
L1	N 43°50′41.0″ E 18°19′50.1″ 509 m above sea level	Standard Plant — *industrial area about 5 km to the west of the city center; the sampling site was located in the area of the plant nearby the main thoroughfare.*
L2	N 43°52′04.0″ E 18°25′22.3″ 645 m above sea level	Hydrometeorological Institute (12 Bardakčije street) — *residential area on a hillside above the city center; the sampling site was located in the area of the meteorological site surrounded by local routes and family houses.*
L3	N 43°53′59.6″ E 18°21′35.7″ 531 m above sea level	Volkswagen Vogošča Plant — *industrial area separated from the city center by a hill; the sampling site was located in the area of the plant close to a parking site without any heavy traffic.*
L4	N 43°51′28.0″ E 18°15′03.4″ 526 m above sea level	Tenax (Vlakovo) Plant — *marble-working plant; the sampling site was located in the area of the plant*
L5*	N 43°45′04.1″ E 18°02′10.6″ 972 m above sea level	Ivan Saddle (reference background locality for Sarajevo) — *background locality in the mountains about 20 km to the west of Sarajevo; sampling site was located in the area of a meteorological site; no impact of traffic and household heating emissions*
b. Tuzla area		
L6	N 44°31′48.4″ E 18°41′08.9″ 249 m above sea level	Tuzla Hotel (2 Vrapče street) — *residential area about 1 km to the east of the city center; the sampling site was located nearby the main thoroughfare*
L7	N 44°31′54.9″ E 18°39′18.3″ 247 m above sea level	Fireworks Station (N.H.M. Trifunoviča street) — the *sampling site was located in the area of the transformer sub-station Elektrodistribucia Tuzla close to the main thoroughfare*
L8	N 44°32′50.1″ E 18°39′55.8″ 253 m above sea level	Tušajn Salt Mine (104 N.H.Hasana Brkiča street) — *abandoned salt mine in a side valley close to the city center*
L9	N 44°31′25.2″ E 18°36′01.6″ 227 m above sea level	Tuzla - Bukinje Power Station — *residential and industrial area; the sampling site was located nearby the power station; the site was surrounded by local routes and family houses*
L10*	N 44°32′31.5″ E 18°41′06.6″ 313 m above sea level	Tuzla Meteorological Site (33 Trnovac street) (reference background locality for Tuzla) — *urban background in a residential zone above the city center the sampling site was located in the area of the meteorological site;. the site was surrounded by local routes and family houses*

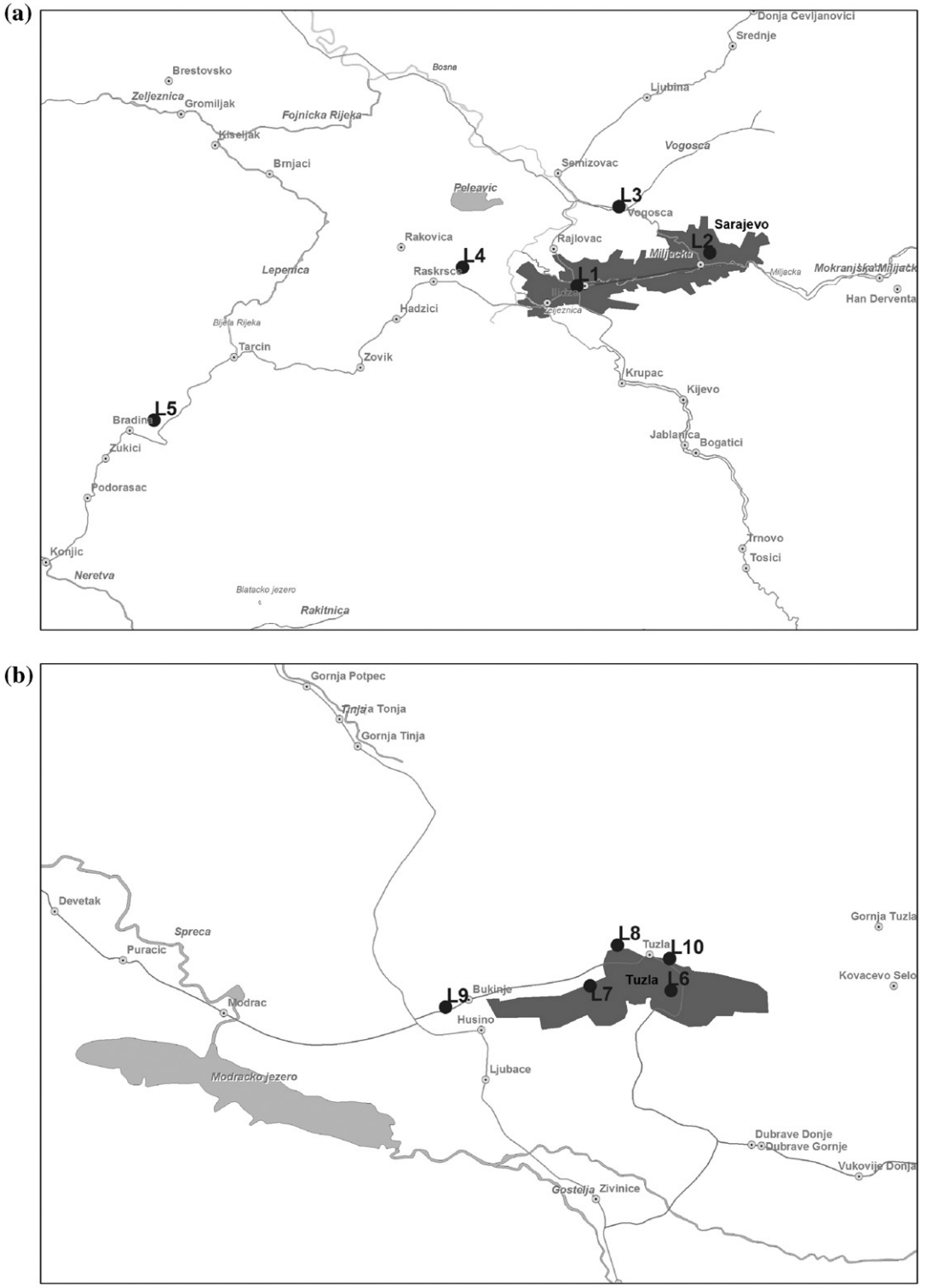

Fig. 1. Maps of Sarajevo area (a) and Tuzla area (b) with the marked sampling localities.

APOPSBAL (ICA2-CT2002-10007). The meteorological situation during the sampling was the following: Sarajevo — daily average temperatures from 9.2 °C to 11.6 °C; west-southwest wind with the daily average velocities from 1.1 m/s to 2.7 m/s; Tuzla — daily average temperatures from 9.4 °C to 13.8 °C; wind of variable directions with the daily average velocities from 0.4 m/s to 1.1 m/s.

2.2. Extraction and chemical analysis

All the samples were extracted with dichloromethane in a Soxhlet extractor (Büchi System B-811 automatic extractor). Surrogate recovery standards (D_8-naphthalene, D_{10}-phenanthrene, D_{12}-perylene) were spiked on each filter prior to the extraction. The extracts were then concentrated under a gentle stream of nitrogen at ambient temperature and divided into one part for the chemical analysis and another part for the genotoxicity testing. The fractionation achieved on a silica gel column (30 cm length, 1 cm i.d.) was then used for the chemical analysis of the contents of 16 priority PAHs according to U.S. EPA (2004). The analysis was performed using a GC-MS system (HP 6890–HP 5972) equipped with an autosampler and J&W Scientific fused silica column DB-5MS (60 m × 0.25 mm, 0.25 μm film thickness) coated with (5%-phenyl)-methypolysiloxane. Samples (1 μl) were injected at 80 °C oven temperature. After 2 min, the temperature was raised at 15 °C/min to 180 °C, then at 5 °C/min to 310 °C, and the final temperature was held for 20 min. The carrier gas was helium at a flow of 0.2 ml/min. The mass spectra were collected in the scan range of 550 m/z for identification purposes. Terphenyl was used as an internal standard. The standard solution PROMOCHEM PAH mix 27 was used for calibration. One laboratory blank and one reference material were analyzed with each set of the samples.

For the genotoxicity testing the individual extracts within one locality were pooled to obtain samples of sufficient amount for each locality. The extracts of GF and PUF were pooled, too. Then the samples were transferred into dimethylsulphoxide (DMSO) and stock sample solutions were adjusted to the final concentration of 2000 m^3/ml.

2.3. Quality assurance/quality control

The regression coefficient (R^2) for calibration curves of individual PAHs ranged from 0.995 to 1.0. The limit of detection (LOD) of 0.8 pg/m^3 and the limit of quantitation (LOQ) of 2.5 pg/m^3 were achieved for all the analytes. The variability of the determination was less than 2%. Recoveries were determined for all samples by spiking with the surrogate standards prior to the extraction. The amounts were similar to the detected quantities of the analytes in the samples. The recoveries were higher than 78% and thus any recovery factors were not applied to any of the data. The recovery of native analytes measured for the reference material varied from 72% to 102% for PAHs. The laboratory blanks were very low. Field blanks consisting of pre-extracted filters were taken on each sampling site. They were extracted and analyzed in the same way as the samples, and the level of PAHs in the field blanks never exceeded 3% of the quantities detected in the samples, which indicates a minimal contamination during transport, storage and analysis.

2.4. SOS chromotest

The genotoxic activity (potency) of the samples was determined using a microplate version of the SOS chromotest with *Escherichia coli* PQ 37 as a bacterial test strain (Quillardet and Hofnung, 1985). The test was performed using a procedure based on Xu et al. (1989). The samples were tested both without and with the addition of the metabolic activation (−S9/+S9). The final concentrations of the tested samples corresponded to 20, 10, 5, 2.5 m^3/ml and they were tested in triplicates. An overnight culture of the test strain, cultivated in LB-medium supplemented with ampicillin (20 μg.ml^{-1}) at 37 °C, was diluted 50-fold with the fresh medium. After further incubation (2 h), its absorbance was adjusted to 0.04 at 600 nm with the fresh medium and then a test mixture was prepared. In the case of the test −S9, the inoculum was mixed with a phosphate buffer in a ratio of 3:1. In the case of the test +S9, the inoculum was mixed with S9 mixture (0.5 ml of $MgCl_2$–KCl solution + 0.125 ml of 1 M glucose-6-phosphate + 1.0 ml of 0.1 M NADP + 12.5 ml of phosphate buffer + 8.875 ml of sterile water + 2.0 ml of S9 fraction) in a ratio of 3:1. The prepared test mixture was pipetted per 990 μl into Eppendorf tubes (1.5 ml) with 10 μl of the sample. In a negative control, the sample was replaced with 10 μl of DMSO. In a positive control, the sample was replaced with a solution of 4-nitroquinoline-N-oxide (4-NQO) (test −S9) or 2-aminoanthracene (2-AA) (test +S9). After a 2-hour incubation at 37 °C, 25 μl of the content of each tube was transferred into wells of one 96-well microplate filled with a P-buffer with p-nitrophenylphosphate (PNPP) (cytotoxicity test) and one 96-well microplate filled with a B-buffer with o-nitrophenyl-β-D-

galactopyranoside (ONPG) (genotoxicity test). Then the activities of alkaline phosphatase and β-galactosidase for each of the tested sample concentrations were quantified with the microplate reader GENIOS (Tecan, Mannedorf, Switzerland) at 420 nm. Using the obtained absorbances, Induction Factors (IFs) were calculated for each of the tested concentrations. The IF shows by how much the response of the detection system is induced by the tested sample in comparison to the negative control, adjusted to the sample cytotoxicity. The IF is valid if the tested concentration of the sample does not show any significant cytotoxicity checked by an alkaline phosphatase activity. The G-factor, the alkaline phosphatase activity ratio between the tested sample and the negative control, must not be less than 0.5. The genotoxic potency of the tested samples was quantified based on the maximum obtained IF with the tested concentrations of samples (Maximum Induction Factor, MIF) and the estimation of the so-called SOS Induction Potency (SOSIP). Those samples showing the dose–response relationship and achieving a MIF higher than 1.5 were classified as genotoxic. Those samples that show the dose–response relationship and their MIF is not higher than 1.5 were classified as probably genotoxic. The samples without any observed effects were classified as non-genotoxic (Quillardet and Hofnung, 1985). The SOSIP, in units per m^3, is in fact a slope factor of the relationship between induced β-galactosidase activity, adjusted to the sample cytotoxicity, and the sample concentration (Quillardet and Hofnung, 1985). The slope factor was estimated from the dose-response curve using a simple linear regression ($R^2 > 0.9$). The β-galactosidase activity was calculated according to the following formula:

$$\beta\text{-galactosidase activity} = \frac{1}{I} \times \frac{\lfloor \Delta A_g(\text{sample}) - \Delta A_g(\text{NC}) \rfloor}{\Delta t} \times 1000$$

$$\text{Inhibition}(I) = \frac{\Delta A_p(\text{sample})}{\Delta A_p(\text{NC})}.$$

Here ΔA_q (sample) corresponds to the β-galactosidase activity induced by the sample solution (an increase in absorbance at 420 nm), ΔA_q (NC) corresponds to the β-galactosidase activity detected in the negative control and Δt corresponds to the time interval between two measurements. The parameter I (inhibition) correcting the cytotoxicity of the tested sample is a ratio of the alkaline phosphatase activity detected for the sample solution (ΔA_p (sample)) and the alkaline phosphatase activity detected for the negative control (ΔA_p (NC)), determined within the time interval Δt.

All the chemicals used for the extraction of the exposed filters, chemical analyses and genotoxicity testing of the samples were at least of *analytical grade* quality.

3. Results and discussion

Samples of the organic air pollution were collected in Sarajevo and Tuzla areas as a part of the APOPSBAL project (APOPSBAL, 2005). From the group of various organic pollutants in the air, an analysis of 16 priority PAHs according to U.S. EPA was performed using the GC-MS system. Average concentrations of the PAHs, sums of 16 PAHs, sums of 8 carcinogenic PAHs (benz(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene) determined during the sampling periods at the localities are presented in Table 2. In both areas the PAHs levels at the urban localities were much higher than the ones at the corresponding reference background localities (L5∗, L10∗). Generally, a much higher level of PAHs pollution was determined in Tuzla than in Sarajevo. The sum of 16 PAHs at the Tuzla urban localities ranged from 70 ng/m^3 to 121 ng/m^3 while in Sarajevo it ranged from 22 ng/m^3 to 50 ng/m^3. The lowest sum from all the localities was detected at the locality L5* (15 ng/m^3). The sum determined at the reference background locality for Tuzla (L10*) (58 ng/m^3) was higher than the ones detected at all the urban localities in Sarajevo. The highest sums from all the localities were observed at the localities L7 (121 ng/m^3) and L6 (100 ng/m^3). In Sarajevo, the highest sums were observed at the localities L4 (50 ng/m^3), L1 (47 ng/m^3) and L2 (44 ng/m^3). The sums of 8 carcinogenic PAHs at the urban localities in Tuzla ranged from 12 ng/m^3 to 22 ng/m^3 and, in Sarajevo, from 3.9 ng/m^3 to 5.9 ng/m^3. Even if the sums of the carcinogenic PAHs at the reference background localities were lower, the sum detected at the background locality L10∗ (8.4 ng/m^3) was again higher than the ones at the urban localities in Sarajevo. The highest sums of the carcinogenic PAHs were detected at the localities with the highest sums of 16 PAHs but they did not fully correlate. The highest sums of the carcinogenic PAHs were observed at the localities L7 (22 ng/m^3) and L6 (19 ng/m^3). In Sarajevo, the highest sums of the carcinogenic PAHs were observed at the localities L2 (5.9 ng/m^3), L4 (5.4 ng/m^3) and L1 (4.6 ng/m^3). The concentrations of benzo(a)pyrene (BaP), an indicator of carcinogenic risk of the PAHs (WHO, 1998), correlate with the levels of the carcinogenic PAHs. The BaP levels at the urban localities in Tuzla ranged from 1.8 ng/m^3 to 3.4 ng/m^3 and in Sarajevo from 0.48 ng/m^3 to 0.75 ng/m^3. The concentrations of BaP at the reference background localities were

lower, but the one determined at the locality L10∗ (1.2 ng/m^3) was again higher than all the concentrations determined at the urban localities in Sarajevo.

Some differences between the sums of 16 PAHs and the carcinogenic PAHs at the localities were observed. For example, even if the sum of 16 PAHs was higher at the locality L4 than at the locality L2, the sum of the carcinogenic PAHs was higher at the locality L2. The higher sums of 16 PAHs at the localities L4 and L1 than at the locality L2 resulted from significantly higher levels of some of the PAHs of low molecular weight at the localities (L1 — acenaphthylene, acenaphthene, fluorene; L4 — phenanthrene, anthracene, fluoranthene, pyrene). The same contrast also concerns the localities L8 and L9. This fact may be explained by different sources of the PAHs pollution at the localities. Besides it is also necessary to consider the positions and distances from the sources. Here, the wind direction plays an important role. The impact of different sources is also apparent from the calculated portions of the sums of carcinogenic PAHs and BaP contents on the sums of 16 PAHs presented in Table 2. For example, the urban locality in Sarajevo with the lowest sum of 16 PAHs was L3, but, on the other hand, this locality showed the highest portion of the carcinogenic PAHs (18%). On the contrary, the locality L4 with the highest sum of 16 PAHs in Sarajevo contained almost the lowest portion of the carcinogenic PAHs (11%). The portions of the carcinogenic PAHs were higher at almost all localities in Tuzla than at the ones in Sarajevo. In Tuzla, the portions of the carcinogenic PAHs were very similar at all localities (16–19%) while in Sarajevo the localities showed higher variability (10–18%). The portion of BaP reached about 3% at the urban localities in Tuzla while in Sarajevo its portion was lower (1–2%). The potential PAHs sources in the studied areas include predominantly traffic emissions, emissions from chemical industries, steelworks, an incineration plant and household heating. In Tuzla, they also cover mining and electricity production. Since the fingerprints of PAHs emissions from different sources usually overlap, it is impossible to identify specific sources of the pollution for individual localities even if e.g. ratios of individual PAHs (fluoranthene/pyrene, pyrene/benzo(a)pyrene or benzo(a)pyrene/benzo(g,h,i)perylene) in the air in relation to different PAHs sources (Holoubek, 1996) are used. Thus the identification of the potential sources is based only on local investigation. The background locality L5* in the mountains, 20 km to the west of Sarajevo, is not significantly affected by any air pollution sources, which was also confirmed by the very low PAHs level detected there. On the other hand,

Table 2
Average concentrations of 16 U.S. EPA priority PAHs in the air [ng/m^3] and portions of the sum of 8 carcinogenic PAHs and the concentration of BaP on the sum of 16 PAHs [%]

	Sarajevo area [ng/m^3]					Tuzla area [ng/m^3]				
Pollutants	L1	L2	L3	L4	L5 [a]	L6	L7	L8	L9	L10 [a]
Naphthalene	2.99	2.71	0.58	0.75	0.43	2.84	2.93	2.30	1.46	1.61
Acenaphtylene	4.04	2.83	1.33	1.66	0.70	9.79	11.84	5.67	2.52	3.79
Acenaphthene	0.70	0.33	0.26	0.26	0.16	0.79	1.07	0.60	0.41	0.53
Fluorene	5.96	3.95	2.48	5.14	2.16	11.77	17.06	9.39	8.41	7.98
Phenanthrene	17.95	15.73	8.34	20.83	6.77	33.37	38.98	26.53	26.52	22.29
Anthracene	1.55	1.83	0.64	2.39	0.50	4.87	5.87	3.53	3.37	2.38
Fluoranthene	5.08	5.33	2.56	7.43	1.95	9.48	10.84	7.46	8.02	6.04
Pyrene	4.45	5.18	1.99	6.60	1.46	8.49	10.28	6.28	6.53	5.11
Benz(a)anthracene	0.55	0.79	0.45	0.60	0.09	2.95	3.36	1.75	1.64	1.18
Chrysene	0.99	1.26	0.79	1.21	0.28	3.57	4.09	2.26	2.43	1.81
Benzo(b)fluoranthene	1.03	1.24	0.97	1.10	0.22	3.28	3.77	2.16	2.80	1.64
Benzo(k)fluoranthene	0.46	0.57	0.38	0.64	0.14	1.56	1.74	1.06	1.17	0.83
Benzo(a)pyrene	0.57	0.75	0.48	0.69	0.11	3.10	3.42	1.87	1.76	1.21
Indeno(1,2,3-cd)pyrene	0.41	0.56	0.35	0.49	0.08	2.22	2.69	1.45	1.22	0.82
Dibenz(a,h)anthracene	0.05	0.05	0.05	0.04	0.01	0.24	0.31	0.17	0.14	0.09
Benzo(g,h,i)perylene	0.55	0.73	0.43	0.60	0.10	2.05	2.99	1.31	1.21	0.85
Sum of 16 PAHs	47.31	43.83	22.08	50.44	15.15	100.36	121.24	73.80	69.61	58.17
Sum of 8 carcinogenic PAHs	4.61	5.94	3.90	5.38	1.02	18.96	22.37	12.04	12.38	8.44
Portion of 8 carcPAHs	10%	14%	18%	11%	7%	19%	18%	16%	18%	14%
Portion of BaP	1%	2%	2%	1%	1%	3%	3%	3%	3%	2%

Carcinogenic U.S. EPA priority PAHs: benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd) pyrene, dibenz(a,h)anthracene, benzo(g,h,i)perylene.

[a] Reference background locality.

the locality L2, where the highest level of the carcinogenic PAHs in Sarajevo was detected, lies in the east of the city center and close to the main thoroughfare. The locality L1, where the level of the carcinogenic PAHs was lower than at the locality L2, is also found close to the city center and affected by traffic emissions. Since the locality L1 is located in the east of the city center, the difference may result from the general wind direction during the sampling period. The high pollution level determined at the locality L4 is related to the local sources (traffic, marble-working plant and household heating). The high portion of the carcinogenic PAHs at this locality is probably caused by traffic. In Tuzla, the localities with the highest levels of PAHs (L6, L7) are also located close to the city center and affected by traffic emissions. The localities L8 and L10∗ are found to the north of the city center. Almost no air flow was determined in this area during the sampling period. The locality L8, close to the abandoned mine, was probably affected only by emissions from heavy trucks operating at the site. The background locality L10∗ is in fact an urban locality in the residential area. The PAH concentration at this locality corresponds to the generally increased level of organic pollution in the air of Tuzla. The local sources cover mainly traffic and household heating. The locality L9 lies in the western suburb of Tuzla where the air is affected more by emissions from traffic and household heating than from the power plant. To summarize, the localities with the highest concentrations of PAHs are located close to the city centers and/or are significantly affected by traffic emissions. There, high levels of the carcinogenic PAHs were observed, too. This is fully in compliance with the fact that traffic is considered the main source of PAHs, including the carcinogenic ones, in the air (Wild and Jones, 1995; WHO, 2000). As expected, the PAHs of high molecular weight were absorbed on the particulate matter while the PAHs of low molecular weight were predominantly in the gas phase. Fluoranthene, pyrene, benz(a)anthracene and chrysene were distributed between both phases (detailed data are not presented in the paper).

None of the analyzed compounds exceeded the maximum allowable limits according to AHEM (1986) or the risk-base concentrations (RBC) stated by U.S. EPA (2004) with the exception of average BaP concentrations at the localities in Tuzla, including the urban background locality L10*. It concerns the maximum allowable limit of 1 ng/m^3 for BaP according to AHEM. This limit is equivalent to the target limit of the average annual BaP concentration established according to the European directives 96/62/EC and 2004/107/EC which must be met by the year 2010. The RBC of 2.02 ng/m^3 for BaP, equal to the stated non-significant risk level, was exceeded at the localities L6 and L7. But generally the determined levels of PAHs in the air fully correspond with the levels determined in the urban air elsewhere and published in literature (Lin et al., 1999; Cigánek et al., 2004; Du Four et al., 2004).

The genotoxic potency of the air samples was tested using the SOS chromotest. In comparison with the frequently used Ames test, the SOS chromotest enables us to analyze quickly genotoxic effects of the samples using only one test strain. In addition, its miniaturized design decreases the consumption of tested samples. Quantification is based on a simple evaluation of the end-point using a spectrophotometer. Samples can be tested both without and with the addition of metabolic activation. The genotoxicity test is very sensitive to a large spectrum of genotoxic compounds and shows high similarity with the Ames test (Quillardet et al., 1985).

The results of the evaluation of genotoxic activity without (−S9) and with the addition of metabolic activation (+S9) are summarized in Table 3. The validity of the genotoxicity data was confirmed by significant responses of the detection system to the standard mutagens used for the positive control. None of the tested samples showed unacceptable cytotoxic effects. In the case of the genotoxicity test −S9, all the tested samples reached IFs higher than 1.5 in the range of the tested concentrations. They showed significant direct genotoxic activity. The samples from the urban localities were more genotoxic than the ones from the reference localities (L5*, L10*). Based on the obtained SOSIPs, a much higher direct genotoxic activity was shown by the samples collected in Tuzla than the ones collected in Sarajevo. The highest direct genotoxic activity was detected at the locality L7 (SOSIP = 0.49). High

Table 3
Determined Maximum Induction Factors (MIFs) and estimated SOS Induction Potencies (SOSIPs) for the air samples at the localities [units/m^3]

Sarajevo area					Tuzla area				
	MIF		SOSIP [unit/m^3]			MIF		SOSIP [unit/m^3]	
Locality	−S9	+S9	−S9	+S9	Locality	−S9	+S9	−S9	+S9
L1	**3.03**	**1.63**	0.15	0.05	**L6**	**5.23**	**2.65**	0.28	0.14
L2	**2.75**	**2.00**	0.13	0.08	**L7**	**6.93**	**2.62**	0.49	0.14
L3	**1.86**	**1.48**	0.08	0.04	**L8**	**5.33**	**2.03**	0.37	0.08
L4	**1.96**	**1.60**	0.07	0.05	**L9**	**4.57**	**2.26**	0.30	0.11
L5[a]	**1.53**	**1.39**	0.05	0.04	**L10**[a]	**3.33**	**2.29**	0.20	0.09

MIF values indicating significant genotoxicity are written in bold.
[a] Reference background locality.

activities were also detected at the localities L8 (SOSIP=0.37) and L9 (SOSIP=0.30). On the other hand, the lowest activity in Tuzla was detected at the locality L6 (SOSIP=0.28) and at the urban background locality L10* (SOSIP=0.20). In Sarajevo, the highest activity was determined for the samples from the localities L1 (SOSIP=0.15) and L2 (SOSIP=0.13). The localities L3 and L4 showed similar low activity which was still twice as high as the direct genotoxic activity detected at the reference background locality L5* (SOSIP=0.05), in the mountains. The direct genotoxic activity determined at the urban localities in Sarajevo were always lower than that detected at the urban background locality L10* in Tuzla.

Even if the direct genotoxic activity in Tuzla was significantly higher than the one detected in Sarajevo, in both cities the highest activities were determined at the localities situated close to the city centers and affected mainly by traffic emissions. The direct genotoxicity of organic air pollution is related to the presence of PAH derivatives, especially nitro-, hydroxy- and oxi-PAHs (Nardini and Clonfero, 1992; Černá et al., 2000; Finlayson-Pitts and Pitts, 2000; Feilberg et al., 2002). But the direct genotoxicity is also shown by a spectrum of polar organic compounds which have not been identified so far (Lewtas et al., 1990; Crebelli et al., 1991; Černá et al., 2000). They are both emitted from the combustion of fossil fuels related to e.g. traffic, household heating and electricity production, and products of the transformation reactions of PAHs and other organic compounds in the atmosphere. Large amounts of nitro-PAHs are also emitted from diesel engines, which explains the high direct genotoxic activity of the air samples from the locality L8. The high direct activity determined at the locality L9 may be related both to the emissions from traffic and the power station. Unfortunately, no information about the PAH derivatives levels at the localities is available because they were not covered by the chemical analyses. But even if the PAHs do not show the direct genotoxicity, some relationship was observed between the parameters. No relationship with the wind direction was observed, and thus the direct genotoxicity is mainly related to the pollution sources at the locality and in its surroundings.

In the case of the genotoxicity test +S9, all the samples showed lower activity than in the test −S9. In contrast to the Ames test, air samples tested with the metabolic activation in the genotoxicity tests based on the SOS response usually show decreased genotoxic activities (Courtois et al., 1988; Schleibinger et al., 1989; Hamers et al., 2000; Du Four et al., 2004). This is explained by metabolic detoxification of toxicants, including direct genotoxins, with enzymes contained in the S9 fraction and complexation effects of proteins of the S9 fraction, which result in lower availability of toxicants for the bacteria (Hamers et al., 2000). However, significant indirect genotoxicity was still detected for all the localities with the exception of the localities L3 and L5*. These localities were classified only as probably genotoxic. Again much higher genotoxic activities were observed at the localities in Tuzla. The highest activity detected in Sarajevo was comparable with the lowest activities detected in Tuzla. The highest indirect genotoxicity in Tuzla was shown at the urban localities L6 and L7 (SOSIP=0.14). The samples from other localities in Tuzla showed a lower activity. The indirect activity detected at the urban background locality L10* (SOSIP=0.09) was slightly higher than the one detected at the locality L8 (SOSIP=0.08). In Sarajevo, the highest activity was observed at the locality L2 (SOSIP=0.08). On the other hand, the lowest indirect genotoxicity was observed at the localities L3 and L5* (SOSIP=0.04).

Increased direct genotoxic activity was observed at the localities affected by traffic emissions and household heating. While in Tuzla comparable indirect genotoxic activity was observed at the localities L6 and L7, in Sarajevo the locality L2 showed higher activity than the locality L1, which may be explained by the impact of the west-eastern air pollution transport on the detected genotoxic activity of the air pollution at the locality L2. No definite correlation was observed between the direct and indirect genotoxic activities at the localities, which is explained by fact that different pollutants induce these activities. In contrast to the direct genotoxicity, the indirect genotoxicity of the organic air pollution is related to the parental PAHs and their alkyl-derivatives (Černá et al., 1999, 2000; Feilberg et al., 2002; Du Four et al., 2004). This relationship was confirmed by the correlation between the carcinogenic PAHs level and the detected indirect genotoxicity at the localities (see below). But there are also other organic pollutants that affect the final activity. The impact of the whole mixture of organic air pollution on the genotoxic activity may explain the higher indirect activity detected at the background locality L10* than the one detected at the locality L8. The background locality L10* is not a real background locality as the reference locality L5* used for Sarajevo. It is an urban locality placed in a residential zone, not far from the city center. The air is affected by both traffic emissions and emissions from household heating there.

The observed similarities between the PAH levels and genotoxic activities at the localities have been

confirmed by the results of an analysis of these relationships using a simple linear regression. The results of the performed comparisons are summarized in Fig. 2. The individual graphs visualize the relationships between the genotoxic activities expressed as SOSIPs obtained with the genotoxicity tests −S9 and +S9, and the sums of 16 PAHs, sums of 8 carcinogenic PAHs and the BaP concentrations. Determined regression coefficients (R^2) prove the significant relationship between the genotoxic potency and the indicators of the organic air pollution. As expected, a closer relationship with the PAHs was determined for the indirect genotoxicity than for the direct genotoxicity, but the differences are small. The R^2 values were slightly higher for the sum of the carcinogenic PAHs ($R^2=0.896$) and the BaP concentration ($R^2=0.886$) than the R^2 value for the sum of 16 PAHs ($R^2=0.849$). So the results confirm the role of the carcinogenic PAHs in the detected indirect genotoxicity. The similar relationship determined for the concentration of BaP and for the sum of the carcinogenic PAHs confirms the usage of BaP as an indicator of the carcinogenic PAHs pollution and its important role, regarding its high genotoxic activity, within the group of the carcinogenic PAHs. On the other hand, in the case of the direct genotoxicity the lowest R^2 was obtained for BaP ($R^2=0.808$). But the relationship is still significant. The R^2 values obtained for the sum of 16 PAHs and the sum of 8 carcinogenic PAHs were slightly higher than for the concentration of BaP. The lower R^2 values for the relationship to the direct genotoxicity result from the fact the observed genotoxic activity was induced by other organic pollutants. The results fully confirm the observed relationship between the concentration of PAHs and the genotoxic activity of the organic air pollution determined at the localities. The similar significant relationships between the genotoxic activity of the air samples detected with the Ames test and the concentrations of PAHs in the air were published by

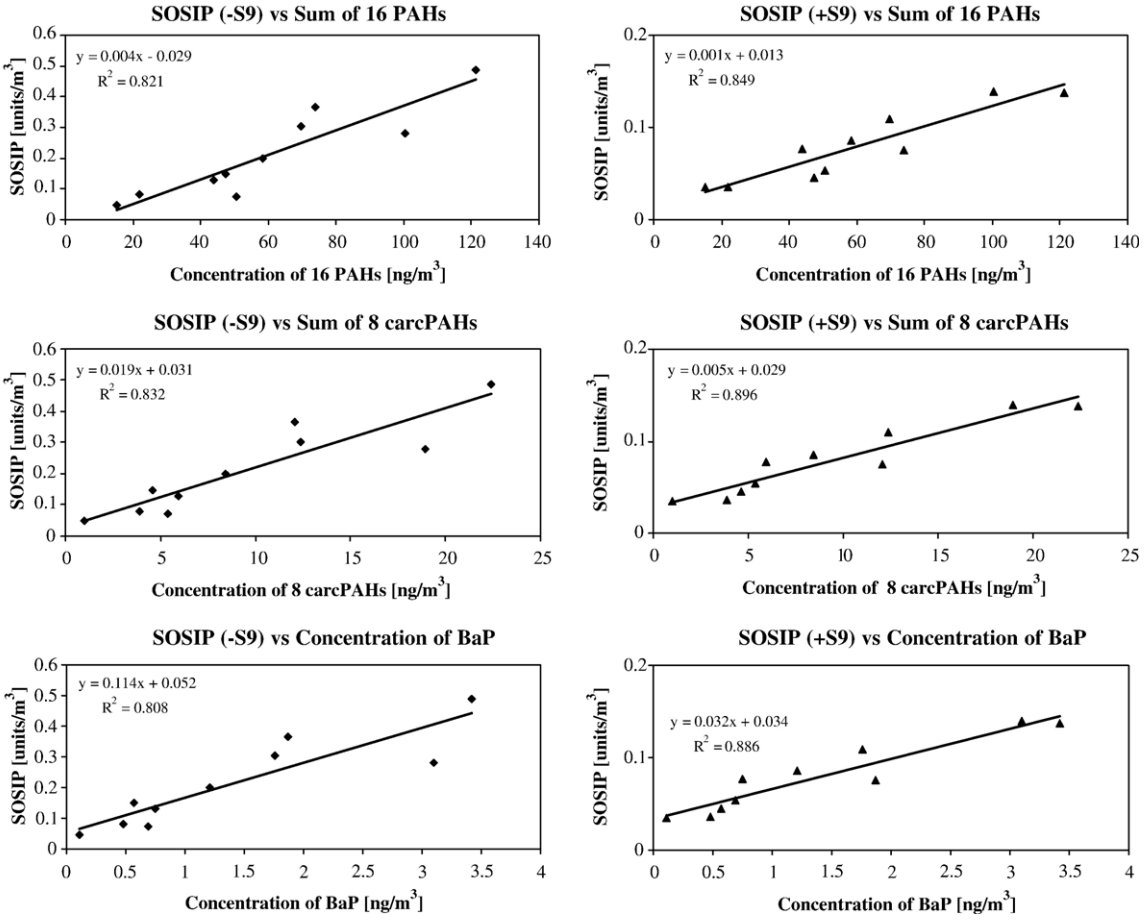

Fig. 2. Relationships between the sum of 16 PAHs, sum of 8 carcinogenic PAHs and BaP concentration, and the SOSIPs for the direct (−S9) and indirect genotoxicity (+S9).

several authors in literature (Černá et al., 1999; Feilberg et al., 2002, Du Four et al., 2004). The study of Feilberg et al. (2002) also showed the significant correlations both for the direct and indirect genotoxicity activities with the BaP content in the urban air.

However, it should be considered that the correlations are not absolute and the total response of the bioassay is also affected by other organic compounds. It concerns the relationship observed for the direct genotoxicity where a mediated interaction is apparent. As mentioned above, the PAHs must be activated to show their genotoxic activity in the genotoxicity test. The explanation of the significant relationship between the direct genotoxic activity and the PAHs level in the air can be found in the co-existence of the PAHs derivatives and other organic pollutants with the direct genotoxic activity with the parental PAHs in the air. They may be emitted by the same sources or they may be products of various transformation reactions of the PAHs in the air. But information about the levels of the PAHs derivatives and the other pollutants is not easy to obtain and so they do not belong among routinely determined pollutants yet.

The results confirm the applicability of the bioassay for indication of the organic pollution level in the air. Therefore, for the determination of the presence of genotoxic pollutants and consequently for the identification of possible health risks or localities with increased air pollution level, the usage of screening genotoxicity tests, such as the SOS chromotest, is highly recommended. Then detailed chemical analyses may be applied on the positive samples for further investigation.

4. Conclusions

An evaluation of the organic air pollution in two important industrial cities, Sarajevo and Tuzla, in Bosnia and Herzegovina, was performed, using chemical analyses (concentrations of 16 U.S. EPA priority PAHs) and genotoxicity testing (SOS chromotest). Both approaches confirmed a higher level of pollution in the much more industrialized Tuzla. But even if the permitted levels for BaP were exceeded at some localities, the concentrations of the PAHs were still comparable with the average concentrations determined in other European cities. The highest pollution levels were determined at localities lying close to the city centers and main thoroughfares that are strongly affected by traffic emissions. The highest levels of the carcinogenic PAHs were also detected there. But the air pollution must be also related with other sources at the localities including the emissions from industrial processes and household heating. The highest direct genotoxic activities were detected at the localities affected by traffic. Indirect genotoxicity was observed, in addition, at the localities affected by the emissions from industries and household heating. The identified localities with the highest potential health risks are the urban localities L6, L7 and L9 in Tuzla and the localities L1, L2 and L4 in Sarajevo. But some genotoxic risks of the air pollution may also be expected at other urban localities. Moreover, since the study was performed in spring, even higher organic pollution and health risks are expected there in winter due to the higher emissions from household heating. The levels of organic pollution may also increase due to reduced dispersion conditions. Due to a quite high organic air pollution level and genotoxic activities determined at the urban background locality L10* in comparison with all the localities from Sarajevo, the only real background locality was the locality L5* in the mountains which is apparently not affected by any air pollution sources. The observed relationship between the PAH levels at the localities and genotoxic activities of the samples was proved by a regression analysis. The strong correlation of the indirect genotoxicity with the carcinogenic PAHs level and BaP concentration was expected as well. But it was not absolute due to the probable presence of other pollutants with the indirect genotoxic activity. The correlation of the direct activity with the PAH level was lower but still significant. However, the correlation with the concentration of BaP was weaker. This fact confirms that a correlation exists between the PAH level and the level of the direct genotoxins, e.g. the PAH derivatives, in urban air. Unfortunately, the contents of PAH derivatives were not determined during the study. The study contributed not only to the assessment of the organic air pollution in the region, where any similar study had not been performed, but it also supports the evaluation of the pollution level using the determination of genotoxic activity as an indicator of the human health risk. The screening genotoxicity tests can be highly recommended for the assessment and monitoring of the organic pollution in the air and the identification of localities with increased organic pollution based on the genotoxic activity.

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10007). See http://www.recetox.muni.cz/projekty/apopsbal/index.php. The study was also supported by EUDG Centre of Excellence EVK1-CT-2002-80012 and the Ministry of Education of the Czech Republic Project MSM 0021622412 INCHEMBIOL.

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Příloha 8

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Assessing the Influence of Meteorological Parameters on the Performance of Polyurethane Foam-Based Passive Air Samplers

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Polyurethane foam (PUF) disk passive air samplers were evaluated under field conditions to assess the effect of temperature and wind speed on the sampling rate for polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and organochlorine pesticides (OCPs). Passive samples integrated over 28-day periods were compared to high-volume air samples collected for 24 h, every 7 days. This provided a large data set of 42 passive sampling events and 168 high-volume samples over a 3-year period, starting in October 2003. Average PUF disk sampling rates for gas-phase chemicals was ~7 m^3 d^{-1} and comparable to previous reports. The high molecular weight PAHs, which are mainly particle-bound, experienced much lower sampling rates of ~0.7 m^3 d^{-1}. This small rate was attributed to the ability of the sampling chamber to filter out coarse particles with only the fine/ultrafine fraction capable of penetration and collection on the PUF disk. Passive sampler-derived data were converted to equivalent air volumes (V_{EQ}, m^3) using the high-volume air measurement results. Correlations of V_{EQ} against meteorological data collected on-site yielded different behavior for gas- and particle-associated compounds. For gas-phase chemicals, sampling rates varied by about a factor of 2 with temperature and wind speed. The higher sampling rates at colder temperatures were explained by the wind effect on sampling rates. Temperature and wind were strongly correlated with the greatest winds at colder temperatures. Mainly particle-phase compounds (namely, the high molecular weight PAHs) had more variable sampling rates. Sampling rates increased greatly at warmer temperatures as the high molecular weight PAH burden was shifted toward the gas phase and subject to higher gas-phase sampling rates. At colder temperatures, sampling rates were reduced as the partitioning of the high molecular weight PAHs was shifted toward the particle phase. The observed wind effect on sampling for the particle-phase compounds is believed to be tied to this strong temperature dependence on phase partitioning and hence sampling rate. For purposes of comparing passive sampler derived data for persistent organic pollutants, the factor of 2 variability observed for mainly gas-phase compounds is deemed to be acceptable in many instances for semiquantitative analysis. Depuration compounds may be used to improve accuracy and provide site-specific sampling rates, although this adds a level of complexity to the analysis. More research is needed to develop and test passive air samplers for particle-associated chemicals.

Introduction

Persistent organic pollutants (POPs) are long-lived in the environment, bioaccumulative, toxic, and prone to long-range transport. International conventions such as the Stockholm Convention on POPs (SC) (*1*) deal with regulating these chemicals in order to reduce their potential to cause environmental and human harm. For effective POP control, information about their sources, distribution, levels, and transport is needed. Signatory countries of the SC are required to conduct source inventories and develop national implementation plans to demonstrate how the obligations of the Convention will be implemented. Coupled with this is the need for a monitoring strategy to show that ambient levels of POPs are declining as a result of control measures.

As part of the "effectiveness evaluation" of the SC, a global monitoring program has been initiated that will use air and human tissues (milk and blood) as core media for assessing trends of POPs (*2*). Air is expected to respond quickly to changes in emissions and is therefore well-suited for this purpose. Also, because air is relatively uniform and abundant throughout the globe, it is well-suited for investigating spatial trends and the regional and global transport of POPs. Guidelines have been established for conducting air monitoring (*3*) that include a combination of conventional high-volume monitoring stations that are supplemented with a higher-resolution network of much more cost-effective, passive air samplers (PASs). Passive samplers are especially useful in situations where electricity is not available or for initial installations (screening efforts) in regions where there is a lack of POPs data for air.

PASs of various designs have been used in a number of studies to derive data on the levels of POPs in the atmosphere (*4–28*). The Global Atmospheric Passive Sampling (GAPS) project has been monitoring POPs at background sites around the globe since 2002 using polyurethane foam (PUF) disks (*22, 25, 29*). Similarly, a regional-scale effort has been initiated at 50 sampling sites in the Czech Republic (including various industrial sources and urban, rural, agricultural, and background areas) and another 50 sites in other countries of central, southern, and eastern Europe to assess the effectiveness of the protective measures on POPs (*30, 31*).

Despite the feasibility and widespread acceptance and use of PUF disk passive air samplers, there exist some operational parameters that need to be more fully characterized in order to improve confidence in the derived, time-weighted average air concentrations. This is required for a comparison of data between sites and for assessing temporal trends of POPs concentrations in the air. One operational parameter requiring further investigation is the extent to which particle-associated compounds are captured by PUF disk samplers. Although many POPs are mainly in the gas phase at ambient temperatures, there are some POPs and related chemicals that are substantially particle-bound at ambient temperatures [e.g., polycyclic aromatic hydrocarbons (PAHs) and polychlorinated dioxins and furans (PCDDs/Fs)]. It is also important to understand the potential changes

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in sampling rates associated with variable environmental conditions, most notably wind speed (*4*) and temperature. Results from laboratory calibrations (*4*) for PUF disk samplers and field deployments where depuration compounds were used (these are isotopically labeled chemicals added to the PUF disks prior to exposure and are used to assess site-specific mean sampling rates) (*15, 24*) suggest that typical sampling rates are in the range of 3–5 m^3 of air per day (*4*). However, larger variations are observed in some cases, between sites and from season to season (*15, 24*). Some studies have investigated the wind effect on PUF disk samplers in the laboratory (*28*) and using 3-D flow models (*27*), but so far no comprehensive field calibration has been undertaken.

In this study, data from the long-term concurrent passive and high-volume air samples over 42 consecutive 28-day periods were used to assess the performance of PUF disk samplers under various meteorological conditions.

Materials and Methods

Sampling Site. Passive and high-volume air samplers were deployed at Košetice observatory of the Czech Hydrometeorological Institute, located in the southern Czech Republic (49° 35′ N, 15° 05′ E). The climatic classification of the region is a moderately warm and moderately humid upland zone with a mean annual temperature of 7.1 °C, a mean annual total precipitation of 621 mm, 60−100 days of snow cover per year, 1800 h of sunshine per year, and prevailing westerly winds. The observatory was established as a regional station of an integrated background monitoring network in the late 1970s, and it is part of the EMEP (European Monitoring and Evaluation Program) monitoring network (*32–34*). Passive air samples were collected for 42 consecutive periods of 28 days starting in October, 2003. During each 28-day integration, four 24-h high-volume samples were collected—one per week.

Air Sampling. Passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5-cm-thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in protective chambers (*4, 11*) were employed in this study. These disks have approximately 30% greater planar surface area compared to PUF disks used in a GAPS network (*21, 25, 29*). Since a diameter of the chamber was larger as well and all the other parameters (gap or overlap of two domes) were kept the same, it did not affect PAS performance. Sampling chambers were washed and solvent-rinsed with acetone prior to installation. All PUF disks were prewashed, cleaned (8 h Soxhlet extraction in acetone and 8 h in dichloromethane), wrapped in two layers of aluminum foil, placed into zip-lock polyethylene bags, and kept in a freezer prior to deployment. Exposed PUF disks were wrapped in two layers of aluminum foil, labeled, placed into zip-lock polyethylene bags, and transported in a cooler at 5 °C to the laboratory where they were stored at −18 °C until analysis. Field blanks were obtained by installing and removing the PUF disks at all sampling sites.

High-volume ambient air samples were collected using a PS-1 apparatus (Graseby-Andersen, U.S.A.; volume 250–400 m^3 per 24 h, $d_{ae} < 50\,\mu m$) with a quartz fiber filter (QFF) for collecting the particle phase (Whatmann, fraction > 2 μm) and a PUF plug (Gumotex Breclav; density 0.030 g cm^{-3}) for trapping gas-phase compounds. All PUF plugs were cleaned before the campaign (8 h Soxhlet extraction in acetone and 8 h in dichloromethane), and QFFs were baked at 450 °C. QFFs and PUFs were treated and stored in the same manner as the PUF disks. Field blanks were obtained by installing and removing the quartz and PUF filters at the sampling sites.

Sample Analysis. All sample media (PUF plugs, QFF, and PUF disks) were extracted with dichloromethane in a Büchi System B-811 automatic extractor. Surrogate recovery standards (*d*8-naphthalene, *d*10-phenanthrene, *d*12-perylene, and polychlorinated biphenyl congeners PCB 30 and PCB 185) were spiked on each sample media prior to extraction. Sample extract volumes were reduced under a gentle nitrogen stream at ambient temperature and divided in two halves for PCB/OCP and PAH analyses. PAH cleanup was achieved on a silica gel column (30 cm length, 1 cm i.d., 5 g of silica, eluted with 10 mL of hexane—discarded, followed by 20 mL of dichlomethane). A sulfuric acid modified silica gel column was used for further cleanup of the PCB/OCP (eluted with 30 mL of 1:1 hexane/dichloromethane). Terphenyl and PCB 121 were applied as internal standards for PAH and PCB analyses, respectively. Air samples were analyzed using a gas chromatograph equipped with an electron capture detector, HP 5890, supplied with a Quadrex fused silica column, 5% Ph, for PCBs and OCPs; a gas chromatograph coupled with a mass spectrometer (GC-MS), HP 5975, with a J&W Scientific fused silica column, DB-5MS, was used for confirmation. A total of 16 United States Environmental Protection Agency (U.S. EPA) PAHs were determined in all air samples using a GC-MS instrument (HP 6890, HP 5972 and 5973) supplied with a J&W Scientific fused silica column, DB-5MS.

Quality Assurance/Quality Control. Recoveries based on surrogate standards added prior to extraction were higher than 71% and 69% for all samples for PCBs and PAHs, respectively. Recovery factors were not applied to any of the data. Recovery of native analytes measured for the reference material varied from 88 to 100% for PCBs, from 75 to 98% for OCPs, and from 72 to 100% for PAHs. Field blanks were extracted and analyzed in the same way as the samples, and the levels in field blanks never exceeded 1% of the quantities detected in samples for PCBs, 1% for OCPs, and 3% for PAHs, indicating minimal contamination during the transport, storage, and analysis. Laboratory blanks were always lower than 1% of the amount found in samples. Due to potential breakthrough during high-volume air sampling, low-molecular-weight PAHs (namely, naphthalene, acenaphthylene, and acenaphthene) were excluded from the analysis of the sampling rates.

Meteorological Parameters. Meteorological information was recorded during the entire period using the professional service of the Czech Hydrometeorological Institute. Wind speed and temperature were monitored continuously at 5 and 10 m above the ground using an automatic weather station (Vaisala HydroMet, Finland). Median values based on the hourly synoptic reports were calculated. Fluctuations of the temperature and wind speed can be seen in Figures 1 and 2, where average values were calculated for the 28-day period that the PUF disks were deployed. Both parameters were significantly negatively correlated—the statistically significant Spearman rank correlation is 0.65 ($p < 0.05$).

Results and Discussion

Uptake Rates. For short deployment periods of 28 days, most of the target compounds will be accumulated by the PUF disks in the linear phase (*21*), and a linear sampling rate R (cubic meters per day) can be derived from high-volume air concentration data. There are two potential problems with this approach that should be acknowledged. First, the high-volume data represent just 4 days (14%) of the 28-day deployment period of the PUF disk. Gouin et al. (*15*) demonstrated, using back-trajectory analysis, that discrepancies between intermittent high-volume samples and continuous passive sampling data are possible and occur when high-volume samples are collected on days that are not typical of the passive integration period. However, due to the large number of passive sampling periods ($n = 42$) in this study, we expect that the net effect of these discrepancies will be diminished. Second, some of the more volatile target compounds (those with low K_{OA} values) will exceed the linear

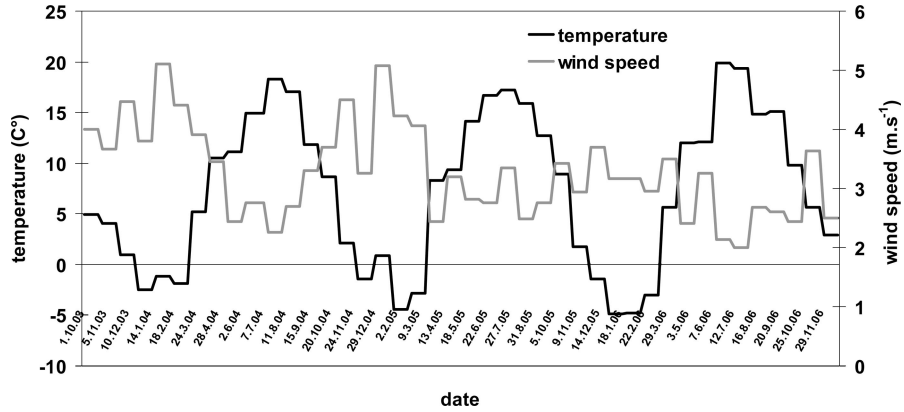

FIGURE 1. The 3-year temperature (°C) and wind speed (m s^{-1}) record at Košetice for the individual passive and high-volume sampling events showing the mean temperature and wind speed (based on hourly data) for each 28-day deployment period for passive samplers. Dates indicate starting days of PAS sampling periods; there were five high-volume samples for each PAS period.

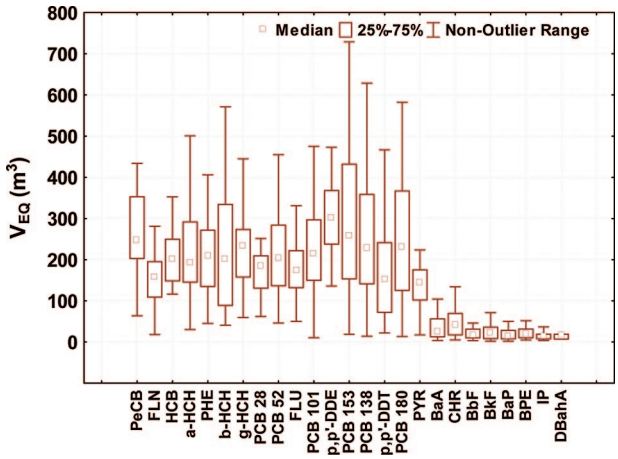

FIGURE 2. Summary of equivalent sample volumes (V_{EQ} values, m^3; calculated using eq 1) for all target compounds (pentachlorobenzene/PeCB; hexachlorobenzene/HCB; α-hexachlorocyclohexane/a-HCH; *β*-hexachlorocyclohexane/b-HCH; *γ*-hexachlorocyclohexane/g-HCH; *p,p′*-DDE; *p,p′*-DDT; PCB 28, 52, 101, 153, 138, and 180; fluorene/FLN; phenanthrene/PHE; fluoranthene/FLU; pyrene/PYR; benz[a]anthracene/ANT; chrysene/CHR; benzo[b]fluoranthene/BbF; benzo[k]fluoranthene/BkF; benzo[a]pyrene/BaP; indeno[1,2,3-c,d]pyrene/IND; dibenz[a,h]anthracene/DBahA; and benzo[g,h,i]perylene/BPE) and 42 passive sampling events at Košetice observatory. Values are arranged according to decreasing volatility (p_L^o values at 25 °C, Pa) with the lowest volatility compounds expected to be particle-bound.

phase as they approach equilibrium between the PUF disks and the atmosphere (*15, 24*). This is more likely to occur at warmer temperatures since K_{OA} decreases (and consequently the PUF−air partition coefficient also decreases, i.e., reduces the capacity of the PUF disk) as the temperature increases. For these chemicals, the net uptake rate is not constant over the entire period. For all target chemicals, the equivalent sample volume of the 28-day passive sample (V_{EQ}, cubic meters) was calculated as

$$V_{EQ} = C_{PD}/C_{AIR} \quad (1)$$

where C_{PD} (nanograms per sampler) is the PUF disk concentration and C_{AIR} (nanograms per cubic meter) is the mean air concentration (gas + particle phase) derived from the high-volume air samplers for each integration period.

Equivalent sample volumes (V_{EQ} values) are summarized in Figure 2 for all target compounds (12 PAHs, 6 PCB congeners, and 7 OCPs) and for all 42 passive sampling periods (besides low-molecular-weight PAHs, also compounds occurring in very low concentrations were excluded from this analysis). Results are presented according to the chemical's volatility (supercooled liquid–vapor pressure) to help distinguish chemicals that are mainly in the gas phase from particle-bound compounds. For mainly gas-phase chemicals, with p_L^o values greater than about 10^{-4} Pa (this result is confirmed by the gas/particle split observed in the results of the high-volume sampling), the derived V_{EQ} values are in the range 150−250 m^3, with a mean of ∼200 m^3. This corresponds to linear range sampling rates of approximately 7 m^3 day^{-1}. This is slightly higher but within the variability of the mean rate derived for more than 30 sites under GAPS, of ∼4 ± 2 m^3 day^{-1}, or for proper comparison, 5.2 m^3 day^{-1} when scaled up to the 30% larger surface area of the Košetice PUF disks. Furthermore, some of the remaining difference may be attributed to the different chamber configurations used under GAPS.

Lower V_{EQ} values of about 20 m^3 (∼0.7 m^3 day^{-1}) are derived for mainly particle-associated chemicals (in this case, only the higher-molecular-weight PAHs), compared to ∼200 m^3 for gas-phase chemicals as shown above. This result suggests that approximately 10% of the ambient particles are sampled by the PUF disk, likely representing the finest particle component. As discussed previously (*35*), these fine particles (likely less than 100 nm) can enter the sampling chamber because they behave much like gas-phase chemicals. This component is important because it is respirable and the least susceptible to deposition (i.e., greatest air transport potential).

Temperature and Wind Effects on Sampling Rates. In assessing the effect of meteorological parameters on sampling rates, gas-phase and particle-phase target compounds are considered separately. Previous studies have investigated these parameters under controlled conditions or using theoretical principles. For instance, Shoeib and Harner showed that the uptake of gas-phase compounds by the PAS sampler is air-side-controlled, and that the mass transfer coefficient in relatively calm air is likely to be similar to the chemicals molecular diffusivity, *D* (*4*):

$$D_a = \{10^{-3}T^{1.75}[(1/m_{air}) + (1/m)^{1/2}\}/P[V_{air}^{1/3} + V^{1/3}] \quad (2)$$

where T is the absolute temperature (Kelvin), m_{air} is the average molecular mass of air (28.97 g mol^{-1}), m is the molecular mass of the chemical (grams per mole), P is the gas-phase pressure (atmospheres), V_{air} is the average molecular volume of the gases in air (20.1 cm^3 mol^{-1}), and V is the molecular volume of the chemical (cubic centimeters per mole).

Since molecular diffusivity is a weak function of temperature, it will increase by only a small factor of $(293/273)^{1.75}$ or 1.13 with a temperature increase of 20 °C. Tuduri et al.

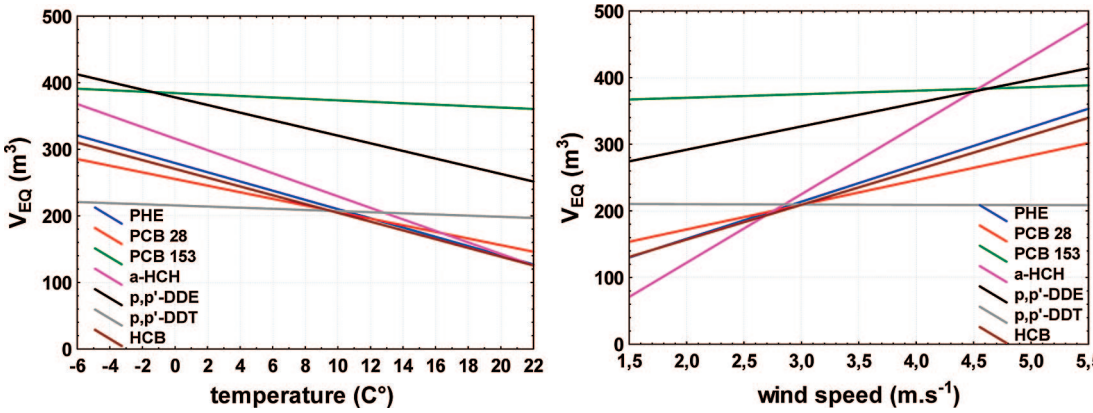

FIGURE 3. Correlation of V_{EQ} (m^3) with temperature (°C) and wind speed (m s^{-1}) for various gas-phase-associated compounds.

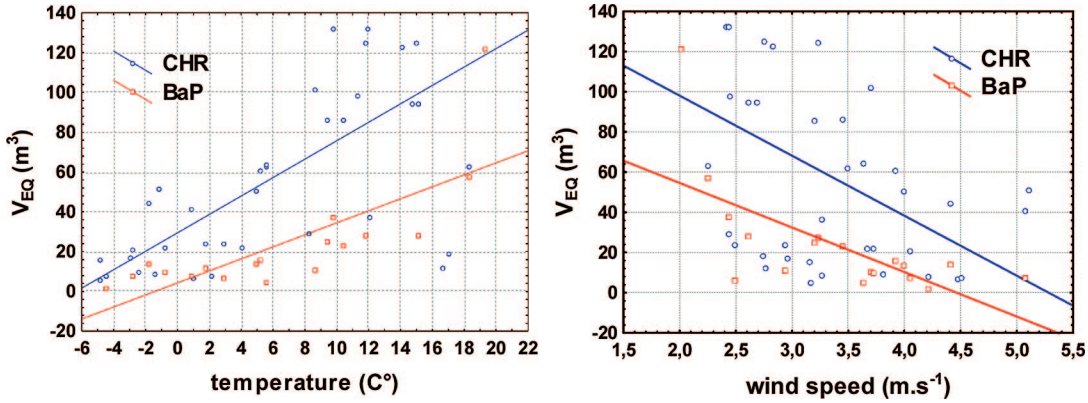

FIGURE 4. Regression lines of correlation of V_{EQ} (m^3) with temperature (°C) and wind speed (m s^{-1}) for particle-bound compounds.

(*28*) and Thomas et al. (*27*) showed that increases in sampling rates occurred when ambient wind speeds exceeded approximately 5 m s^{-1}.

Figure 3 shows the correlation of V_{EQ} values for mainly gas-phase compounds against temperature (left panel) and wind speed. Contrary to theoretical predictions discussed above, the V_{EQ} value is negatively correlated with temperature and ranges from a high of about 300−400 m^3 at −6 °C (267 K) to approximately 150−250 m^3 at +22 °C (293 K). However, the higher sampling rates at colder temperatures may be partly explained by higher wind speeds at colder temperatures (see Figure 1) and the dependence of the sampling rate on wind speed (*27, 28*). This is confirmed by Figure 3 (right panel), which shows a fairly steep and positive correlation of V_{EQ} against wind speed.

On the basis of these findings, we expect PUF disk sampling rates to be slightly higher (up to a factor of about 2) during colder periods. It is necessary to account for this increase when interpreting and comparing seasonal data derived from PUF-disk samplers or when comparing data from samplers deployed in different climates. In many cases, PAS-derived air concentrations are interpreted semiquantitatively, and this magnitude of variability is not a concern. However, in instances where greater confidence is required, for instance, for a more quantitative comparison, we recommend the use of depuration compounds. Although this adds an additional step and cost to the sample analysis, it does allow for site-specific sampling rates (*15, 24, 25*) that account for the wind and temperature effects on the sampling rate.

Figure 4 shows the correlation of V_{EQ} values against temperature (left panel) and windspeed for two mainly particle-phase PAHs, chrysene and benzo[a]pyrene. This behavior is typical for other high-molecular-weight PAHs. In this case, the V_{EQ} values range over larger magnitudes and are positively correlated with temperature (increase with increasing *T*) and negatively correlated with windspeed (decrease with higher windspeeds)—opposite to what was observed for gas-phase compounds. This behavior is driven by the fact that, at warmer temperatures, high-molecular-weight PAHs begin to partition more to the gas phase. For instance, applying the Junge−Pankow model for chrysene for rural air (*36, 37*), we can predict that it will be almost entirely particle-bound at −6 °C while at 22 °C it splits between both phases with about 10–20% on particles. Benzo[a]pyrene, which is less volatile, is expected to be 80–90% particle-bound at 22 °C and entirely particle-bound at −6 °C. This analysis is consistent with the observed V_{EQ} values for chrysene and benzo[a]pyrene with greater temperature sensitivity observed for chrysene, due to its greater presence in the gas phase at warmer temperatures. The increase in sampling rates at lower wind speeds (right panel) is largely a result of the strong negative correlation of windspeed with temperature; that is, higher winds tend to occur when temperatures are colder, and high-molecular-weight PAHs are partitioned more to particles, leading to low sampling rates.

The correlation of theoretical sampling volumes with other meteorological parameters (wet precipitation, vapor pressure, atmospheric pressure, and sunshine) was studied as well. No significant correlation was observed for these parameters.

In summary, this is the first comprehensive study to investigate the effect of temperature and wind on PUF disks passive air samplers under field conditions. The results provide new information that can be used to interpret and compare passive sampler derived air concentrations. Overall, the variability in sampling rates for mainly gas-phase compounds is expected to be fairly low (within a factor of about two) over typical field conditions. When greater quantitative power or confidence is required, depuration compounds should be used. For chemicals that are split

between the gas and particle phases, sampling rates are much more variable and increase substantially with increasing temperature as chemicals partition more to the gas phase and are subject to the much higher gas-phase sampling rate. Consequently, care must be taken when deriving air concentrations for chemicals that are split between the particle and gas phases over the range of sampling temperatures. Furthermore, sampling rates derived from depuration compounds cannot be applied as they are only valid for gas-phase chemicals. More research is needed to investigate passive air samplers for particle-associated chemicals, as many compounds of interest fall into this category. Future field assessments should also strive to do passive and air sampling concurrently and continuously, using low-volume active air samplers, to ensure better comparability of the results.

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Which compounds contribute most to elevated airborne exposure and corresponding health risks in the Western Balkans?

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ABSTRACT

A majority of ongoing monitoring of persistent organic pollutants (POPs) is currently focused on chemicals emphasized in the Stockholm Convention. Quantitative detection of other substances (especially those with numerous anthropogenic sources such as polyaromatic hydrocarbons (PAHs)) is, however, also needed since their concentrations are usually several orders of magnitude higher. A goal of this study was to determine how various groups of compounds contribute to total human health risks at the variety of sampling sites in the region of Western Balkan. Distribution of the risks between the gas and particulate phases was also addressed. Results showed that inhalation exposure to organochlorine pesticides (OCPs) does not represent a significant risk to humans, while polychlorinated biphenyls (PCBs) re-volatilized to the atmosphere from contaminated soils and buildings can pose a problem. PCB evaporation from primary sources (currently used PCB-filled transformers or non-adequate storage facilities) generally resulted in much higher atmospheric concentrations than evaporation from the secondary sources (soils at the sites of war destructions). A majority of the human health risks at the urban sites were associated with PAHs. Between 83 and 94% of the cumulative risk at such sites was assigned to chemicals sorbed to particles, and out of it, PAHs were responsible for 99%.

1. Introduction

A potential impact of polluted air on human health has been a subject of numerous investigations recently. It has been suggested that air pollution is likely to increase mortality and hospital admissions (Brunekreef and Holgate, 2002). The inhalation exposure can result in a range of effects from breathing difficulties to development of a lung cancer (Boffetta and Nyberg, 2003; Vineis and Husgafvel-Pursiainen, 2005). In addition to respiratory tract, vital functions of other organs can be affected as well (Cohen et al., 2005; Kunzli and Tager, 2005). A quantification of such harmful effects is complicated by the fact that ambient air is a complex mixture of components with variable chemical composition, physicochemical properties, persistence in the environment, long-range transport potential, toxicity and carcinogenicity.

Several regulations on production, marketing, application and disposal of persistent toxic substances have been introduced in recent years but many of these compounds persist in the environment at significant levels long after they have been banned. To evaluate the effectiveness of recently introduced regulations on production, marketing, application and disposal of persistent toxic substances; reliable air monitoring programs are needed worldwide. As many of these compounds persist in the environment long after they have been banned, such programs should provide information on present concentrations and their long-term trends (Middleton, 1997). Ambient air concentrations derived from the regular monitoring of POPs are also a source of data for evaluation of the long-term population exposure and related effects, in particular for pollutants for which ambient air represents a dominating exposure pathway.

A majority of ongoing monitoring of persistent organic pollutants (POPs) is currently focused on chemicals emphasized in the Stockholm Convention (UNEP, 2001) which regulates emissions of compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDDs/Fs), and organochlorine pesticides (OCPs). Quantitative detection of other substances, (especially those with numerous anthropogenic sources such as PAHs) is, however, also needed since their concentrations are usually several orders of magnitude higher (PCDDs/Fs: pg m^{-3} or less, PCBs and OCPs: tens to hundreds of pg m^{-3}, PAHs up to hundreds of ng m^{-3}) (Menichini et al., 2007). Even though reduction of the human exposure to POPs in ambient air has been a primary focus of the public health policy, the lack of available data often prevents the authorities from the adequate actions.

Countries of the former Yugoslavia represent a European region with very limited information on the levels of atmospheric pollution. As many industrial sites and energy installations were damaged in the bomb attacks and missile strikes during the Balkan war conflicts in the late 1990s, and large amounts of hazardous substances were released into the environment (Picer and Holoubek, 2003), contamination of

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the former Yugoslavia has been a subject of numerous investigations in the last decade (Rapsomanikis et al., 2002; Picer and Holoubek, 2003; Klánová et al., 2007a,b; Ruzickova et al., 2008). The scientific attention was focused mostly on determination of the PCB concentrations in soil and air, while other classes of pollutants were largely overlooked. A first study investigating toxicity of the air samples from this region, however, showed that toxic effects were much stronger (Skarek et al., 2007) in the air samples from industrial sites contaminated with PAHs than in the samples from PCB hotspots.

A contribution of various classes of toxic compounds (PCBs, OCPs, PAHs), both particle-bound and gas phase-associated, to total human health risks was assessed in this study. Large number of ambient air samples from background, urban and industrial sites including hot spots in Croatia, Serbia, Bosnia and Herzegovina was collected. Ambient air concentrations of selected pollutants and related human health risks associated with the inhalation exposure were determined for all samples.

2. Materials and methods

2.1. Sampling locations

A total number of 127 ambient air samples were collected in the countries of former Yugoslavia (Croatia, Serbia, Bosnia and Herzegovina) in the early summers (May–June) of 2003 and 2004 under comparable meteorological conditions at all sites (median temperature 19 °C). In 2003, ten high volume samples were collected from each of five sampling sites in Croatia. Four samplers were positioned in the city of Zadar (damaged transformer station, industrial zone, historical center, meteorological station). Background sampler was 150 km north on mountain Velebit at the Zavizan location. Similarly, five samples were taken from each of five sampling sites in Sarajevo area (industrial zones, residential areas, background site) and another five sampling sites in Tuzla region in Bosnia and Herzegovina (service and storage place for damaged capacitors, transformer station, salt mine, residential area, background site). Ivan Sedlo meteorological station served as a background site for Bosnia and Herzegovina. In Serbia, Kragujevac (Zastava factory, university), Pancevo (Petrochimika, refinery, center) and Novi Sad (refinery, residential part, center) were the cities of interest, each providing three sampling sites and three high volume samples from each site.

2.2. High volume air sampling

The high volume air samplers PS-1 (Graseby-Andersen, USA, flow: 20–25 m^3 h^{-1}, volume: 250–300 m^3 per 24 h) and two types of adsorbents were used: a Whatmann quartz filter (fraction dae <50 μm) for a collection of particles, and a polyurethane foam filter (Gumotex Břeclav, density 0.03 g m^{-3}) for a gaseous phase sampling. A sampling duration was 24 h. All filters were cleaned before the campaign: PUF filters were extracted with acetone and dichloromethane in a Soxtec extractor, quartz filters were heated to 450 °C.

2.3. Passive air sampling

To assess the seasonal variability of the air concentrations of investigated POPs, passive air sampling technique has been employed at all sampling sites for the next six months following the active air sampling. Passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5 cm thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in protective chambers (Shoeib and Harner, 2002; Klanova et al., 2006) were employed in this study. Sampling chambers were washed and solvent-rinsed with acetone prior to installation. All PUF disks were prewashed, cleaned (8 h Soxhlet extraction in acetone and 8 h in dichloromethane), wrapped in two layers of aluminum foil, placed into zip-lock polyethylene bags and kept in a freezer prior to deployment. Exposed PUF disks were wrapped in two layers of aluminum foil, labeled, placed into zip-lock polyethylene bags and transported in cooler at 5 °C to the laboratory where they were stored at −18 °C until analysis. Field blanks were obtained by installing and removing the PUF disks at all sampling sites.

2.4. Sample analysis

All samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. One laboratory blank and one reference material were analyzed with each set of samples. Surrogate recovery standards (D8-naphthalene, D10-phenanthrene, D12-perylene for PAHs analysis, PCB 30 and PCB 185 for PCBs analysis) were spiked on each filter prior to extraction. Terfenyl and PCB 121 were used as internal standards for PAHs and PCBs analyses, respectively. Volume was reduced after extraction under a gentle nitrogen stream at ambient temperature, and fractionation achieved on a silica gel column (30 cm length, 1 cm i.d.); a sulphuric acid modified silica gel column was used for PCB/OCP samples. Samples were analyzed a GC-MS instrument (HP 6890–HP 5975) supplied with a J&W Scientific fused silica column DB-5MS for PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180), OCPs (α-HCH, β-HCH, γ-HCH, δ-HCH, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT) and 16 US EPA PAHs. Analytical details and Quality Assurance/Quality Control measures have been published previously (Klánová et al., 2007a,b).

2.5. Risk assessment

According to EPA (EPA, 1998), a human exposure depends on a Chronic Daily Intake (CDI) of every single contaminant inhaled by the receptor. The CDI value (mg kg^{-1} day^{-1}) can be derived from Eq. (1) (EPA, 1992; EPA, 1996):

$$CDI = CA \cdot IF, \quad (1)$$

where CA is a compound concentration (mg m^{-3}) and IF is an Intake Factor (m^{-3} kg^{-1} day^{-1}).

Intake Factor is derived from Eq. (2):

$$IF = \frac{IR\text{–}A \cdot EF \cdot ED \cdot ET}{BW \cdot AT}, \quad (2)$$

where IR-A (Inhalation Rate) is a breathing rate (m^3 h^{-1}), EF (Exposure Frequency) is a number of exposures per year, ED (Exposure Duration) is a duration of exposure in years, ET (Exposure Time) is a number of hours per exposure, BW (Body Weight) is a default weight of the receptor body (kg), and AT (Averaging Time) is an average exposure extent over a lifetime (35,500 days for carcinogenic exposure). Appropriate default exposure parameters were obtained from EPA (EPA, 1998) [IR-A = 20 m^3/day; EF = 365 days; ED = 70 years; ET = 24 h/day; BW = 70 kg]. CDI for carcinogenic substances is called Life Averaged Daily Dose (LADD).

Human health risk related to contaminated air depends on the extent of exposure as well as on the toxic effects of chemicals. The chemical-specific risks were calculated from the Life Averaged Daily Dose and the Slope Factor (SF) (1/mg kg^{-1} day^{-1}) using the linear low-dose cancer risk equation (Eq. (3)):

$$Risk = LADD \cdot SF \quad (3)$$

A Slope Factor is a plausible upper-bound estimate of probability of the response per unit chemical intake over the lifetime. It is used to estimate an upper-bound probability of the individual developing a cancer as a result of the lifetime exposure to certain level of potential carcinogen. Cancer potency factors for chemicals of concern were obtained from EPA, OEHHA (OEHHA, 2002; EPA, 2003). Cancer

Table 1
Cancer potency factor expressed as inhalation unit risk [(μg m^{-3})$^{-1}$] for all assessed chemical compounds (OEHHA, 2002; EPA, 2003).

Compound	IUR
Naphthalene	3.40E−05
Acenaphtylene	1.10E−06
Acenapthene	1.10E−06
Fluorene	1.10E−06
Phenanthrene	1.10E−06
Anthracene	1.10E−05
Fluoranthene	1.10E−06
Pyrene	1.10E−06
Benz(a)anthracene	1.10E−04
Chrysene	1.10E−05
Benzo(b)fluoranthene	1.10E−04
Benzo(k)fluoranthene	1.10E−04
Benzo(a)pyrene	1.10E−03
Indeno(123cd)pyrene	1.10E−04
Dibenz(ah)anthracene	1.20E−03
Benzo(ghi)perylene	1.10E−05
PCB 28	1.00E−04
PCB 52	1.00E−04
PCB 101	1.00E−04
PCB 118	1.00E−04
PCB 153	1.00E−04
PCB 138	1.00E−04
PCB 180	1.00E−04
alpha-HCH	1.80E−03
beta-HCH	5.30E−04
gamma-HCH	3.10E−04
p,p′-DDE	9.70E−05
p,p′-DDD	6.90E−05
p,p′-DDT	9.70E−05
HCB	4.60E−04

potency factors for inhalation exposure are expressed as Inhalation Unit Risk (IUR). The IUR values used in this risk assessment are presented in Table 1.

The final SF values were calculated according to Eq. (4):

$$SF\left[\frac{\text{mg}}{\text{kg*day}}\right]^{-1} = \frac{IUR\left[\frac{\mu g}{m^3}\right]^{-1} * 70[\text{kg}] * 1000\left[\frac{\mu g}{\text{mg}}\right]}{20\left[\frac{m^3}{\text{day}}\right]} \quad (4)$$

A final cumulative health risk related to each sampling site was calculated as a sum of the partial risks of the individual pollutants.

A carcinogenic benchmark level is an exposure that poses an upper-bound lifetime excess cancer risk of 1E−6 (EPA, 2003). Exposure for which the risk factor exceeds 1E−6 (i.e. one occurrence over 1 million people) is then scored as significant.

3. Results and discussion

3.1. Site-to-site variability

Determination of the human health risks was based on the atmospheric concentrations measured in summer using an active air sampling technique. The cumulative cancer risk values for 25 sampling sites are presented in Table 2 and Fig. 1. Partial risk levels for the gas phase associated and particle-bound chemicals are presented separately in order to identify a fraction that most of the risk-posing chemicals associate with (Lee et al., 1995; Mader and Pankow, 2000; Halsall et al., 2001; Mader and Pankow, 2001). As expected, the lowest risks (entirely associated to the gas phase) were found in Croatia as the Zadar area is a seashore resort. Previously damaged Zadar transformer station demonstrated a risk several times higher than other sites (4.3E−7) but still it barely reached a half of the significant risk level. All sites in Serbia posed higher hazards than the Zadar transformer station and, at the same time, a major risk contribution was assigned to particle-bound chemicals. The only exception was the Zastava car factory in Kragujevac. It was the only site where the acceptable risk level of 1E−6 was exceeded 4.5 times, but only less than 7% of the risk was connected to particle-bound fraction. Fig. 2 distinguishes not only between the phases but also between the individual classes of compounds responsible for human health risks. As can be seen from Fig. 2, PCBs were the compounds responsible for elevated risks associated with the gas phase in Kragujevac. Volatilization from the PCB-filled transformer which is still in use at this site was a source of high atmospheric concentrations. The overall health risk more than one order of magnitude higher than anywhere else was, however, found in Bosnia and Herzegovina (Fig. 1) where the only site with low risks was the background station Ivan Sedlo. For all the others, a significant risk level was reached or overdrawn. It was exceeded 6.5 times in Tuzla fire station where volatilization from the storage place of the old electrical equipment

Table 2
Overall summary of the health risks at the individual sampling sites (CRO — Croatia, BIH — Bosnia and Herzegovina, SER — Serbia, CZE — Czech Republic).

Locality	Country	PUF					GF					GF/(GF + PUF) ratio
		PAH	PCB	HCH	DDT	Σ RISK	PAH	PCB	HCH	DDT	Σ RISK	
Zadar CZ	CRO	2.02E−08	5.75E−09	3.15E−08	1.53E−09	5.90E−08	1.36E−08	8.50E−10	3.45E−09	2.98E−10	1.81E−08	0.24
Zadar CM	CRO	1.94E−08	9.70E−09	5.72E−08	5.23E−09	9.15E−08	2.13E−08	8.60E−10	2.66E−09	2.49E−10	2.51E−08	0.22
Zadar CT	CRO	2.35E−08	2.66E−07	7.57E−08	7.06E−09	3.72E−07	3.58E−08	4.22E−09	3.34E−09	3.20E−10	4.37E−08	0.11
Zadar CA	CRO	5.51E−08	1.24E−08	7.90E−08	5.71E−09	1.52E−07	5.22E−08	1.26E−09	1.48E−09	4.18E−10	5.54E−08	0.27
Zadar CV	CRO	5.36E−08	1.24E−08	6.23E−08	2.99E−09	1.31E−07	4.18E−08	1.26E−09	2.87E−09	5.15E−10	4.65E−08	0.26
Sarajevo IS	BIH	3.41E−08	1.00E−08	2.14E−08	1.17E−09	6.68E−08	1.88E−07	2.82E−09	2.48E−10	1.75E−10	1.91E−07	0.74
Sarajevo HMI	BIH	1.50E−07	5.90E−09	1.77E−08	1.13E−09	1.74E−07	1.25E−06	1.21E−08	1.86E−09	8.70E−10	1.27E−06	0.88
Sarajevo ST	BIH	1.63E−07	1.55E−08	2.41E−08	1.46E−09	2.04E−07	9.70E−07	1.08E−09	1.57E−09	1.30E−10	9.73E−07	0.83
Sarajevo VL	BIH	1.08E−07	1.72E−08	1.96E−08	1.33E−09	1.46E−07	1.13E−06	3.06E−09	3.80E−10	1.36E−10	1.13E−06	0.89
Sarajevo VW	BIH	4.52E−08	1.04E−08	1.88E−08	2.33E−09	7.67E−08	8.40E−07	1.10E−09	1.57E−09	2.99E−10	8.43E−07	0.92
Tuzla BU	BIH	1.72E−07	1.15E−08	2.48E−08	2.33E−09	2.11E−07	2.87E−06	1.26E−09	7.82E−10	2.74E−10	2.87E−06	0.93
Tuzla FI	BIH	2.85E−07	5.64E−07	1.74E−08	1.87E−08	8.85E−07	5.46E−06	1.65E−07	3.18E−10	9.68E−09	5.63E−06	0.86
Tuzla HO	BIH	2.62E−07	1.37E−08	2.64E−08	1.73E−09	3.03E−07	4.83E−06	1.74E−09	4.34E−10	4.65E−10	4.83E−06	0.94
Tuzla MI	BIH	2.11E−07	1.09E−08	1.78E−08	2.27E−09	2.42E−07	2.98E−06	1.74E−09	2.12E−10	4.65E−10	2.98E−06	0.92
Tuzla ME	BIH	1.42E−07	5.98E−09	3.76E−08	4.05E−09	1.90E−07	1.95E−06	2.02E−09	1.24E−10	4.63E−10	1.95E−06	0.91
Novi Sad CA	SER	4.87E−08	2.01E−08	6.71E−08	5.43E−09	1.41E−07	3.49E−07	3.33E−10	2.07E−10	6.47E−11	3.50E−07	0.71
Novi Sad KG	SER	4.98E−08	4.80E−08	6.73E−08	4.98E−09	1.70E−07	3.69E−07	2.00E−10	0.00E+00	6.47E−11	3.69E−07	0.68
Novi Sad NIS	SER	5.45E−08	1.86E−08	6.35E−08	4.05E−09	1.41E−07	3.04E−07	2.00E−10	0.00E+00	9.70E−11	3.04E−07	0.68
Pancevo CH	SER	1.10E−07	1.80E−07	7.78E−08	8.92E−09	3.77E−07	3.45E−08	1.40E−09	1.55E−10	0.00E+00	3.60E−08	0.09
Pancevo NIS	SER	1.01E−07	1.67E−07	7.13E−08	8.08E−09	3.48E−07	3.00E−07	4.90E−08	3.35E−08	9.70E−11	3.82E−07	0.52
Pancevo PE	SER	4.07E−08	1.03E−08	6.37E−08	5.80E−09	1.20E−07	2.81E−07	5.50E−10	3.10E−10	4.85E−11	2.82E−07	0.70
Kragujevac ZG	SER	7.39E−08	3.97E−06	3.25E−07	2.97E−08	4.40E−06	2.56E−07	4.85E−08	0.00E+00	2.20E−09	3.07E−07	0.07
Kragujevac ZF	SER	9.53E−08	2.70E−07	1.21E−07	4.85E−09	4.91E−07	3.32E−07	8.13E−09	1.03E−10	1.04E−09	3.41E−07	0.41
Kragujevac UK	SER	6.34E−08	1.07E−08	1.16E−07	1.88E−09	1.92E−07	1.97E−07	5.33E−10	1.03E−10	0.00E+00	1.97E−07	0.51
Kosetice	CZE	1.11E−08	4.78E−09	2.65E−08	3.17E−09	4.55E−08	3.14E−08	5.67E−10	2.69E−10	1.89E−10	3.24E−08	0.42

Partial risk levels for the gas phase associated chemicals (GF) and the particle-bound chemicals (PUF) are presented separately showing also contributions of the individual groups of compounds.

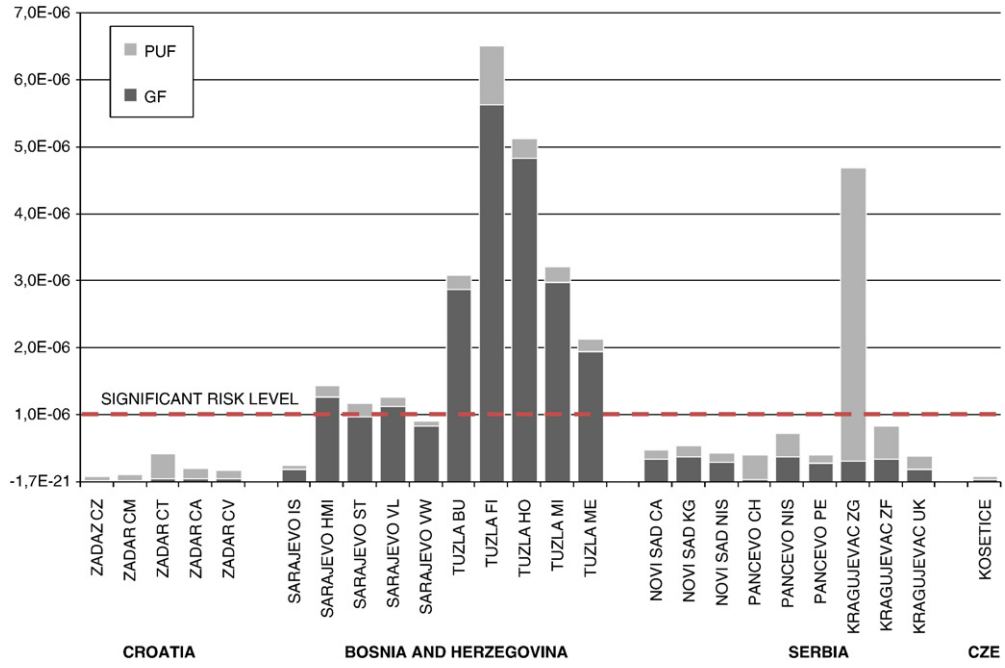

Fig. 1. Contributions of the gas phase- and particle phase-associated chemicals to overall health risks at the individual sampling sites.

increased the atmospheric concentrations of PCBs and significantly contributed to the gas phase associated overall health risks (Figs. 1 and 2). On the other hand, new results confirmed previously published findings on elevated levels of atmospheric PAHs and toxicity of the high volume air samples from Bosnia and Herzegovina (Skarek et al., 2007). In this study, between 83 and 94% of the cumulative risk was assigned to particle-associated compounds, and out of it, PAHs were responsible for 99% (Fig. 2). This is not surprising considering that majority of the higher molecular weight PAHs are particle-bound (Rehwagen et al., 2005; Omar et al., 2006; Cincinelli et al., 2007), and that 5 and 6-ring PAHs manifest the highest cancer potency. This also corresponds to the highest potency factors of individual PAHs relative to benzo(a)pyrene as a most active compound in PAH mixture (WHO, 2001). PAHs are among the substances of a great interest as they appear to be significant contributors to the genotoxicity and carcinogenity of air pollution in the urban environments. Oxidative stress has emerged as a mechanism that underlies the toxic pulmonary effects of atmospheric particles. Experimental evidence (Ohyama et al., 2007) showed that redox-active transition metals, redox-cycling quinoids and PAHs contained in aerosols act synergistically, producing reactive oxygen species leading to oxidative DNA damage. Current evidence also indicates that PAHs are transformed enzymatically to active metabolites that react with DNA to form adducts that result in mutations (Ran et al., 2008).

It can be concluded that inhalation exposure to OCPs does not represent a significant risk to humans in the Western Balkans while PCBs re-volatilized to the atmosphere from contaminated soils and buildings can pose a problem at certain sites. Elevated atmospheric concentrations of PCBs were found at the sites of war destructions (Zadar, Pancevo, and Kragujevac), but PCB evaporation and leakage from

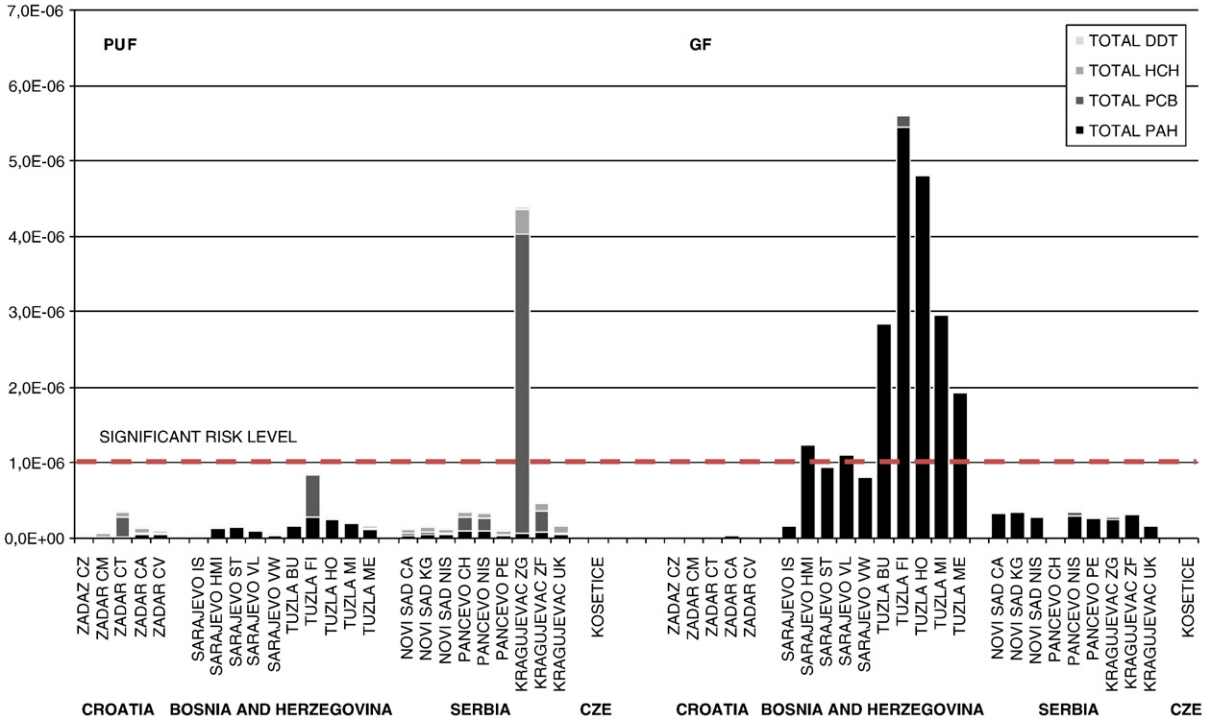

Fig. 2. Contributions of the individual groups of POPs to overall health risks at all sampling sites.

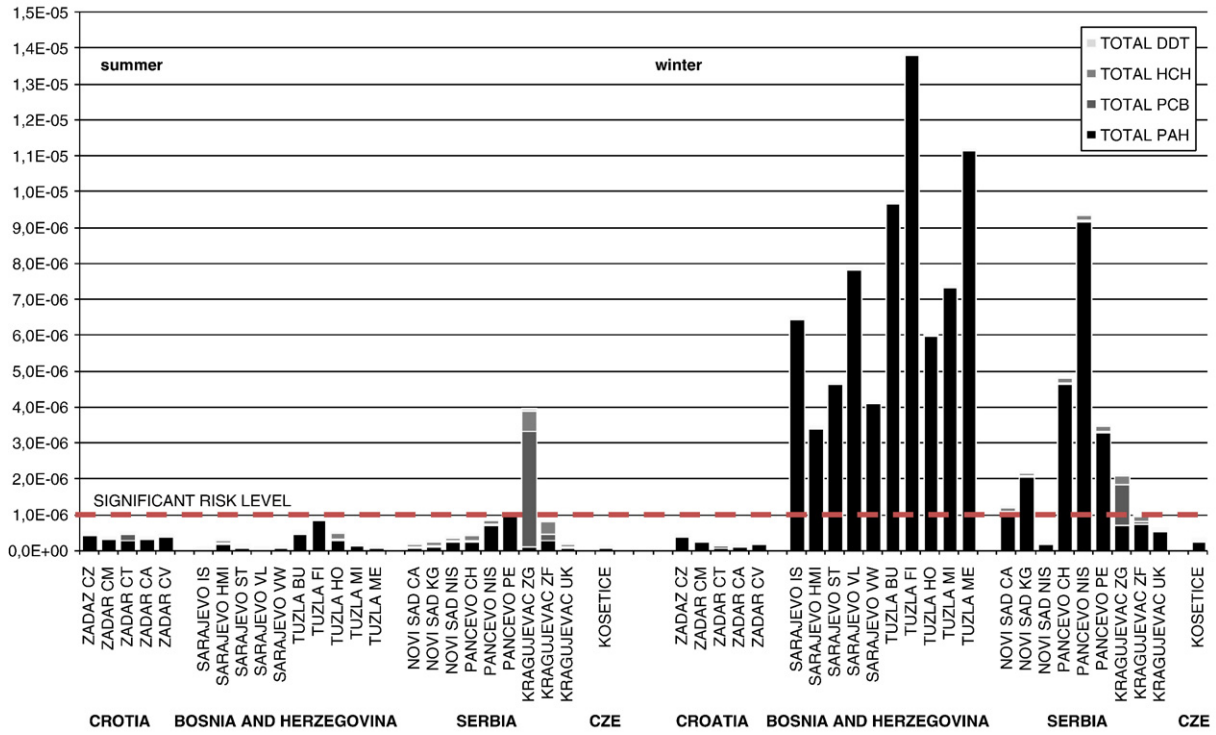

Fig. 3. Contributions of the individual groups of POPs to overall health risks in summer and winter season.

currently used PCB-filled transformers or from the non-adequate storage facilities for the old and damaged equipment resulted in much higher atmospheric levels (Tuzla, Kragujevac). A special attention deserves PAH contamination which was responsible for a majority of human health risks in Bosnia and Herzegovina. Significant risk level was exceeded at most of the sites in this country and the fact that some 90% of the risks were assigned to particulate matter indicated serious contamination with atmospheric particles.

Zencak et al. (2007) suggested recently that non-regulated incineration can significantly affect PAH emissions in the Western Balkans. According to their study on the source apportionment of the atmospheric contaminants in this region the biomass burning was responsible for 50–60% of the atmospheric PAHs both, in the summer and winter seasons. Improper waste management, common burning of waste, wood and other non-fossil fuels for heating purposes as well as frequent forest fires contribute to this high percentage. It is to be expected that such non-regulated incineration also generates great amounts of particulate matter and can be responsible for elevated human health risks found in this study.

3.2. Seasonal variability

When estimating the overall human health risks at various sites it has to be considered that all air samples were collected between May and June, i.e. during the season with lowest emissions from various combustion sources. Since the atmospheric concentrations of PAHs are a subject of seasonal variability it has to be expected that the risk levels found in this study (and already exceeding the safety limits) can be another two orders of magnitude higher in winter.

To assess a seasonal variability of the risk levels at the sites of interest, passive sampling technique was applied. Passive air sampling proved to be the most relevant technique when addressing the seasonal trends since it provides the averaged concentrations integrated over the sampling period. In the other hand, a main disadvantage is a low efficiency of passive sampler in sampling the particulate matter. Based on the field calibration of the passive sampler at the background monitoring station it has been estimated that the gas phase chemicals are sampled with the rate of 7 m^3 per day, while the same rate is only 0.7 m^3 per day for the particle-bound substances (Klánová et al., 2008). Such sampling rate for the atmospheric particles is of course just empirical value that may not be generally applicable since it probably varies from site to site according to specific size and material distribution of the particulate matter. It also differs among the individual compounds because the chemicals with higher vapor pressure can partition between the particles and the gas phase where they are a subject of higher sampling rates. Passive sampling efficiency for such chemicals must be highly variable because it depends not only on the physicochemical properties of the compounds but also on the meteorological parameters.

While the high volume air sampling campaign organized in early summer lasted only 3–5 days at each site, passive air sampling campaign lasted 5 months between July and December. To derive the values of inhalation risk, PAS data were transferred to the atmospheric concentrations using different rates for the gas phase- and particle phase-associated chemicals as cited above. Results for the summer (left) and winter (right) seasons were compared in Fig. 3.

Theoretically, the left part of Fig. 3 (summer risk levels) should be identical to Fig. 1 (substances connected to the gas and particle phases cannot be separated in this case) but it is not in reality. Results of the active and passive sampling campaigns were of course comparable in Croatia since the atmospheric pollutants at these sites were associated with the gas phase and could be sampled effectively by passive samplers. Also the Serbian sites gave similar information for both sampling techniques: risks were split between PCBs and PAHs at all sites except for Zastava Kragujevac where PCBs were responsible for the risk exceeding a level of significant risks 4 times. Risks based on the passive air sampling technique at the sites in Bosnia and Herzegovina were, however, quite different from the values derived from the high volume campaigns. They corresponded much better to the results from Fig. 2 (left) showing the gas phase associated chemicals only. This indicates that the efficiency of PAS in sampling of the particulate matter was much lower than estimated for the background sites (Klánová et al., 2008). Particle-bound PAHs (contribution of which is critical for determination of total risks) were found at very low concentrations, and often even below detection limits which caused the underestimation of the risk values.

Keeping in mind that risks derived from passive air samplers are underestimated, we can still assess the seasonality. As can be seen from Fig. 3, PAH related human health risks increased by several orders of magnitude between the summer and winter seasons indicating that winter inhalation exposure can significantly affect the health of the population.

3.3. Uncertainties of the risk estimations

We have to be aware of the uncertainties of our results and the possibility of underestimation or overestimation of the risk factors. Chronic cancer overestimation arises from the application of the EPA's unit risk values based on 95% upper confidence limits of cancer slopes. On the other hand, health risks may have been underestimated due to the fact that inhalation was the only kind of exposure considered in this study. Ingestion exposure via food consumption, for instance, can be very significant exposure scenario for both, organochlorine pesticides and PCBs. Application of the simple additive model avoiding all chemical interactions and synergic effects is yet another source of potential errors.

Most important, however, is a fact that this analysis is based on the number of high volume samples collected from each site within a period of several weeks. Such sampling design does not assure collecting typical samples representing the site and season. This may neglect potential temporal variations that can significantly influence continuous exposures over a 70-year lifespan (Kim et al., 2002).

To reduce a risk of such failure, a feasibility of deriving information on the seasonal variability from the passive air measurements was explored in this study. Previously published sampling rates for the gas and particle phase associated compounds were applied when calculating the atmospheric concentrations and associated risks. Since the particle-bound substances usually represent only a minor part of the total air

concentrations, their underestimation due to the sampling technique did not cause much of a problem for estimation of total PAH concentrations. In case of their application for the risk assessment, however, they led to serious bias in the risk values. As the particle-associated PAHs play a dominant role in the health effects, the underestimation of their total concentrations resulted in underestimation of the human health risks by an order of magnitude. Therefore, passive air samplers alone are not fit for the risk assessment of low-volatility compounds and should always be combined with active air measurements. Even with these limitations, however, they can provide valuable information on seasonal fluctuations at the sites where continuous active air monitoring is not feasible.

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Příloha 10

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Monitoring of persistent organic pollutants in Africa. Part 1: Passive air sampling across the continent in 2008

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A 6-month air sampling survey was conducted at 26 sites across the African continent with the aim to establish baseline information on contamination of ambient air with persistent organic pollutants (POPs) as a reference for future monitoring programs in the region. Sampling sites included continental, rural and urban backgrounds, agricultural and industrial sites as well as waste and obsolete pesticide dumps. Concentrations of polycyclic aromatic hydrocarbons, polychlorinated dibenzo-*p*-dioxins and furans, polychlorinated biphenyls and organochlorine pesticides were low at most of the rural background sites, but they raise some concern in big cities. The large temporal variability in the pesticide concentrations suggested seasonal application of γ-HCHs and endosulfans; levels of *p*,*p*′-DDT were often much higher than those of *p*,*p*′-DDE and indicated recent application of DDT.

Introduction

The history of application of persistent organic pollutants (POPs) in the African countries dates back to the 1940s and it is closely connected to pesticides. They were used in the agricultural production of food crops such as maize, sorghum and millet as well as cash crops for export such as cocoa, rubber, cotton and timber. They were applied also for disease vector control, especially for mosquito (malaria) and tsetse fly (trypanosomiases).[1] POP pesticides have been generally imported but pesticide formulation plants exist in many countries. 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl) ethane (*p*,*p*′-DDT), endosulfan, chlordane, lindane (γ-HCH), heptachlor, toxaphene, hexachlorobenzene (HCB) and aldrin were identified as the most frequently used pesticides. The Republic of South Africa, Nigeria, Ivory Coast, Kenya, Ethiopia, Ghana, Sudan, Tanzania, Egypt, Algeria, Mozambique, and Mali were among the top users. The most serious problem the African region faces is an issue of stocks and reservoirs of obsolete POP pesticides. It has been estimated that there might be more than 40 000 metric tons of these chemicals stocked or discarded over the African continent.[1,2] In addition, banned pesticides continue to be used in practice without any control of the authorities.

DDT stands for a very sensitive problem in nowadays Africa. Based on the reports from the Global Environment Facility, the global production of DDT for vector control was estimated to be 6269 metric tons in 2005.[3] DDT is currently being produced in two countries; India and China. The production in India was estimated to be 4250 metric tons of active ingredient in 2005 based on information on the domestic use of DDT for vector-borne disease control alone. China has produced a total amount of 4458 metric tons of DDT between 2003 and 2005, 55% of which was used as an intermediate in the production of dicofol or as an additive for production of anti-fouling paints. The remaining 45% were exported to the South Africa, Ethiopia, Eritrea, Namibia and Djibouti. DDT is being formulated in Ethiopia and South Africa from the ingredients imported from China. South Africa further exports some of the formulated material to other African countries. Ethiopia, Mozambique, Zambia and Zimbabwe reported recent increases in DDT use. Various sources indicate that at least seven other countries are considering re-introduction of DDT for disease vector control. Indoor residual spraying of insecticides (IRS) is expanding in

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Environmental impact

This is a first comprehensive study reporting levels of persistent organic pollutants (POPs) in the atmosphere over the African continent. Baseline atmospheric levels of POPs at the variety of background sites (continental, rural, urban, and industrial backgrounds) were established during 6 sampling campaigns covering a period of 6 months. At the same time, strong primary and secondary sources of the atmospheric pollution were identified. Such results are important for global inventories of POPs, and for development of the Global monitoring plan under the Stockholm Convention on POPs. All these findings should be considered not only when planning future monitoring programs but also when preparing the national regulations, and implementation plans of the international conventions.

Africa and more countries are considering the introduction of IRS in the future. Pilot IRS programs have already started in Uganda and preparatory work is being conducted in Malawi, Cameroon and Nigeria.

Regarding production of other POPs, especially polychlorinated biphenyls (PCBs), polychlorinated dibenzo-*p*-dioxins and furans (PCDDs/Fs), and polycyclic aromatic hydrocarbons (PAHs), industrial production and generation of electric power have been used as criteria to rank the countries. Based on this evaluation, the countries releasing the largest POP emissions were Egypt, South Africa, Nigeria, Zimbabwe, Ghana, Kenya, Democratic Republic of Congo, Zambia, Ivory Coast, Sudan, and Cameroon.[1,2] Exhaust emissions from combustion of fossil fuels in vehicles and electric power generators are probably the main source categories for PAHs. Burning of domestic, hospital, and industrial waste may be a dominant source of dioxins and furans but this is the least known factor in production of POPs in Africa. Based on the population figures (2001 estimates), the largest potential emitters of dioxins from the waste burning are Egypt, Nigeria, Ethiopia, Democratic Republic of Congo, South Africa, Algeria, Tanzania, Sudan, Kenya, Uganda, Ghana, and Mozambique.

There are no consistent monitoring programs focused on POP levels in air, human tissues and other environmental matrices in Africa. One of the few active air sampling campaigns in Africa was performed in the Lake Victoria watershed. High volume air samplers were stationed at two Ugandan locations and samples were analyzed for organochlorine pesticides (OCPs), currently used pesticides (CUPs) and polychlorinated biphenyls (PCBs). PCBs had the highest mean concentrations ranging between 30 and 1729 pg m^{-3}, and they were detected in all samples. Levels of DDTs ranged from 7 to 733 pg m^{-3}. Other detected POPs included (maxima in pg m^{-3}): aldrin (1.6), dieldrin (109), endrin (22), HCB (10), heptachlor (9), mirex (0.7), α-chlordane (113) and γ-chlordane (3.7). α-endosulfan (maximal concentration of 247 pg m^{-3}) and γ-HCH (220 pg m^{-3}) were the most abundant compounds from the group of currently used pesticides.

The first long-term measurement of POP levels in ambient air was performed at four sampling sites of the Global Atmospheric Passive Sampling (GAPS) study.[4] Levels of OCPs, PCBs, and polybrominated diphenyl ethers (PBDEs) in the passive air samples (PAS) from 2005 collected quarterly (January-December, seasons I–IV) were published recently.[4] PCBs were detected only in the first two sampling periods in Ghana (35 pg m^{-3} in period 1) and the Republic of South Africa (252 pg m^{-3} in period 1, 50 pg m^{-3} in period 2). Endosulfan I and II were the most abundant pesticides found in the samples. Their levels were lowest in period 1 when they were detected only in Ghana (564 and 358 pg m^{-3} for endosulfan I and II, respectively) and the Republic of South Africa (325 and 23 pg m^{-3} for endosulfan I and II, respectively). On the contrary, they were detected at all sites and their concentrations were highest in period 2 (3 712 and 1 417 pg m^{-3} for 1 and 2 in Ghana, 162 and 10 pg m^{-3} for I and II in Malawi, 305 and 12 pg m^{-3} for I and II in Kalahari, and 182 and 4 pg m^{-3} for I and II in the Republic of South Africa). *p*,*p*′-DDT was not detected at any of the sampling sites and *p*,*p*′-DDE was detected only in South Africa in periods 1 and 2 (2–44 pg m^{-3}). HCHs also had higher concentrations in the first two sampling periods: α-HCH was most abundant in period 1 in the Republic of South Africa (114–117 pg m^{-3}), γ-HCH was found in all four periods in Ghana (44–95 pg m^{-3}) and three periods in South Africa although the levels were lower than those of α-HCH (10–68 pg m^{-3}). Among the remaining pesticides, dieldrin was detected in Ghana (10–37 pg m^{-3} in periods 1, 3 and 4), Malawi (141 pg m^{-3} in period 2) and the Republic of South Africa (16 pg m^{-3} in period 2). Chlordanes were found occasionally (especially in period 2) at the picogram per cubic meter levels. XAD-based PAS were deployed at three African sites in 2005 and four sites in 2006. By sampling air for one year, XAD resin-based PAS provided annually averaged concentrations of organic pollutants. Detected concentrations were similar to the ones measured using PUF-PAS.

Based on their unquestionable advantages, passive samplers were recommended by the Technical Working Group for the Global Monitoring Plan of the Stockholm Convention as a suitable tool for the global monitoring of POPs in ambient air. The sampling campaign reported here was conducted between January and June, 2008 with the aim to screen POP levels across Africa. Based on the pilot survey, a new monitoring network addressing long-term trends of the continental and intercontinental backgrounds is proposed. The design of this network is introduced in a companion paper.[5]

Methods and materials

Sampling sites

Sampling sites for the MONET_AFRICA project have been selected in cooperation with the local partners in all 15 participating countries (Table 1). Each country was represented by one site, background site was preferred wherever possible as a potential candidate for the background monitoring for the effectiveness evaluation of the Stockholm Convention. In Mali and Kenya, four additional sites were selected to investigate the gradient of pollution. Samples from more than one site were also collected in Ghana and the Republic of South Africa.

Air sampling

Passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5 cm thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in the protective chambers were employed in this study. The theory of passive sampling using similar devices was described elsewhere.[6,7] The sampling chambers were prewashed and solvent-rinsed with acetone prior to installation. All PUF disks were prewashed, cleaned (8 hours extraction in acetone and 8 hours in dichloromethane), wrapped in two layers of aluminium foil, placed into zip-lock polyethylene bags and kept in the freezer prior to deployment. Exposed disks were wrapped in two layers of aluminium foil, labeled, placed into zip-lock polyethylene bags and transported in cooler at 5 °C to the laboratory where they were kept in the freezer at −18 °C until the analysis. Field blanks were obtained by installing and removing the PUF disks at all sampling sites.

One passive air sampler was exposed at each sampling site and filters were exchanged every 28 days for six months, from January to July. These six disks were used to assess temporal variability in the air concentrations of PCBs, HCHs, DDTs,

Table 1 List of the passive air sampling sites in Africa (from north to south)

			COORDINATES				METEOROLOGY		
SAMPLING SITE	COUNTRY	CODE	LATITUDE	LONGITUDE	ALTITUDE	SITE TYPE	Temp. (°C)	Humidity (%)	Precip. (mm) Rain (days)
Tunis	Tunisia	TUN	36.839278	10.219333	3	Urban background	12.8–24.9	65–83	0–57 0–12
Cairo	Egypt	EGY	30.071142	31.315089	50	Urban industrial	12.6–29.3	41–60	0–10 0–6
Khartum	Sudan	SUD	15.564167	32.513639	390	Urban industrial background	23.1–35.1	18–57	0–3 0–1
Tombouctou	Mali	MAL	16.731083	−2.997861	200	Urban background	19.1–35.7	16–33	0–9 0–1
Bamako Centre			12.630000	−8.022000	335	Urban, waste dump	22.8–31.4	23–74	0–111 0–9
Bamako Int. airport			12.533333	−7.950000	366	Suburban background	—	—	— 0–7
Koutiala			12.383333	−5.466667	366	Agricultural, cotton	22.3–32.5	30–75	0–174 0–10
Niono			14.283333	−5.133333	295	Agricultural, Niger basin	20–32	—	0–8 —
Dakar	Senegal	SEN	14.668097	−17.440486	441	Urban industrial	22.7–26.2	55–91	0–2 0–1
Sheda	Nigeria	NIG	8.881000	7.062167	229	Agricultural	—	—	—
Koumakonda	Togo	TOG	6.950000	0.616667	576	Agricultural background	26.4–29.4	53–90	0–246 0–13
Kwabenya	Ghana	GHA	5.676389	−0.186667	76	Urban background	—	70–89	— 19–31
East Legon			5.651944	−0.174167	77	Urban background	—	—	—
Asela	Ethiopia	ETH	7.950000	39.116667	2372	Urban background	16.8–18.9	25–44	0–94 0–17
Mt. Kenya	Kenya	KEN	−0.030000	37.220000	3678	Mountain background	—	—	—
Kabete			−1.249444	36.742500	1841	Urban background	—	—	—
Kitengela			−1.444549	36.988564	1525	Pesticide dumpsite	18.7–20.9	0–48	2–193 1–12
Industrial site			−1.306573	36.874924	1623	Industrial	—	—	—
Dandora			−1.243074	36.906166	1625	Municipal waste dump.	—	—	—
Orstom de Brazzaville	Congo	CON	−4.281250	15.243639	298	Urban	23.6–26.7	88–93	0–125 0–10
Kinshasa	DR Congo	DRC	−4.4186111	15.3086111	437	Urban	—	—	—
Lusaka IA	Zambia	ZAM	−15.316667	28.450000	1150	Urban background	16.9–27.3	—	— 0–25
Reduit	Mauritius	MAU	−20.233203	57.498492	310	Background	24.3–27.9	79–84	66–338 8–18
Molopo Nature res.	South Africa	SAF	−25.883333	22.883333	1001	Background	13.6–28.1	29–38	7–73 1–8
Barberspan			−26.533333	25.600000	1366	Background	—	—	—
Vanderbijl Park			−26.716667	27.883333	1454	Industrial	13.2–23.3	47–60	8–247 2–5

HCB, pentachlorobenzene (PeCB) and PAHs. Additional two samplers were deployed at one site from each participating country. One of these samplers was meant for analysis of PCDDs/Fs, the second for extended selection of pesticides (aldrin, dieldrin, endrin, heptachlor, isodrin, methoxychlor, mirex, *cis*-chlordane, *trans*-chlordane, nonachlor, endosulfan I and II). As most of these compounds were expected to be found at the low levels, these passive samplers were deployed for 3 months. It means that two samples (January–March, April–June) were collected from each site in six months, similar to the GAPS sampling scheme. 3-month samples were not available from Democratic Republic of the Congo and from the second sampling period in Zambia.

Weather conditions

Below average temperatures prevailed throughout the continent since January until February. While central and southern Africa continued to have lower temperatures until May, the northern part was up to 4 °C above the long-term average in March and April.

Precipitation was above average in January but below average since February until April throughout the continent. In May, the eastern parts of southern Africa were dryer than average, but the western parts were wetter. June was mostly dry, above average precipitation was only observed in the northern tropics from Senegal to the western Nigeria, in southern Sudan and South Africa. The rainy season in January brought floods to southeast Africa (Zambia, Zimbabwe, Malawi, Mozambique) as well as heavy rainfalls in March to the south-east of the continent (Namibia, Angola, Kenya and Tanzania), and in June to Kenya. Meteorological data measured at or near the sampling sites during the campaign are included in Table 1.[5]

Sample analysis

All 4-week samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. One laboratory blank and one reference material were analyzed with each set of ten samples. Surrogate recovery standards (d_8-naphthalene, d_{10}-phenanthrene, d_{12}-perylene for PAHs analysis, PCB 30 and PCB 185 for PCBs analysis) were spiked on each PUF disk prior to extraction. Terphenyl and PCB 121 were used as internal standards for polycyclic aromatic hydrocarbons (PAH), and polychlorinated biphenyls (PCB)/organochlorine pesticides (OCP) analyses, respectively. The volume was reduced after extraction under a gentle nitrogen stream at ambient temperature, and fractionation achieved on a silica gel column; a sulfuric acid modified silica gel column was used for PCB/OCP samples. Samples were analyzed using GC-MS (HP 6890-HP 5975) supplied with a J&W Scientific fused silica column DB-5MS for PCBs: PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, and OCPs: α-HCH, β-HCH, γ-HCH, δ-HCH, 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene (*p*,*p*′-DDE), 1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethane (*p*,*p*′-DDD), *p*,*p*′-DDT, *o*,*p*′-DDE, *o*,*p*′-DDD, *o*,*p*′-DDE, HCB, and PeCB. 16 US EPA polycyclic aromatic hydrocarbons were determined in all samples using GC-MS instrument (HP 6890-HP 5973) supplied with a J&W Scientific fused silica column DB-5MS. 3-month samples were spiked with the isotopically labeled standards and extracted with toluene. Extracts were cleaned-up on a silica column, fractionated on an alumina and further purified on a carbon column. PCDDs/Fs were analyzed using a GC-HRMS. GC-EI-MS/MS and GC-NCI-MS were applied for the analysis of pesticides.

Quality assurance/Quality control

Recoveries were determined for all samples by spiking with the surrogate standards prior to extraction. Amounts were similar to detected quantities of analytes in the samples. Recoveries were higher than 76% and 71% for all samples for PCBs and PAHs, respectively. Recovery factors were not applied to any of the data. For PCDDs/Fs, recoveries of the isotopically labeled standards were 51–74%. Recovery of native analytes measured for the reference material varied from 88 to 103% for PCBs, from 75 to 98% for OCPs, from 72 to 102% for PAHs. Laboratory blanks were under the detection limits for selected compounds. Field blanks consisted of pre-extracted PUF disks and they were taken on each sampling site. They were extracted and analyzed in the same way as the samples, and the levels in field blanks never exceeded 3% of quantities detected in samples for PCBs, 1% for OCPs, 3% for PAHs, indicating minimal contamination during the transport, storage and analysis.

Results

Concentrations of selected POPs in the air samples from six months (6 sampling periods between January and July of 2008, 28 days each) of the African screening campaign in 15 countries are presented in Fig. 1 and 2. Each country is represented by one site, industrial and waste dumping sites are not shown in the maps. All results are presented in Tables 2–6.

Extremely high levels of **HCHs** in PAS (median 2450, maximum 9310 ng sample^{-1} for the sum) corresponding to median air concentrations between 10 and 20 ng m^{-3} (the sampler deployed for 28 days collects the amount of chemicals equivalent to 100–200 m^3 of air)[8] were measured near the obsolete pesticide dump site in Kenya. At all the other sites (with the exception of one period in Tunis), concentrations were quite uniform with the background sites in Zambia, South Africa, Mauritius or Mali being at the lower end (below 5 ng sample^{-1}), and Egypt, Togo and Tunisia at the higher end. This range between 3.5 and 47 ng per sample means the atmospheric concentration between 35 and 470 pg m^{-3}. It corresponds to the GAPS results[4] where the sum of α- and γ-HCH ranged from traces to hundreds of pg m^{-3}.

A similar distribution was observed for **DDTs**. The highest air levels (median 2580 ng sample^{-1}, maximum 8970 ng sample^{-1} for the sum of *p*,*p*′- and *o*,*p*′-DDT, DDE and DDD) were again found at the Kitengela pesticide dump site. Hundreds of nanograms per sample were found at the sampling sites in Ethiopia, Mali and Senegal, the lowest concentrations (1–2 ng sample^{-1}) were measured at Mount Kenya, Tombouctou in Mali or the background sites in the Republic of South Africa. It means that most samples ranged between tens and hundreds of pg m^{-3} for the sum of DDTs while there were also few samples with concentrations above 1 ng m^{-3}. DDT levels in samples from background sites measured under the GAPS study were in tens of

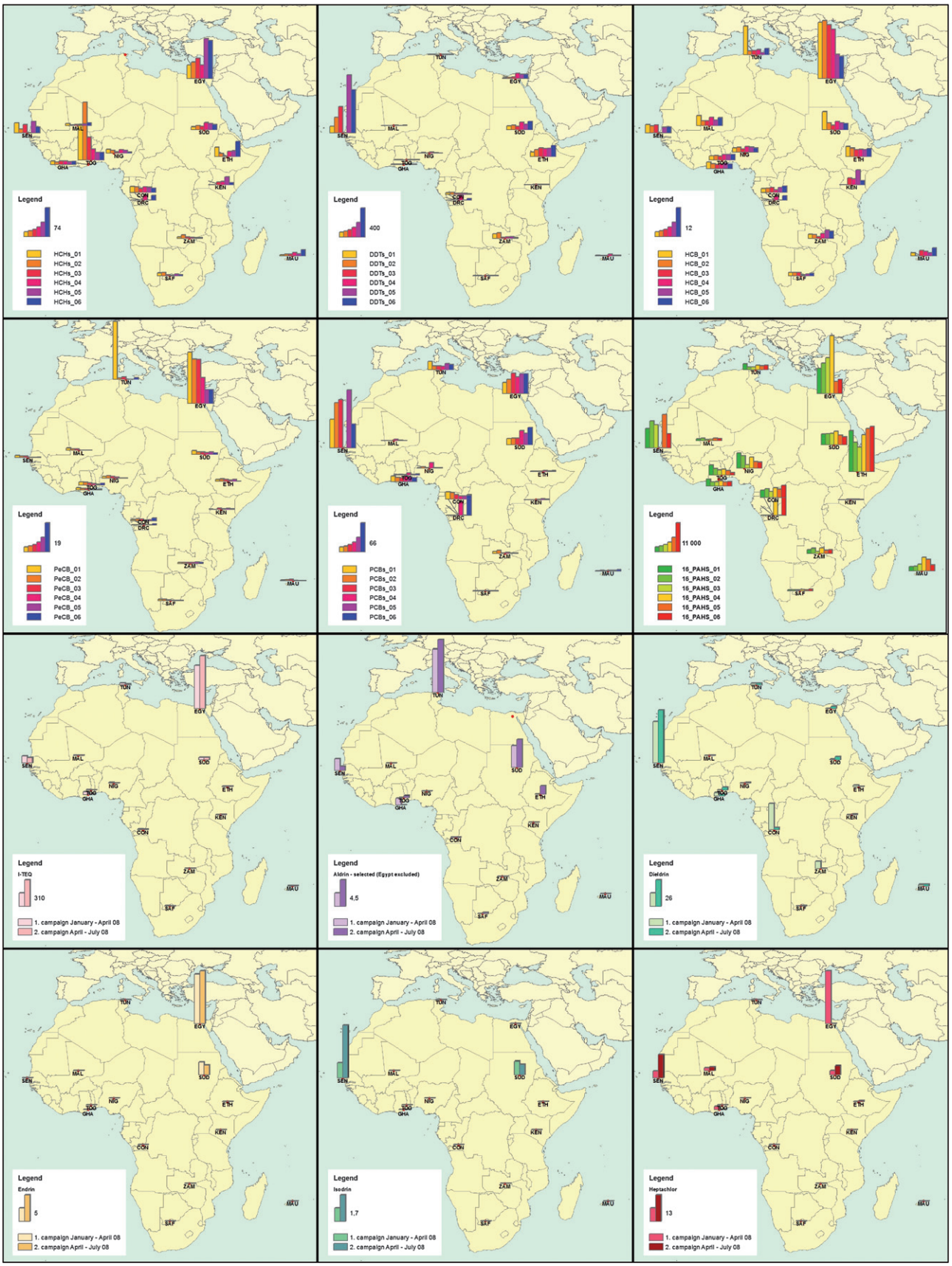

Fig. 1 POP levels (PAS, ng sample^{-1}) in ambient air of Africa. Sum of 4 HCHs (Tunis excluded), DDT including metabolites, HCB, PeCB, sum of 7 PCBs, sum of 16 PAHs, TEQ of PCDDs/Fs (pg sample^{-1}), Aldrin (Egypt excluded), Dieldrin, Endrin, Isodrin, Heptachlor, January-July, 2008.

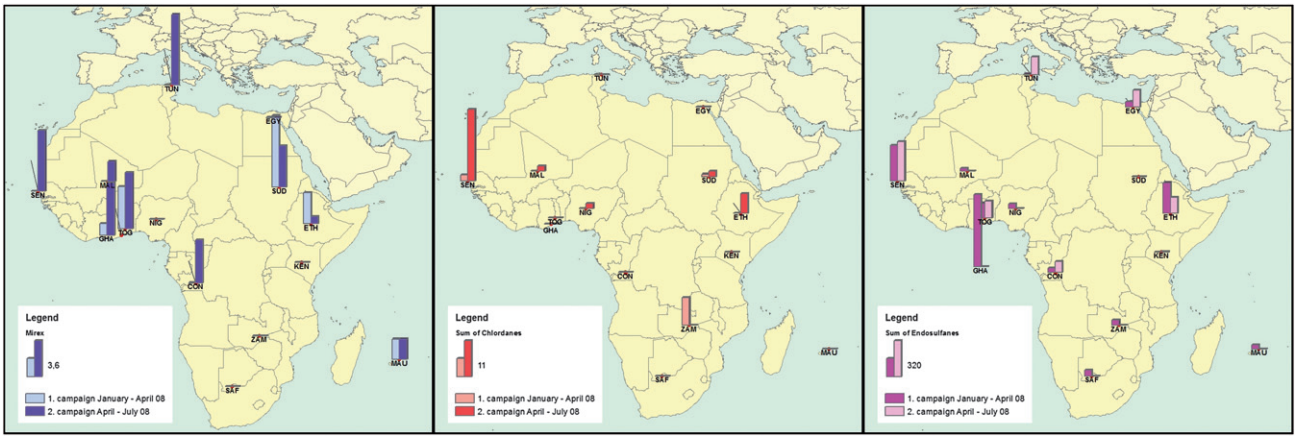

Fig. 2 POP levels (PAS, ng sample^{-1}) in ambient air of Africa. Mirex, sum of chlordanes, sum of endosulfanes, January–July, 2008.

pg m^{-3} which corresponds to the background levels found in the MONET_AFRICA study.

DDE/DDT ratio in ambient air was very variable between the countries. DDE was more abundant at the sampling sites in Congo, Ghana, Mali or Sudan while DDT had higher concentrations in Ethiopia or Zambia. In Senegal and Kenya, the levels of DDE and DDT were comparable; DDT prevailed in the summer samples from the sites in Kenya.

HCB concentrations in air were low and uniform except for Egypt where the levels were one order of magnitude higher than anywhere else (18 ng sample^{-1} with the maxima of 23 ng sample^{-1}). Levels of PeCB as a degradation product of HCB followed closely the pattern of HCB. Egypt showed PeCB concentrations one order of magnitude higher that all the other countries, but the Kitengela dumpsite was also quite high for PeCB.

The highest median **PCB** level was found in the passive air samples from Dakar, Senegal (104 ng per sample with the maxima of 132 ng for the sum of 7 indicator congeners). It means that PCB level measured in Senegal represented the ambient air concentration of 500 pg–1 ng m^{-3}. Higher median levels were also found in Cairo, Egypt (42 ng sample^{-1}), the industrial sites in Kenya (36 ng sample^{-1}) and Kinshasa, Democratic Republic of the Congo (30 ng sample^{-1} with the maxima of 102 ng). The lowest PCB levels (around 1 ng per sample) were measured at the background sites in Ethiopia, Mali, Mauritius, Kenya and South Africa, but also at the rural sites in Mali or Nigeria, while the urban sites in Congo, Ghana, Mali, Sudan or Tunisia had levels around 10 ng per sample. PCB levels from units to hundreds of ng per filter corresponded to the air concentrations between tens of pg and 1 ng m^{-3}. This is again in a good agreement with the

Table 2 Statistical evaluation of HCH and DDT concentrations

MIN–MAX (MEAN, MEDIAN)	HCHs ng sample^{-1}	DDTs ng sample^{-1}
Congo	10.2–14.8 (12.9, 13.4)	8.9–27.7 (16.9, 14.3)
DRCongo	6.8–16 (12.6, 13.8)	19.5–85.6 (44.7, 36.5)
Egypt	34.1–102 (60.3, 47.1)	10.6–70.1 (38.3, 38.9)
Ethiopia	1.2–38.5 (16.5, 13.9)	61.5–152 (108, 111)
Ghana Kwabenya	6.3–9.2 (8.1, 8.4)	4.5–6.1 (5.2, 5.1)
Ghana East Legon	3.3–17.7 (9.1, 7.9)	7.9–20.6 (12.1, 10.5)
Kenya Mt. Kenya	4.9–19.7 (9.9, 7.5)	1.3–2.4 (1.9, 2)
Kenya Kabete	4.6–30.9 (10.5, 7)	3.5–12.6 (7.7, 7.1)
Kenya Kitengela	1940–9 310 (4520, 4510)	1970–8970 (3980, 2580)
Kenya Industrial	1.7–18.8 (9.1, 8.3)	19.8–98.6 (54.2, 48.7)
Kenya Dandora	1.5–23.6 (14, 13.5)	14.7–40.1 (25, 20.1)
Mali Tombouctou	1.9–7.4 (4, 3.5)	0.6–3 (1.8, 1.8)
Mali Bamako Center	6.9–45.1 (22.3, 17.8)	68.6–164 (113, 101)
Mali Bamako Airport	4.9–29.4 (14.1, 9.1)	2.8–139 (37.7, 15.4)
Mali Koutiala	3.4–17.7 (10.4, 10.2)	23.4–74.1 (37.3, 28.8)
Mali Niono	3–7.2 (4.5, 4.3)	3.7–24.4 (10.9, 9.3)
Mauritius	2.3–15.6 (6.8, 4.9)	3.8–21.3 (9.3, 7.8)
Nigeria	3.6–10.1 (6.9, 7.5)	1.7–14.9 (6.4, 4.7)
Senegal	9.9–29.1 (20.1, 21.2)	92.7–797 (411, 360)
S. Africa Molopo Reserve	2–8.9 (4.7, 4)	1.1–3.1 (1.8, 1.5)
S. Africa Barberspan	2.3–12.3 (7.1, 4.9)	1–5.5 (2.9, 2.1)
S.Africa Vanderbijl Park	8.7–43.7 (23.4, 21.4)	1.5–6.5 (3.5, 2.5)
Sudan	7.5–18 (12.4, 12)	45.2–11.9 (75.3, 68.8)
Togo	19.7–147 (60.8, 43.3)	2.8–6.5 (4.6, 4.5)
Tunisia	16.8–17 014 (2 860, 34)	1.5–5.7 (3.4–3.4)
Zambia	2–9.3 (4.2, 3.5)	13.2–77.8 (36.5, 23.3)

Table 3 Statistical evaluation of HCB and PeCB concentrations

MIN–MAX (MEAN, MEDIAN)	HCB ng sample^{-1}	PeCB ng sample^{-1}
Congo	1–2.5 (1.7, 1.8)	0.3–1.7 (0.9, 0.9)
DRCongo	0.9–2.9 (1.7, 1.6)	0.1–0.8 (0.4, 0.3)
Egypt	9–23.4 (17.8, 20.9)	8.7–33 (20.7, 22.5)
Ethiopia	2.8–4.1 (3.2, 3.1)	0.1–1.5 (0.9, 1.1)
Ghana Kwabenya	1.7–2.6 (2.0, 1.9)	0.3–1.5 (0.8, 0.7)
Ghana East Legon	1.2–2.8 (1.7, 1.5)	0.1–1.2 (0.7, 0.8)
Kenya Mt. Kenya	1.6–6 (3.2, 2.5)	0.4–1 (0.6, 0.5)
Kenya Kabete	0.4–2.3 (1.5, 1.8)	0.1–1.2 (0.5, 0.3)
Kenya Kitengela	3.7–6 (5.2, 5.4)	6.6–13.8 (11.0, 11.4)
Kenya Industrial	3.6–4.6 (4.2, 4.2)	1.5–3.2 (2.2, 2.1)
Kenya Dandora	4.5–12.4 (7.9, 7.9)	2.6–13.0 (6.9, 6.0)
Mali Tombouctou	2.0–4.0 (2.8, 2.7)	0.1–1.5 (0.6, 0.5)
Mali Bamako Center	1.4–4.7 (2.6, 2.6)	0.3–3.3 (1.3, 0.9)
Mali Bamako Airport	1.2–2.9 (2.1, 2.2)	0.1–1.2 (0.6, 0.5)
Mali Koutiala	1.3–2.8 (2.0, 1.9)	0.1–1.7 (0.8, 0.6)
Mali Niono	0.9–3.2 (1.7, 1.6)	0.1–1.8 (0.6, 0.4)
Mauritius	0.1–3.2 (1.6, 1.5)	0.1–0.9 (0.4, 0.4)
Nigeria	1.2–2.2 (1.8, 1.8)	0.1–1.5 (0.7, 0.8)
Senegal	2.3–3.4 (2.8, 2.8)	0.1–1.4 (0.7, 0.9)
S. Africa Molopo Res.	0.3–1.3 (1.0, 1.1)	0.1–0.9 (0.4, 0.2)
S. Africa Barberspan	0.1–2.0 (1.1, 1.0)	0.1–0.9 (0.5, 0.5)
S.Africa Vanderbijl P.	1.4–3.8 (2.8, 2.7)	0.1–1.2 (0.6, 0.8)
Sudan	1.8–7.3 (3.4, 2.8)	1.0–2.4 (1.6, 1.5)
Togo	1.6–2.3 (2.0, 2.0)	0.3–1.9 (1.1, 1.2)
Tunisia	0.8–11.6 (3.3, 1.8)	0.1–37.4 (6.9, 1.0)
Zambia	0.8–3.4 (2.1, 1.8)	0.5–0.9 (0.7, 0.7)

GAPS study where PCB concentrations between 35 and 252 pg m^{-3} were found at background sites (there was, however, more than 7 indicator congeners analyzed in the GAPS study).

The highest levels of **PAHs** were measured at the urban background in Ethiopia (15.5 μg sample^{-1} corresponding to 80–150 ng m^{-3} for the sum of 16 EPA PAHs), at the industrial sites in Egypt (11 μg sample^{-1}) and Senegal (9 μg sample^{-1}), municipal dumpsite in Kenya or cotton growing region in Mali. Air levels were also higher at the urban sites in Congo and DRCongo, Ghana, South Africa or Sudan. Very clean samples came from Mountain Kenya, Tombouctou in Mali, and both background sites in South Africa.

It has to be noted that PAH compounds in ambient air partition between the gas and particle phases. As passive air samplers are designed to sample the gas phase only (although the finest particle fraction cannot be prevented from entering samplers), particle fraction in the atmosphere tends to be underestimated. It does not represent a significant error in terms of concentrations since the amount of particle-associated PAHs is usually an order of magnitude lower than the one of gas phase-associated PAHs. It can, however, cause a serious underestimation of the risks since the particle-bound PAHs are significantly more toxic.[9]

Table 5 Statistical evaluation of PCDD and PCDF concentrations

MIN–MAX	PCDDs pg sample $^{-1}$	PCDFs pg sample $^{-1}$
Congo	246–268	335–524
Egypt	5700–6550	30 600–38 300
Ethiopia	80–102	205–286
Ghana	373–1070	808–2 150
Kenya	22	25
Mali	15–65	3–52
Mauritius	52–78	63–87
Nigeria	39–286	554–1 200
Senegal	2220–3430	4260–7310
South Africa	23–31	22–23
Sudan	630–1 130	923–1290
Togo	46–210	103–259
Tunisia	368–917	440–1200
Zambia	82	92

Table 4 Statistical evaluation of PCB and PAH concentrations

MIN–MAX (MEAN, MEDIAN)	PCBs ng sample^{-1}	PAHs ng sample^{-1}
Congo	6.8–16.2 (10.3, 8.6)	2330–4750 (3390, 3340)
DRCongo	23.1–102 (39.1, 30.4)	4050–13 150 (6420, 5670)
Egypt	25.3–46.4 (38.8, 41.6)	4810–22 950 (11 640, 11 060)
Ethiopia	1.2–2.5 (1.8, 1.7)	9730–17 920 (14 550, 15 450)
Ghana Kwabenya	8.2–12.6 (10.1, 9.7)	1650–2790 (1950, 1750)
Ghana East Legon	6.9–20.3 (12.8, 11.4)	2970–4160 (3380, 3340)
Kenya Mt. Kenya	0.7–2.4 (1.4, 1.3)	145–212 (185, 191)
Kenya Kabete	1.6–2.8 (2.2, 2.1)	1570–2310 (1940, 2010)
Kenya Kitengela	3.0–5.8 (4.8, 5.0)	818–1320 (1120, 1180)
Kenya Industrial	11.7–71.3 (40.9, 36.0)	6210–8 450 (7150, 6810)
Kenya Dandora	22.2–34.7 (26.0, 24.7)	6740–9680 (8150, 8110)
Mali Tombouctou	0.7–3.2 (1.2, 0.8)	362–905 (688, 711)
Mali Bamako Center	14.9–26.1 (20.0, 19.4)	3400–6310 (5040, 5280)
Mali Bamako Airport	3.5–25.6 (12.0, 9.7)	1100–2790 (1730, 1620)
Mali Koutiala	2.0–5.0 (3.7, 3.9)	5830–16 280 (9260, 6780)
Mali Niono	0.9–2.8 (1.4, 1.2)	581–3460 (1890, 1590)
Mauritius	1.1–3.5 (1.8, 1.7)	1790–5430 (3130, 2530)
Nigeria	1.4–12.9 (3.7, 1.5)	1410–6030 (3670, 3550)
Senegal	54.3–133 (93.3, 104)	5670–13 290 (9280, 9150)
S. Africa Molopo Res.	0.8–6.1 (1.8, 1.2)	303–640 (423, 413)
S. Africa Barberspan	0.9–1.9 (1.4, 1.4)	233–564 (385, 402)
S.Africa Vanderbijl P.	1.0–4.5 (3.1, 3.1)	3520–7800 (5430, 5220)
Sudan	13.8–39.2 (23.6, 20.5)	3170–5270 (4230, 4330)
Togo	0.7–5.3 (2.4, 2.3)	993–3 980 (2310, 2200)
Tunisia	8.1–19.1 (11.7, 10.2)	1090–2360 (1610, 1640)
Zambia	1.5–7.9 (3.5, 2.5)	834–2180 (1620, 1580)

Table 6 Statistical evaluation of OCP concentrations

ng sample^{-1}	Aldrin	Dieldrin	Endrin	Isodrin	Heptachlor	Methoxy chlor	Mirex	Trans- chlordane	Cis- chlordane	Nonachlor	Endosulfan I	Endosulfan II
Congo	<0.6	2.2–26	<0.2	<0.3	<0.3	<1.4	<0.5–4.3	<0.2	<0.3	<0.8	40–69.5	<1.3–27.9
Egypt	<0.6–192	<0.2–2.2	9.4–10	<0.3	<0.3–26	<1.4	<0.5	<0.2	<0.3	<0.8	48–152	<1.3
Ethiopia	<0.6–1.4	<0.2–1.4	<0.2	<0.3	<0.3–0.6	<1.4	0.7–3.1	<0.2–0.9	<0.3–3.9	<0.8–1.4	99.6–190	44–85
Ghana	<0.6–1.1	2.2–3.3	<0.2	<0.3	<0.3–1.5	<1.4	1.1–5.2	<0.2	<0.3	<0.8	<0.8–450	<1.3–192
Kenya	<0.6	<0.2–0.5	<0.2	<0.3	<0.3	<1.4	<0.5	<0.2	<0.3	<0.8	<0.8	2.3
Mali	<0.6	<0.2–0.3	<0.2	<0.3	1.3–1.9	<1.4	<0.5–2	<0.2	<0.3	<0.8–1.6	<0.8–24	<1.–3
Mauritius	<0.6	1.4–1.7	<0.2	<0.3	<0.3	<1.4	2	<0.2	<0.3	<0.8	<0.8–35.2	<1.3
Nigeria	<0.6	<0.2–0.8	<0.2	<0.3	<0.3	<1.4	<0.5	<0.2	<0.3	<0.8–1.7	<0.8–33	<1.3–11
Senegal	0.7–2	41–52	<0.2	1.0–3.5	3.4–11.6	<1.4–42.2	<0.5–6.1	<0.2–2	<0.3–17.9	<0.8–5	182–210	111–171
S. Africa	<0.6	<0.2	<0.2	<0.3	<0.3	<1.4	<0.5	<0.2	<0.3	<0.8	<0.8–52	<1.3
Sudan	3.7–4.8	0.6–3	1.8–2.4	0.7–0.9	2.1–4.5	4.5–33.7	4.1–7.2	<0.2	<0.3	0.8–1.8	<0.8	<1.3
Togo	<0.6	<0.2–3	<0.2	<0.3	<0.3	<1.4	4.2–5.6	<0.2	<0.3	<0.8	100–152	<1.3–33
Tunisia	7.4–9	1.3–1.3	<0.2	<0.3	<0.3	<1.4	<0.5–7.1	<0.2	<0.3	<0.8	5.5–88.7	<1.3–68.5
Zambia	<0.6	<0.2–7.2	<0.2	<0.3	<0.3	<1.4	<0.5	3.9	5	<0.8	46	4.2

PCDD/F contamination of the air samples from Cairo, Egypt was at least one order of magnitude higher than contamination of samples from any other site. It is connected to the industrial character of the site but especially to frequency of combustion and open burning processes. I-TEQ concentrations in two 3-months samples were 505 and 616 pg of I-TEQ per sample (providing that the theoretical sample volume was 300–600 m^3 in 3 months, these values should roughly correspond to the atmospheric concentrations around 1 pg of I-TEQ m^{-3}. Concentrations of PCDFs in these samples were almost half order of magnitude higher than concentrations of PCDDs. PCDD/F levels in the range between 10 and 100 pg of I-TEQ per sample were also found at other industrial (Senegal) and urban (Ghana, Sudan and Tunisia) sites in this study. On the contrary, the lowest levels of PCDDs/Fs (around 1 pg of I-TEQ per sample) were found at Mt. Kenya, in Mauritius, Zambia or Tombouctou. As in the case of PAHs, partitioning between the gas and particle phases plays an important role for PCDDs/Fs as well. It is expected that PAS-derived PCDD/F concentrations are somewhat underestimated. This, however, deserves further investigation.

A broad selection of pesticides was also analyzed in two 3-month samples. Great variability between the levels of the individual pesticides in 14 countries was found.

Aldrin was detected in Senegal, Sudan and Tunisia in the first campaign, in the second campaign also is Egypt, Ethiopia, Ghana, South Africa, and Togo. The only site with the high levels was Cairo, Egypt, where the concentration reached 191 ng sample^{-1} in the second sampling period while all the other sites were below 10 ng. **Dieldrin** was detected in Congo, Ghana, Mauritius, Senegal, Sudan, and Tunisia in the first campaign while they were found in all countries in the second period. The highest levels were measured in Senegal (52 ng sample^{-1}, 85–170 pg m^{-3}) and Congo (26 ng sample^{-1}, 45–90 pg m^{-3}). Under the GAPS study, dieldrin was detected in Ghana, Malawi and South Africa between 16 and 141 pg m^{-3}. **Endrin**, isodrin, heptachlor and methoxychlor were below the limit of quantification in most countries, they were found more frequently in the second sampling period. The only two countries where endrin was detected were Egypt and Sudan, levels in Egypt in the second period reached 10 ng sample^{-1}. Heptachlor had also the highest levels in Egypt (26 ng sample^{-1}) and Senegal (12 ng sample^{-1}). Isodrin and methoxychlor were only found in Senegal and Sudan: isodrin stayed always below 3.5 ng sample^{-1}, methoxychlor reached 42 and 34 ng sample^{-1} in Senegal and Sudan, respectively. In the first sampling period, **mirex** was detected in four countries only (Ethiopia, Ghana, Sudan, Togo) while it was found at most sites in the second period. The levels, however, stayed always below 7 ng sample^{-1}.

Sum of **chlordanes** (*cis*-chlordane, *trans*-chlordane and nonachlor) reached the highest levels in Senegal (23 ng sample^{-1}, 35–70 pg m^{-3}), Zambia (9 ng sample^{-1}, 15–30 pg m^{-3}) and Ethiopia (6 ng sample^{-1}, 10–20 pg m^{-3}). Concentrations of the individual compounds at background sites in the GAPS study were in units of pg m^{-3}, the highest sum was measured in Malawi (11 pg m^{-3}). **Endosulfans** (sum of I and II) were found at the highest levels from all pesticides: 645 ng sample^{-1} in Ghana (1 000–2 000 pg m^{-3}), 353 ng sample^{-1} in Senegal (600–1 200 pg m^{-3}), and 275 ng sample^{-1} in Ethiopia (450–900 pg m^{-3}). Their sum was over 100 ng sample^{-1} also in Egypt, Togo and Tunisia. This corresponds very well with

the GAPS study where up to 3 712 and 1 417 pg m^{-3} of endosulfan I and II, respectively, were detected in samples from Ghana. Similar to the GAPS study, the levels were always significantly higher in the second sampling period.

Discussion

The aim of this African survey was not only to screen current atmospheric levels of selected compounds but also to find most suitable sites for the monitoring programs at various levels. In order to address this question, sites have to be grouped according to pollution sources.

Continental backgrounds

Mt. Kenya was the sampling site in **Kenya**, 3600 m above the sea level. It could serve as a continental background for the region of western Africa as the only source of POPs at Mt. Kenya is long-range transport. Results confirmed the lowest concentrations of PCDDs/Fs and PAHs, but also DDTs and PCBs. Levels of HCB were uniform across Africa, and Mt. Kenya manifested results comparable to remaining sites. The α-HCH/γ-HCH ratio was variable with γ-HCH prevailing most of the time, *p*,*p*′-DDT was prevalent in some samples while *p*,*p*′-DDE in others. From the pesticides other than DDT and HCH, only traces of dieldrin and endosulfan were detected at Mt. Kenya.

Similarly, **Tombouctou in Mali** was a candidate for the continental background site representing eastern Africa. The site was located in the suburban area far from industrial activities. Median PCB concentration was the lowest in this survey, two orders of magnitude lower than the ones measured at industrial sites in Senegal, Egypt or Kenya. Levels were also low for HCHs and DDTs; PAH concentrations were the lowest after Mt. Kenya and two background sites in South Africa. Traces of dieldrin, heptachlor, mirex and chlordanes were detected, endosulfan was found to have the highest concentration from all measured pesticides. It was an order of magnitude higher than concentration measured at Mt. Kenya, but still more than an order of magnitude lower that the ones detected at other sites.

Molopo Nature Reserve and **Barberspan** were the background sites in **South Africa** with no industrial sources. Their results were almost identical. Levels of PCBs, HCHs and DDTs were comparable to those measured at Mt. Kenya or Tombouctou. For PCBs, the highest concentration was found in July (only the less chlorinated congeners were detected most of the time), for HCHs (γ-HCH higher than α-HCH) and DDTs (*p*,*p*′-DDT generally higher than *p*,*p*′-DDE) in January. Traces of aldrin and dieldrin, and somewhat higher levels of endosulfans were detected. Levels of PAHs and PCDDs/Fs were the lowest after Mt. Kenya.

Reduit represented the background of **Mauritius.** PCB and HCH, as well as HCB and PeCB levels were low, similar to other background sites in Africa. DDT concentrations were 3–5 higher than those typical for the background sites; it was more comparable to the suburban sites in Kenya or Mali. Traces of dieldrin and mirex were also detected together with endosulfan which was higher than HCHs or DDTs. Low levels of PAHs and PCDDs/Fs corresponded to the rural site not affected by industry or transport. The medical waste incineration or open waste burning did not seem to have a significant impact.

Kenya, Mali, and South Africa belonged to countries with more than one sampling site. In Kenya, remaining sites represented the urban background, industrial site, municipal waste dumpsite, and obsolete pesticide storage. Two agricultural sites, rural and urban backgrounds were sampled in Mali. Industrial site was investigated also in South Africa. Such design allowed for evaluation of the overall situation at the continent and assessment of an impact of various sources.

Rural and agricultural sites

Sheda was a site in the agricultural area of **Nigeria**. PCB levels were accordingly low except for the fourth period when they grew ten times. HCHs and DDTs were very low as well, with dominant γ-HCH, and variable *p*,*p*′-DDE/*p*,*p*′-DDT ratio.

Traces of dieldrin, chlordanes and higher levels of endosulfans were detected. PAH concentrations were several times higher than those measured at background sites but not very high. On the contrary, PCDD/F levels (resulting probably from the bush and waste burning) belonged to higher in this study.

The agricultural background area of **Togo** was sampled in **Koumakonda** where various pesticides were used for coffee and cocoa production. HCH levels were among the highest in this study (aside from the pesticide dumpsite in Kenya) and much higher in the first three months of the year. γ-HCH was responsible for this increase suggesting a fresh application of lindane. Similar concentrations were measured also for endosulfans while aldrin, dieldrin and mirex were only found in traces. PCB, PAH and PCDD/F concentrations were quite low.

Bamako international airport was the suburban site in **Mali**. PCB concentrations were at the urban levels, similar to HCH concentrations. γ-HCH was up to ten times more abundant than α-HCH. DDTs were elevated when compared to most of other countries and *p*,*p*′-DDE was higher than *p*,*p*′-DDT. HCHs and HCB followed the uniform distribution common in most countries, and PAHs were at the low level typical for sites not affected by massive combustion.

Koutiala was a rural site in the agricultural, cotton growing region of **Kenya** affected by pesticide application and the cotton oil factory. Levels of PCBs, HCB and PeCB were quite low but HCH and DDT concentrations belonged to higher ones. The mean PAH concentrations were the fourth highest (third maximum) in the survey. **Niono** was another agricultural site in Kenya affected by pesticide application. PCB concentrations were very low again, but the levels of HCHs and DDTs as well as HCB, PeCB, and PAHs were not very high either. At both sites, γ-HCH was more abundant than other isomers, and *p*,*p*′-DDE concentration was higher than that of *p*,*p*′-DDT. In general, none of rural and agricultural sites was heavily contaminated and they were all suitable for monitoring purposes.

Urban backgrounds

Kabete was the urban background site in **Kenya**, not far from Nairobi City. Possible sources of POPs included agricultural activities and car emissions. PCBs were slightly higher than at Mt. Kenya and so were DDTs and PAHs. On the contrary, HCHs and HCB were found at lower levels.

Tunis capital city represented the urban background of **Tunisia**. PCB and DDT concentrations corresponded to the urban character of the site but HCH levels were elevated. Extremely high concentration of HCHs (two times higher than those found at Kitengela pesticide storage in Kenya) with a high fraction of δ-HCH was measured in the first sampling period. From the group of other pesticides, aldrin, dieldrin, mirex and endosulfans (highest median level from all investigated pesticides) were detected. PAHs were low in Tunis but PCDDs/Fs were high (fifth highest I-TEQ).

There were two urban background sampling sites in **Ghana. Kwabenya** was the suburban residential area to the north-east of the Accra city center and close to the Akwapim Mountains. **East Legon** was the residential area close to Accra international airport. PCBs were found at the levels typical for the urban sites. Concentrations of HCHs and DDTs were only 2–3 times higher than those at background sites. Levels of HCB and PeCB were low, and aldrin, dieldrin, heptachlor and mirex were only detected in traces. While endosulfans stayed below detection limit in the first sampling period, their concentrations were the highest from all sites in the second period. PAH concentrations were again only 2–3 times higher than those at the backgrounds, PCDD/F level was, however, quite high: maximal I-TEQ was the third highest in this survey after Egypt and Senegal.

Asela was the urban background site to the south-east of Addis Abeba, **Ethiopia**. It is a residential area with no industrial or traffic emission sources. As it is 2 372 m above the sea level, possible sources of POPs include long-range transport and atmospheric deposition. PCB levels were very low, at the level of background sites. HCHs and DDTs were higher, detected median level of DDTs was the third highest from all investigated sites. DDT concentrations grew gradually from January to June and levels of *p*,*p*′-DDT were 2–4 times higher than those of *p*,*p*′-DDE. From the other pesticides, aldrin, dieldrin, heptachlor, mirex, chlordanes and endosulfans were detected. Level of endosulfans was the third highest after Ghana and Senegal. Median concentration of PAHs in Ethiopia was the highest measured in this study; it was 30 times higher than the one at background sites and even higher than the level in Egypt. Interestingly, PCDD/F levels were very low; the highest I-TEQ concentration was less than 1% of what was measured in Egypt.

The sampling site in **Lusaka** served as the urban background for **Zambia**. It was located close to the Zambia's largest international airport, far from industrial emissions. Levels of PCBs, HCHs, HCB and PeCB were low but DDT levels were higher than those found at most of the other sites. All chlorinated compounds had significantly higher concentrations in January and February than in the later months. More volatile PCBs and γ-HCH dominated, *p*,*p*′-DDT was 2–3 times more abundant than *p*,*p*′-DDE. Dieldrin, chlordanes and endosulfans were also detected in the air samples. Both, PAHs and PCDDs/Fs were measured at low concentrations.

Urban and industrial sites

The urban sampling site in **Egypt** was located to the south of **Cairo** at Eltebin Institute, in the vicinity of various industries (cement, steel and iron). 40% of the total amount of industrial facilities in Egypt can be found in the greater Cairo which is surrounded by agricultural land and receives winds contaminated with pesticides coming from the south. This sampling site was the most contaminated with PCDDs/Fs in the whole African survey. Maximal I-TEQ concentration was an order of magnitude higher than the second most contaminated site (Dakar, Senegal) and almost three orders of magnitude higher than background sites. Also PAH levels were high and confirmed severe influence of combustion on the air quality. Median PCB concentration was the second highest. HCH level was also the highest apart from the obsolete pesticide storage site in Kenya. α-HCH was found at concentration levels 2–4 times higher than γ-HCH in most of the sampling periods and *p*,*p*′-DDT and *p*,*p*′-DDE were comparable. Median levels of HCB and PeCB were at least an order of magnitude higher than any other investigated site. Even higher were the concentrations of aldrin and endosulfan. Dieldrin, endrin, heptachlor and mirex were detected as well.

Urban industrial site in **Dakar**, **Senegal** was also one of the most contaminated sites. The median level of atmospheric PCBs was the highest in the survey and concentrations of more chlorinated congeners (especially PCB 153) were significantly higher than those of more volatile PCB congeners. Also level of DDTs was very high, second highest after the obsolete pesticide storage in Kenya. *p*,*p*′-DDE and *p*,*p*′-DDT were found at the same concentrations. The second highest air concentration was measured also for endosulfans while aldrin, dieldrin, heptachlor, isodrin, methoxychlor, mirex and chlordanes were detected at lower levels. Median concentration of PAHs was the third highest after Ethiopia and Egypt, and maximum I-TEQ of PCDDs/Fs was the second highest after Egypt.

Bamako center was the urban sampling site with a waste dumping and open fire incineration near by. It was the most contaminated site in Mali. PCB levels were comparable to industrial sites in Kenya, Sudan or Congo. Higher chlorinated congeners (especially PCB 153) were again found at higher concentrations than more volatile congeners. HCH levels were also elevated; γ-HCH was up to ten times more abundant than α-HCH. DDT concentrations were the fourth highest measured in the survey (after the obsolete pesticide storage in Kenya, Senegal and Ethiopia) and *p*,*p*′-DDT was 2–4 times higher than *p*,*p*′-DDE. HCB and PeCB were detected in similar quantities as those measured at most of the other sites while PAHs were almost an order of magnitude higher. **Orstom de Brazzaville** in the **Republic of the Congo** was the urban site. Possible sources of POPs included stocks of obsolete pesticides, car emissions, sugar cane, cement and petroleum industries, as well as open municipal waste burning. PCB levels were quite high and comparable to other big cities. HCHs were elevated, *p*,*p*′-DDE concentration was somewhat higher than the one of *p*,*p*′-DDT. Dieldrin, mirex and endosulfan were detected in the air samples, both, dieldrin and endosulfan were found at similar or greater levels than HCHs and DDTs. PAH and PCDD/F levels were similar to other urban sites. The air samples from the **Democratic Republic of Congo** were collected in the **University of Kinshasa**, in the urban area with some industrial facilities. PCB levels were among the highest measured in this campaign. HCH concentration was similar to Congo (prevalent γ-HCH) but DDTs were almost three times higher (*p*,*p*′-DDT and *p*,*p*′-DDE were comparable). Median PAH levels were almost two times higher than in Congo.

Urban industrial background was also sampled in **Khartum, Sudan**. Concentration of PCBs was among the highest values, close to industrial sites in Kenya and Mali. Elevated levels were found for HCHs and even more so for DDTs. For all chlorinated compounds the levels in the last three months were about two times higher than those in the first three months. γ-HCH was up to one order of magnitude higher than α-HCH while *p*,*p*′-DDE was 3–5 times higher than *p*,*p*′-DDT. From the group of other pesticides, aldrin, dieldrin, endrin, heptachlor, isodrin, mirex and chlordanes were found at low levels, methoxychlor was about one order of magnitude higher. On the contrary, endosulfans were not detected. PAH concentrations were at the levels typical for urban sites but the fourth highest I-TEQ concentration was found indicating significant PCDD/F air pollution. It can be a result of combustion processes in households and small industries, emissions from the asphalt mixing station, road traffic, and frequent solid waste burning.

The Industrial site in **Kenya** was positioned in a heart of the industrial area. Main sources of POPs were supposed to be industrial activities, open burning of used tires and vehicle exhausts. PCB concentrations were the third highest found in this campaign. Also DDT and PAH contamination was high, an order of magnitude higher than backgrounds for DDTs and up to 30 times higher than backgrounds for PAHs. HCHs and HCB levels did not differ from other sites in Kenya.

Vanderbijl Park was the industrial site in South Africa. PCB levels were 2–3 times higher than those at the background sites in South Africa, DDTs were almost the same but HCHs were half order of magnitude higher with the highest levels between January and March. γ-HCH was up to one order of magnitude higher than α-HCH while *p*,*p*′-DDE/*p*,*p*′-DDT ratio was variable. Median PAH level was high, similar to the industrial sites in Kenya and Mali. Sources included the iron and steel manufacturing, petrochemical plant, power plant and coal mining in the vicinity.

Dumping sites

Kitengela was the obsolete pesticide storage in Kenya. For pesticides, it was the most contaminated site found in Africa. DDTs (2584 ng filter^{-1}) and HCHs (2451 ng filter^{-1}) were measured at concentrations 2–3 orders of magnitude higher than most of the other sites. Levels of α-HCH were similar or higher than those of γ-HCH, β-HCH reached only about 20% of their values. Most surprising were the levels of δ-HCH, 2–4 times higher that those of α-HCH or γ-HCH. *p*,*p*′-DDT and *p*,*p*′-DDE were found at similar levels in air but *p*,*p*′-DDT was an order of magnitude higher than *p*,*p*′-DDE in the soil samples. Levels of PCBs were quite low, PAHs were at the same level as at the other urban or rural sites. HCB was slightly elevated but level of PeCB was an order of magnitude higher than at any other site except for Egypt.

Dandora was the sampling site located close to the municipal dumpsite. Main source of POPs was expected to be a waste burning. Elevated levels of PCBs, HCHs and DDTs were found, HCH concentrations were several times higher in summer. Level of DDTs was also much higher in the warm months when *p*,*p*′-DDT dominated. Level of *p*,*p*′-DDT in soil was slightly higher than the one of *p*,*p*′-DDE but concentration of *o*,*p*′-DDT reached up to 60% of the *p*,*p*′-DDT concentration. For PAHs, the median air concentration was the fourth highest found in this survey.

Conclusions

The air samples from 26 sampling sites in 15 countries of the African continent were collected during the 6 months of the MONET_AFRICA survey. They ranged from continental, rural and urban backgrounds to contaminated sites heavily affected by industrial activities or old burdens. The levels of investigated chemicals have been shown to range over several orders of magnitude. A number of strong primary and secondary sources of the atmospheric pollution were identified. The industrial site in Cairo, for instance, introduced a problem of PCDD/F contamination of ambient air in this urban area of Egypt. Together with the Ethiopian site, the one in Egypt drew the attention also to extremely high atmospheric levels of PAHs associated with combustion processes. A site heavily polluted with PCBs, PAHs and PCDDs/Fs was also found in Dakar, Senegal. The Kitengela site in Kenya with very high atmospheric levels of DDTs, HCHs and HCB revealed a problem related to obsolete pesticide storages and dumping sites in Africa. Low *p*,*p*′-DDE/*p*,*p*′-DDT ratio at many sampling sites indicated the recent application of DDT. Several sites showed a month-to-month variability of the pesticide concentrations ranging over an order of magnitude and suggesting fresh applications, especially of the currently used pesticides, such as lindane or endosulfan. Although the atmospheric levels of the investigated organic pollutants were low in the continental and in the majority of rural backgrounds, it has to be noted that they may represent serious health risks at some of the urban sites. All these findings should be considered not only when planning future monitoring programs but also when preparing the national regulations, and implementation plans of the international conventions.

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Příloha 11

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Can pine needles indicate trends in the air pollution levels at remote sites?

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Pine needle monitoring is a feasible tool for an assessment of temporal trends in the atmospheric contamination.

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ABSTRACT

Data from ten years of integrated monitoring were used here to evaluate whether pine needles are a feasible tool for an assessment of long-term trends of the atmospheric contamination. Pine needles collected once a year were compared to high volume air samples collected for 24 h, every 7 days, and passive air samples integrated over 28-day periods. Results showed the same concentration patterns of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and organochlorine pesticides (OCPs) captured in needles and high volume samples. Passive air samplers were less efficient in sampling the particle-bound compounds. Theoretical air volume equivalent to each needle sample (V_{EQ}) was calculated as a ratio of the needle concentration over the mean air concentration. Results indicated different equivalent volumes for PAHs and organochlorines, possibly due to the faster degradation rates of PAHs in needles. The most important finding is that in the long term a needle monitoring gives very similar information on temporal trends of the atmospheric pollution as does a high volume air monitoring.

1. Introduction

Persistent organic pollutants (POPs) in the atmosphere are conventionally monitored by an active air sampling technique (Holoubek et al., 2007a). Various passive air sampling devices based on semipermeable membranes or polyurethane foam have been developed in the last years and utilized for spatial and time integrated monitoring of POPs (Bartkow et al., 2004; Farrar et al., 2005; Harner et al., 2006; Klánová et al., 2006). A monitoring of vegetation, however, remains a cheapest and best available tool for estimation of the atmospheric contamination levels at remote and poorly accessible locations like the high mountains. POPs as a group of compounds with a very low water solubility, high *n*-octanol–water partition coefficient, and low vapor pressure tend to accumulate in lipidic tissues. That includes a wax layer on the surface of plants, which can efficiently scavenge and accumulate POPs from the atmosphere over the long time periods (Viskari et al., 2000; Wyrzykowska et al., 2007) and in doing so, serve as an integrative passive air sampler.

Plants selected for such monitoring have to show a high affinity towards compounds of interest while tolerating high concentrations of these substances accumulated in their tissues (Guidotti et al., 2003). Most common matrices used as indicators of the atmospheric contamination (Holoubek et al., 2000; Di Guardo et al., 2003; Lehndorff and Schwark, 2004; Piccardo et al., 2005) are coniferous needles, along with lichens and mosses (Riget et al., 2000; Guidotti et al., 2003; Fuga et al., 2008). A surface of needles is covered by a 1–5 μm thick cuticle consisting of cutin (a polyester of di- and trihydroxy fatty acids) and cuticular wax (lipids, free fatty acids, *n*-alkanes, *n*-alkenes, primary alcohols, α,ω-diols, ketones, and ω-hydroxyacids) (Dolinova et al., 2004). The epicuticular wax layer is a barrier between the plant and its environment: it acts as a protective layer against desiccation and ultraviolet radiation and it is believed to play an important role in the plant defense against the bacterial and fungal pathogens (Kunst and Samuels, 2003).

An advantage of conifers as evergreen species is that – unlike broadleaf species – they can accumulate atmospheric pollutants for several years (Di Guardo et al., 2003). A major disadvantage is a poor characterization of a performance of such sampler. Many species with different uptake characteristics are used for the passive air sampling e.g. *Picea abies*, *Pinus nigra* (Di Guardo et al., 2003), *Pinus uncinata* (Grimalt and van Drooge, 2006), *Pinus pinaster* (Piccardo et al., 2005), *Pinus sylvestris* (Holoubek et al., 2000; Keymeulen et al., 2001) or *Cedrus deodara* (Chen et al., 2006). A sampling rate as well as an extent to which the plant sequesters certain chemicals depends on the plant species, age of needles, their structure (number and accessibility of resin channels, for instance) (Di Guardo et al., 2003), or a terpene content of the wax.

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The external stresses such as acid rains or mechanical abrasion, low humidity or a presence of moulds may cause important reductions or alterations of the wax layers similar to the natural process of the needle aging, and such needles exhibit much lower uptake rates. Moreover, a degradation and loss of the cuticle leads to the loss of previously accumulated compounds (Piccardo et al., 2005). Geographical and meteorological factors are influential, too. More volatile organochlorines as α-HCH, γ-HCH, aldrin and α-endosulfan positively correlated with the altitude, while DDTs were inversely correlated (Wang et al., 2006), and Ockenden et al. (1998) demonstrated that lower temperatures enhanced the accumulation rates of PCBs.

Pine needles are probably the most frequently used biomonitoring tool because needles from 1 to 3 years old are found at the same branch and their age can be easily determined (Piccardo et al., 2005). Physicochemical properties of investigated POPs and their ambient air concentrations in both, gaseous and aerosol phases (Di Guardo et al., 2003) are also important. Piccardo et al. (2005) investigated an accumulation of nine polycyclic aromatic hydrocarbons (PAHs) in pine needles of different ages (6–30 months). The bioconcentration of more volatile compounds (phenanthrene, anthracene, fluoranthene, pyrene) was more efficient than the one of less volatile PAHs (benzo(*a*)anthracene, chrysene, benzofluoranthenes, benzo(*a*)pyrene). Low molecular weight PAHs diffuse and accumulate more readily in the inner compartments, while particle associated chemicals very likely linger in the surface compartment where they are exposed to the effects of external environmental factors like temperature, solar irradiation or atmospheric ozone. Liu et al. (2006), however, manifested that pine needles accumulated higher molecular weight PAHs as well. A time-dependent accumulation behavior of organochlorines was also investigated in the field experiments with pine needles of different ages (4, 16 and 22 months) in the vicinity of various emission sources (Wenzel et al., 2000). The concentration increased with the age of needles for low chlorinated PCBs. For highly chlorinated PCBs, HCH isomers, DDT and its metabolites, however, the bioconcentration was not related to the age of needles. Explanation of this surprising result can be found in the work of Kylin and Sjodin (2003) who observed differences in accumulation behavior of HCHs and *p*,*p*′-DDT. He demonstrated that levels of POPs accumulated in needles are consequently a subject of seasonal fluctuations due to the annual cycle of terpene content in the wax with winter minima (1%) and summer maxima (10%). Observed seasonal variation of POP concentrations in needles was significantly larger than the overall accumulation. Underlying the prominent seasonal variation there still was a clear continuous accumulation of POPs which could, however, be easily overlooked if needles from several years were not analyzed in parallel (Kylin and Sjodin, 2003). This natural process could be responsible for the false conclusions about the rapid equilibria and exhausted hydrophobic capacity in older needles reported earlier. In fact, Kylin and Sjodin (2003) observed an accumulation of HCHs and *p*,*p*′-DDT in Scots pine during the entire life span of the needles and hydrophobic capacity did not seem to be exceeded at any time.

Even though needles are undoubtedly useful as an indicator of the atmospheric pollution, a quantitative data interpretation is complicated by the lack of comparative studies. A quantitative statement can be made if the same methods have been used. A variety of employed coniferous species and needle ages, sampling, analytical and reporting techniques, however, prevent a reasonable comparison of the results from various studies. A standardization of the methods and approaches is highly desirable. The biological processes in the plants further complicate the matter and cause such difficulties that we may never be able to calculate absolute air concentrations of the contaminants (Hellstrom et al., 2004) from their levels in needles. A question, whether these levels can be used for establishment of the long-term trends of atmospheric contamination, still has to be answered. To do so, a long-term study has to be designed where needles are collected and analyzed yearly, at exactly the same time of the year. Concentration levels and patterns have to be compared to the continuous air pollution data derived from the high volume air monitoring.

The attempts to compare the sampling efficiency of spruce needles (*P. abies*) to the results of active and passive air measurements have been made recently (Kirchner et al., 2006; Levy et al., 2007). The homologue patterns of polychlorinated dibenzo-*p*-dioxins and furans accumulated in 0.5, 1.5 and 2.5 year-old needles were compared to the ones in semipermeable membrane devices and high volume air samples. Needles were capable of the uptake from both, gas and particulate phases, and the fingerprint of PCDDs/Fs captured in needles correlated very closely with the one obtained from the active sampler. On the other hand, SPMDs appeared to be a very sensitive tool for lower chlorinated PCDDs/Fs due to their ability to sequester compounds from the gas phase. Such data are, however, not yet available on polyurethane foam (PUF) based devices that are capable of fine particle sampling (Klanova et al., 2008).

In the attempt to fill these gaps, data from the long-term integrated monitoring project in the background observatory Kosetice, Czech Republic (Holoubek et al., 2007a; Váňa and Holoubek, 2007) were analyzed. A multimedia sampling of ambient air (active and passive), wet deposition, surface water, sediment, soil, and biota, as the key components of the environmental system, has been performed since 1988 as a part of the European Monitoring and Evaluation Programme (EMEP) and provided an extensive data base. Results of the comparative study focused on polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs) and polyaromatic hydrocarbons (PAHs) and the efficiency of their sequestration in the pine needles, active and passive samplers are reported here.

2. Materials and methods

2.1. Sampling methods

Air samples have been collected in the background station once a week (52 samples per year) since 1996 using a high volume ambient air sampler PS-1 (Graseby-Andersen, USA, flow: 12–18 $m^3\ h^{-1}$, volume: 250–400 m^3 per 24 h) and two types of adsorbents: a Whatmann quartz filter (fraction $d_{ae} < 50\ \mu m$) for collection of particles, and a polyurethane foam (PUF) filter (Gumotex Břeclav, density 0.03 g cm^{-3}) for collection of the gaseous phase. Duration of sampling was 24 h; quartz filter field blanks and PUF filter field blanks were collected each month (Holoubek et al., 2007a).

Passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5 cm thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in protective chambers were employed with the sampling duration of 28 days (13 samples per year) since 2003. All PUF disks were pre-washed, cleaned (8 h Soxhlet extraction in acetone and 8 h in dichloromethane), wrapped in two layers of aluminum foil, placed into zip-lock polyethylene bags and kept in a freezer prior to deployment. Field blanks were obtained by installing and removing the PUF disks at all sampling sites (Klanova et al., 2008).

Pine needles (*P. sylvestris)* used in this project were collected annually in early September from five sampling sites in the vicinity of the background station. 3.5-year-old needles (*P. sylvestris*) were handpicked, transported to the laboratory wrapped in the aluminum foil (Holoubek et al., 2007b), and air-dried before the analysis.

2.2. Chemical analysis

All filters as well as needles were extracted with dichloromethane in a Bühi System B-811 automatic extractor. Surrogate recovery standards (d_8-naphthalene, d_{10}-phenantrene, d_{12}-perylene for PAH analysis; PCB 30 and PCB 185 for PCB analysis) were spiked on each sample prior to extraction. Terphenyl and PCB 121 were used as internal standards for PAH and PCB analyses, respectively. The volume was reduced after the extraction under a gentle nitrogen stream at ambient temperature, and fractionation was achieved on a silica gel column; a sulfuric acid modified silica

gel column was used for PCB/OCP samples. Gel permeation chromatography was used as an additional clean-up step for needles. Samples were analyzed using a GC-ECD (HP 5890) supplied with a Quadrex fused silica column 5% Ph, and a GC–MS (HP 6890 – HP 5975) with a J&W Scientific fused silica column DB-5MS for PCBs (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180), and OCPs (α-HCH, β-HCH, γ-HCH, δ-HCH, *p,p′*-DDE, *p,p′*-DDD, *p,p′*-DDT). 16 US EPA polycyclic aromatic hydrocarbons were determined in all samples using a GC–MS instrument (HP 6890 – HP 5972 and 5973) supplied with a J&W Scientific fused silica column DB-5MS. Analytical details and the quality control measures have been described elsewhere (Holoubek et al., 2007a,b).

3. Results and discussion

Long-term trends of the POP levels in ambient air, wet deposition, surface water, sediment, soil and biota at this background station were assessed previously (Holoubek et al., 2007a,b). Most of selected compounds exhibited decreasing trends of the atmospheric concentrations in the last decade. In this study, we compared the POP concentrations determined in pine needles (*P. sylvestris*) to the ones derived from the active air sampling in order to evaluate a feasibility of employment of the needle analyses as a tool for determination of the long-term trends.

To confirm the findings of Kylin and Sjodin (2003) who reported an accumulation of HCHs and *p,p′*-DDT in Scots pine during the entire life span of the needles, pine needles from five individual sites in the vicinity of the observatory were collected in September, 2007. Beside a regular collection of the 3.5-year-old needles, 0.5 and 1.5 old needles were handpicked and analyzed in parallel. Needles from all sampling locations exhibited the same phenomenon: an increase of concentration with needle age was observed for all investigated compounds (Fig. 1). While this method does not exactly confirm a steady increase of concentration over time because the needles of the different ages were taken in one point of time, it can, however, be assumed that the accumulation over time behaves very likely in a similar manner as indicated by the concentrations of the three needle age classes taken at one point of time. These results advocate a use of 3.5-year-old pine needles in this long-term project.

For every year and needle sample, a mean atmospheric concentration of POPs during the life span of needles (3.5 years) was calculated from 182 corresponding high volume air samples. A temporal variability of these mean air concentrations in the last decade (black square points) can be observed for selected compounds in Fig. 2. Grey bars represent the corresponding dry weight concentrations of POPs in pine needles. It can be seen that needle levels of the gas phase associated chemicals (as HCB or γ-HCH) corresponded to the mean gas phase air concentrations as measured in the PUF filter of an active sampler (left column). Total air concentrations (PUF + QF, right column) of these compounds showed similar correlation since they are almost 100% in the gas phase.

Generally decreasing trends were observed for the air and needle concentrations of PCBs and HCHs. On the contrary, HCB and DDT showed increasing trends of the air concentrations until 2005 (reflected also in needles). This increase, as well as some minor temporal fluctuations of other organochlorines in the last decade may reflect the major flood events in the Czech Republic in 1997 and 2002. A large area in the eastern and southern part of the country was flooded in 1997, including chemical factories with a history of pesticide production, agricultural enterprises, and storage facilities, causing the large amounts of pesticides to escape to the environment. Similarly, the central part of the country was flooded in 2002. Several large chemical enterprises were severely damaged and variety of chemicals escaped to surface waters and was distributed with the flood. The floods were followed by extremely hot summer. Since semi-volatile persistent organic compounds can easily re-evaporate from flooded soils during the warm season, this could be the source of elevated atmospheric concentration of chlorinated POPs in the years following these disasters (Holoubek et al., 2007a). A reason for a steep decline of the organochlorine concentrations in the last sampling campaign remains unknown and has to be further confirmed in the following seasons.

Lower molecular weight PAHs as phenanthrene exhibited similar behavior: decreasing trend has been observed in needles as well as in the atmospheric concentrations since 1996 followed by a minor increase in 2003. This effect probably reflects the local economic situation in the Czech Republic where growing prices of gas in 2001 brought back the coal and wood combustion in local heating systems (Holoubek et al., 2007a). Needles corresponded with the gas phase concentrations. Particle-bound PAHs as benzo(*a*)pyrene gave a different picture. After the initial decrease, their levels in needles peaked significantly in 2003. As expected, in this case the needle concentrations corresponded better to the total air concentrations or the particle phase concentrations than to the gas phase concentration. Chrysene is a compound which partitions between both phases, being more in the gas phase in the summer and more on the particles in the winter season. While the gas phase atmospheric concentrations follow generally decreasing trend, the total air concentrations can explain a sudden increase in the needle level in 2005. Elevated particle-bound concentrations of chrysene in the wintertime are probably responsible for this peak.

Presented results confirm that pine needles can be a valuable tool not only for relative comparison of the sampling sites, but also for an assessment of the temporal trends in the atmospheric pollution. Needle derived temporal variability corresponds very well to data obtained from the high volume samplers.

To compare a fingerprint of compounds sequestered in pine needles to those of other sampling devices, a passive air sampler with polyurethane foam was applied beside a high volume sampler. Even though passive samplers were developed for sampling the gas

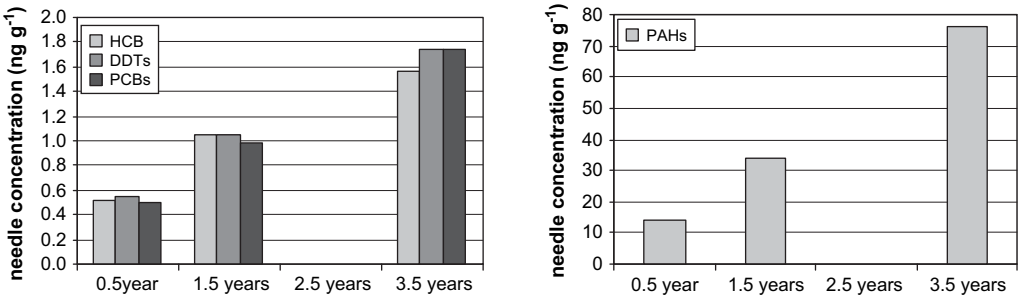

Fig. 1. Dry weight POP concentrations (HCB, sum of *p,p′*-DDE, DDD and DDT, sum of 7 indicator PCBs, sum of 16 EPA PAHs) determined in pine needles of various ages (0.5, 1.5, 3.5 years).

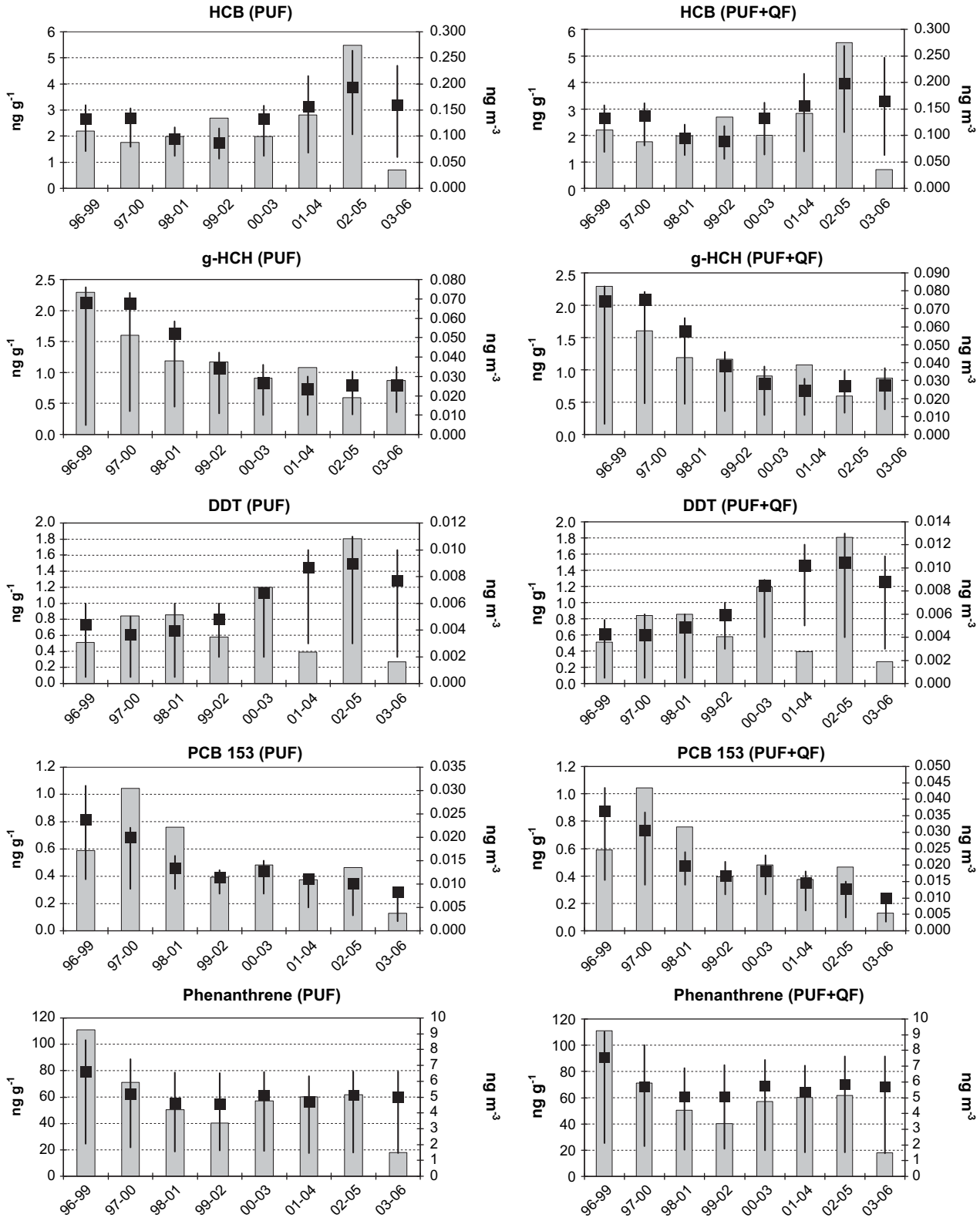

Fig. 2. A comparison of the mean POP concentrations measured in pine needles (grey bars, ng g^{-1}, left axis, d.w.) and ambient air (black square points, 25–75 quartile, ng m^{-3}, right axis) in the last decade (1996–2006, $n = 182$). Left column compares needles to the gas phase concentrations, right column to the total air concentrations.

phase associated chemicals only, they are known to sample a fine fraction of the particulate matter (Klanova et al., 2008). PAS were co-employed with high volume samplers in Kosetice station since 2003, and they integrated over 28-day periods. Using a similar design as described in the previous paragraph, we compared the amounts of individual chemicals captured in needles to the ones determined in high volume (Fig. 3, left) and passive (Fig. 3, right) air samples. A mean concentration of each compound in 182 high

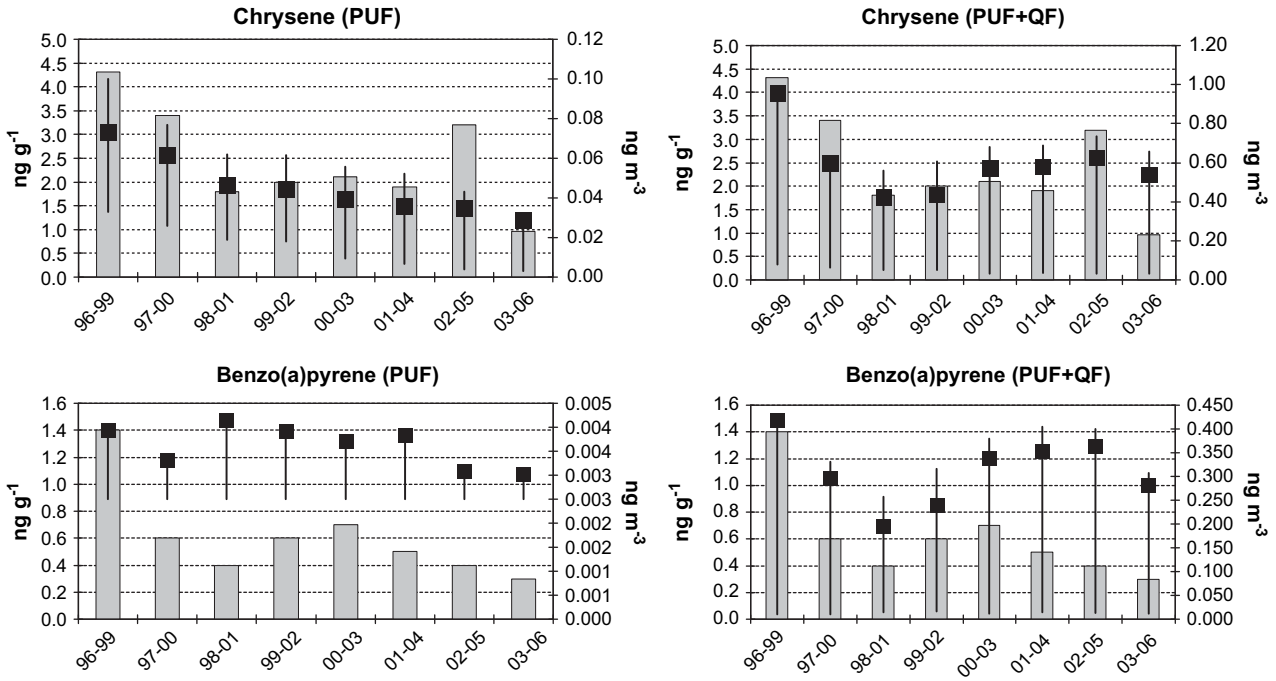

Fig. 2. (*continued*).

volume and 42 passive air samples corresponding to the age of needles was used to create a model fingerprint.

As can be seen in Fig. 3, the fingerprints of chemicals sequestered in the pine needles corresponded very well to the ones measured in the active air samples (left). This is true for both, the gas phase, and particle phase associated chemicals. Phenanthrene was the most abundant PAH in both matrices, followed by fluoranthene, naphthalene and pyrene. From the group of PCBs, the lower chlorinated congeners as PCB 28, 52, 153 and 101 were found in the somewhat higher concentrations. To the right, needle concentrations were compared to the mean values determined in passive samplers. As expected, particle-bound chemicals have been

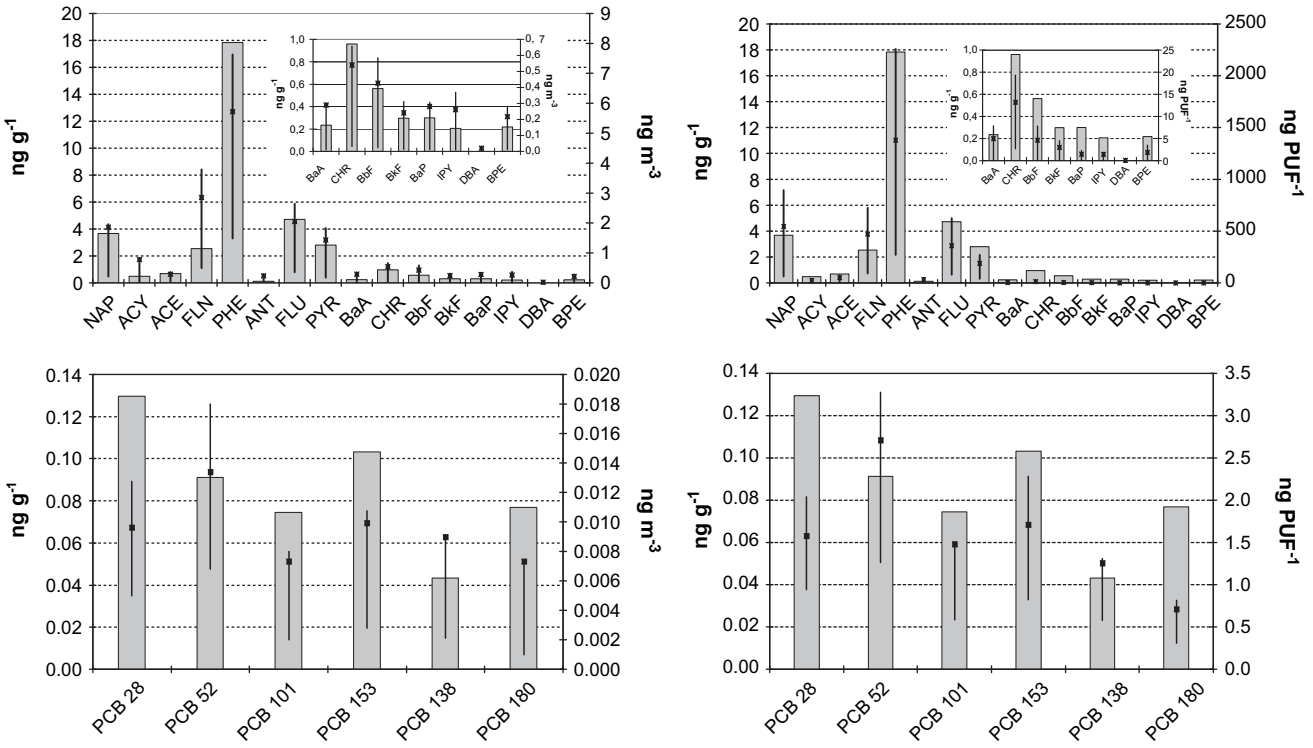

Fig. 3. A mean concentration of the individual PAHs (upper row) and PCBs (lower row) in three media over the period of 3.5 years was used for a comparison. Left: a fingerprint of POPs captured in pine needles (grey bars, ng g^{-1}, left axis, d.w.) and high volume air samplers (black square points, 25–75 quartile, ng m^{-3}, right axis). Right: a fingerprint of POPs captured in pine needles (grey bars, ng g^{-1}, left axis, d.w.) and PUF based passive air samplers (black square points, 25–75 quartile, ng PUF^{-1}, right axis).

also captured in PAS although less efficiently than by the high volume device.

While Fig. 3 only compares the patterns of POPs in three media, it tells nothing about the absolute air concentrations and the efficiency of sequestration in needles for the main groups of POPs. Based on the consistent results described above, a quantification of factors predicting the atmospheric concentrations from the POP levels found in needles was attempted.

For the passive air samplers, an equivalent sample volume (V_{EQ}, m^3) was adopted as a parameter for deriving the air concentration from passive sampling data (Klanova et al., 2008). It is calculated as,

$$V_{EQ} = C_{PD}/C_{AIR}, \quad (1)$$

where C_{PD} (ng sampler^{-1}) is the PUF disk concentration, C_{AIR} (ng m^{-3}) is the mean air concentration (gas + particle phase) derived from the high volume air samplers for each integration period.

Using the same idea, the equivalent sample volume calculated as a ratio of the needle concentration over the mean air concentration was determined for all needle samples from the last decade. Results indicated that the amount of individual PAHs contained in 1 g of dry needles was equivalent to the amount in some 3–10 m^3 of ambient air for the gas phase associated PAHs, and 1–2 m^3 for particle-bound PAHs. Interestingly, the equivalent sample volume of organochlorines was higher – between 20 and 30 m^3 per 1 g of needles for PCB congeners and most of the pesticides.

This was further confirmed by comparison of needles to PAS. The amounts of individual gas phase associated PAHs captured in PAS during the 28-days exposition period were equivalent to the amounts found in 50–100 g of pine needles. On the contrary, the amounts of particle-bound PAHs found in PAS after 28 days equaled to the PAH quantity contained in less than 10 g of needles. This is in a good agreement with previously published equivalent sampling volumes for the same PAS (200 m^3 per 28 days for the gas phase, and 20 m^3 for particle-bound chemicals) (Klanova et al., 2008). For organochlorines, the equivalent sample amounts varied between 10 and 20 g of needles for one passive air sample.

While for passive air samplers the equivalent sampling volumes of the individual compounds corresponded with their vapor pressures (Klanova et al., 2008) (V_{EQ} of organochlorines and gas phase associated PAHs were comparable and volumes of the particle-bound compounds were about ten times lower), these volumes seemed to compare differently for needles. There was not so much difference between the gas phase and particle phase PAHs (approximately factor of 3) since needles sample particles more efficiently than PAS do. However, there is a difference between various gas phase associated compounds. Even though vapor pressures of lower molecular weight PAHs are comparable to more volatile organochlorines, their equivalent sampling volumes for needles were lower (factor of 3). This could possibly be explained by the shorter life times of PAHs in needles. We have to consider that 28 days of exposure was used for PAS while polyurethane foam as a sampling media was efficiently protected from solar radiation by the chamber (Bartkow et al., 2006). Naturally, we can expect a significant degradation of the compounds captured in the wax of coniferous needles which are exposed to meteorological conditions for several years without a shield. Degradation rates of POPs in waxes are not available but we can draw some assumptions from the atmospheric and soil half-lives. While an estimated half-life in the atmosphere varies between several hours and several days (Mackay et al., 2006) for PAHs, it extends up to one year for highly chlorinated PCBs or hexachlorobenzene (Sinkkonen and Paasivirta, 2000; Mackay et al., 2006). This difference is even more significant in soils: from 2 months to 2 years for PAHs and between 4 and 40 years for organochlorines (Sinkkonen and Paasivirta, 2000; Meijer et al., 2001; Mackay et al., 2006). Photodegradation experiments with semipermeable membrane devices (SPMDs) filled with triolein as a sampling media can provide certain guidance on the photochemical behavior of POPs in needles. Bartkow et al. (2006) performed a degradation study with SPMDs exposed for 29 days in chambers of various designs. It has been demonstrated that SPMDs in the cage chamber where the light intensity was about 100 μW cm^{-2}, lost about one half of the captured PAHs in 29 days when compared to the bowl chamber (similar to the one used in this study). Such study is not available for organochlorines but lower degradation rates are to be expected.

4. Conclusion

In summary, this is the first long-term comprehensive study comparing the pine needles as an indicator of the atmospheric pollution to the polyurethane foam based passive air samplers and high volume samplers. It has been demonstrated that pine needles are capable of the uptake of both, gas and particulate phase associated chemicals. They can serve as a passive air sampler integrating the air pollutants over the years and providing averaged information about the contamination level. Fingerprints of compounds (PCBs, OCPs and PAHs) accumulated in needles were compared to the ones determined in the active and passive air samples. While needle samples very closely reflected a pattern of the high volume samples, passive samplers were less efficient in sampling the particle associated compounds.

With the goal to provide a tool for estimation of the air concentrations from the POP levels in needles, we estimated the equivalent sample volumes of needles. These volumes varied an order of magnitude between the groups of investigated compounds. As needles integrate the air pollutants over the period of several years providing the mean information about the contamination level, we can speculate that such results can be significantly altered by the photochemical degradation of the chemicals trapped in the surface wax (much faster for PAHs than organochlorines). Our estimation that 1 g of dry 3.5-year-old needles roughly corresponded to 20–30 m^3 of ambient air for organochlorines while it was equivalent to 3–10 m^3 for the gas phase and 1–2 m^3 for the particle phase associated PAHs is a very preliminary result that calls for further investigation. The most important finding is that in the long run the needle monitoring gives very similar information on the temporal trends as does the high volume monitoring, providing that needle samples are collected at the same time of the year. A standardization of the sampling, analytical and reporting procedures is crucial for a success of such assessment.

We have to consider advantages and disadvantages of three samplers for the purpose of each project. A high volume air sampler provides precise ambient air concentrations of POPs at the time of sampling. Since both, polyurethane foam filters and quartz filters, are used, the gas phase and particle phase associated chemicals can be distinguished. A major disadvantage is a sampling duration: it is usually short due to the high expenses, and it gives no information on the average situation or temporal fluctuations. This is exactly a kind of information the passive air sampler provides. While incapable of providing precise concentrations, it gives very useful mean values, seasonal variations, long-term trends and relative comparisons. It is impossible to distinguish between the gas phase and particle-bound compounds, latter ones are, however, also captured to some extent. Needles cannot provide any information on the seasonal variability of the air concentrations since fluctuations in needle concentrations are more influenced by the biological processes than by the air concentrations (Kylin and Hellstrom, 2003). On the other hand, they are still very useful for a relative

comparison of the sampling sites and – as has been shown in this project – an assessments of the long-term trends. Both of these applications have a great value especially for the background monitoring of the atmospheric contamination at remote and poorly accessible locations.

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Soil burdens of persistent organic pollutants – Their levels, fate and risk. Part I. Variation of concentration ranges according to different soil uses and locations

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Due to its large retention capacity for hydrophobic compounds, carbon-rich mountain soil showed higher concentrations for several persistent organic pollutants.

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ABSTRACT

Detailed soil screening data from the Czech Republic as a typical Central European country are presented here. Determination of a wide selection of organic and inorganic pollutants as well as an assessment of specific soil parameters allowed us to study the soil contamination in relation to the land use and soil properties. While HCHs and HCB were found at highest levels in arable soils, the higher concentrations of PCDDs/Fs, PCBs, PAHs and DDTs were observed in high altitude forest soils. Concentrations of these compounds strongly correlated with the soil organic carbon content. Several possible reasons have been suggested for the observed higher concentrations in mountain forest soils but the impact of each of these influencing factors remains to be identified. An inventory of the soil contamination is needed as a first step in our effort to estimate an extent to which the secondary sources contribute to the enhanced atmospheric levels of POPs.

1. Introduction

A widespread production and use of persistent organic pollutants (POPs) in industrialized regions together with their environmental persistence led to the intensive contamination of soils (Jones and de Voogt, 1999) representing potential ecological and human health risks (Skowronski et al., 1990; Nessel et al., 1992; Dor et al., 2000; Roos et al., 2004). Soil plays an important role in the global fate and distribution of POPs. Due to its large retention capacity for hydrophobic compounds, it has been identified as an effective sink of these chemicals. It, however, has to be regarded as a long-term archive of the atmospheric deposition rather than an indicator of the actual inputs (Schmid et al., 2005). A net POP content in soils is given by the balance between inputs and losses (Sweetman et al., 2002). The main input (beside a direct application) is wet and dry atmospheric deposition (including a part of deposition sequestered in vegetation and deposited consequently with a litter fall) and the principal loss pathways are volatilization, degradation and leaching. Based on the physicochemical properties of the individual POP, on the sorption capacity of soil, temperature and other meteorological conditions (Hippelein and McLachlan, 2000; Sinkkonen and Paasivirta, 2000; Valle et al., 2005), the soil bodies may currently represent a long-term storage system for these chemicals. Soil was found to be a sink for highly chlorinated PCB congeners while for lower molecular weight PCBs, as well as for HCHs, soil tends to be a source of pollution to the air, especially during summer (Ruzickova et al., 2008). As re-volatilization is one of the most important elimination pathways, contaminated soils should be considered an important source of POPs to the atmosphere (Wild and Jones, 1995; Harner et al., 2001; Meijer et al., 2002, 2003a,b; Ockenden et al., 2003; Hassanin et al., 2005), especially for the POPs primary sources of which have been largely eliminated. A fact, that significant shifts in the soil–air equilibrium status are to be expected with potential climate changes, further emphasizes a need for a detailed assessment of soil as a secondary source of POPs to the atmosphere.

The Czech Republic, as a model country for this study, is considered to be one of the most industrially developed among new member states of the European Union. Simultaneously, it is also a country whose industrial development in the last forty years has led to a variety of negative environmental implications. Polychlorinated biphenyls (PCBs) were produced in former

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Czechoslovakia between 1959 and 1984 as well as high amounts of organochlorine pesticides (OCPs). Both technical hexachlorocyclohexane (HCH) and lindane (γ-HCH) were produced between 1958 and 1976, together with DDT and hexachlorobenzene (HCB). Due to their long-term application, all these POPs are still detectable in abiotic and biotic environmental compartments, including humans. Despite of decreasing trends, PCB contamination of the food chains and the human population (including breast milk) in the Czech Republic is among the highest in Europe.

At the same time, the Czech Republic is a country with vast information on contamination of the environmental matrices. POPs have been monitored on the regular basis in the background station of the Czech Hydrometeorological Institute in Kosetice. This observatory is a part of the Environmental Monitoring and Assessment Programme (EMEP) as the only EMEP station in the Central, Southern and Eastern Europe measuring persistent organics. It is also the only EMEP site that is focused not only on the atmospheric concentrations but on the POP levels in a variety of environmental matrices (ambient air, wet deposition, surface water, sediment, soil, moss, and needles). POPs monitoring program was established in Kosetice in 1988, and since then, data have been used for an assessment of long-term trends in the levels of POPs in the European background (Holoubek et al., 2007a,b). This unique data set has been also used for the field validation of the POP transport models in the EMEP MSC-East (Shatalov et al., 2000, 2001; Holoubek et al., 2001). This comparison of modeling results with monitoring data indicated a significant underestimation of the POP concentrations in all measured compartments (Shatalov et al., 2001). EMEP MSC-EAST model is based on the emission factors derived from a measurement at major industrial sources leaving out a contribution of secondary sources (re-volatilization from constructions, soils and water). Thus, we can hypothesize that this omission results in the observed bias in predicted POP concentrations. To confirm this hypothesis, an inventory of the secondary sources (contamination levels of the soil and water bodies) as well as a quantification of associated volatilization fluxes is needed.

To quantify a contribution of non-industrial sources to contamination of the atmosphere by persistent, toxic substances, and to assess associated risks, we have to accomplish several steps: (1) a countrywide inventory of soils, their properties and contamination levels based on available monitoring data, (2) a model estimating total burdens and associated risks for human and wildlife, (3) laboratory experiments on quantification of volatilization fluxes from soils with various properties and contamination levels, (4) a model estimating a contribution of contaminated soils to ambient air pollution based on the measured soil burdens and laboratory-derived emission factors. This paper summarizes our efforts and achievements in the first step.

2. Materials and methods

2.1. Soil sampling

Soil samples analyzed in this study originate from two complementary sources. A monitoring of agricultural soils has been performed by the Central Institute for Supervising and Testing in Agriculture in the network of 190 representative sites including arable soils and grasslands since 1992. From this large database, all 38 arable soil and 6 grassland soil samples, where the POP levels have been measured annually, were selected for the purpose of this study. Remaining 59 soils came from the RECETOX soil survey project focused on the inventory of the POP burdens. Sampling sites were carefully selected to complement the agricultural set and to provide samples from various geographical regions, altitudes, soil qualities and land uses. This set included 8 arable soils, 28 grasslands, and 23 forest soils (14 from the highlands and 9 from the border mountains). A soil profile and basic soil parameters were described at all sites at the beginning of each project.

Each site was represented by ten sub-samples collected from the area of 25 × 25 m (about 1.5 kg of weight). The mixed plough layer (0–25 cm) was sampled on arable soil, while only the top 10 cm soil layer was collected on grassland and forest soils. In the forest, the litter (O_L horizon) was carefully removed before sampling, so the sample was a mixture of the overlaying organic horizons $O_F + O_H$. At the sampling sites with the organic horizon layers thinner than 10 cm, this mixture also contained a fraction of mineral horizon A_H. This sampling strategy results in high variability of the physicochemical properties of the soil samples. On the other hand, it is the only strategy enabling to compare the POPs concentrations in the top soil layers countrywide, and to estimate the burdens, fluxes and risks in the next steps.

All soil samples were transported to the laboratory in polyethylene bags, air-dried at laboratory temperature, and sieved through 2-mm mesh. Such sample preparation technique has been shown not to alter the POP content in soils significantly. A map of the sampling sites is presented in Fig. 1.

2.2. Sample analyses

Basic soil parameters were determined according to the standard operational procedures: total organic carbon content TOC (ISO 14235, 1998), total soil nitrogen N_{tot} (ISO 11261, 1995), soil pH_{KCl} and pH_{H_2O} (ISO 10390, 2005), particle size analyses (ISO 11277, 1998). Humic compounds were extracted by sodium pyrophosphate and separated to humic and fulvic acids (HA and FA). Cation exchange capacity CEC was calculated as a sum of chemical equivalents of H^+ (from pH),

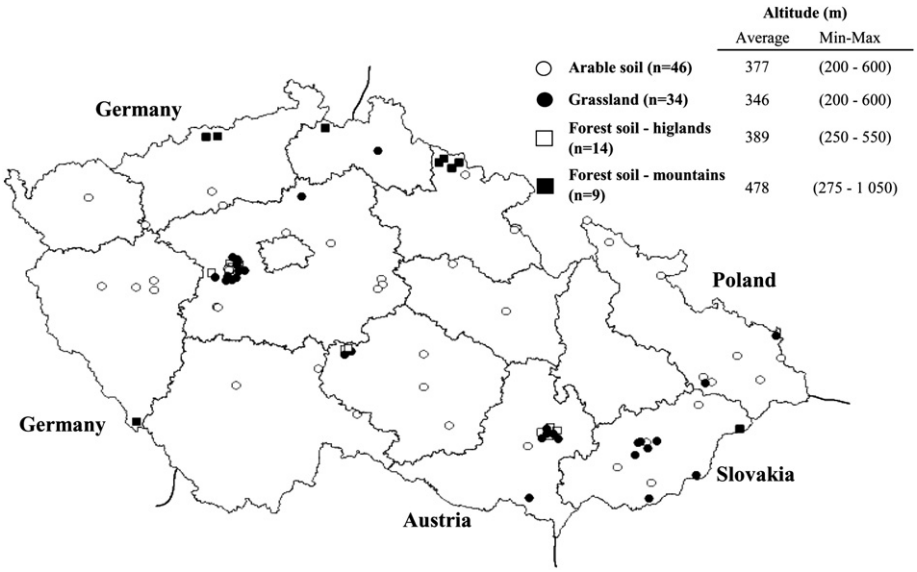

Fig. 1. Sampling network in the Czech Republic.

Ca^{2+}, Mg^{2+} (by FAAS), and K^{+} (by FAES) in soil extracts by Mehlichs II extractant. Base saturation (BS) was calculated as a fraction of basic cations (Ca^{2+}, Mg^{2+} and K^{+}) in CEC. A soil concentration of heavy metals was determined after aqua regia extraction (ISO 11466, 1996) of the soils using FAAS, ETA-AAS (Cd), ICP-OES (As), and mercury analyzer (Hg).

For POP analyses, all soil samples were extracted with dichloromethane. Fractionation was achieved on a silica gel column; a sulfuric acid modified silica gel column was used for PCBs/OCPs samples. Samples were analyzed using a GC–ECD and a GC–MS for 16 US EPA PAHs, seven indicator PCB congeners (PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180), *p,p′*-DDT, *p,p′*-DDD, and *p,p′*-DDE, α-, β, γ-, δ-hexachlorocyclohexanes and hexachlorobenzene (Holoubek et al., 2007a,b). Laboratory blank and reference material was analyzed with every set of samples. Recoveries of PCBs, OCPs and PAHs were determined by spiking all samples with the surrogate standards prior to extraction. Recoveries were higher than 73% and 71% for PCBs and PAHs, respectively.

A toluene extraction was used for PCDDs/Fs analysis. Samples were cleaned-up using a sulfuric acid/silica – sodium hydroxide/silica – activated silica mixed column. A fractionation of PCDDs/Fs from PCBs was achieved on an alumina column. Each sample was spiked with $^{13}C_{12}$-labeled surrogates of all 17 2,3,7,8-substituted PCDDs/Fs congeners and three di or trichlorinated DDs/Fs. PCDDs/Fs were quantified using an HRGC/HRMS system tuned to 10 000 resolving power and running in selected ion monitoring mode (Green et al., 2001). For PCDD/Fs recoveries, five sub samples were fortified with unlabeled 2,3,7,8-substituted PCDDs/Fs at different levels. Measured values for spiked soils were between 87 and 125% of the anticipated values for all congeners (mean value 98%) (Green et al., 2001).

2.3. Data processing and statistical methods

A total number of 103 soil samples were analyzed for their content of POPs and heavy metals. Data records were digitized and a validity of the database can be regarded as high due to a double control of the key variables. Criteria for the exclusion of a record from the analysis included inconsistent retrospective data on contamination (3 arable soils, 2 grasslands), missing information on important covariates, especially TOC (3 arable soils, 10 grasslands, 5 forest soils) or inconsistency in other parameters (1 arable soil). A resulting file consisted of 79 soil samples (39 arable soils, 22 grasslands, 18 forest soil samples). Such validation of the database and exclusion of the inconsistent records changed neither the range, nor the mean values of contaminants.

A standard set of summary statistics was applied (mean, standard deviation, median, percentiles). Sample frequency distributions were examined prior to statistical analyses, and robust summary statistics were used to describe the distribution patterns in primary data (median, 5– 95th percentile range). One-way ANOVA model was applied as the only parametric test to examine a relative contribution of various soils to the overall experimental variability. A log transformation $\ln(X+1)$ of all chemical and abiotic parameters was verified as effective in reaching the normality (goodness-of-fit test and Shapiro–Wilk's test). This transformation also sufficiently stabilized a variability of parameters (Bartlett's test, Levene's test) that further facilitated an ANOVA model. Pearson's product–limit correlation (log transformed variables) was applied as a measurement of association among the chemical parameters and the organic carbon content in soils. Probabilistic level of $\alpha = 0.05$ was accepted as the critical level of statistical significance for all analyses. The analyses were performed using Statistica for Windows 7.1. (StatSoft Inc., 2005) and SPSS 12.0.1 (SPSS Inc., 2003).

3. Results and discussion

3.1. Soil properties

To correlate the soil levels of various compounds with a variety of soil properties, arable, grassland and forest soils were assessed separately. Forest soils were further aggregated according to their geographical position. Czech Republic is surrounded by a ring of mountains (maximum altitude of 1622 m) while the inland goes from highlands to lowlands. A majority of forested areas are highlands and mountains. Since a forest can play an active role in the scavenging of the atmospheric pollution, differentiation between the border mountain forests which can be more affected by the long range transport (especially along the north-western border due to the prevailing wind direction), and other forests was important. These groups also differed in the organic carbon content, median of which was much higher in the set of mountain soils (37.4%) than in inland soils (5.0%) from several reasons. While deciduous woods are more common in lowlands, mountain forests are almost exclusively coniferous. Since decomposition of needles is a much slower process than decay of leaves, the annual carbon loss is much lower in the litter covering the coniferous forest floor than it is in the deciduous forest (Qualls et al., 2003). The lower temperature also contributes to the slower turn-over of mountain sites. This results in the thicker layer of organic horizon in mountain soils, as well in generally higher organic carbon content. It means that the organic layers of coniferous forests represent a longer time of pollutant input than those of the deciduous forests. It has to be also noted that due to low depth of organic horizons in the deciduous forests and unclear or gradual transition between O_H and A_H, we cannot exclude that small part of the mineral soil below was sampled together with the organic layers. This would result in decrease of C_{org} (and concentrations of contaminants as well) in such samples by dilution.

Table 1
Abiotic characteristics of soil samples.

Parameter	C_{org} [%]	pH_{H_2O}	pH_{KCl}	N_{tot} [%]	FA [%]	HA [%]	CEC [meq kg^{-1}]	Base saturation [%]	Clay particles <0.01 mm [%]
Arable soils[a]									
Min/Max	1.0–2.7	5.4–8.2	4.8–7.6	0.09–0.28	0.18–0.28	0.16–0.25	257.8–568.8	88.2–98.5	12.7–33.5
Percentiles 5–95%	1.0–2.3	6.1–8.1	5.5–7.6	0.09–0.26	0.20–0.24	0.19–0.24	313.8–521.7	94.4–97.2	15.6–31.8
Median	1.5	7.1	6.6	0.16	0.21	0.24	371.7	95.7	22.6
Mean	1.5	7.1	6.6	0.15	0.22	0.22	400.9	94.9	23.1
Grassland soils ($n = 22$)									
Min/Max	1.2–7.1	5.0–7.9	3.8–7.4	0.11–0.48	0.14–0.82	0.15–0.53	75.0–868.5	39.2–98.6	5.7–29.4
Percentiles 5–95%	1.5–5.0	5.4–7.9	4.2–7.3	0.13–0.48	0.15–0.79	0.15–0.52	110.5–756.9	53.4–98.6	7.0–29.4
Median	3.0	7.2	6.4	0.23	0.36	0.30	262.5	92.4	15.5
Mean	3.2	7.0	6.3	0.24	0.39	0.31	337.4	87.2	16.3
Forest soils – highlands and hilly country ($n = 9$)									
Min/Max	3.8–18.7	4.5–7.9	3.8–7.3	0.22–0.49	0.53–1.19	0.33–1.02	156.4–1121.9	37.6–98.8	5.7–23.4
Percentiles 5–95%	4.3–10.4	5.7–7.5	5.0–7.1	0.25–0.48	0.72–1.00	0.41–1.00	203.3–761.8	67.7–96.7	6.9–20.4
Median	5.0	6.9	6.4	0.31	0.95	0.55	478.2	91.8	18.2
Mean	7.1	6.5	5.9	0.34	0.90	0.66	503.1	79.4	15.0
Forest soils – border mountains ($n = 9$)									
Min/Max	8.1–41.8	3.1–3.9	2.7–3.1	0.41–1.62	1.22–2.74	1.18–6.99	203.3–468.9	10.0–30.5	4.7–16.5
Percentiles 5–95%	30.5–38.9	3.4–3.8	2.7–3.0	1.26–1.59	1.97–2.47	3.53–5.41	332.4–410.6	10.5–18.2	5.3–13.4
Median	37.4	3.6	2.9	1.41	2.12	4.82	354.2	14.3	8.6
Mean	33.8	3.6	2.9	1.33	2.14	4.39	353.5	15.7	9.1

[a] Monitoring of arable soils did not include all abiotic characteristics in all sites: $n = 39$ for C_{org}, pH and N_{tot}; $n = 6$ for other parameters.

Similarly, the content of humic substances (humic and fulvic acids) or total nitrogen increased along the line arable – grassland – highland forest – mountain forest while pH, base saturation or clay particle content decreased along the same line. An introductory set of soil parameters can be found in Table 1. The organic carbon content was lower than the Czech national average (Sáňka et al., 2002) for arable and grassland soils in this study. Mean and median values of pH, total nitrogen, and a content of humic compounds corresponded to the reference values derived from the national soil monitoring systems, a higher value of a cation exchange capacity is typical for fertile soils. Base saturation corresponds to the individual land use exceeding 90% in agricultural soils and being much lower in mountains soils (14%). Regarding the content of clay particles, a majority of selected soils were of the light to medium texture category.

3.2. Heavy metals

A summary statistics of heavy metals is presented in Table 2. The reference values (Sáňka et al., 2002) of the soil content for heavy metals (and POPs in the following tables) are included for comparison. A reference value is defined as an upper boundary (95th percentile) of natural and diffuse anthropogenic variability of the soil level, and it is used in legislation as a safe level of toxic substances in soils. Selection of the sampling sites including contaminated areas resulted in the wide range of measured soil levels. It is also a reason why the average values exceeded the national average in the Czech Republic (Sáňka et al., 2002), while the medians stayed close to the national medians for all elements with the exception of cadmium (measured arable: 0.47 mg kg^{-1}, national median 0.27 mg kg^{-1}), lead (measured arable: 29.1 mg kg^{-1}, national median 20.4 mg kg^{-1}) and zinc (measured arable: 93.5 mg kg^{-1}, national median 62.0 mg kg^{-1}). Similarly, the median concentration of heavy metals was lower than the reference value in all arable soils, while the arithmetic mean exceeded the reference value for Hg, Cd, Cr, Pb, Zn, and As for arable soil, and Zn for grassland. All of these elements are associated with anthropogenic activities. Heavy metals in forest soils had generally higher median values than in arable and grassland soils. Median soil concentrations exceeded the reference values for Hg, Cd and Pb, for lead in mountains almost three times. It probably reflects an accumulation of lead in the surface soil horizon over the years when a leaded gasoline was used in the Czech Republic.

3.3. Organochlorine pesticides

From a group of organochlorine pesticides (Table 3, reference levels are available only for DDTs and HCHs), the widest ranges as well as the highest levels were found for DDT and its metabolites (0.6–1509.8 μg kg^{-1} for DDT and 0.6–599.8 μg kg^{-1} for DDE). These levels are comparable to the ones published in Europe for Romania (Covaci et al., 2001), for China (Feng et al., 2003; Gong et al., 2003; Chen et al., 2005), or for US (Aigner et al., 1998). The median concentration of DDT in mountain soils (46 μg kg^{-1} dw) was twice as high than the one in arable soils (20.4 μg kg^{-1} dw), and it exceeded the reference value (30 μg kg^{-1} dw). For DDE, median values in arable and mountain soils were comparable and none was higher than the reference level.

DDT in soil is a subject of microbial degradation to more stable and toxic metabolites (DDE and DDD). The degradation rate is, however, very slow, and it depends on several factors including a soil type, temperature, moisture and organic carbon content (Hitch and Day, 1992; Boul et al., 1994; Harner et al., 1999). The concentration ratio of the parent compound to metabolites is often used to determine the age of the soil contamination or to detect fresh inputs of DDT to the environment. Various combinations can be found in the literature with DDT/DDE or DDT/DDE + DDD ratios being used most often. In general, low values of the DDT/DDE ratio indicate the old DDT burdens while high values indicate a fresh application. In reality, this problem is more complex because degradation rates differ greatly from soil to soil. The ratio is influenced by longer life times of pesticides in soils with a high organic carbon content (Diamond and Owen, 1996; Chen et al., 2005), by soil pH values (Gong et al., 2003) as well as by different volatilization rates of DDT and its metabolites. The median value of DDT/DDE ratio found in mountain soils (3.16) was 2–3 times higher than the one in arable and grassland soils (1.07 and 1.62, respectively). The high ratio in mountain soils can be probably assigned to the application of DDT in Germany in early 1980s. It was the latest large-scale application of DDT in this part of Europe, impact of

Table 2
Heavy metals in examined soil samples [mg kg^{-1}].

Parameter	Hg(tot)	Cd(AR)	Cr(AR)	Ni(AR)	Pb(AR)	Zn(AR)	As(AR)	Be(AR)	Co(AR)	Cu(AR)	V(AR)	Mo(AR)
Ref. value[a]	0.3	0.5	50.0	50.0	60.0	120.0	20.0	2.0	30.0	60.0	130.0	
Arable soils ($n = 39$)												
Min/Max	0.05–0.78	0.14–6.25	11.9–517.7	5.9–106.0	14.3–825.2	40.3–1012.4	4.2–518.3	0.03–10.51	2.53–26.77	6.6–227.1	15.5–131.3	0.10–3.19
Percentiles 5–95%	0.05–0.78	0.19–5.23	19.4–394.8	9.4–68.1	14.4–813.5	45.2–477.0	4.8–340.3	0.55–3.15	3.16–25.52	10.1–108.6	25.5–98.3	0.14–1.23
Median	0.13	0.47	38.2	23.9	29.1	93.5	11.8	1.19	11.53	23.9	48.7	0.41
Mean	1.11	1.15	63.7	27.5	118.3	164.7	41.9	1.50	12.44	33.2	51.9	0.53
Grassland soils ($n = 22$ for Hg to As; $n = 13$ for other heavy metals)												
Min/Max	0.07–0.89	0.13–1.98	10.6–129.0	5.2–44.2	16.1–124.0	62.5–1208.0	4.0–14.3	0.37–1.33	2.96–23.20	9.1–43.7	19.1–133.0	0.10–1.37
Percentiles 5–95%	0.09–0.38	0.16–0.77	13.1–92.9	11.7–42.6	17.1–55.8	77.2–347.0	5.9–13.9	0.47–1.00	7.26–15.10	12.1–26.7	24.9–74.1	0.10–0.65
Median	0.14	0.39	33.3	29.0	31.4	87.8	9.1	0.70	9.79	23.7	4.7	0.43
Mean	0.20	0.46	41.9	27.5	36.6	159.9	9.2	0.74	11.61	22.4	54.2	0.46
Forest soils – highlands and hilly country ($n = 9$ for Hg to As; $n = 6$ for other heavy metals)												
Min/Max	0.14–0.53	0.30–1.12	10.1–92.7	7.4–65.9	39.7–108.0	61.8–282.0	5.7–18.6	0.20–1.30	2.59–26.70	9.7–39.1	19.9–143.0	0.20–0.83
Percentiles 5–95%	0.17–0.52	0.37–0.84	13.5–85.5	13.7–55.0	45.0–106.0	67.2–140.0	8.5–18.4	0.37–0.83	10.60–24.20	13.5–32.5	29.6–124.0	0.30–0.70
Median	0.25	0.53	46.6	36.3	48.5	121.0	11.4	0.73	17.80	25.5	82.8	0.44
Mean	0.28	0.61	49.4	33.8	61.6	122.1	12.6	0.69	16.62	24.3	80.4	0.48
Forest soils – mountains ($n = 9$)												
Min/Max	0.18–1.02	0.15–0.63	7.9–22.7	5.7–15.0	54.7–372.0	36.5–69.5	5.1–32.7					
Percentiles 5–95%	0.58–0.78	0.18–0.45	8.6–21.1	5.9–13.5	115.0–251.0	36.8–57.0	5.7–28.7					
Median	0.66	0.24	14.6	7.5	146.0	46.8	12.2					
Mean	0.66	0.32	14.5	8.8	170.9	48.1	14.8					

[a] Reference value (Sáňka et al., 2002).

Table 3
Selected organic pollutants in examined soil samples [μg kg^{-1}].

Parameter	HCB	α-HCH	β-HCH	γ-HCH	δ-HCH	ΣHCHs	α/γ HCHs
Ref. value[a]	–	–	–	–	–	10.0	–
Arable soils ($n = 39$)							
Min/Max	0.60–16.60	0.43–1.00	0.03–1.00	0.13–1.13	0.01–1.00	0.65–4.00	0.44–3.80
Percentiles 5–95%	0.70–6.80	0.48–1.00	0.03–1.00	0.18–1.00	0.01–1.00	0.74–4.00	0.44–3.80
Median	3.40	1.00	1.00	1.00	1.00	4.00	2.12
Mean	5.41	0.93	0.86	0.91	0.85	3.54	2.15
Grassland soils ($n = 22$)							
Min/Max	0.02–6.28	0.05–1.00	0.03–1.00	0.08–1.00	0.01–1.00	0.38–4.00	0.20–3.26
Percentiles 5–95%	0.50–5.92	0.06–1.00	0.03–1.00	0.09–1.00	0.01–1.00	0.38–4.00	0.20–3.23
Median	1.52	0.57	0.09	0.29	0.02	0.88	2.50
Mean	2.99	0.51	0.24	0.34	0.11	1.18	1.92
Forest soils – highlands and hilly country ($n = 9$)							
Min/Max	0.47–3.40	0.06–0.71	0.03–1.76	0.14–0.45	0.01–0.04	0.55–2.34	0.15–3.08
Percentiles 5–95%	0.82–1.88	0.16–0.69	0.03–1.10	0.16–0.43	0.01–0.02	0.59–1.75	0.36–2.95
Median	1.36	0.63	0.10	0.33	0.01	1.09	2.12
Mean	1.55	0.50	0.39	0.32	0.02	1.18	1.81
Forest soils – mountains ($n = 9$)							
Min/Max	0.50–2.92	0.12–0.94	0.03–0.39	0.08–0.67	0.01–0.01	0.26–1.66	0.62–1.72
Percentiles 5–95%	1.00–2.21	0.14–0.66	0.03–0.14	0.15–0.67	0.01–0.01	0.31–1.48	0.71–1.40
Median	1.41	0.35	0.03	0.26	0.01	0.65	0.94
Mean	1.57	0.38	0.09	0.37	0.01	0.86	1.06

Parameter	pp′-DDT	pp′-DDE	pp′-DDD	ΣDDTs	DDT/DDE + DDD	DDT/DDE
Ref. value[a]	30.0	25.0	20.0	–	–	–
Arable soils ($n = 39$)						
Min/Max	1.88–516.40	1.76–599.80	0.32–52.10	4.0–1018.3	0.28–0.78	0.58–4.12
Percentiles 5–95%	2.40–375.20	4.00–316.70	2.00–43.30	11.1–228.6	0.31–0.74	0.59–3.07
Median	20.30	13.80	2.49	34.2	0.46	1.07
Mean	58.36	48.46	6.85	113.7	0.48	1.39
Grassland soils ($n = 22$)						
Min/Max	1.03–19.60	0.55–11.90	0.19–3.03	2.04–28.20	0.27–0.72	0.49–4.84
Percentiles 5–95%	1.20–15.90	0.65–11.78	0.19–2.81	2.19–26.25	0.30–0.70	0.51–3.59
Median	5.31	3.19	0.89	9.34	0.56	1.62
Mean	6.53	4.37	1.29	12.19	0.52	1.79
Forest soils – highlands and hilly country ($n = 9$)						
Min/Max	0.63–852.79	1.34–26.50	0.25–75.58	2.2–954.9	0.28–0.89	0.47–2.71
Percentiles 5–95%	3.36–32.66	2.49–19.63	0.48–6.73	6.3–59.0	0.51–0.66	1.22–1.66
Median	4.63	3.37	0.76	8.5	0.54	1.45
Mean	102.15	7.61	9.72	119.5	0.56	1.46
Forest soils – mountains ($n = 9$)						
Min/Max	5.02–1509.76	2.31–265.30	1.48–133.28	8.8–1908.3	0.57–0.79	2.17–5.69
Percentiles 5–95%	30.24–345.86	6.59–123.05	5.68–48.11	42.5–517.0	0.60–0.71	2.19–4.59
Median	46.04	15.23	9.14	69.2	0.67	3.16
Mean	236.81	52.48	25.93	315.2	0.67	3.54

[a] Reference value (Sáňka et al., 2002).

which has been observed in surrounding regions (Heinisch et al., 1997). Prevailing western winds enhance a chance that mountains at the Czech-German border were affected as well. High elevation, enhanced vertical and horizontal wet deposition, scavenging effect of coniferous forests, and high retention capacity of soils can all contribute to elevated concentrations of DDT in the mountain soils. Arable soils (a ratio from 0.58 to 4.12) corresponded to the values published by Harner et al. (1999) for 36 Alabama agricultural soils where DDT/DDE ratio ranged from 0.39 to 1.5 in six regions. DDT/DDE + DDD ratio published for US soils was smaller than 0.1 (Pereira et al., 1996), while it was higher in countries like China, where DDT production was banned later (1983). Chen et al. (2005) presented results for 43 arable soil samples with a DDT/DDE + DDD ratio higher than 2 in some samples, and even higher than 14 in two cases. In our case, the same ratio ranged between 0.28 and 0.89 with the highest median in mountain soils again (Table 3).

On the contrary, the highest median levels of more volatile pesticides (HCHs and HCB) were determined in arable soils (Table 3). For HCB, the arable soil median concentration was 3.4 μg kg^{-1}, while it only reached 1.3 μg kg^{-1} in highland and 1.4 μg kg^{-1} in mountain forest soils. The levels of HCB in grassland soils (0.02–6.3 μg kg^{-1} dw) and both types of forest soils (0.47–3.4 and 0.5–2.9 μg kg^{-1}) were comparable to data presented in the global background survey (0.01–5.2 μg kg^{-1}), but HCB levels in arable soils were two orders of magnitude higher than those of the global survey (Meijer et al., 2003b). The increased levels might be a result of the wide use of HCB-based fungicides for a crop seed treatment together with an ongoing HCB production related to the combustion of chlorinated wastes.

A similar trend was observed for all HCH isomers (Table 3): median concentrations were 2–4 times higher in arable soils (median level 4 μg kg^{-1} for the sum of HCHs) when compared to mountain soils (0.7 and 1.1 μg kg^{-1} for highland and mountain forest soils, respectively). Concentration levels of HCHs in Czech forest soils (0.55–2.34 and 0.26–1.66 μg kg^{-1} for highland and mountain soils, respectively) were at least one order of magnitude lower than those observed in Germany (4.4–82.4 μg kg^{-1}) (Wenzel et al., 2002). Similarly, the medians of arable soil levels of HCH

isomers were 1–3 orders of magnitude lower than the ones measured in the agricultural regions of China (Feng et al., 2003; Gong et al., 2003; Chen et al., 2005), India (Babu et al., 2003), Romania (Covaci et al., 2001) or US (Aigner et al., 1998).

Various HCH ratios are often used to assign the sources. When the α/γ-HCH ratio is higher than 1, it indicates an application of technical mixtures while the lindane application is most probable at the sites with the ratio lower than 1 (Ballschmiter and Wittlinger, 1991; Haugen et al., 1998; Kim et al., 2002; Barra et al., 2005). The actual concentrations and concentration ratios of all HCH isomers are affected by their different physico-chemical properties. Since the estimated half-life of lindane in upper soils is approximately 2 months (depends on the soil parameters) (Turnbull et al., 1997), a prevalence of γ-HCH isomer may indicate a recent input. A predominance of β-HCH can be a result of the isomerization of α-HCH to β-HCH in agricultural upper soil or γ-HCH to β-HCH via α-HCH. This isomer is energetically most favorable and thus, stable in soils (Wu et al., 1997). It has also the significantly longer half-time, and the bioconcentration factor two orders of magnitude higher than those of the other isomers. The fact that α-HCH is more prone to volatilization from soil than γ-HCH or β-HCH, while γ-HCH is the most water soluble and thus, prone to leaching (Wang et al., 2006) than α-HCH or β-HCH, can also alter final ratios. The median value of α/γ-HCH ratio in Czech soils varied between 0.94 (mountain soils) and 2.50 (grasslands) with observed maxima of 3.8. A β/γ ratio ranged from 0.03 to 5.33, with a median of 0.3 (Table 3).

3.4. Polyaromatic hydrocarbons

The median values of PAH concentrations for arable (729.9 μg kg^{-1} dw), grassland (661.7 μg kg^{-1} dw) and highland (523.9 μg kg^{-1} dw) soils were similar (Table 4) but these levels were about half order of magnitude higher in mountain soils (3713 μg kg^{-1} dw). We can speculate about the sources of elevated concentrations, however, it is probably a combination of the local heating sources, traffic as well as long-range transport. Phenanthrene, fluoranthene and pyrene were the most abundant compounds. While for arable and forest soils the mean and median

Table 4
PAHs, PCBs [μg kg^{-1}] and PCDDs/Fs [ng kg^{-1}] in examined soil samples.

Parameter	PAHs	PCBs	Σhomologous PCDFs	Σhomologous PCDDs
Ref. value[a]	1000.0	20.0		
Arable soils ($n = 39$ for PAHs and PCBs; $n = 32$ for dioxins)				
Min/Max	139.40–2436.00	3.50–42.10	16.53–726.31	16.82–746.77
Percentiles 5–95%	204.20–2306.60	3.50–34.60	26.00–502.86	19.72–679.42
Median	729.90	3.60	90.81	71.69
Mean	846.90	6.86	129.95	130.57
Grassland soils ($n = 22$)				
Min/Max	123.10–15 284.30	2.01–29.21	33.40–338.35	21.30–646.00
Percentiles 5–95%	234.70–10 052.20	4.10–24.67	41.00–316.21	25.70–204.20
Median	661.70	6.31	145.44	66.79
Mean	2510.56	9.34	158.13	100.10
Forest soils – highlands and hilly country ($n = 9$)				
Min/Max	148.90–1435.60	3.42–13.37	111.90–1061.02	76.00–324.30
Percentiles 5–95%	229.30–704.40	7.60–13.13	221.48–916.70	78.18–267.70
Median	523.90	8.40	435.70	110.81
Mean	538.73	8.87	500.59	157.40
Forest soils – mountains ($n = 9$)				
Min/Max	1693.80–8187.50	7.90–36.18	888.82–5987.39	316.48–2395.25
Percentiles 5–95%	2089.90–4775.90	15.32–34.72	1156.07–3502.34	461.74–1667.62
Median	3713.20	22.64	2132.82	983.41
Mean	3767.93	22.76	2594.00	1141.54

Parameter	Σ2378-PCDFs	Σ2378-PCDDs	Σ2378-PCDDs/Fs	TEQ Σ2378-PCDFs	TEQ Σ2378-PCDDs	TEQ Σ2378-PCDDs/Fs
Ref. value[a]	–	–	–	–	–	1.0
Arable soils ($n = 32$)						
Min/Max	5.20–165.16	7.38–661.18	15.99–722.15	0.28–10.28	0.17–7.92	0.56–14.26
Percentiles 5–95%	6.33–164.41	10.03–483.11	17.58–497.92	0.36–3.03	0.20–3.97	0.57–8.96
Median	11.91	34.50	51.09	0.77	0.53	1.41
Mean	30.97	83.09	114.06	1.42	1.07	2.49
Grassland soils ($n = 22$)						
Min/Max	4.90–50.66	7.90–483.93	12.80–522.42	0.33–2.35	0.15–2.73	0.47–4.06
Percentiles 5–95%	8.54–45.43	14.10–128.33	23.70–178.99	0.51–2.29	0.30–1.87	0.82–3.49
Median	20.75	28.87	50.03	1.17	0.76	2.00
Mean	21.53	57.51	79.04	1.24	0.87	2.11
Forest soils – highlands and hilly country ($n = 9$)						
Min/Max	26.41–156.80	21.01–149.00	47.41–305.80	1.68–11.85	0.87–4.52	2.59–16.37
Percentiles 5–95%	27.40–145.00	31.02–113.70	65.20–258.70	2.12–10.31	0.91–3.28	2.99–13.59
Median	38.14	51.69	96.27	2.84	1.40	4.24
Mean	66.15	62.90	129.05	4.85	1.92	6.77
Forest soils – mountains ($n = 9$)						
Min/Max	253.93–2255.86	171.85–1137.49	425.78–3393.35	14.68–106.67	4.49–34.91	19.17–141.58
Percentiles 5–95%	294.92–822.32	238.11–747.86	533.03–1525.41	17.58–51.87	6.28–21.04	23.86–72.91
Median	575.88	523.91	1041.19	34.05	12.41	46.09
Mean	756.88	561.80	1318.68	42.43	15.26	57.69

[a] Reference value (Sáňka et al., 2002).

Table 5
Main types of soil sites compared in one-way ANOVA analysis: abiotic characteristics and content of heavy metals.[a]

Type of soil site	C_{org}		Clay particles		CEC		pH_{H_2O}	
	(%)[b]	ANOVA[c]	(%)[b]	ANOVA[c]	(meq kg^{-1})[b]	ANOVA[c]	(pH units)[b]	ANOVA[c]
Arable soils ($n = 39$)	1.5 (1.4–1.6)[e]	89.1%	21.8 (14.5; 32.6)[d,e]	29.7%	385 (277; 533)[d,e]	7.8%	7.0 (6.8; 7.2)[e]	80.4%
Grasslands ($n = 22$)	3.0 (2.5–3.5)[e]	($p < 0.001$)	14.7 (11.8; 18.3)[e]	($p = 0.004$)	283 (212; 377)[e]	($p = 0.372$)	6.9 (6.5; 7.3)[e]	($p < 0.001$)
Forest soils – highlands ($n = 9$)	6.3 (4.1–9.2)[e]		13.25 (7.8; 22.4)[e]		408 (211; 789)[e]		6.4 (5.3; 7.7)[e]	
Forest soils – mountains ($n = 9$)	36.8 (34.2–39.8)[e]		8.53 (6.3; 11.5)[e]		346 (290; 412)[e]		3.5 (3.3; 3.7)[e]	
	N_{tot}		**Hg**		**Cd**		**Cr**	
	(%)[b]	ANOVA[c]	(mg kg^{-1})[b]	ANOVA[c]	(mg kg^{-1})[b]	ANOVA[c]	(mg kg^{-1})[b]	ANOVA[c]
Arable soils ($n = 39$)	0.15 (0.13; 0.16)[e]	80.2%	0.15 (0.11; 0.22)[e]	19.9%	0.64 (0.45; 0.89)[e]	12.3%	42.5 (33.6; 53.4)[e]	22.0%
Grasslands ($n = 22$)	0.23 (0.19; 0.27)[e]	($p < 0.001$)	0.16 (0.13; 0.21)[e]	($p = 0.001$)	0.37 (0.28; 0.49)[e]	($p = 0.019$)	34.6 (26.2; 45.8)[e]	($p < 0.001$)
Forest soils – highlands ($n = 9$)	0.32 (0.24; 0.43)[e]		0.25 (0.18; 0.36)[e]		0.57 (0.41; 0.77)[e]		38.5 (20.4; 72.5)[e]	
Forest soils – mountains ($n = 9$)	1.25 (0.90; 1.74)[e]		0.61 (0.42; 0.88)[e]		0.28 (0.19; 0.42)[e]		13.3 (9.5; 18.6)[e]	
	Ni		**Pb**		**Zn**		**As**	
	(mg kg^{-1})[b]	ANOVA[c]	(mg kg^{-1})[b]	ANOVA[c]	(mg kg^{-1})[b]	ANOVA[c]	(mg kg^{-1})[b]	ANOVA[c]
Arable soils ($n = 39$)	23.3 (19.4; 28.0)[e]	29.8%	45.3 (30.7; 66.9)[e]	20.0%	117.3 (91.9; 149.7)[e]	16.5%	16.0 (11.2; 23.1)[e]	8.8%
Grasslands ($n = 22$)	25.1 (20.1; 31.2)[e]	($p < 0.001$)	32.5 (26.4; 39.9)[e]	($p = 0.001$)	113.2 (85.2; 150.5)[e]	($p = 0.004$)	8.7 (7.6; 10.1)[e]	($p = 0.073$)
Forest soils – highlands ($n = 9$)	27.8 (16.1; 48.1)[e]		57.6 (43.4; 76.4)[e]		109.2 (75.4; 158.1)[e]		11.8 (8.8; 15.8)[e]	
Forest soils – mountains ($n = 9$)	8.2 (6.2; 10.9)[e]		151.4 (100.9; 227.3)[e]		47.1 (40.0; 55.4)[e]		12.3 (7.5; 20.1)[e]	

[a] All variables were transformed prior to the analysis: $X_{tr} = \ln [X + 1]$.
[b] Geometric mean with 95% confidence interval (in parenthesis), expressed in original scale.
[c] Outcomes of one-way ANOVA models: variance ratio (% of total sum of squares attributed to the influence of soil type); p value of global F test.
[d] There is a limited sample size in case of arable soils ($n = 6$ sites, see also Table 1).
[e] Soil types marked by the same letter are not statistically significantly different ($p < 0.05$; Tukey HSD test for unequal N).

PAH values stayed close, it is not true for grassland soils. The mean PAH concentration was about four times higher than the median in this group due to some heavily contaminated soils in the study set. The mean value of grassland soils exceeded the reference value 2.5 times, and both mean and median levels in mountains were almost 4 times higher than the reference value. According to classification of the soil contamination published by Maliszewska-Kordybach (1996), this reference level is also a threshold for heavily contaminated soils. This classification was derived from the results of PAH screening in European soils, as well as from the estimation of human exposure risks, and it indicates that Czech soils ranged from weekly contaminated to contaminated, with mountain soils being contaminated heavily. The mountain levels (1694–8188 μg kg^{-1}) were four times higher than the ones measured in Poland (4.4–1906 μg kg^{-1} in Holy Cross Mountains). For comparison, the statistical values for PAH contents in upper organic horizons of forest soils in Bavaria varied between 396 and 8189 μg kg^{-1} (median value 2214 μg kg^{-1}) in the areas with population density > 300 inhabitants/km^2, 205–6844 μg kg^{-1} (median value 1710 μg kg^{-1}) in the areas with population density > 150 and ≤300 inhabitants/km^2, and 208–9304 μg kg^{-1} (median value 2526 μg kg^{-1}) in the areas with population density ≤ 150 inhabitants/km^2 (Joneck et al., 2006).

3.5. Polychlorinated biphenyls

A distribution with lower levels and narrower range was observed for PCBs (Table 4). PCB levels increased from arable and grassland to highland and mountain forests. Those in the mountain

Table 6
Main types of soil sites compared in one-way ANOVA analysis: main groups of organic pollutants.[a]

Type of soil site	HCB		ΣHCH		α/γ HCHs		ΣDDTs	
	(μg kg^{-1})[b]	ANOVA[c]	(μg kg^{-1})[b]	ANOVA[c]	(ratio)[b]	ANOVA[c]	(μg kg^{-1})[b]	ANOVA[c]
Arable soils ($n = 39$)	3.61 (2.64; 4.95)[d]	16.3%	3.21 (2.69; 3.82)[d]	55.0%	1.79 (0.81; 3.96)[d]	3.2%	45.3 (29.9; 68.7)[d]	21.0%
Grasslands ($n = 22$)	1.57 (0.84; 2.92)[d]	($p = 0.004$)	0.91 (0.67; 1.25)[d]	($p < 0.001$)	1.27 (0.75; 2.17)[d]	($p = 0.727$)	11.4 (5.6; 23.3)[d]	($p = 0.005$)
Forest soils – highlands ($n = 9$)	1.36 (0.89; 2.09)[d]		1.06 (0.73; 1.54)[d]		1.34 (0.60; 2.97)[d]		16.4 (4.2; 63.1)[d]	
Forest soils – mountains ($n = 9$)	1.42 (0.96; 2.10)[d]		0.69 (0.40; 1.20)[d]		1.00 (0.77; 1.31)[d]		96.0 (29.7; 310.3)[d]	
	DDT/DDE + DDD		**DDT/DDE**		**ΣPAHs**		**ΣPCBs**	
	(ratio)[b]	ANOVA[c]	(ratio)[b]	ANOVA[c]	(μg kg^{-1})[b]	ANOVA[c]	(μg kg^{-1})[b]	ANOVA[c]
Arable soils ($n = 39$)	0.47 (0.43; 0.51)[d]	17.3%	1.20 (1.02; 1.43)[d]	21.1%	657 (517; 835)[d]	27.5%	5.24 (4.29; 6.39)[d]	36.1%
Grasslands ($n = 22$)	0.51 (0.45; 0.57)[d]	($p = 0.002$)	1.49 (1.11; 1.98)[d]	($p < 0.001$)	1004 (554; 1821)[d]	($p < 0.001$)	7.63 (5.78; 10.07)[d]	($p < 0.001$)
Forest soils – highlands ($n = 9$)	0.54 (0.43; 0.68)[d]		1.90 (0.78; 4.62)[d]		442 (265; 738)[d]		8.33 (6.14; 11.32)[d]	
Forest soils – mountains ($n = 9$)	0.67 (0.62; 0.72)[d]		3.36 (2.60; 4.35)[d]		3393(2352; 4895)[d]		20.89 (14.58; 29.93)[d]	
	Σ2378-PCDDs/Fs		**Σhomologous PCDFs**		**Σhomologous PCDDs**			
	(ng kg^{-1})[b]	ANOVA[c]	(ng kg^{-1})[b]	ANOVA[c]	(ng kg^{-1})[b]	ANOVA[c]		
Arable soils ($n = 32$)	64 (45; 92)[d]	57.5%	84 (60; 117)[d]	66.7%	80 (57; 112)[d]	53.4%		
Grasslands ($n = 22$)	53 (38; 76)[d]	($p < 0.001$)	130 (96; 176)[d]	($p < 0.001$)	69 (49; 97)[d]	($p = 0.001$)		
Forest soils – highlands ($n = 9$)	107 (66; 172)[d]		416 (246; 702)[d]		137 (90; 208)[d]			
Forest soils – mountains ($n = 9$)	1116 (698; 1383)[d]		2247 (1448; 2489)[d]		977 (604; 1109)[d]			

[a] All variables were transformed prior to the analysis: $X_{tr} = \ln [X + 1]$.
[b] Geometric mean with 95% confidence interval (in parenthesis), expressed in original scale.
[c] Outcomes of one-way ANOVA models: variance ratio (% of total sum of squares attributed to the influence of soil type); p value of global F test.
[d] Soil types marked by the same letter are not statistically significantly different ($p < 0.05$; Tukey HSD test for unequal N).

forests (22.6 μg kg^{-1}) were three times higher than PCB levels in highland soils (8.4 μg kg^{-1}). Although some individual values exceeded the reference levels in arable, grassland and forest soils, these levels were never exceeded by medians or arithmetic means. Since there are no primary sources of PCBs in rural and mountain regions, evaporation from the secondary sources as contaminated buildings, equipment and soil is the most probable source of the soil pollution. Re-volatilized chemicals are then a subject to long-range transport in the atmosphere and scavenging by vegetation. Coniferous needles have a high capacity to accumulate atmospheric pollutants from the gas phase, fine particle phase and wet deposition, and to transfer them to soil with litter. Due to their low degradation rates in soils, PCBs are still found in fairly high concentrations. The levels found in this study are in the same order of magnitude as the ones from the Global PCB soil survey (0.026–97 μg kg^{-1}) (Meijer et al., 2003b), higher when compared to the PCBs levels in the Swiss soil monitoring (1.1–12 μg kg^{-1}) (Schmid et al., 2005), and lower than surveys in Poland (2.3–38 μg kg^{-1}) (Falandysz et al., 2001), Slovakia (Kocan et al., 1999) or France (Motelay-Massei et al., 2004).

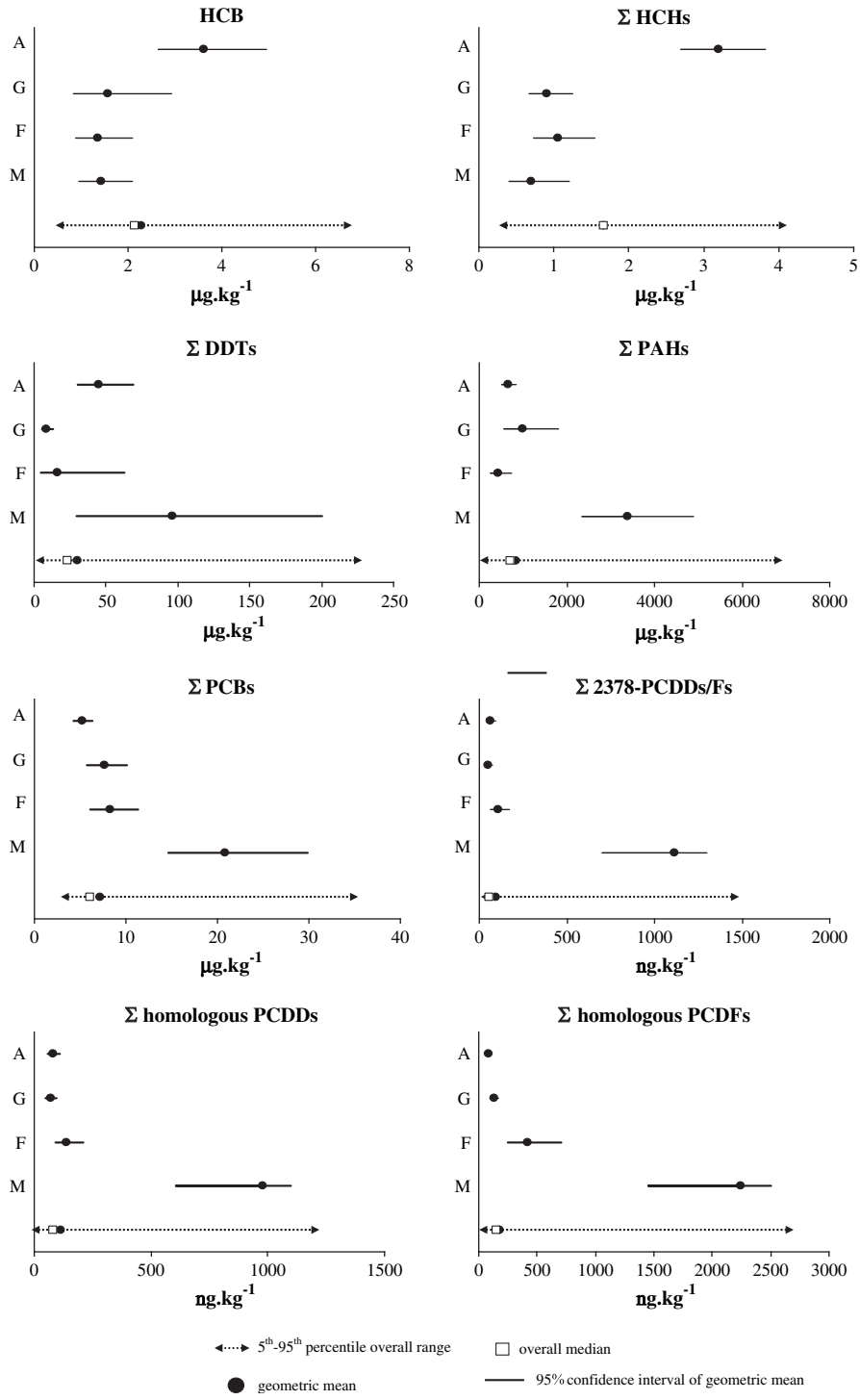

Fig. 2. POP levels in soils as the range plots (A: arable soils; G: grassland; F: forest soils in highlands; M: forest soils in mountains).

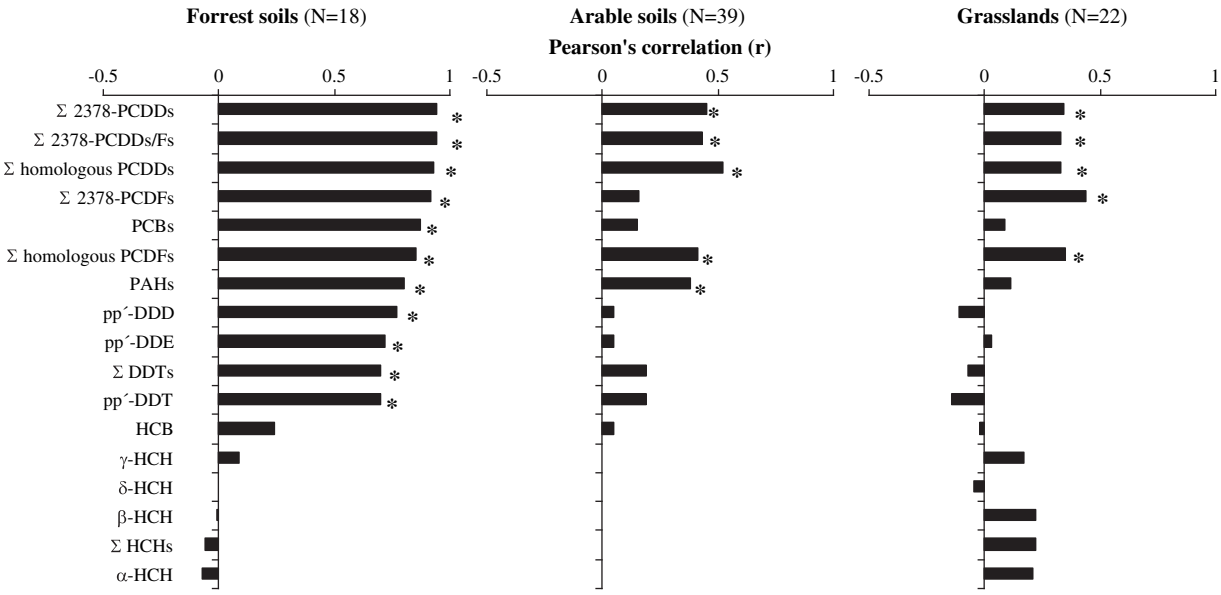

Fig. 3. Rank order of Pearson's correlation coefficients between the POP levels and organic carbon content in soils. * Mark for statistically significant correlation ($p < 0.05$). Rank ordered according to correlations in forest soils.

3.6. Polychlorinated dibenzo-p-dioxins and dibenzofurans

Among all substances, the group of PCDDs and PCDFs manifested the greatest difference between the concentration levels in arable and grassland soils, and mountain forest soils. The median value for the sum of 2378-substituted PCDDs/Fs in mountain soils was as high as 1041 ng kg^{-1} (ranging from 426 to 3393 ng kg^{-1} for the individual soils) which was 20 times higher than in arable soils (51 ng kg^{-1}, ranging from 16 to 722 ng kg^{-1}) or grasslands. A comparison of the concentration levels for PCDDs/Fs homologous groups (mono- to hepta-) gave similar results: the mountain soil median reached 983 ng kg^{-1} for PCDDs (ranging from 317 to 2395 ng kg^{-1}), and 2133 ng kg^{-1} for PCDFs (889–5987 ng kg^{-1}), while for arable soils the same median was only 72 ng kg^{-1} for PCDDs (17–747 ng kg^{-1}) and 91 ng kg^{-1} (17–726 ng kg^{-1}) for PCDFs. The reference value for the Czech soils is only available for TEQ (1 ng kg^{-1}). This was exceeded in all soils, both by medians and arithmetic means. The observed range of PCDDs/Fs concentrations in arable (16–722 ng kg^{-1}), grassland (13–522 ng kg^{-1}) and highland forest soils (47–306 ng kg^{-1}) were similar to the levels found in the Swiss national soil monitoring (72–703 ng kg^{-1}) (Schmid et al., 2005). Concentrations expressed as TEQ were comparable as well: 0.6–14 ng TEQ kg^{-1} for arable, 0.5–4 ng TEQ kg^{-1} for grassland, and 2.6–16 ng TEQ kg^{-1} for highland forest soils in the Czech Republic, and 1.1–11 ng TEQ kg^{-1} in Swiss soils. PCCD/F levels in the Czech mountain forest soils were, however, one order of magnitude higher (426–3393 ng kg^{-1}). While ranges between 0.2 and 2.7 ng TEQ kg^{-1} were published for the UK and Norway soil survey (Hassanin et al., 2005) and 4.1 ng TEQ kg^{-1} for US soils (Rogowski and Yake, 2005), a median TEQ value for Czech mountain soils was as high as 46 TEQ kg^{-1}.

There was a strong predominance of homologous PCDFs in all soils when compared to homologous PCDDs, most obviously for forest soils: ΣPCDFs/ΣPCDDs ratio was 1.27 for arable, 2.18 for grassland and 3.93 for forest soils. It was not the same for 2378-substituted congeners: in mountain soils, ΣPCDFs/PCDDs ratio for homologous groups equaled to 2.17, and 2378-PCDFs/Σ2378-PCDDs ratio was only 1.11. For arable, grassland and highland forest soils, 2378-substituted PCDDs were prevalent: Σ2378-PCDFs/Σ2378-PCDDs ratio was 0.35 for arable, 0.72 for grassland, and 0.74 for forest soils. When we expressed 2378-PCDDs/Fs concentrations as a toxic equivalent of 2378-TCDD, PCDFs strongly prevailed again: TEQ PCDFs/TEQ PCDDs was equal 1.45 in arable, 1.54 in grassland, 2.03 in highland, and 2.74 in mountain forest soils. A median toxic equivalent value TEQ PCDDs/Fs increased along the line arable (1.41 TEQ kg^{-1}), grassland (2 TEQ kg^{-1}), highland (4.24 TEQ kg^{-1}) and mountain forest soils, with latter one being one order of magnitude higher (46.09 TEQ kg^{-1}) than all remaining soils.

3.7. ANOVA modeling and correlation analyses

One-way ANOVA models discriminating different types of land use according to the levels of pollutants are documented in Tables 5 and 6 and also in Fig. 2. Many persistent compounds clearly separated three soil categories (arable, grassland, forest). A high discrimination potential had the sum of HCHs. Elevated HCH levels are still associated with former field application and increased HCH concentrations in arable soils exhausted 55% of the total data variance (Fig. 2). On the contrary, PCDDs and PCDFs, as the chemicals produced un-intentionally and associated with the long-range transport, were most abundant in forest soils. Their elevated forest soil levels exhausted 53–67% of a total experimental variance. The indication indices derived from DDTs (DDT/DDE, DDT/DDE + DDD) also highly significantly separated low values in arable and/or grassland soils from increased values in forests, namely mountain forests, unlike α/γ HCH ratio which did not contribute to the soil discrimination. A low discrimination potential was also found for rather ubiquitous PAHs and HCB, even though a separation of arable and mountain soils was still significant for both.

A relationship between the soil organic carbon content and the level of contamination was confirmed. Except for the most volatile compounds (HCB and HCHs) which did not correlate with TOC in any soil category (Fig. 3), all the other organic pollutants correlated to some extent. A strong correlation of PAHs, DDTs, PCBs, and PCDDs/PCDFs was observed in the carbon-rich forest soils. PCDDs and PCDFs correlated significantly also in grassland and arable soils (Fig. 3).

4. Conclusion

Data on the country-wide screening of the soil contamination in the Czech Republic as a representative of the Central European

region is presented here. A wide selection of organic and inorganic pollutants as well as a detailed assessment of a variety of the soil parameters allowed us to study the soil contamination in relation to the land use (cropland, grassland, forest), locations (highland and mountain forest soils), and specific soil properties.

Results clearly differentiated between arable, grassland and forest soils, and showed that due to the global atmospheric transport, the mountain ecosystems can reach the contamination levels higher than the ones found in urban and industrial regions. It is most significant for a group of PCDDs/Fs, where concentrations in mountain forests were 20 times higher than those in arable and grasslands soils.

There are several reasons for the observed higher concentrations in mountain forest soils. Frequent fogs and high wet deposition together with a high capacity of coniferous needles to scavenge and accumulate atmospheric pollutants and to transfer them to soil with a litter are just a few factors possibly responsible for these findings. The high retention capacity of carbon-rich soils is another reason, as is the different vegetation cover of the highland and mountain soils (deciduous and coniferous). The litter of these forest types differs widely with coniferous litter having a clearly longer turn over time than the deciduous one. This also means that the organic layer of coniferous forests represents a longer time period of pollutant input. The impact of each of the possible influencing factors on the observed concentration differences between the studied soils of various uses and locations remains to be identified.

Even though there has been a number of regulations and international measures successfully lowering the atmospheric levels of toxic compounds in recent years, we cannot expect to see similar fast decline of POPs in the mountain soils. Due to the very low degradation rates of these compounds in soils it will probably take several more years before we detect any improvement.

A variability of the POP concentrations in soils of different uses was discussed so far. For the assessment of the soil burdens and associated risks, however, large differences in the soil properties (different soil densities, history of input, turn-over and accumulation) become increasingly important. Chemical burdens in various soils can only be compared when the total pollutant masses bound in the soils (on a per square meter basis) are estimated. To achieve that, information on the individual soil densities is needed in addition to the concentrations in the various soil layers of the profile. The fact that bulk density of overlaying organic horizons (about 0.1–0.2 g cm^{-1}), for instance, is very low when compared to mineral horizons (1.2–1.4 g cm^{-1}), can significantly influence a final distribution of burdens.

Detailed information on the extent of contamination as well as on the soil quality may also serve as a basis for an assessment of the volatilization fluxes of POPs from polluted soils to air, and to estimate an extent to which soil, as a secondary source of the pollution, is responsible for elevated atmospheric levels of POPs. To complete such model, the volatilization fluxes of organic compounds have to be quantified for soils with various land use, organic carbon content and level of contamination. Since the Czech Republic represents a typical industrial country of the mild climate, such information on both, soil burdens and atmospheric fluxes, would significantly contribute to the on-going inventories of persistent toxic compounds in the environment.

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Soil burdens of persistent organic pollutants — Their levels, fate and risks Part III. Quantification of the soil burdens and related health risks in the Czech Republic

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ABSTRACT

A total number of 471 soil samples collected during the period of 1996–2006 from the agricultural and forest areas of the Czech Republic were analyzed for their content of persistent organic pollutants (POPs). Spatial variability of the POP concentrations was assessed using an IDW spatial GIS model analysis. For every grid of the network, resulting modeled levels of contamination allowed for estimation of the total burden of POPs in soils. Potential risks associated with contaminated soils were assessed as well. Database of the old ecological burdens counting 3061 sampling sites was used to adjust the model and incorporate the risks of heavily contaminated sites. The high levels of health risks were only found at less than 1% of the area of interest. The IDW modeling proved to be a useful tool for screening of the health risks in the large areas with scarce monitoring data. Presented approach can be applied in the risk management, to support an efficient targeting of the risk reduction measures, or to improve a design of the national monitoring.

1. Introduction

Persistent organic pollutants (POPs) represent a diverse group of toxic substances, which are semi-volatile, mobile in the environment, and prone to long-range transport, accumulation in abiotic matrices as well as bioaccumulation in living bodies. They encompass several classes of organic contaminants including polychlorinated dibenzo-*p*-dioxins and furans (PCDDs/Fs), polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), and other industrial and agricultural chemicals. Polycyclic aromatic hydrocarbons (PAHs), although much less persistent and bioaccumulative, are sometimes included due to their great potential for long-range transport.

POPs have various physicochemical properties, which are responsible for their specific environmental behavior (Lohmann et al., 2007), efficiency of degradation or bioaccumulation. One of the matrices that acts as a natural sink of these toxic chemicals (Meijer et al., 2002, 2003a,b; Hassanin et al., 2004, 2005) is soil. A total POP burden in soils is a function of the balance between their inputs and losses (Sweetman et al., 2002). POPs can be accumulated in forest, grassland, agricultural and urban soils as a result of various anthropogenic activities (pesticide application, emissions from industry and traffic, application of sewage sludge or compost, spills, and contaminated water irrigation). As POPs are often released to the atmosphere where they are a subject to long-range transport and soil–air exchange processes, background soils can be contaminated as well (Dalla Valle et al., 2007). As the accumulation takes place preferably in horizons rich in organic matter, it is important to sample and analyze both, the overlaying organic horizons (O_L, O_F and O_H) and the first mineral horizon (A_H) with a high organic matter content in natural (forest) soils. In grasslands, the top 10–15 cm is usually enriched in organic matter. The organic horizon is missing in arable soils, and the top mineral horizons are homogenized by ploughing, which levels out the POPs concentration in about the top 25 cm (WRB, 2006). The urban soil concentrations are subject to great variability, which makes it difficult to estimate their contribution to the land mass budget. In temperate industrialized zones, soils generally contain concentrations that are factor of 5–10 above the rural levels. On the other hand, urban areas represent just a small fraction of the global surface (0.05%).

Once deposited to surface soils, POPs often persist for many years and affect soil quality, accumulate in plants and enter food chains. They are subject to further redistribution (erosion, percolation, re-emission, and bioaccumulation) and transformation processes. Understanding their fate in soils, though, becomes increasingly important (Armitage et al., 2006). In order to assess probability of POPs release to other environmental compartments, and to quantify this process, information is needed not only on the POP levels, but also on their total pools in the top soil layers. Both, the concentration and the pool, are influenced by

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many factors, especially land use, soil type, type of vegetation and climatic conditions.

Data on the pools of POPs in soils are still quite scarce. Based on data from the global background soil survey (208 undisturbed sites throughout the world), for instance, it has been estimated that 21 000 metric tons of PCBs are still present in the background surface soils (0–5 cm) globally (Ockenden et al., 2003). Number of studies assessing the risks resulting from the soil pollution and providing a spatial distribution of such risks is also limited.

Relevant human exposure pathways include inhalation of particles (Nessel et al., 1992), skin contact (Skowronski et al., 1990; Dor et al., 2000) or ingestion. All of these intake routes contribute to the overall exposure, but the extent of such contribution depends on the physiology and biochemistry of each individual with respect to contaminant uptake and processing (Roos et al., 2004), person's occupational and recreational activities, and age-specific behavior.

Historically, risks were assessed qualitatively, rating sites simply as 'high', 'medium' or 'low' risk. This approach was outmoded by quantitative assessments giving a numerical evaluation of the risks. A deterministic risk assessment uses a single point value for each variable in the risk model, e.g. the average soil concentration and the average bodyweight of receptor to produce an 'average' risk measure (Gay and Korre, 2006). An effective and widely applied tool for the environmental modeling is a Geographic Information System (GIS); (Tristan et al., 2000; Korre et al., 2002; Gay and Korre, 2006). This tool can combine data from various sources, present them spatially and analyze their relations and interactions. It supports development of predictive models and provides support for decision-making.

To assess the risks connected to the POP burdens in soil, we proceeded in steps. In the first step, a country wide inventory of soils, their properties and contamination levels was performed based on available monitoring data (Holoubek et al., submitted for publication). As a second step, we developed a model estimating total burdens and associated potential risks for human and wildlife. Spatial GIS data models were applied to facilitate a spatial modeling. Priority regions where additional measurements would be most beneficial for significant improvement of current work have been identified.

2. Material and methods

2.1. Available data

The Czech Republic stretches over the area of 78 870 km^2, out of which 54.35% is agricultural land (arable land 39.31%, grassland 12.01%), and 33.4% are forests.

A total number of 471 soil samples originating from two monitoring programs were analyzed for their contents of POPs (selected PCBs and OCPs). The first group consisted of 237 samples of agricultural soils and represented the basal monitoring of soils of the Ministry of Agriculture of the Czech Republic (Sáňka et al., 1998). The second group consisting of 243 grassland and forest soil samples represented a database of the screening projects of the Research Centre for Environmental Chemistry and Ecotoxicology (RECETOX) of Masaryk University. This database includes 63 forest and 180 agricultural soil samples. Although these two programs served different purposes and collected the samples from different land use areas, the sampling strategies and procedures as well as analytical methods were compatible (Holoubek et al., submitted for publication; Sáňka and Paterson, 1995; Sáňka et al., 1998). Moreover, an inventory of the old ecological burdens (SESEZ) (Gruntorád, 2006) containing classified information on the risk levels from 3061 heavily contaminated sites was applied in the final step of the risk evaluation. This database is supervised by the Ministry of Environment and collects administrative, geological, hydrogeological, and geochemical information on contaminated sites. Nowadays, it contains about 7000 sites, mostly the old dumps and brownfields. All sampling sites are presented in Fig. 1.

2.2. Soil sampling and analysis

Detailed sampling and analytical procedures have been published previously (Holoubek et al., 2009). In short: The mixed plough layer (0–25 cm) was sampled on arable soil. The top 10 cm soil layer enriched in organic matter was collected on grasslands. In the forest, the litter (O_L horizon) was carefully removed before sampling; the

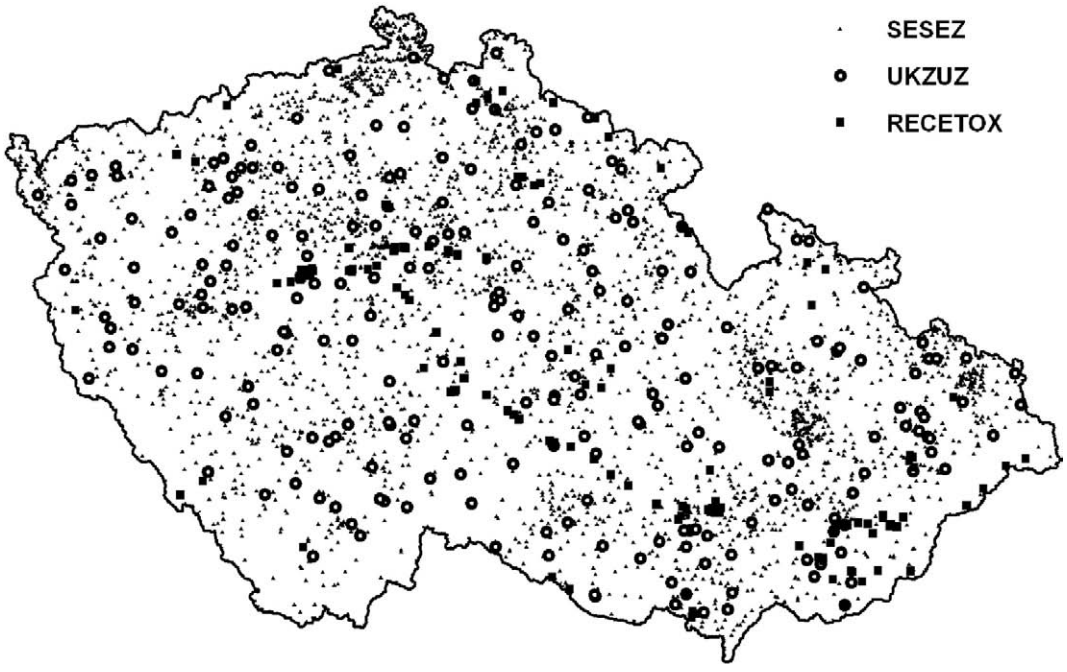

Fig. 1. Location of the soil sampling sites.

overlaying organic horizons $O_F + O_H$ and the mineral horizon A_H were sampled separately. This sampling strategy results in a high variability of the physicochemical properties of the soil samples. On the other hand, it is the only strategy enabling to compare the POPs concentrations in the top soil layers countrywide, and to estimate the burdens and risks in the next steps.

All soil samples were transported to the laboratory in polyethylene bags, air-dried at laboratory temperature, sieved through 2-mm mesh, and analyzed as described earlier (ČSN-ISO, 1998; Holoubek et al., submitted for publication).

2.3. Concentration modeling

As a base for calculation of the POP burdens in soils, the POPs concentration data were processed differently for agricultural and forest soils. The Inverse Distance Weighting (IDW) method was applied to the agricultural sites since they are well distributed over the country forming a dense network. On the contrary, there were not enough sites for application of IDW method on forest soils, and arithmetic means were used to cover the area both, for the organic and mineral horizons. The IDW is a method for multivariate interpolation used usually for estimation of the environmental levels in areas with no sampling data (Cheng et al., 2007; Zhou et al., 2007; Holoubek et al., submitted for publication). The values assigned to such points are determined by the mathematical interpolation of the values of their neighbors. Assuming that concentration values at locations that are close to one another will be more alike than those far apart, IDW will use the measured values surrounding the site of interest to predict an unmeasured value. It means that the weight of each measured value applied in prediction is decreasing as a function of distance. A general form for interpolation of certain value using IDW is:

$$Z = \frac{\sum_{i=1}^{N} \frac{Z_i}{d_i^p}}{\sum_{i=1}^{N} \frac{1}{d_i^p}} \quad (1)$$

where Z is a value of the interpolated point, Z_i is a known value, N is a total number of the known points used in the interpolation ($N = 10$ for our study), d_i is the distance between the interpolated point and the point with a known concentration, and p is a positive real number called the power parameter. As the weight decreases with increasing distance between the points, greater values of p assign a greater influence to concentration values closest to the point of interest. The optimal p-value can be determined by minimizing a root mean square prediction error (RMSPE). In order to calculate a minimal RMSPE, and to identify an optimal p-value, a number of different power parameters have been tested using an ArcGIS Geostatistical Analyst (Johnston et al., 2001). All available concentration data were used (Johnston et al., 2001) as an input to the IDW modeling.

An average POP concentration in soil was used for estimation of the forest soil burdens. As the POP concentration was determined separately for the upper organic layer (with a thickness h_{fO}) and the mineral layer with humus content (with a thickness h_{fA}), the average POP concentration was calculated for each sampling site according to the following equation:

$$C_f = \frac{C_{fO} \cdot h_{fO} + C_{fA} \cdot h_{fA}}{h} \ [\mu g\ kg^{-1}] \quad (2)$$

where:

- C_{fO} is a POP concentration in O horizon of forest soil at the site of interest ($\mu g\,kg^{-1}$)
- h_{fO} is a thickness of upper (organic) horizon of forest soil ($= 0.05$ m)
- C_{fA} is a POP concentration in A horizon of forest soil at the site of interest ($\mu g\,kg^{-1}$)
- h_{fA} is a thickness of humus (Ah) horizon of forest soil ($= 0.20$ m)
- h is a total thickness of O and Ah horizons of forest soil ($= 0.25$ m)

2.4. Calculation of the total pool of POPs in the top soil layer

The loading of POPs in background surface soils is a function of cumulative atmospheric deposition minus losses due to volatilization, biodegradation and percolation (Wania and McLachlan, 2001). Land use, soil depth and turnover, bulk density, stoniness and a content of the soil organic matter (SOM) were found to be important factors affecting the POP persistence and behavior in soils (Mackay, 2001).

A pool of POPs in the top soil layer was calculated for each km^2 according to following equations:

2.4.1. Agricultural soil

$$Pool = C_a \cdot \rho_a \cdot h_a \ [kg\,m^{-2}] \quad (3)$$

where:

- C_a is a concentration of POPs at the specific site on agricultural soil ($kg\,t^{-1}$)
- ρ_a is a bulk density of Ap horizon of agricultural soil ($t\,m^{-3}$)
- h_a is a thickness of Ap horizon of agricultural soil (m)

2.4.2. Forest soil

An average bulk density ρ_f for the top organic and mineral layers was calculated according to:

$$\rho_f = \frac{\rho_{fO} \cdot h_{fO} + \rho_{fA} \cdot h_{fA}}{h} \ [t\,m^{-3}] \quad (4)$$

where:

- ρ_{fO} is a bulk density of upper (organic) horizon of forest soil ($t\,m^{-3}$)
- h_{fO} thickness of upper (organic) horizon of forest soil ($= 0.05$ m)
- ρ_{fA} bulk density of humus (Ah) horizon of forest soil ($t\,m^{-3}$)
- h_{fA} thickness of humus (Ah) horizon of forest soil ($= 0.20$ m)
- h is a total thickness of O and Ah horizons of forest soil ($= 0.25$ m)

Average values of bulk densities ρ_{fO} and ρ_{fA} for the organic and mineral horizons, respectively, were taken from Macků (2006) (Table 1).

Stoniness was included as an important factor correcting the POP concentration values measured in sieved soils to the stone content to assess the POP pool values. Coefficients of stoniness were derived for all soil types from the taxonomic classification system of Czech soils (Nemecek and Kozak, 2003), and they varied between 0 and 1 according to the average content of gravel and stones in the top 0.25 m of soil.

Pool of POPs in forest soils was calculated according to:

$$Pool = C_f \cdot \rho_f \cdot h_f \cdot S \ [kg\,m^{-2}] \quad (5)$$

where:

- C_f is a POP concentration at the specific forest site ($kg\,t^{-1}$)
- ρ_f is an average bulk density for the top organic and mineral layers ($t\,m^{-3}$)
- h_f is a thickness of the top organic and mineral layers (m)
- S is a stoniness of O and Ah horizons of forest soil (unitless)

Table 1
Parameters used for determination of the pool of POPs in the individual polygons.

Parameter	Symbol	Unit	Value
Concentration of POPs at the specific site in O hor. of forest soil	C_{fO}	μg kg^{-1}	Specific
Concentration of POPs at the specific site in A hor. of forest soil	C_{fA}	μg kg^{-1}	Specific
Concentration of POPs at the specific site on forest soil	C_f	μg kg^{-1}	Specific
Concentration of POPs at the specific site on agricultural soil	C_a	μg kg^{-1}	Specific-IDW model
Bulk density of upper (O) horizon of forest soil	ρ_{fO}	t m^{-3}	0.1
Bulk density of humus (Ah) horizon of forest soil	ρ_{fA}	t m^{-3}	1
Stoniness of O and Ah horizon of forest soil	S	Index 0–1	Specific
Thickness of upper (organic) horizon of forest soil	h_{fO}	m	0.05
Thickness of humus (Ah) horizon of forest soil	h_{fA}	m	0.2
Bulk density of Ap horizon of agricultural soil	ρ_a	t m^{-3}	1.4
Thickness of Ap horizon of agricultural soil	h_a	m	0.25

2.5. Risk assessment

The soil screening level (SSL) model was adopted for estimation of the human intake of soil contaminants and consequent risks. This method is based on the risk assessment procedure developed by US EPA (EPA, 2001). Soil screening levels represent the risk-based soil concentrations derived for the individual chemicals of concern from equations combining exposure assumptions with toxicity criteria.

For each chemical, SSL is back-calculated from the target risk level, whereas an excess lifetime cancer risk (ELCR) is 1×10^{-6} for the soil exposure. The following equations are used to calculate SSL values for a residential population exposed to hazardous chemicals via all three exposure pathways. Default exposure parameters are provided whenever site-specific data are not available (Moya and Phillips, 2002; EPA, 2009 update).

2.5.1. SSL based on non-carcinogenic risks

$$C = \frac{THQ \cdot BW_c \cdot AT_n}{EF_r \cdot ED_c \left[\left(\frac{1}{RfD_o}\cdot\frac{IRS_c}{10^6\ \mathrm{mg/kg}}\right) + \left(\frac{1}{RfD_o}\cdot\frac{SA_c \cdot AF_c \cdot ABS}{10^6\ \mathrm{mg/kg}}\right) + \left(\frac{1}{RfD_i}\cdot\frac{IRA_c}{VF_s \text{ or } PEF}\right)\right]} \quad (6)$$

where:

C	Contaminant concentration (SSL) (mg kg^{-1})	Chemical-specific
THQ	Target hazard quotient	1
BW_c	Body weight, child (kg)	15
AT_n	Averaging time, non-carcinogens (days)	ED × 365
EF_r	Exposure frequency, resident (day yr^{-1})	350
ED_c	Exposure duration, child (years)	6
IRS_c	Soil ingestion rate, child (mg day^{-1})	200
RfD_o	Oral reference dose (mg kg^{-1} day^{-1})	Chemical-specific
SA_c	Dermal surface area, child (cm^2 day^{-1})	2800
AF_c	Soil adherence factor, child (mg cm^{-2})	0.2
ABS	Skin absorption factor (unitless)	Chemical-specific
IRA_c	Inhalation rate, child (m^3 day^{-1})	10
RfD_I	Inhalation reference dose (mg kg^{-1} day^{-1})	Chemical-specific
VF_s	Volatilization factor for soil (m^3 kg^{-1})	Chemical-specific
PEF	Particulate emission factor (m^3 kg^{-1})	Chemical-specific

2.5.2. SSL based on carcinogenic risks

$$C = \frac{TR \cdot AT_c}{EF_r \left[\left(\frac{IFS_{adj}\cdot CSF_o}{10^6\ \mathrm{mg/kg}}\right) + \left(\frac{SFS_{adj}\cdot ABS\cdot CSF_o}{10^6\ \mathrm{mg/kg}}\right) + \left(\frac{InhF_{adj}\cdot CSF_i}{VF_s \text{ or } PEF}\right)\right]} \quad (7)$$

where:

C	Contaminant concentration (SSL) (mg kg^{-1})	Chemical-specific
TR	Target cancer risk	1E−06
AT_c	Averaging time, carcinogens (days)	25 550
EFr	Exposure frequency, resident (day yr^{-1})	350
IFS_{adj}	Age-adjusted soil ingestion factor ([mg yr^{-1}]/[kg day])$^{-1}$	114
CSF_o	Oral cancer slope factor (mg kg^{-1} day^{-1})	Chemical-specific
SFS_{adj}	Age-adjusted dermal factor ([mg yr^{-1}]/[kg day^{-1}])	361
ABS	Skin absorption factor (unitless)	Chemical-specific
$InhF_{adj}$	Age-adjusted inhalation factor ([m^3 yr^{-1}]/[kg day^{-1}])	11
CSF_i	Inhalation cancer slope factor (mg kg day)$^{-1}$	Chemical-specific
VF_s	Volatilization factor for soil (m^3 kg^{-1})	Chemical-specific
PEF	Particulate emission factor (m^3 kg^{-1})	Chemical-specific

Calculated SSL values (presented in Table 2) were further compared to concentrations of contaminants measured in soils. In case of the exposure to multiple chemicals, total risk is calculated as an additive value according to following equation:

$$Site\,Risk = c_1 / SSL_1 + c_2 / SSL_2 + \ldots + c_i / SSL_i.$$

Resulting ratio smaller than 1 indicates that the POP concentrations measured at the site are unlikely to result in an adverse health impact.

The analysis was performed using a maximal concentration of each chemical found at the individual monitoring site during the whole sampling period (1998–2006). This approach guarantees the worst

Table 2
Calculated SSL (mg kg^{-1}) values for the individual POPs.

Compound	p,p′-DDT	p,p′-DDD	p,p′-DDE	o,p′-DDT	o,p′-DDD	o,p′-DDE	
SSL (mg kg^{-1})	1.72	2.44	1.72	1.72	2.44	1.72	
risk type	ca	ca	ca	ca	ca	ca	
Compound	HCB	α-HCH	β-HCH	γ-HCH			
SSL (mg kg^{-1})	0.304	0.0902	0.316	0.437			
risk type	ca	ca	ca	ca			
Compound	PCB118	PCB101	PCB52	PCB28	PCB180	PCB153	PCB138
SSL (mg kg^{-1})	0.112	0.112	0.112	0.112	0.112	0.112	0.112
risk type	nc	nc	nc	nc	nc	nc	nc

Risk type ca — carcinogenic; nc — non-carcinogenic.

Table 3
Risk categories and risk factors of the sampling sites in the SESEZ database.

Risk level	Risk category	Distance	Risk factor
Extreme	Local	1 km	2.00
		3 km	1.80
		5 km	1.50
	Point	1 km	2.00
High	Regional	1 km	1.70
		3 km	1.56
		5 km	1.35
		20 km	1.14
	Local	1 km	1.70
		3 km	1.56
		5 km	1.35
	Point	1 km	1.70
Medium	Regional	1 km	1.40
		3 km	1.32
		5 km	1.20
		20 km	1.08
	Local	1 km	1.40
		3 km	1.32
		5 km	1.20
	Point	1 km	1.40
Low	Regional	1 km	1.20
		3 km	1.16
		5 km	1.10
		20 km	1.04
	Local	1 km	1.20
		3 km	1.16
		5 km	1.10
	Point	1 km	1.20

case scenario within the consequential risk assessment. Agriculture locations were analyzed separately from the forest soil sites.

Applied methodology combined a quantitative human health risk assessment with spatial GIS methods in order to assess the spatially resolved human health risks originating from contaminated soil providing measures of uncertainty at the same time.

To take in the influence of the hot spots on the overall risks, the primary risk calculations were adjusted using the SESEZ database. In the SESEZ database, the sites were divided into several categories (point, local or regional impact) with various intensities of the effects (extreme, high, medium or low). The final risk was derived from the primary calculated risk value multiplied by an appropriate risk factor based on the SESEZ effect classification (Table 3, Gruntorád, 2006).

3. Results and discussion

3.1. Estimation of the burdens

The IDW method was applied for a multivariate interpolation of the POP levels in soils. Based on available POP concentration data, a total pool of each chemical in the top 0.25 m layer of soil was estimated for each polygon of 1×1 km. A summary of the statistical values of the pools for the individual POPs per square kilometer is shown in Table 4 for both, agricultural and forest soils. Total pools of the individual POPs in the top soil layers of the Czech Republic are presented in Table 5. As can be seen from Table 5, DDT and its metabolites represent a group of POPs with the largest pool, representing some 1669 metric tons of chemicals while PCBs and HCHs were estimated to represent a pool of 280 and 303 tons, respectively.

This method presents a new approach to modeling of the environmental burdens of POPs because until now, estimation of the soil body burden has been solely based on available data on the POP inputs to soils (e.g. fertilizers application). A comparison of both approaches demonstrated a good agreement between the method presented in this paper and the simple calculation of the POP levels from the production and application estimates for various chemicals in the Czech Republic (Holoubek, 2003) and their half-lives. It has been estimated, for instance, that some 15 665 tons of DDTs and 61 680 tons of lindane (gamma HCH) were used in the Czech Republic. Estimated half-lives are up to 6 years for DDT, and 2 years for HCH (Holoubek, 2004). Provided that DDTs and HCHs have been banned since 1980s, their environmental burdens should be around 1000 tons of DDTs or 100 tons of lindane today. Estimation of the total pools of POP in soils based on the IDW model used in present study gave very similar results suggesting that the IDW modeling offers a very useful tool for an assessment of the environmental burdens and associated risks.

3.2. Estimation of the risks

A map of the IDW predicted concentrations can also serve as an input for the exposure assessment. As can be seen from Fig. 2, the sites with elevated risks can be found in the vicinity of potential sources of POPs (mainly industrial areas). Nevertheless, a significant risk level was determined only at the very small fraction of the investigated region.

Table 4
Statistical values of the pools of the individual POPs (in metric tons per square kilometer) in agricultural and forest soils.

Compound	Agriculture soil			Forest soil		
	Min	Max	Avg	Min	Max	Avg
	t km^2			t km^2		
*p,p′*DDT	6.00E−05	8.60E−01	1.60E−02	2.20E−03	4.40E−03	3.50E−03
*p,p′*DDD	1.70E−05	2.00E−01	7.20E−04	3.40E−04	6.80E−04	5.40E−04
*p,p′*DDE	3.50E−04	5.40E−01	9.80E−03	1.00E−03	2.00E−03	1.60E−03
o,p′-DDT	6.30E−05	1.30E−01	3.00E−03	8.20E−06	1.60E−05	1.30E−05
o,p′-DDD	2.00E−05	9.80E−03	3.10E−04	8.40E−05	1.70E−04	1.30E−04
o,p′-DDE	2.30E−05	1.60E−02	4.30E−04	2.20E−05	4.40E−05	3.60E−05
DDT's	6.20E−04	1.40E+00	3.00E−02	3.70E−03	7.30E−03	5.80E−03
HCB	8.00E−06	2.00E−01	2.20E−03	2.50E−04	4.90E−04	3.90E−04
α-HCH	4.20E−05	3.40E−02	1.20E−03	3.20E−04	6.30E−04	5.10E−04
β-HCH	6.60E−06	1.50E+00	1.60E−03	1.90E−04	3.90E−04	3.10E−04
γ-HCH	3.80E−05	2.20E−02	2.20E−03	2.60E−04	5.30E−04	4.20E−04
δ-HCH	2.40E−06	1.50E−02	4.90E−04	1.50E−06	3.10E−06	2.50E−06
HCHs	1.10E−04	1.50E+00	5.40E−03	7.70E−04	1.50E−03	1.20E−03
PCB118	1.30E−05	2.20E−02	3.10E−04	8.50E−05	1.70E−04	1.40E−04
PCB101	1.70E−05	3.10E−02	4.60E−04	6.40E−05	1.30E−04	1.00E−04
PCB52	1.60E−05	8.80E−03	2.60E−04	5.70E−05	1.10E−04	9.10E−05
PCB28	1.40E−05	9.30E−03	2.40E−04	3.70E−05	7.40E−05	5.90E−05
PCB180	2.30E−05	5.30E−01	1.20E−03	1.30E−04	2.70E−04	2.10E−04
PCB153	4.10E−05	1.50E−01	1.00E−03	2.30E−04	4.60E−04	3.70E−04
PCB138	3.60E−05	4.50E−01	1.50E−03	2.20E−04	4.30E−04	3.50E−04
PCBs	1.90E−04	1.20E+00	4.90E−03	8.20E−04	1.60E−03	1.30E−03

Table 5
Total pools of the individual POPs (in metric tons) in the top soil layers of the Czech Republic.

Compound	*p,p′*-DDT	*p,p′*-DDD	*p,p′*-DDE	*o,p′*-DDT	*o,p′*-DDD	*o,p′*-DDE	DDTs	
Total pool	897.18	51.43	529.64	149.32	19.22	22.32	1669.11	
Compound	HCB	α-HCH	β-HCH	γ-HCH	δh-HCH	HCHs		
Total pool	120.96	71.85	88.33	118.89	24.16	303.23		
Compound	PCB118	PCB101	PCB52	PCB28	PCB180	PCB153	PCB138	PCBs
Total pool	19.06	25.48	15.52	13.57	63.25	61.39	82.44	280.7

The target carcinogenic risk limit as a standard risk protection factor was set to 10^{-6} for the purpose of this study. Target risk levels decide a degree of the human health protection to be achieved by the risk-based soil cleanup standards. In the interest of the conservative nature of the risk assessment calculations, there is an apparent perception that firmer acceptable risk levels should be applied. There are several approaches to utilization of the target risks: a single specified acceptable risk level of 10^{-5} or 10^{-4}, a range of acceptable risk levels (e.g. 10^{-4} to 10^{-6}) or specific risk levels depending on the weight-of-evidence classification of each chemical (i.e., 10^{-6} for A carcinogens, and 10^{-5} for B and C carcinogens, or 10^{-5} for A carcinogens and 10^{-4} for B and C carcinogens). Calculation of the human risk levels using any of these approaches results in the conclusion that situation in the studied area is much better and contamination levels represent negligible human health risk.

3.3. Identification of uncertainties

Identification and quantification of uncertainties is a crucial step in characterization of potential risks (EPA, 1989). In our study, an uncertainty is defined as a function of the grid centroid distance from the monitored sampling site which means that increasing distance from the sampling site results in increasing uncertainty. This method can identify uncertainties at various stages of the whole interpolation model and allows for an assessment of the net homogeneity of the monitoring sites (Fig. 3). Using this approach, we can alter distribution of additional sampling sites to improve significantly the accuracy of estimation of the environmental levels.

There is a continuous need to eliminate population health risks associated with an exposure to the environmental contaminants. In order to do so, it is necessary to identify the sites with high population density and high contamination level at the same time. In most of the previous studies, however, only the hot spots were identified. An example of such analysis can be found in Fig. 4 presenting the fractions of the whole investigated area where the risks fall to given risk categories. It is apparent from the picture that the highest risk (a value greater than 2) was found on less than 1% of the studied area.

On the other hand, the areas with high population density represent the highest hazards despite of medium potential risk levels. An assessment of the most hazardous areas was performed in the next step. The value of hazard was determined as a function of predicted risk level multiplied by population density (Fig. 5). This approach can identify the areas with the most urgent need to decrease the health risks by decreasing the exposures. Similar maps can provide a powerful tool for the risk managers, enabling efficient targeting of the risk reduction measures to specific regions, as well as improvement of the regional monitoring networks.

4. Conclusions

The IDW model represents a smooth transition between the sampled sites in the non-sampled area. This character of modeling

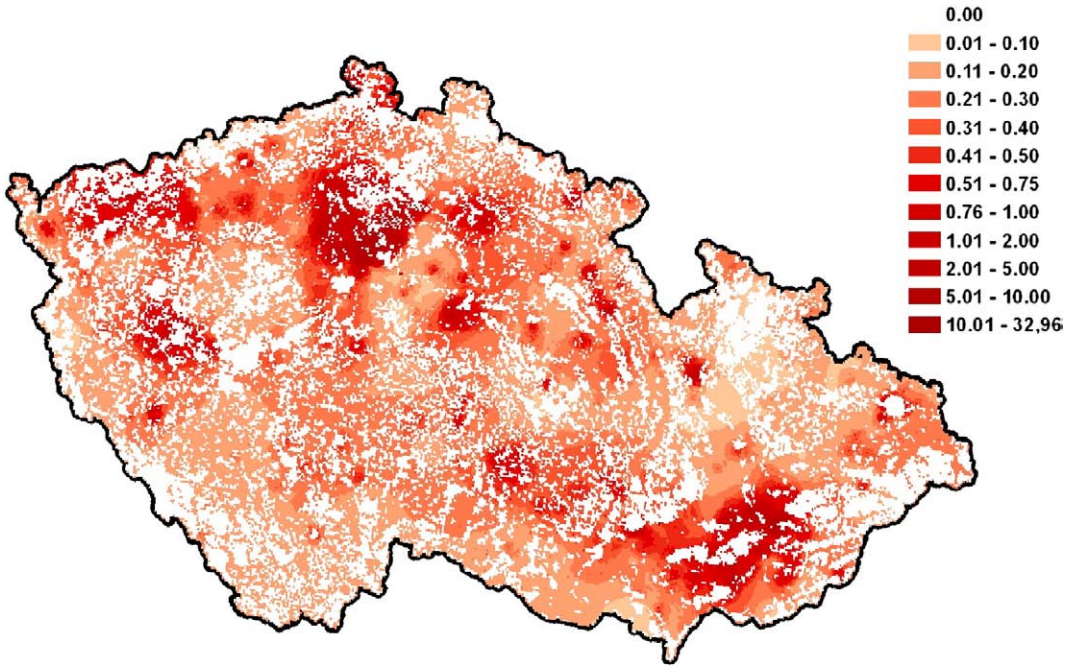

Fig. 2. Spatially resolved (grid of 1 × 1 km) potential risk (unitless, EPA) from the POP (selected PCBs and OCPs) exposure via contaminated soil based on the IDW interpolation.

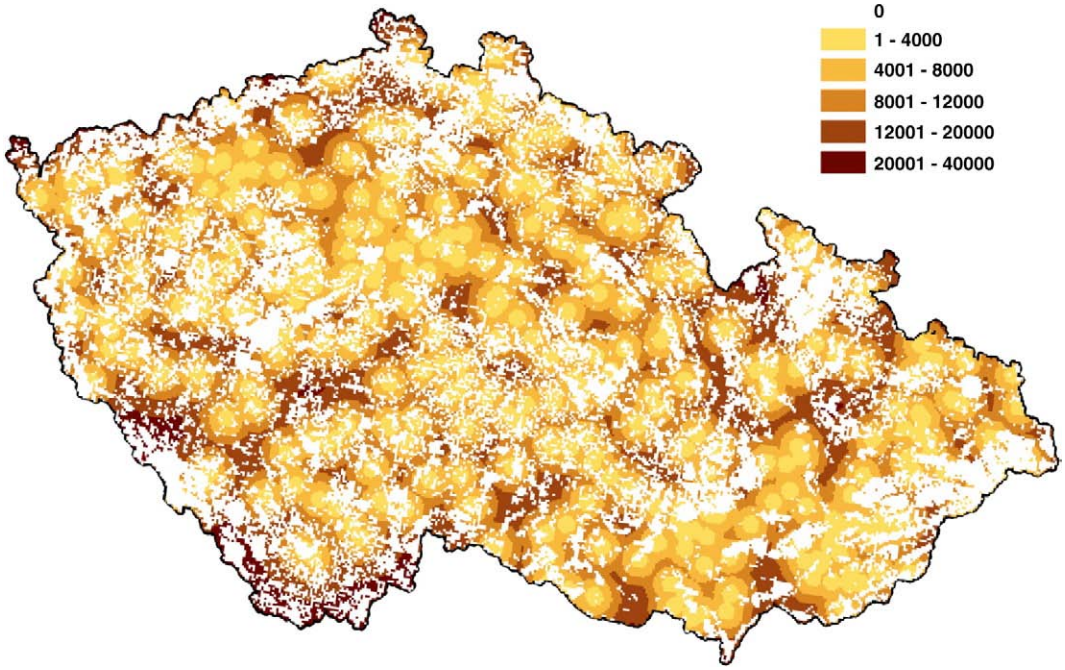

Fig. 3. Relative uncertainty of the POP concentration map (a function of distance in metres from the monitored site = analysis of monitoring sites net homogeneity).

seems to be very efficient in avoiding the underestimation of the risk levels as a possibility of the secondary contamination of the areas adjacent to hot spots is considered (Xu et al., 2001; Hunová et al., 2003; Cheng et al., 2007). Based on results of the IDW modeling, pools of the individual POPs were estimated for agricultural soils in the Czech Republic. The POPs pools for forest soils were estimated based on the average concentration values in the following step. This allowed for an assessment of total pools of POPs in soils over the whole area of the country, as well as for comparison of these values to the ones derived from the consumption/emission data on POPs.

It has been found that DDT and its metabolites represent a group of compounds with the largest pool (1669 metric tons) in the Czech soils. The pools calculated in this work from the POP concentrations obtained in the country wide monitoring programs corresponded well to the amounts estimated from the past usage of these compounds in the Czech Republic.

The IDW-derived levels were also applied for assessment of the risks related to contaminated soils. Only less than 1% of the studied area was affected by the high levels of the health risks coming from the POP contaminated soil. The IDW modeling seems to be a very useful tool for the prediction of the health risks connected to the soil pollution in the large areas with a scarce sampling network. Such simple screening model can be used for setting the priority goals for the second level of the risk assessment procedure. It is successful in identification of both, the regions with the highest risks, as well as the areas with the highest uncertainties (due to very low density of the sampling points) where additional screening is required in order to improve reliability of the model.

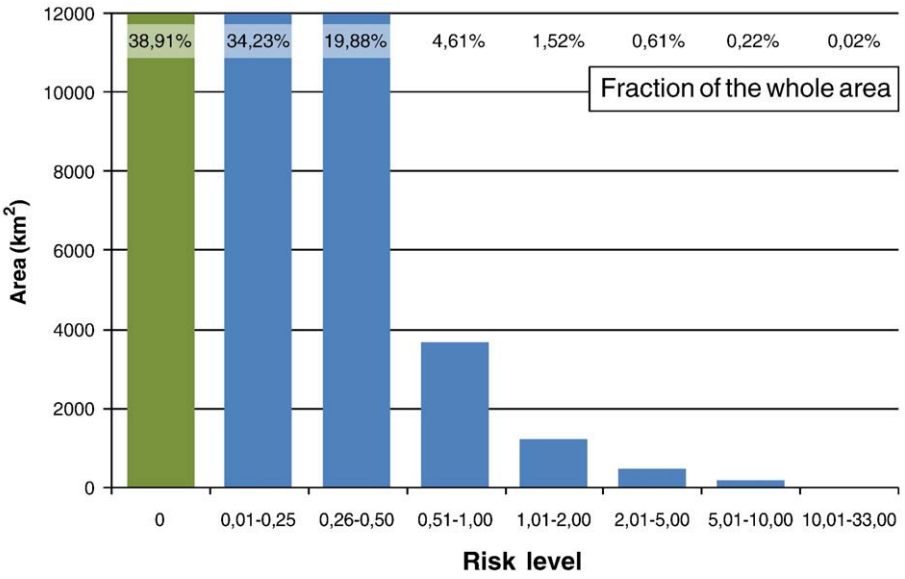

Fig. 4. Percentage of the studied area falling into the specified ranges of the risk levels.

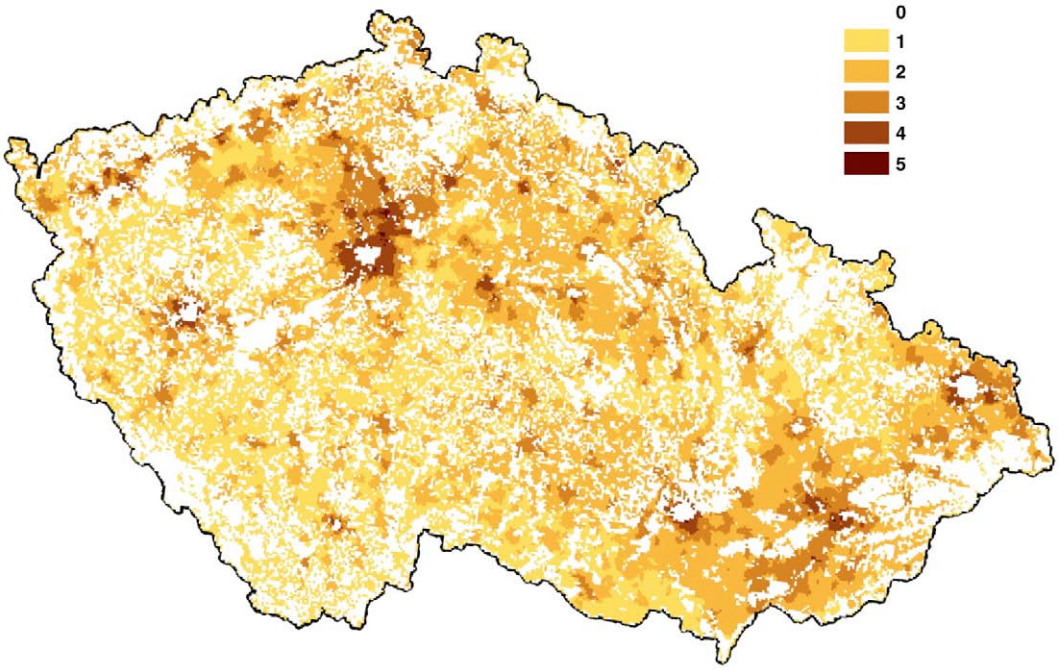

Fig. 5. Identification of the areas with elevated risk in relation to the high population density. The value of hazard was determined as a function of predicted risk level multiplied by population density (categorization is based on "natural breaks" in GIS).

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Příloha 14

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AREA 6.4 • PERSISTENT CHEMICALS • RESEARCH ARTICLE

Distribution pattern of PCBs, HCB and PeCB using passive air and soil sampling in Estonia

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Abstract
Background, aim, and scope Passive air sampling survey of the Central and Eastern Europe was initiated in 2006. This paper presents data on toxic organic compounds such as polychlorinated biphenyls (PCB 28, 52, 101, 118, 153, 138, and 180), hexachlorobenzene (HCB), pentachlorobenzene (PeCB), hexachlorocyclohexane compounds (α-HCH, β-HCH,γ-HCH, δ-HCH), and dichloro-diphenyl-trichloroethane (DDT) compounds (*p*,*p*′DDE, *p*,*p*′DDD, *p*,*p*′DDT, *o*,*p*′DDE, *o*,*p*′DDD, and *o*,*p*′DDT) determined in ambient air and soil samples collected at Estonian monitoring stations.
Materials and methods Ambient air and soil samples were collected in five sites in northern Estonia. Passive air samplers were deployed four times over 4-week periods covering the period April–August 2006. Samples were analyzed using gas chromatography–electron capture detector (HP 5890) supplied with a Quadrex fused silica column 5% Ph for organochlorine pesticides (OCPs). Local ground-boundary wind field was modeled for each monitoring station and sampling period on the basis of observed wind data from the nearest meteorological station with a high quality of time series and compared with upper air (at 850- and 500-hPa level) data from Tallinn-Harku aerological station.
Results Median levels of PCB at Estonian stations varied between 3 and 9 ng/filter, although the maximum in Kohtla-Järve reached as high as 28 ng/filter. Sampling rates about 3.5 m^3/day were determined by empirical measurements, making approximately 100 m^3 for a 28-day sampling cycle. In general, OCP levels in soil were at the limit of detection, except Tallinn site and Muuga Port affected mainly by local sources. However, the atmospheric PCB concentrations are in agreement with the soil analyses where highest PCB levels were found in the soil sample for Tallinn (12.0 ng/g dry weight). For HCB, the atmospheric distribution was quite uniform, with the background levels sometimes higher than the urban ones. HCB and PeCB concentrations were very low in May and June when meridional airflow from the southern sector dominated, and concentrations were slightly higher in July and August, most probably due to revolatilization of adsorbed HCB (with PeCB impurities) from former industrial applications during the summer month and possibly enhanced by forest fires in Russia. Also, the highest summary HCH and DDT levels (63.5 and 2.5 ng/filter, respectively) in Estonian monitoring stations were determined at the end of July and beginning of August when the ground-boundary wind direction was from NE with relatively high speed (4–7 m/s). The highest DDT levels in ambient air

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(3.5 ng/filter) were determined in the spring samples. For DDT and HCH, long-range atmospheric transport clearly dominates persistent OCP, atmospheric input to Estonia as well as for the Scandinavian countries. The DDE/DDT ratio was >1, indicating no fresh input.

Discussion The passive air sampling demonstrates uniform distribution of OCPs. In the regional context, there is no indication of increased levels of concentrations of OCPs in the industrial Northeast Estonia where the oil shale processing causes certain pollution impacts. Though the passive sampling does not apply for monitoring of short-term fluxes, the method is capable of reflecting background levels in long-term prospective for potential effect on human health due to long-term exposition of OCPs.

Conclusions PCB and its congeners, HCB, PeCB, HCH, and DDT were very low in Estonia. None of the persistent organochlorine pesticides have ever been produced in Estonia, and as of today, all old OCP stocks in the country have been destroyed. Highest concentrations could be expected in March and April when southwestern airflow is still strong and dominant, but air humidity is lower and deposition takes place far from the place of origin of OCPs. In summer, the share of locally formed organic compounds increases and deposition depends strongly on weather conditions. In some cases in Tallinn and Muuga where local anthropogenic impact occurs, HCB and PeCB stem from revolatilization of industrial application.

Recommendations and perspectives The passive air sampling could be employed more widely to explore long-term human exposure to OCP deposition and assess potential health risks. The survey based on passive air sampling could be extended from Central and Eastern Europe to other European regions to get methodically adjusted cross-European data coverage. Based on the results of the survey, the Lahemaa reference station is a feasible option to represent background monitoring of persistent organic pollutants.

Keywords Air · DDT · Estonia · HCB · HCH · Passive sampler · PCB · Pentachlorobenzene · Pollution · Soil · Ground-boundary wind fields · Revolatilization

1 Background, aim, and scope

The contamination of environment by hazardous substances such as persistent organochlorine pesticides (OCPs) and other persistent organic pollutants (POPs) is a worldwide public health concern (WHO 2003; The Stockholm Convention 2004).

The 16 POPs listed by the UNECE protocol (UNECE 1998), including also the 12 Stockholm Convention POPs (UNEP 2001), can be categorized into three groups:

- Intentionally produced substances, which are intended to be removed [aldrin, chlordane, chlorodecon, dichlorodiphenyl-trichloroethane (DDT), dieldrin, endrin, heptachlor, hexabromobiphenyl, hexachlorobenzene (HCB), mirex, polychlorinated biphenyls (PCB) and toxaphene].
- Intentionally produced substances whose use is restricted (DDT).
- Unintentionally produced substances of which reduction of emissions compared to the reference year is required [polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), HCB, and polycyclic aromatic hydrocarbons (PAH)].

The production and use of these compounds is governed by a series of international conventions, among them the Stockholm Convention and the Persistent Organic Pollutants Protocol of the Convention on Long-Range Transboundary Air Pollution.

Persistent organic pollutants are a group of toxic and persistent chemicals whose effect on human health and on the environment includes dermal toxicity, immunotoxicity, reproductive effects and teratogenicity, endocrine disruption effects, and carcinogenicity (UNEP 2001; GEF 2003). OCPs are acutely toxic, persistent, and bioaccumulative. For that reason, emission quantities do not necessarily have to be very high before the initial effects of accumulations can be seen.

Because of slow rates of chemical, photochemical, and biological degradation, persistent organic pollutants such as PCB, HCB, DDT, HCH, etc. provide excellent model compounds to survey atmospheric transport process of organic pollutants (Gioia et al. 2007; Jaward et al. 2004; Klanova et al. 2006; Roots and Sweetman 2007).

The European Community and its member states have recently proposed pentachlorobenzene (PeCB) to be added to the list in Annex A, B, and/or C to the Stockholm Convention, and the POPs Reviewing Committee have finalized their evaluation and recommended it to be listed. PeCB is formed as a by-product in industrial processes (Heinisch et al. 2006; POPRC Stockholm Convention 2007a, b) and combustion processes and as an intermediate in industrial processes. PeCB is moderately toxic to humans, but is very toxic to aquatic organisms. PeCB is persistent in the environment and is bioaccumulative. It can be transported over long distances (POPRC Stockholm Convention 2007a, b).

The monitoring of POPs in the environment is a costly and highly time-consuming process. The Environmental Chemistry and Ecotoxicology Group of Lancaster University, UK, Meteorological Service of Canada, Environment Canada (Gioia et al. 2007; Jaward et al. 2004) and Research Centre for Environmental Chemistry and Ecotoxicology of Masaryk University, Brno (RECETOX; Klanova et al.

2006; Kohoutek et al. 2006) confirmed that passive air samplers are sensitive enough to indicate even micro-level differences, which makes them feasible for monitoring of spatial, seasonal, and temporal variations. Passive air samplers are suitable for measurements of long-term average concentrations at various levels. Passive air sampling is a cost-efficient screening method for comparison of contamination at various sites or for verification of information obtained by active samplers.

Model monitoring network in the Czech Republic has been operated since 2005. Aiming to expand geographically sampling, passive air sampling survey of the Central and Eastern Europe (CEE) was initiated in 2006 when CEE partners joint passive air monitoring network (MONET_CZ). A design of the study, including spatiotemporal features of the sampling set, was synchronized with the program in Czech Republic which provides continuous data coverage. The RECETOX of Masaryk University in Brno, Czech Republic has completed an exclusive full-scale CEE air and soil sampling campaign within the framework of the project "Pilot study for development of the monitoring network in the Central and Eastern Europe (MONET_CEEC)". Samplers were deployed and soil samples were collected at remote, rural, and urban locations, in total 58 stations in eight countries—Czech Republic, Bosnia and Herzegovina, Estonia, Latvia, Lithuania, Romania, Serbia, and Slovakia. In Estonia, five stations, Tallinn (Kopli), Muuga Port, Lahemaa, Kunda, and Kohtla-Järve, were employed in the survey. Passive air samplers are used for the evaluation of point sources in the scale of several square kilometers—from the local plants to diffusive emissions from transportations or household incinerators—as well as for the evaluation of diffusive emissions from secondary sources (Gioia et al. 2007; Klanova et al. 2006). The import of OCP was banned in Estonia from October 21, 1967. None of OCPs have ever been produced in Estonia (Müür 1996), and as of today, all POPs stockpiles have been destroyed.

During recent years, our attention has also been drawn to other toxic and persistent pollutants such as polychlorinated naphthalene and polybrominated diphenyl ether, which were analyzed in the ambient air in Estonia for the first time in 2002 (Jaward et al. 2004; Roots and Sweetman 2007) and 2005 (Gioia et al. 2007).

The aim of this article was to assess the levels of POPs in Estonian air in 2006. To assess micro- and mesoscale impact of meteorological conditions on monitoring stations, ground-boundary wind field was modeled at sampling plots. Also, the article compares the sampling data with similar European surveys for quality control and verification.

2 Materials and methods

2.1 Sampling locations

Five sampling sites, including the metropolitan, industrial, urban, suburban, and background sites, were situated in northern Estonia along the shoreline of the Gulf of Finland. The sampling geographical profile was designed in the west-eastern direction for the region-wide coverage of pollution and wind conditions (Table 1).

This paper presents data on a range of different toxic organic compound, as, PCB (28, 52, 101, 118, 153, 138, and 180), HCB, PeCB, hexachlorocyclohexane compounds (α-HCH, β-HCH, γ-HCH, δ-HCH), and DDT compounds (*p*,*p*′DDE, *p*,*p*′DDD, *p*,*p*′DDT, *o*,*p*′DDE, *o*,*p*′DDD, *o*,*p*′DDT) determined in ambient air and soil samples collected at Estonian stations.

Due to slow rates of chemical, photochemical, and biological degradation, these pollutants provide excellent

Table 1 Characteristics of monitoring sites in Estonia

Site	Location	Target	Site specifics
Tallinn EE01	59°27′22″ N 24°41′23″E	Urban impacts	Near the car park
Muuga Port EE02	59°29′40″ N 24°55′51″ E	Industrial impacts, at the Muuga Sea Port monitoring station	Suburban site, 17 km east of Tallinn, near the main cargo harbor for the port of Tallinn. Nearly 75% of the cargo loaded in Muuga port consisted of crude oil and oil products in 2005–2006.
Lahemaa EE03	59°30′55″ N 25°55′41″ E	Long-range impacts; the EMEP background monitoring station	80 km east of Tallinn. Major pollution source is supposed to be a long-range transport.
Kunda EE04	59°30′10″ N 26°33′28″ E	Industrial impacts, in a suburban area of the industrial town	120 km east of Tallinn. Major pollution sources of the cement and pulp-mill industries, and of transportation.
Kohtla-Järve EE05	59°24′35″ N 27°16′43″ E	Industrial impacts; in a suburban area of the industrial town	160 km east of Tallinn. Major pollution source is chemical industry—oil shale processing, production of fertilizers or benzolic acid—and manufacturing of construction materials

model compounds to study atmospheric transport process of organic pollutants. Above the Baltic Sea, the concentrations of airborne pollutants proved to be higher with southwest winds (Roots 1992; Agrell et al. 2001). In particular, this endangers Estonian islands Saaremaa and Hiiumaa, since these winds prevail on the Baltic Sea. This problem was the reason why, at the beginning of 1990s, the long-range transportation of OCPs to Estonia became the focal item of this survey (Roots and Sweetman 2007).

2.2 Air sampling

Passive air samplers containing polyurethane foam disks (15 diameter, 1.5 cm thick, density 0.030 g/cm^3, type N 3038; Gumotex Breclav, Czech Republic) housed in the protective chambers were employed in this study (Kohoutek et al. 2006). The relationship between the amount of POPs captured on a polyuretane foam filter and their concentrations in the sampled air has not been fully characterized yet mathematically. Thus, only empirically estimated information (for example, based on parallel active and passive measurements) is available to evaluate and interpret the results. Sampling rates about 3.5 m^3/day were determined by empirical measurements, making approximately 100 m^3 for a 28-day sampling cycle. Passive air samplers were deployed over 4-week periods: 21 March–19 April, 19 April–17 May, 17 May–12 July, and 12 July–08 August 2006.

2.3 Soil sampling

Soil samples were collected in the same sites as the air samples. In order to reduce the number (and costs) of samples for analysis, soil samples were mainly composited. Soil chemistry plot was divided into 10×10-m subplots (Manual 1998). According to soil classification based on soil genesis and morphology (FAO and WRB 2006 classifications), soils at Tallinn monitoring site are dominated by luvic gleysol, at Muuga by hyposolic fluvisol, at Kunda and Kohtla-Järve by eutric gleysol, and at Lahemaa monitoring site by calcaric cambisol (Reintam et al. 2000). The humus layer was sampled separately with a steel cylinder of known diameter. The sample included only the 0- to 10-cm topsoil layer. About six to ten small subsamples (approximately 0.3–0.5 kg) from different randomly placed subplot points were taken for one composite sample. The above-ground plant and litter material were excluded. All subsamples were placed in one black plastic bag, were mixed, labeled, and transported to the lab within 24 h. Then, samples were dried (at room temperature on lyophilized filter paper on trays), thoroughly mixed, and sieved through 2-mm mesh to prepare the material for chemical analyses (Klanova et al. 2006; Manual 1998).

2.4 Sample analyses

All samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. One laboratory blank and one reference material were analyzed with each set of ten samples. Surrogate recovery standards PCB 30 and PCB 185 for PCB analyses were spiked on each filter prior to extraction. PCB 121 was used as internal standard for PCB/organochlorine pesticide analyses, respectively. Volume was reduced after extraction under a gentle nitrogen stream at ambient temperature and fractionation achieved on a silica gel column; a sulfuric-acid-modified silica gel column was used for PCB/OCPs samples. Samples were analyzed using gas chromatography–electron capture detector (HP 5890) supplied with a Quadrex-fused silica column 5% Ph for PCBs and OCPs (Klanova et al. 2006).

2.5 Quality assurance/quality control

Recoveries were determined for all samples by spiking with the surrogate standards prior to extraction. Amounts were similar to detected quantities of analytes in the samples. Recoveries were higher than 76% for all samples for PCBs, respectively. Recovery factors were not applied to any of the data. Recovery of native analytes measured for the reference material varied from 88% to 103% for PCBs and from 75% to 98% for OCPs. Laboratory blanks were under the detection limits for selected compounds. Field blanks consisted of pre-extracted polyuretane foam disks and were taken on each sampling site. These disks were extracted and analyzed in the same way as the samples, and the levels in field blanks never exceeded 3% of quantities detected in samples for PCBs and 1% for OCPs, indicating minimal contamination during the transport, storage, and analysis (Klanova et al. 2006).

2.6 Wind field analysis

Ground-boundary wind field was modeled for each monitoring station, Jõhvi, Väike-Maarja, Kunda, and Tallinn-Harku, and sampling period on the basis of observed wind data from the nearest meteorological station of the Estonian Meteorological and Hydrological Institute with a high quality time series. Wind Atlas Analysis and Application Program (WAsP) with standard procedure (Troen and Petersen 1989) was used to eliminate any effect of orography, land use, and nearby obstacles (hedges, buildings) from observed wind data in order to create undisturbed regional wind climate. Based on reverse calculation, all local effects by nearby obstacles, orography, and land use at monitoring sites were added to regional wind to produce local wind field at measurement sites for each sampling period. In addition,

statistical data of upper air winds (at 500- and 850-hPa level) from Tallinn-Harku aerological station was used.

3 Results and discussion

All four sampling periods of the current study (Mar. 21–Apr. 19, Apr. 19–May 17, May 17–Jul. 12, and Jul. 12–Aug. 08, 2006) had significantly different ground-boundary wind (Fig. 1), but it followed a main course of long-term seasonal wind speed at ground level (Kull and Laas 2003) and at 850- or 500-hPa level (Keevallik and Soomere 2008). Average ground-boundary wind speed (3.7–4.2 m/s) and maximum wind speed (10.5 m/s, SSW wind) was highest in early spring (March 21–April 19, 2006). In this period, winds from the south (strongest) and southwest sector dominated, while almost no airflow (less than 2% per sector and mean wind speed <2 m/s) was coming in from the north and northeastern sector. In Apr. 19–May 17, 2006, mean ground-boundary wind speed was slightly lower (3.4–4.2 m/s); strongest wind were blowing from the west, but the most dominant wind directions were SE and NNE associated with relatively low wind speed (2.0–3.7 m/s). During the following monitoring periods, mean and maximum wind speed steadily decreased, achieving values typical for the summer period (2.5–3.5 m/s) where average zonal wind component (u=4.8 m/s) slightly exceeds meridional wind (v=2 m/s) at 500-hPa level. In May 17–Jul. 12, 2006, ground-boundary winds from SW and N–NW sector clearly dominated, both associated with winds of speed above average, while winds from the eastern sector had lowest occurrence (2–3% per sector) and speed (<2.5 m/s). During

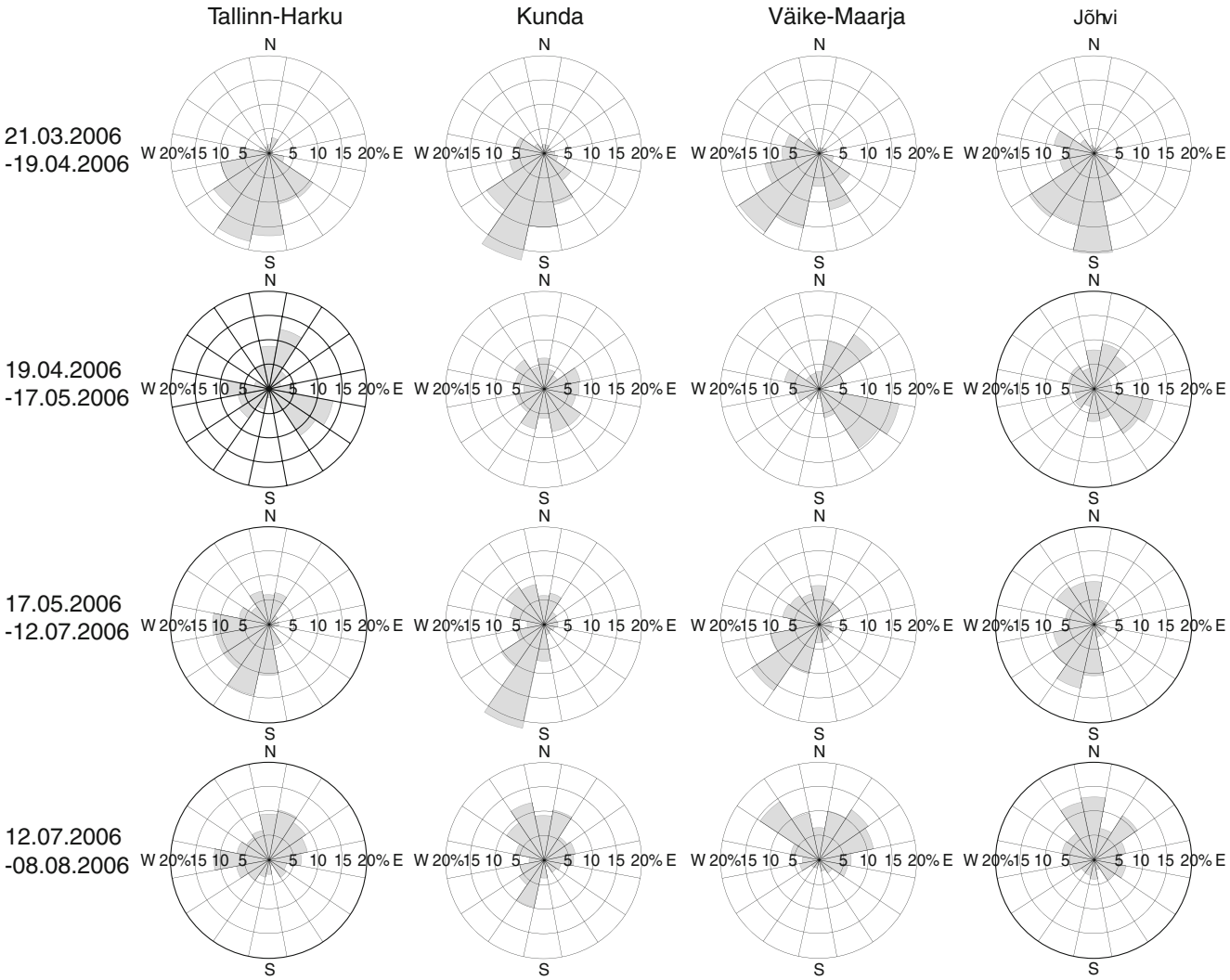

Fig. 1 Distribution of ground-boundary wind direction at regional level where Tallinn-Harku meteorological station corresponds to Tallinn and Muuga Port monitoring site, Kunda meteorological station to Kunda, Väike-Maarja to Lahemaa and Jõhvi to Kohtla-Järve monitoring site

Jul. 12–Aug. 08, 2006, different from any previous monitoring periods, meridional circulation type of upper air (at 500-hPa level) occurred, indicating a dominance of *Grosswettertyp East* which is associated in Estonia with dry and warm weather (Keevallik and Rajasalu 2000). In this period, there was very low occurrence of wind from southern sector, while highly dominant were relatively strong winds blowing from the NW to NE sector.

Unlike any other monitoring station covered by the current study, the ground-level wind at Kunda station is influenced by clearly formed breeze phenomenon which causes a higher share of wind from the southern (land breeze) and northern (sea breeze) sectors, especially in summer. It has an effect on the deposition and transport of locally formed and long-range airborne pollutants both through diurnally changing local air movement and due to periodically changing air temperature and humidity.

3.1 Distribution of PCBs, HCB, and PeCB

In this study, lowest PCB concentrations were measured in Lahemaa, which is a European Monitoring and Evaluation Program (EMEP) background station. Median levels at Estonian stations varied between 3 and 9 ng/filter, although the maximum in Kohtla-Järve reached as high as 28 ng/filter. PCB 28 was the most abundant congener in all samples, followed by PCB 52. PCB concentrations were highest in Tallinn, on the levels of one order of magnitude higher than at all the other four sites. This level (maximum 70 ng/filter, median 44 ng/filter, which corresponds to 700 and 440 pg/m^3, respectively), however, is comparable to other urban and industrial sites in CEE (Gioia et al. 2007; Jaward et al. 2004). It shows also clear regional trend related to dominant wind direction where higher concentrations are characteristic of the westernmost monitoring site at Tallinn, where southern and westerly winds are prevailing, while PCB concentrations are lowered at eastward-located monitoring sites where shares of westerly winds are decreased and, in contrast, southern winds are increased. This is associated with dominant zonal circulation type in upper air (at 850- and 500-hPa level) where zonal wind velocity (u=7–11 m/s at 500 hPa) clearly prevails over meridional wind speed components (v=−4–1 m/s). The atmospheric PCB concentrations are in agreement (Table 2) with the soil analyses where highest PCB levels were found in the soil sample for Tallinn, near the car park, and for Muuga Port (Table 3).

For HCB, the atmospheric distribution was quite uniform, with the background levels occasionally higher than the urban ones, corresponding to other European surveys. Seasonal trends are well correlated with the atmospheric levels of pentachlorobenzene (see Table 2). HCB and PeCB concentrations were very low in May and June due to meridional airflow from the southern sector. The concentrations were marginally higher in July and August due to the forest fires in Russia. HCB levels in soil were at the limit of detection, except Tallinn having a significant, continuous technogenic impact (see Table 3).

Table 2 Temporal variations of each PCBs, HCBs, and PeCB summary of concentrations in the ambient air (ng/filter) at the sampling sites in Estonia

Sampling site/Compound	Tallinn EE01	Muuga Port EE02	Lahemaa EE03	Kunda EE04	Kohtla-Järve EE05
Sampling date			21.03.–19.04.06		
Sum of PCB	18.8	2.6	2.1	5.0	7.9
HCB	8.0	<LOQ	6.6	5.9	8.7
PeCB	3.2	2.3	2.2	2.3	2.8
Sampling date			19.04.–17.05.06		
Sum of PCB	57.3	12.3	4.6	4.0	27.8
HCB	8.3	7.3	5.3	5.6	6.7
PeCB	3.2	2.5	1.6	1.7	2.2
Sampling date			17.05.–12.07.06		
Sum of PCB	29.9	5.6	1.9	1.4	8.0
HCB	2.9	2.9	2.5	2.2	2.8
PeCB	0.8	0.7	0.6	0.6	0.5
Sampling date			12.07.–08.08.06		
Sum of PCB	69.9	8.1	3.3	3.5	9.5
HCB	5.5	<LOQ	4.6	4.6	<LOQ
PeCB	1.6	1.2	1.0	1.3	2.0

LOQ limit of quantitation

Table 3 PCBs, HCB, and PeCB summary of concentrations in soil (ng/g dry weight) at the sampling sites in Estonia (July 12, 2006)

Sampling site/Compound	Tallinn EE01	Muuga Port EE02	Lahemaa EE03	Kunda EE04	Kohtla-Järve EE05
PCB 28	0.2	<LOQ	<LOQ	<LOQ	<LOQ
PCB 52	0.6	0.6	<LOQ	<LOQ	<LOQ
PCB 101	1.1	0.2	<LOQ	<LOQ	0.1
PCB 118	3.6	0.6	<LOQ	<LOQ	0.3
PCB 153	2.4	0.3	0.1	<LOQ	0.2
PCB 138	2.7	0.3	0.1	<LOQ	0.2
PCB 180	1.3	0.1	0.1	<LOQ	<LOQ
Sum of PCBs	12.0	2.1	0.3	<LOQ	0.8
HCB	0.5	0.1	0.1	<LOQ	0.1
PeCB	0.1	0.1	<LOQ	<LOQ	0.0

Pacyna et al. (2003) focused on European HCB emissions, concluding that emissions had been reduced from a total of 200 t/year in 1970 to 40 t/year in 1993. By the mid-1990s, the greatest contributors to the European inventory were Russia, Spain, the Ukraine, France, and Germany. In the data of Jaward et al. (2004), the highest value was obtained at a location in the southern part of rural Germany where local agricultural usage of HCB in glasshouses has been high.

Industrial HCB was emitted in the 100,000-t scale (Barber et al. 2005) in history containing PeCB as an impurity and were distributed in the environment. HCB additionally were produced as industrial by-products in some organochlorine production in the 1,000-t scale from single facilities documented, e.g., for Ukraine or Czech Republic (Weber et al. 2008a, b) and Russia, East Europe, East Germany/Germany (Heinisch et al. 2006). Also, this so-called HCB waste certainly includes PeCB and was often landfilled from where it can slowly be released from these deposits into the environment (Heinisch et al. 2006; Weber et al. 2008a, b). A part of HCB (as other POPs) already reached the arctic, while a part is adsorbed in soil (Beck and Hansen 1974; Klanova et al. 2007; Sajwan et al. 2008) and also adsorbed by vegetation such as the forest (Horstman and McLachtan 1998; Trapp et al. 2001). Chlorobenzenes are also formed by waste incineration processes and from other thermal processes, e.g., in metal industry or biomass combustion. In these processes, PeCB (and TetraCB) are formed in higher concentrations compared to HCB (Ballschmitter et al. 1988; Weber and Hagenmaier 1999; Weber et al. 2008a).

In our study, we found a relatively consistent ratio of HCB/PeCB, ranging between 2.5 and 5.6. Therefore, the HCB and PeCB do not stem from incineration processes but mainly from the revolatilization of industrial HCB with a minor impact of PeCB. The relatively higher impact of PeCB in the air compared to the industrial product can be explained by higher revolatization rates of the more volatile PeCB. The revolatilization theory is consistent with the finding of higher HCB and PeCB values in the hot months (July and August) of our study. To which extent revolatilization of the forest fires in Russia could have contributed cannot be clarified with the limited data set. Also, it is unclear if and to which extent revolatilization from deposited HCB waste stockpiles from organochlorine production in East Europe and Russia have contributed. To clarify these important questions, monitoring of the known deposits and tracking of unknown industrial deposits should be carried out.

Also, the ratio of HCB/PeCB which differs considerably throughout European countries should be surveyed in more detail (e.g., the ratio of HCB/PeCB, ranging between 2.5 and 5.6 in the present study, is two to three times higher in Central Europe; Dvorska et al. 2008).

Table 4 Each HCH and its summary of concentrations in soil (ng/g dry weight) at the sampling sites in Estonia (July 12, 2006)

Sampling site/Compound	Tallinn EE01	Muuga Port EE02	Lahemaa EE03	Kunda EE04	Kohtla-Järve EE05
Alpha-HCH	0.2	0.3	<LOQ	0.3	<LOQ
Beta-HCH	0.2	0.3	0.1	0.2	0.2
Gamma-HCH	<LOQ	0.5	0.1	<LOQ	0.2
Delta-HCH	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
Sum of HCHs	0.4	1.1	0.2	0.5	0.4

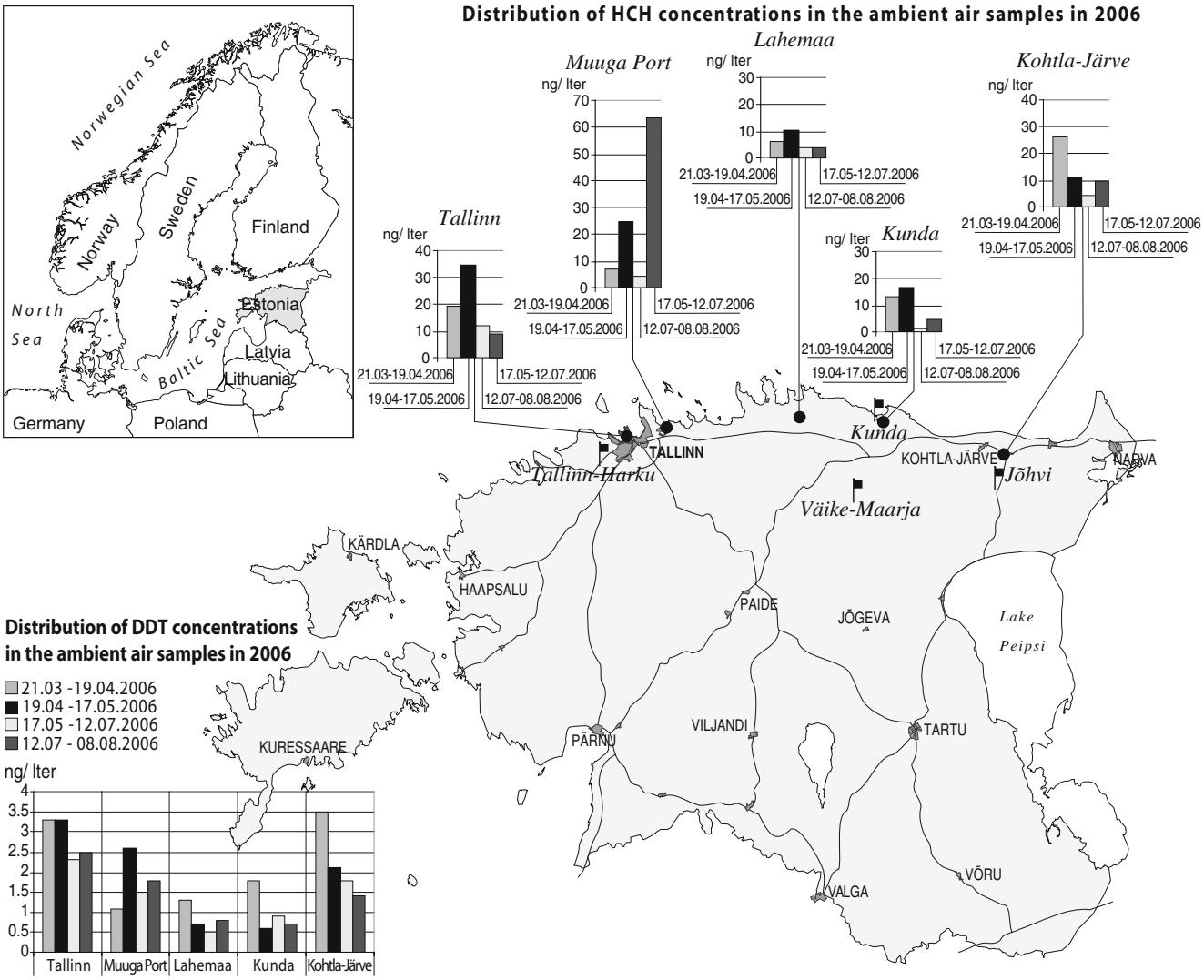

Fig. 2 Location of sampling sites, meteorological stations (marked by flags), distribution of HCH (sum of α-, β-,γ-, and δ-HCH) and DDT in the ambient air (ng/per filter) March–August 2006 in Estonia

3.2 Distribution of HCHs

For HCHs, the levels in Estonian sites were generally low (Table 4; Fig. 2); the highest median values were determined in Tallinn (near the car park) and Muuga Port, but each site had a different seasonal pattern. While in Tallinn the levels were highest in the spring, in Muuga Port, these were highest in the last summer period. Lower levels (one or two thirds) were found at the other three sites, all of them showing the spring maximum. Interest-

Table 5 Each DDT and its summary of concentrations in soil (ng/g dry weight) at the sampling sites in Estonia (August 2006)

Sampling site/Compound	Tallinn EE01	Muuga Port EE02	Lahemaa EE03	Kunda EE04	Kohtla-Järve EE05
*o,p′*DDE	0.1	<LOQ	<LOQ	<LOQ	<LOQ
*p,p′*DDE	5.7	0.9	0.1	0.3	0.3
*o,p′*DDD	0.8	0.5	<LOQ	<LOQ	0.1
*p,p′*DDD	4.6	2.1	<LOQ	<LOQ	0.2
*o,p′*DDT	<LOQ	0.1	<LOQ	<LOQ	<LOQ
*p,p′*DDT	0.8	0.1	<LOQ	<LOQ	<LOQ
Sum of DDTs	11.9	3.6	0.1	0.4	0.5

ingly, α-HCH was more abundant than γ-HCH in most air measurements.

HCH concentrations in all soil samples were very low, highest in Muuga Port (see Table 4). It is directly related to high HCH concentration in the ambient air at Muuga in July and August. The steady ground-boundary wind with low speed from the northeast to the eastern sector blowing directly from Sea Port toward the monitoring station (42.1% of the monitoring period) was dominating. This allows us to suggest that HCH deposited in this period at Muuga site was of local origin.

3.3 Distribution of DDT

DDT levels were very low in Estonia, slightly elevated in Tallinn area (Tallinn and Muuga Port) and also in Kohtla-Järve compared to the background levels in Lahemaa. Similar to HCHs, the highest DDT levels were determined in the spring samples, although concentrations were relatively uniform throughout the summer period. The highest DDT levels in the soil samples were observed in Tallinn and Kohtla-Järve, one order of magnitude higher than the other sites (Table 5). *p*,*p*′DDE was most abundant in both the air and soil samples. Considering relatively high wind speed (3.8–4.2 m/s) and dominant wind from SW, S and SE sectors in spring, it can be assumed that part of the DDT has a transboundary origin. The assumption is supported by the survey of Jaward et al. (2004) indicating the highest levels of these compounds in Russia and Italy, while the lowest values of the DDT were observed in the north and west of Europe. In both cases, low *p*,*p*′DDE/*p*,*p*′DDT ratios were measured, suggestive of a fresh *p*,*p*′DDT signal.

As DDTs were banned in Estonia since 1967, we could summarize OCPs atmospheric input domination in Estonia (Agrell et al. 2001; Roots and Sweetman 2007) as well as in the Scandinavian countries (Gioia et al. 2007). In general, DDE/DDT ratio was >1, indicating no fresh input (Agrell et al. 2001; Gioia et al. 2007; Roots and Sweetman 2007). When the winds blew mainly from the northeastern or southeastern directions, the highest summary HCH and DDT levels in Estonian monitoring stations were determined. The wind direction at the end of July and beginning of August was from NE with relatively high speed (4–7 m/s)—brought in air from the Russian side where several big wild forest fires were in the northwestern part at this time. There is no indication of increased levels of concentrations of the above-listed isomers in northeastern Estonia where the oil shale processing causes certain pollution impacts (Roose and Roots 2005). Though the passive sampling does not apply for the monitoring of short-term fluxes, the method is capable of reflecting background levels in long-term prospective for potential effect on human health due to long-term exposition of OCPs.

4 Conclusions

PCB and its congeners, HCB, PeCB, HCH, and DDT, were very low in Estonia. None of the persistent organochlorine pesticides have ever been produced in Estonia, and as of today, all the old OCP stocks in the country have been destroyed. Atmospheric input of OCPs to Estonia has a clear seasonal pattern based on wind climate. Long-range atmospheric transport is most important in the cold season from October to March when strong and steady airflow from the south, southwest, and west occurs, and zonal wind velocity component clearly dominates over meridional wind velocity component (u=7–11 m/s and v<=2 m/s at 500-hPa level). Highest concentrations could be expected in March and April when southwestern airflow is still strong and dominant, but air humidity is lower and deposition takes place far from the place of origin of OCPs. The highest share of local sources prevails in summer (from May until August) when ground-boundary wind speed is low, long-range transport is limited, and locally formed convective airflows may temporarily increase the concentration of PCB, especially in the vicinity of industrial and urban areas. In soil, the OCP levels were at the limit of detection, except in some cases in Tallinn and Muuga where local anthropogenic impact occurs.

Based on the results for persistent organic pollutants in this article and the data from Sajwan et al. (2008) in a limited number of air and soil samples from Estonia, the contamination level of these contaminants seem to be relatively low.

Results of the passive air sampling in Estonia in 2006 confirmed that the method is sensitive in detecting even small-scale differences, which makes them feasible for monitoring the spatial, seasonal, and temporal variations. Based on the results of the MONET_CEEC campaign, Lahemaa background station seems to be an appropriate candidate for the continuous background monitoring of persistent organic pollutants. Passive air sampling could be employed more widely to explore long-term human exposure to OCP deposition and assess potential health risks. The survey based on passive air sampling could be extended from Central and Eastern Europe to other European regions to get methodically adjusted cross-European data coverage.

In future, the inclusion of PeCB in monitoring of the different environments is necessary to understand sources and the environmental fate of this POPs proposed to be included in the Stockholm Convention. Here, one evaluation parameter is the ratio of HCB/PeCB which differs between the European countries and should be assessed for other regions. In addition, ground-boundary wind conditions should be related to tropospheric trajectories of air masses to upgrade surveys of several previous studies (Jaward et al. 2004; Gioia et al. 2007).

Future monitoring of POPs in Estonia including the currently proposed new POPs is ultimately necessary to

conclude on their relevance for the country and the impact of transport from the regions in this area.

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Fifteen years of monitoring of POPs in the breast milk, Czech Republic, 1994–2009: trends and factors

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Abstract
Background, aim and scope The breast milk has been recommended to carry out as a monitoring tool for effectiveness evaluation of the Stockholm Convention on Persistent Organic Pollutants (POPs). Polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloro-ethane (DDT) and its metabolites (DDX), hexachlorbenzene (HCB) and isomers of hexachlocyklohexane (HCHs) have been monitored in the breast milk of nursing mothers in the Czech Republic since 1994 as a part of The Environmental Health Monitoring System. Knowledge about long-term POPs distribution and accumulation in the human body is crucial to understanding uptake, degradation and subsequent effects as well as to conduct risk assessments. The main aim of this study is to evaluate 15-years long-term trends of selected POPs in human milk in the Czech Republic and to elucidate the questionnaire information about the age, parity and social habits, to the final concentrations. This effectiveness evaluation of POPs restriction is quite precisely after 15-years monitoring campaigns.
Materials, methods and results The human milk samples (4,753 samples) were analysed for a number of chlorinated organic chemicals including PCBs and selected chlorinated pesticides (OCPs, HCB, HCHs, DDX). The relative change of concentration per year for all chemicals was analysed. The remaining percentages of POPs in breast milk in comparison to 1994 are also expressed. Czech population half-lives of POPs in breast milk, derived from either linear or exponential models were computed.
Conclusions and perspectives The long-term data indicates a continuation of a decreasing trend of POPs concentrations on breast milk. Our study did not confirm lactation and parity as an important outflux resulting in the decrease in concentrations in mothers, which is in the antagonism with most of the studies. The higher BMI was associated with higher amounts of HCB and lower amounts of higher chlorinated PCBs. The results confirm the effectiveness of restrictions of POPs usage in the Czech Republic. This ongoing long-term study is very useful tool for parametric effectiveness evaluation of Stockholm Convention.

Keywords Human breast milk · Long-term trends · POPs monitoring · Half-lives of POPs in breast milk · Czech Republic · Effectiveness evaluation · Stockholm Convention

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1 Background, aim and scope

Persistent organic pollutants (POPs), as lipophilic chemicals, accumulate in the environmental lipids. In humans, they tend to be stored in the adipose tissues. In mothers, just before the birth of a child, when the lactation starts, amount of fat in the blood rises and chemicals are mobilised from lipid tissues (Wang and Needham 2007). Chemicals are believed to be transported across the mammary epithelial membrane into milk by transcellular pathways driven by

passive diffusion (Hausner et al. 2008). The very first information about the environmental chemicals in the human milk appeared in the 1950s (Laug et al. 1951).

Due to their toxicity, high resistance to biodegradation, bioaccumulation and long-range transport ability, persistent organic pollutants were restricted or banned (Lignell et al. 2009) in international parties by ratification of the Stockholm Convention (Albers et al. 1996) in March 2004. National Implementation Plans have been established and breast milk has been recommended to carry out as a monitoring tool for effectiveness evaluation of the SC in the time period following its ratification in all of actual 173 parties (UNEP 2010, WHO 2007). Selected persistent chlorinated organic pollutants polychlorinated biphenyls (PCBs), dichlorodiphenyltrichloroethane (DDT) and its metabolites (DDX), hexachlorbenzene (HCB) and isomers of hexachlocyclohexane (HCHs) have been monitored in the breast milk of nursing mothers in the Czech Republic since 1994 as a part of The Environmental Health Monitoring System (EHMS) in the Czech Republic based on the Government Decree No. 369/1991 (Černá et al. 2007). Former Czechoslovakian production of chlorinated POPs was permitted in the mid 1980s (Holoubek 2004).

The study aims to summarize the whole long-term sampling period in the Czech Republic and elucidate the questionnaire information about the age, parity and social habits, to the final concentrations. Now, 15 years later, we can evaluate the effect of POPs restriction and interpret the results of monitoring quite precisely.

2 Materials and methods

2.1 Population in the study

In the period 1994–2003, monitoring was conducted in four areas; two of them (Plzeň and Ústí nad Labem) have a higher level of industrialization and traffic load, whereas the remaining two (Benešov and Žďár nad Sázavou) represent rather rural areas (3,544 individuals), although they also have industrial zones on the outskirts. The protocol of the monitoring was approved by the Ethical Committee of the National Institute of Public Health in Prague

Since 2005, the monitored urban areas with a potential of increased exposure included the capital city of Prague and Ostrava—a city in northern Moravia. The less polluted urban areas are Liberec, Kroměříž and Uherské Hradiště (1,209 individuals; see Fig. 1).

A short questionnaire is used to obtain information on age, weight, height and stay in the locality, medication, occupational exposure and lifestyle habits, and above all smoking (Černá et al. 2007). Results were partially presented earlier by Černá et al. (2011, 2003).

2.2 Sample preparation and chemical analysis

Samples were pooled from every single mother during 1 week; thus “weeks after delivery” are used instead of days. The milk samples collected in glass vessels were stored frozen (−18°C) until they were analysed. Isotope

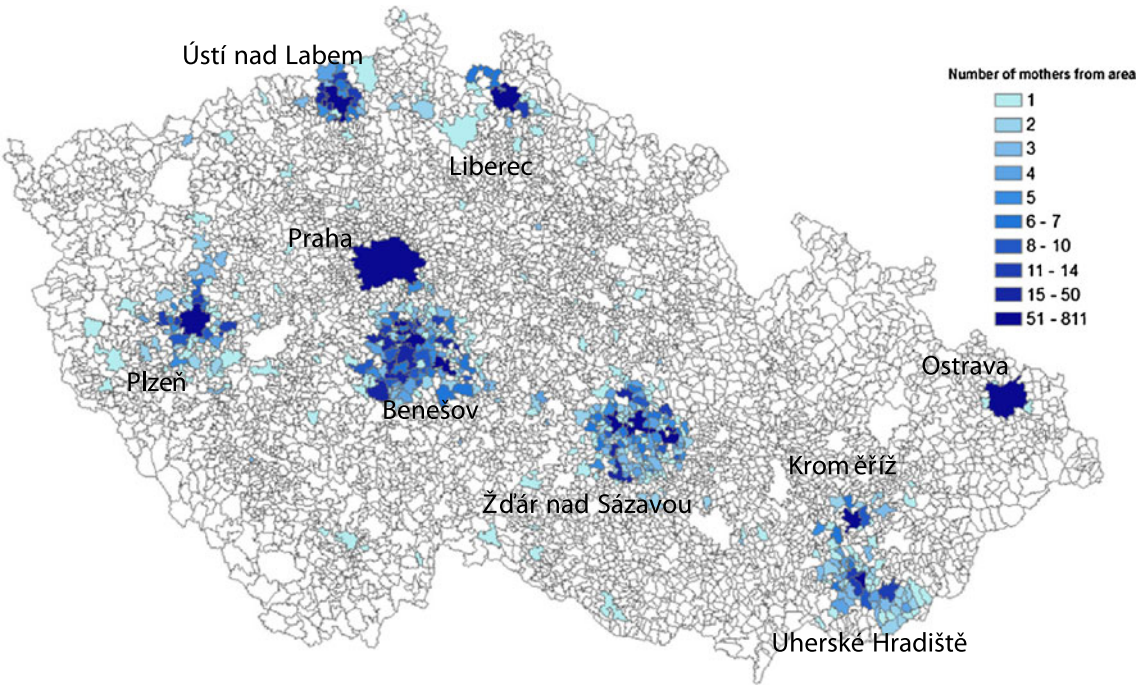

Fig. 1 Number of participating mothers from the smallest NUTS 5 we used (Nomenclature of Territorial Units for Statistics- in the Czech Republic) within different ages of the participants

dilution method was used for quantification of the compounds. Samples were spiked with internal standards (^{13}C labelled γ-HCH, *p,p′*-DDE, PCB 52, 101, 153, 138, 180). Organochlorine pesticides and PCBs were extracted repeatedly with the mixture of diethylether–hexane (1:1) three times in a shaker and centrifuged by 3,000 rpm. Samples were dissolved in hexane and cleaned up on a silica gel column (5 g of H_2SO_4 deactivated silica gel, with 50 ml of *n*-hexane). The syringe standard was added and samples were concentrated to a final volume of about 100 μl of heptane under gentle stream of nitrogen. Samples were analysed by the GC/MS/MS on capillary column DB5-ms (30 m×0.25 mm×0.25 μm).The lipid content was determined gravimetrically after evaporation of solvents.

The analysing laboratory is accredited by Czech Accreditation Institute in accordance to ISO17025. The method was validated using certified reference materials NIST 1588a and BCR430 with properties similar to the samples. Process blank samples are analysed with each series of sample (ca. 30–40 samples). In accordance with standard operating procedures, the recoveries of internal standards have to range between 50% and 130%. When unsatisfactory recoveries obtained, the analysis is repeated. The limits of quantification (LOQs) for OCPs and PCBs ranged from 0.01 to 0.5 and 0.01 to 0.03 ng/g lipd weight (lw), respectively.

2.3 Statistical analysis

Data processing and all analyses were carried out using the following software programmes: STATISTICA version 9.1. and IBM SPSS Statistics version 19.

In order to avoid misinterpretation of the results, half of the detection limit was used in all summations. For computation of ratios, only cases with concentration higher than the detection limit in all elements were involved.

Concentrations of lower chlorinated PCBs (28, 52 and 101) are very often below or on the limit of detection (up to 59% of cases). Higher chlorinated PCBs can be bioaccumulated and are recalcitranting to degradation in comparison to the lower chlorinated PCBs. This causes that the ratio of the PCBs in the diet can vary in comparison with the ratio present in milk (Watanabe et al. 1979).

Due to high skewness of practically all parameters, the statistical results were conducted via non-parametric tests. For correlations, Kendall's statistics was used; Kruskall–Wallis ANOVA was used to evaluate the differences between groups with post hoc test (Tukey HSD).

3 Results

Within the last 15 years, an extensive database of breast milk samples has been made. The samples collected from volunteers in the various regions of the Czech Republic covered in total 4,753 mothers originating from various municipalities; with about 300 individuals per year.

3.1 Demographic and social background

Summary statistics are gathered in Table 1 together with overall concentrations. Mothers were mostly primiparae (75.6%), 20.4% were delivering for the second time and only 4% had more than two children. The median age of these women is 26 years; however, the year of the delivery is of crucial importance, since the median in 1994 was 24.6 and the median in 2009 was 29.4. Statistically significant linear relationship was found between a mother's age and a year of delivery, with 0.33 as the slope factor (i.e. every year the average age of mothers increases of about 4 months compared with the previous year). On the other hand, the age of the oldest mothers has been stable for several years. Week of the sampling in our study ranged between 1st–41st week.

Maternal body mass index was 24.09 and the standard deviation was 3.52 resulting in the assumption of a fairly homogeneous somatotype of mothers. The median fat content of the milk was 1.93%. Smoking habits were divided into four groups to explore the contribution of present smokers (7.2%), former smokers(27.1%), non-smokers with smoking at home influence (25.7) and non- smokers (40%).

4 Discussion

Correlation coefficients between chemicals were highest between higher chlorinated PCB's, suggesting a similar exposure pathway and similar behaviour. Other inter-chemical variations were mostly significant ($p<0.01$), with alpha-HCH being an exception with a significant correlation with other HCHs isomers and lower chlorinated PCBs only.

Some of the chlorinated pesticides were measured only for several years (two to four) and their time trends are not discussed (alpha-HCH, o,p′-DDD, p,p′-DDD, o,p′-DDE, o,p′-DDT). PCB 170 started to be measured in 2005. The remaining chemicals were tested for time trends (Figs. 2, 3, 4 and S1–S9). In all cases, a significant decrease ($p<0.001$) in their concentrations was proved, which corresponds with other studies (Albers et al. 1996, Fitzgerald et al. 2001, Lignell et al. 2009, Polder et al. 2009, Schade and Heinzow 1998). The year of the sampling is the most important parameter since the Czech population lives in the postban (after the end of primary emissions) situation. Table 2 shows a relative change per year for all chemicals with sufficient data and measuring period, expressed as a $k_{dec,year}$, which refers to the decline in concentrations (nanograms per gram per day) derived from either linear or exponential models. See Table S1 for whole equations. HCB and gama-HCH

Table 1 Summary of the statistical data concerning the general characteristics of women who took part in the survey and their overall concentrations presented as median, mean, standard deviation, 5 and 95 percentiles

	Percentile 5	Median	Percentile 95	Mean	Standard deviation
Age	20	26	34	26	4
Number of delivery	1	1	2	1	1
Weight (kg)	55	68	89	69	11
Height (m)	157	168	177	167	6
BMI (kg/m^2)	20.07	24.09	31.23	24.81	3.52
Milk fat%	0.58	1.93	4.9	2.23	1.36
Concentrations ng/g LW					
PCB 28	1.46	2.5	35	9.57	23.95
PCB 52	0.12	2.5	138.7	25.71	66.83
PCB 101	0.36	1.5	15	3.86	11.15
PCB 118	1.5	12.23	38.5	15.19	14.53
PCB 138	46	132	347	159.5	136.1
PCB 153	67.38	182	498	221.8	167.6
PCB 180	45.2	137	388.5	168.6	139.2
PCB 170	21.7	59.1	153	71.52	67.71
HCB	26.1	115	642.3	198.7	245.1
Alpha-HCH	0.06	0.5	2.25	0.72	0.69
Beta-HCH	1.5	20.3	75.2	27.65	31.43
Gama-HCH	0.47	2.5	19.8	5.94	10.61
p,p′-DDD	0.19	0.81	3.1	1.1	1.07
o,p′-DDE	0.04	0.25	0.8	0.32	0.3
p,p′-DDE	108	330	880	394	306.4
o,p′-DDT	0.22	0.65	2.09	0.86	0.78
p,p′-DDT	1	16.8	98	29.97	46.59

LW lipid weight

concentration changes have an exponential character. The remaining percentages of chemicals in population in 2009 in comparison to the those in the 1994 are expressed in the second column, Czech population half-lives ($t_{1/2,popul}$) derived from the same models as $k_{dec,year}$ are shown in the third column. Parent compound p,p′-DDT is present in the samples in a low amounts, while p,p′-DDE, as a dominant metabolite, persists. The ratio between the parent compound and the metabolite can be used as a rough estimate of the period of its application. A steady increase in the ratio has been observed throughout the years (see Fig. 5). Other chlorinated pesticides are still present in the breast milk, but in much lower amounts which are closer to the limit of detection.

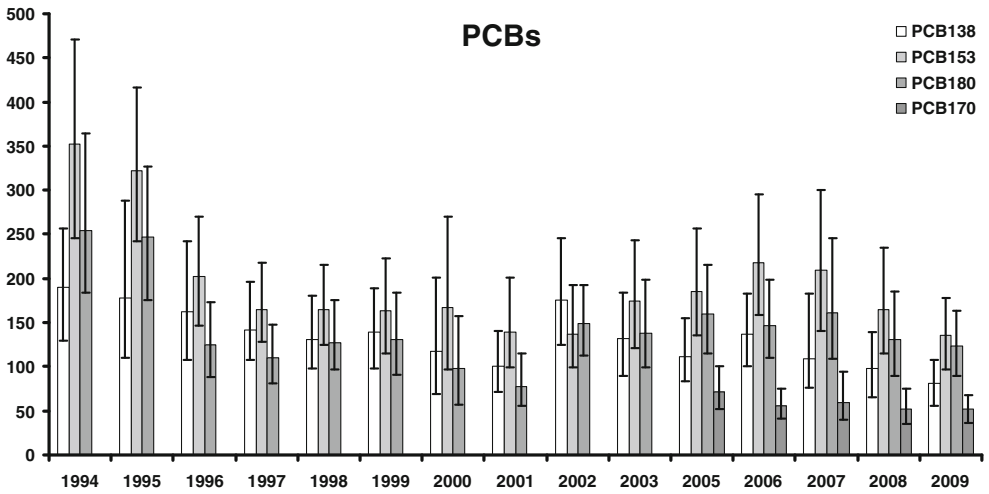

Fig. 2 Time trend of PCBs. Median concentrations in nanograms per gram of lipids in milk with 75% and 25% percentile

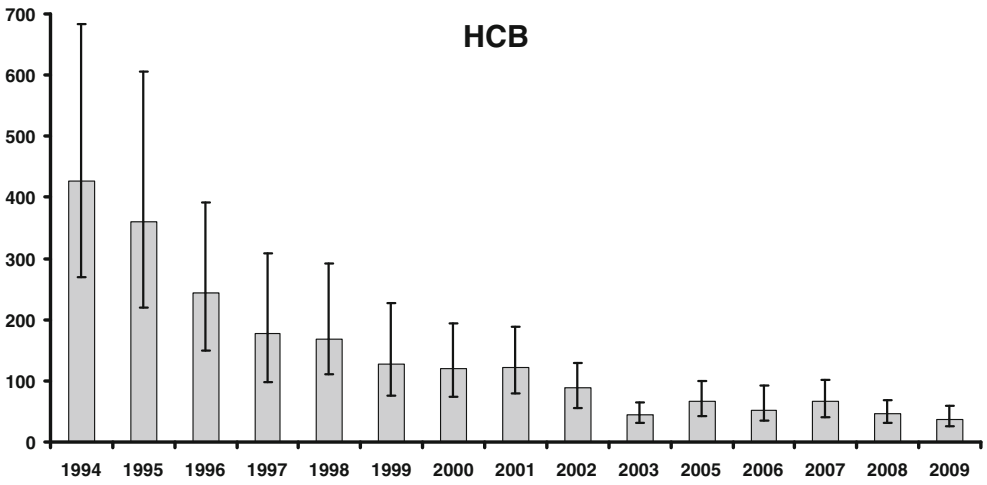

Fig. 3 Time trend of HCB. Median concentrations in nanograms per gram of lipids in milk with 75% and 25% percentile

High concentrations of PCBs were detected in the Czech Republic in the past (Černá and Bencko 1999) and confirmed to be one of the highest in Europe in the WHO-coordinated Exposure Study (WHO/ECEH 1996). Later restrictions are the possible reason for elevated exposure in comparison with other European countries (Černá et al. 2003, Holoubek 2004).

To test the influence of parameters other than the year of sampling, which is the most important, we have performed standardisation in the Statistica programme according to Eq. 1

$$C_{\text{std}} = \frac{C_{\text{original}} - \overline{C}_{\text{year}}}{\text{SD}} \quad (1)$$

where C_{std} stands for the standardised value, C_{original} is the original concentration in the breast milk sample, $\overline{C}_{\text{year}}$ is the mean concentration of a chemical in a specific year and SD is the standard deviation in that particular year. This equation was used for every chemical separately.

The age of mothers is another significant determinant influencing final concentrations. Relative change with the age of the mother for all chemicals with sufficient data is expressed as a $k_{\text{dec,age}}$, which refers to the decline in concentrations (nanograms per gram per age) derived from either linear or exponential models. (see Table 2). The data have shown an increasing trend connected with the increasing age; in agreement with other studies (Albers et al. 1996, Fitzgerald et al. 2001, Lignell et al. 2009, Polder et al. 2009). This trend is clearer especially when an adjustment of the year is applied. The results are summarized in Table 2, and expressed as the increase in the level of POPs in the term of 1 year of mothers. HCB and beta-HCH are expressed in the exponential rather than the linear way.

Even though no samples have been collected from the same participant repeatedly, the number of mothers sampled every year allows us to perform statistical analysis of the lactation period influence on final concentrations. Overall scientific opinion presumes a decline in POPs level during the course of lactation (Harris et al. 2001, Pan et al. 2010, Schecter et al. 1996, Thomsen et al. 2010, Trapp et al. 2008, US-EPA 2009). Our results, on the population bases, do not support such hypothesis. A recent study done by LaKind et

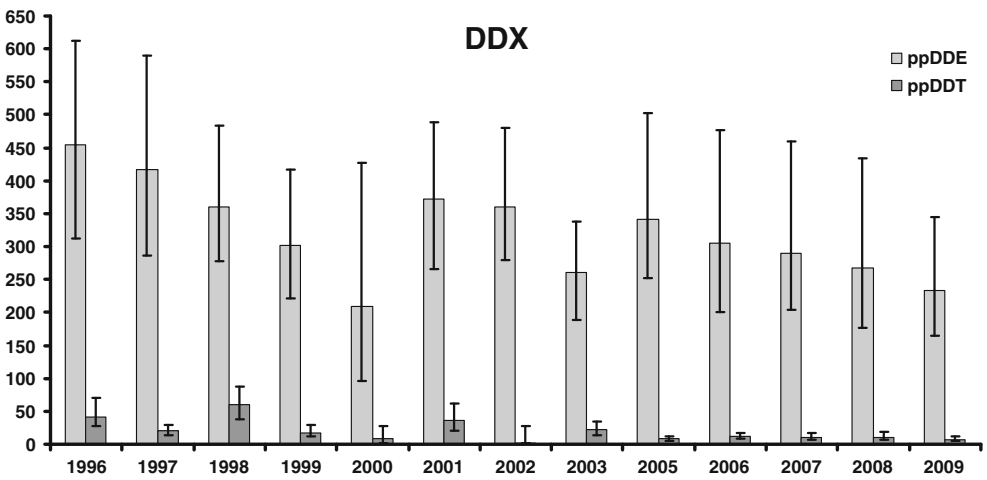

Fig. 4 Time trend of DDX. Median concentrations in nanograms per gram of lipids in milk with 75% and 25% percentile

Table 2 Summary of trends of chemicals within the years and age of the mothers

	$k_{dec,year}$ (ng/g/year)	Remaining%	$t_{1/2,popul}$ (years)	$k_{dec,age}$ (ng/g/age)
PCB 118	0.89	34.41	11.43	0.67
PCB 138	5.53	53.33	16.07	5.49
PCB 153	7.70	54.65	16.54	9.32
PCB 180	4.01	66.09	22.12	9.00
PCB 170	4.34	43.14	13.19	3.25
HCB	$e^{(0.164\times year)}$	8.53	4.22	$e^{(0.080\times age)}$
Beta-HCH	1.43	33.53	11.28	$e^{(0.058\times age)}$
Gama-HCH	$e^{(0.178\times year)}$	6.95	3.90	0.0003
p,p′-DDE	6.97	72.03	26.81	15.24
p,p′-DDT	2.75	7.75	8.13	0.90

$k_{dec,year}$ relative change per year for all chemicals with sufficient data, *Remaining%* remaining percentages of chemicals in 2009 in comparison to 1994 situation, $t_{1/2,popul}$ population based half life, $k_{dec,age}$ relative change of chemicals concentration within different ages of the participants

al. (2009) has shown that chlorinated organics do not consistently decrease during lactation and they proposed that this decline should no longer be assumed.

Statistically significant ($p<0.001$) declination is observed only for p,p′-DDT simultaneously with the increase in the DDE/DDT ratio. Parent p,p′-DDT seems to be quite rapidly metabolise into p,p′-DDE. HCB, beta-HCH and gama-HCH showed higher concentrations ($p<0.001$) in the first week, but no difference in the following weeks. Other compounds do not show any decline with further weeks of the sampling. The influence of the week of sampling is even lower when results are standardised. Hereby, we agree with LaKind et al. (2009) that additional studies that include large cohort should be conducted to confirm these results.

The theory about decrease of life-stored POPs during the course of lactation should support another theory of mothers with higher parity who had already lost some proportion of chemicals during their previous motherhood (Albers et al. 1996, Fitzgerald et al. 2001, Kostyniak et al. 1999). Higher age of the multiparas can be sometimes responsible for no decrease of concentrations in contrast to primiparas (Czaja et al. 2001).

If the time between subsequent children is not too long, such decline should be observed. Mothers up to their third child with less than 4 years from their last delivery were chosen for comparison. Only PCB 153 have shown a downward trend with the rising parity ($p<0.05$), other relationships act inconsistently and were not significant.

Consumption of the fat-rich food, which often contains higher concentration of the organics (Campoy et al. 2001), could be typically linked to the higher body mass index (BMI). On the other hand, large fat depot can serve as a dilution reservoir (Polder et al. 2009). No direct association between BMI and concentration was found (Sullivan et al. 1991); it is rather the diet that seems to influence adipose concentrations (Campoy et al. 2001, Ramos et al. 1997). The data for the dietary habits gathered in the questionnaire are insufficient. BMI was used to compare different somatotypes of mothers and possible different fat depots. Higher chlorinated PCBs (153, 180 and 170) were negatively correlated with the BMI, unlike HCB ($p<0.001$). The same results were observed by Polder et al. (2009). Opposite trends of industrial PCBs and HCB may indicate various origins of the exposure pathway and different chemical–physical

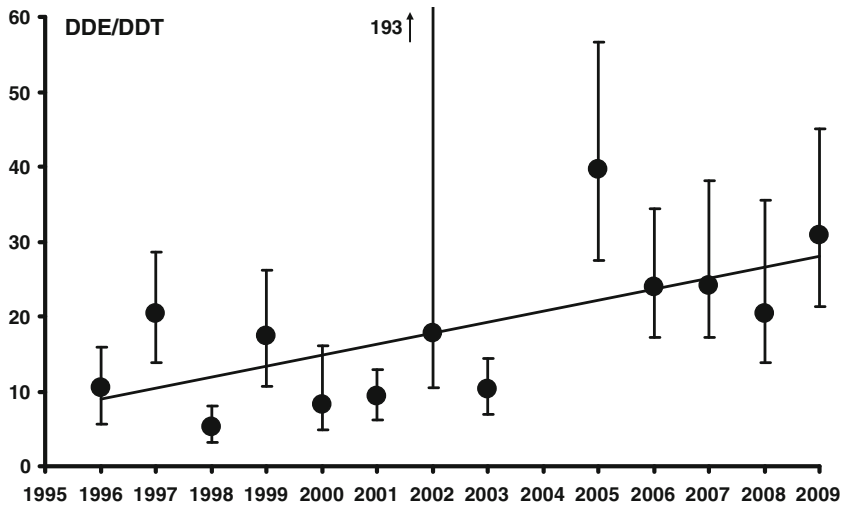

Fig. 5 Time trend of DDE/DDT ratio in milk with 75% and 25% percentile

properties (HCB is smaller and more soluble in water, but still very lipophilic).

Smoking habits of the mothers have been proved to be linked with the heightened concentration of some POPs by several authors (Hong et al. 1994, Rogan et al. 1986), while other studies have not found any statistically significant relationship (Bates et al. 1994, Harris et al. 2001). The mothers were divided into four "smoking" groups according to the questionnaire (smoker, former smoker, smoking at home, non-smoker) with the declining probability of exposure to cigarette smoke. There were no differences between those groups without normalization. The data after normalization have shown slightly lower (in units of percents) concentrations of higher chlorinated PCBs, HCB, beta-HCH and p,p′-DDE for the less probable exposed groups, in agreement with Polder et al.(2009). No connection was found to number of confessed cigarettes per day ($p<0.001$).

5 Conclusions

The sufficiently long sampling period with high number of cases can reveal long-term trends much better than shorter period with few data. As expected, in the postban period the year of the sampling was a very important determinant, because the declining trends are leading to a fraction of original concentrations in comparison to 1994. With gama-HCH, HCB and p, p′-DDT vanishing fastest (7–9% remains nowadays of 1994 concentrations), while the amount p,p′-DDE is about 72% of the 1994 levels. Population based half-lives were determined from the long-term trends. It was proven that age of the participant also strongly influences the concentrations of monitored chlorinated compounds. Older mothers have higher levels than younger ones; this trend is clearer especially when a year adjustment is applied. The population based results from our database did not confirm lactation as an important outflux resulting in the decrease in concentrations in mothers, which is in the strong antagonism with other studies. A similarly expected trend, the connection between the parity and lower levels of POPs, was observed only for PCB 153. The higher BMI was associated with higher amounts of HCB and lower amounts of higher chlorinated PCBs. The normalization gives evidence of the influence of cigarette smoke exposure and concentration of several chlorinated POPs.

Our results can reopen some discussions about factors influencing the concentrations in breast milk. The results confirm the effectiveness of restrictions of POPs usage in the Czech Republic. This ongoing long-term study is very useful for parametric effectiveness evaluation of Stockholm Convention.

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Obsolete pesticide storage sites and their POP release into the environment—an Armenian case study

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Abstract Organochlorinated pesticides were widely applied in Armenia until the 1980s, like in all former Soviet Union republics. Subsequently, the problem of areas contaminated by organochlorinated pesticides emerged. Environmental, waste and food samples at one pesticide burial site (Nubarashen) and three former pesticide storage sites (Jrarat, Echmiadzin and Masis) were taken and analysed on the content of organochlorinated pesticides, polychlorinated dibenzo-*p*-dioxins and furans and dioxin-like polychlorinated biphenyls. Gradient sampling and diffusivity-based calculations provided information on the contamination release from the hot spots on a local scale. A risk analysis based on samples of locally produced food items characterised the impact of storage sites on the health of nearby residents. All four sites were found to be seriously contaminated. High pesticide levels and soil and air contamination gradients of several orders of magnitude were confirmed outside the fence of the Nubarashen burial site, confirming pesticide release. A storage in Jrarat, which was completely demolished in 1996 and contained numerous damaged bags with pure pesticides until 2011, was found to have polluted surrounding soils by wind dispersion of pesticide powders and air by significant evaporation of lindane and β-endosulfan during this period. Dichlorodiphenyltrichloroethane-contaminated eggs, sampled from hens roaming freely in the immediate surroundings of the Echmiadzin storage site, revealed a significant health risk for egg consumers above 1E−5. Although small in size and previously almost unknown to the public, storage sites like Echmiadzin, Masis and Jrarat were found to stock considerable amounts of obsolete pesticides and have a significant negative influence on the environment and human health. Multi-stakeholder cooperation proved to be successful in identifying such sites suspected to be significant sources of persistent organic pollutants.

Keywords Obsolete organochlorinated pesticides · Armenia · Gradient sampling · Emissions · Diffusivity-based calculations · Health risks · Stockholm Convention

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Introduction

Ten years after the adoption of the Stockholm Convention (SC) on Persistent Organic Pollutants (POPs), major primary emission sources are still poorly documented and secured,

including large POP waste stockpiles (Breivik et al. 2002b; Klánová et al. 2011; Vijgen et al. 2011). This, together with a general lack of POP emission factor data and information on production and consumption, leads to major uncertainties in POP emission inventories (Barber et al. 2005; Bailey 2001; Breivik et al. 2002a; Denier van der Gon et al. 2007; Pacyna et al. 2003; Vijgen et al. 2011). Further, factors controlling important re-emissions from secondary sources (soils, vegetation, water bodies and sediments) are not yet fully understood (Nizzetto et al. 2010). This poses key challenges and research requirements, as cost-effective exposure reductions are based on the control of POP sources (Klánová et al. 2011).

Inventories list the amount of obsolete pesticides (many of them POPs) stocked in developing countries and economies in transition to be about 290,000 t (FAO 2011). The donation or purchase of pesticides in excessive amounts due to commercial interests or the lack of inadequate aid coordination, the donation or purchase of unsuitable pesticides, poor stock management and no disposal of banned and expired pesticides are the main reasons for the accumulation of obsolete pesticides (FAO 2011; Haylamicheal and Dalvie 2009). Numerous research papers (e.g. Dalvie et al. 2006; Dasgupta et al. 2010; Galuszka et al. 2011) state the worldwide urgent need for the elimination of these dangerous stockpiles and present detailed local and national inventories and cleanup experiences. This paper focuses on the situation in the former USSR, specifically Armenia.

There are about 180,000 t of obsolete pesticides estimated as located in dumps, landfills, warehouses and stockpiles in the territory of the former USSR (FAO 2011). As in all republics of the former USSR, organochlorinated pesticides (OCPs) were widely applied in Armenia until their ban in the 1980s. Subsequently, the problem of OCP-contaminated areas (agricultural lands, former pesticide storehouses, pesticide burial, dumps etc.) emerged. The Republic of Armenia ratified the SC on October 22, 2003 (Republic of Armenia 2005).

As POPs possess toxic properties, resist degradation, bioaccumulate and are subject to local, regional and global transport (Stockholm Convention 2001), the SC requires the disposal of POPs in a way that the chemical content is destroyed or irreversibly transformed in such a way that they do not exhibit the characteristics of persistent organic pollutants. POPs are also permitted to be disposed of in an environmentally sound manner when destruction or irreversible transformation does not represent an environmentally preferable option or when the persistent organic pollutant content is low (Stockholm Convention 2001). The SC regulates the following pesticides: chlordecone, α-hexachlorocyclohexane (α-HCH), β-hexachlorocyclohexane, γ-hexachlorocyclohexane (in 99 % purity referred to as lindane), pentachlorobenzene, dichlorodiphenyltrichloroethane (DDT), aldrin, chlordane, dieldrin, endrin, endosulfan, heptachlor, hexachlorobenzene, mirex and toxaphene (Stockholm Convention 2010, 2011).

Several sites in Armenia suspected of being contaminated by obsolete pesticides were inspected in the past (Ritsema et al. 2006). In the study presented here, four sites considered of utmost urgency for a detailed assessment and identified during a previous onsite visit were chosen for sampling. Among them, the pesticide burial site Nubarashen is the largest and best known obsolete pesticides hot spot in Armenia. This site has been previously subject to the interest of the non-governmental organisation (NGO) Armenian Women for Health and Healthy Environment (AWHHE), the Food and Agriculture Organisation, the Organization for Security and Co-operation in Europe (OSCE), the International HCH and Pesticides Association and the International POPs Elimination Network (IPEN). The other sites, three former pesticide storage sites (Jrarat, Echmiadzin and Masis), had not previously been subject to detailed investigations.

There is evidence indicating that POP waste stockpiles, landfills, contaminated sites and reservoirs are an ongoing source important for the assessment of human exposure and environmental impact (Klánová et al. 2011; Weber et al. 2008, 2011). Various mechanisms of off-site transport have been described (for summaries, see Vijgen et al. 2011; Weber et al. 2008) and several cases of POP release from stockpiles documented (e.g. Elfvendahl et al. 2004; Jit et al. 2011; Marco and Kishimba 2005). In this study, off-site transport is addressed by investigating three aspects, i.e. emissions into air, POP concentration gradients in surrounding soils and toxic levels in local food.

This paper presents the results of a screening field study based on a joint Czech–Armenian NGO project, with assistance by scientific workers. Despite financial, temporal and personal limitations, the sampling plan reflects the Czech rules of risk analysis for contaminated areas (Ministry of Environment 2005) to the best possible extent. Cheap and simple tools were used for assessing POP hot-spot effects on a local and regional level, including passive air samplers, a simple human exposition model coupled with a health risk analysis and diffusivity-based calculations in combination with an atmospheric transport pattern analysis to discuss POP releases into air.

Methods

Sampling

Four sites (Fig. S1–S12, supplementary information) located in the administrative territory of Yerevan and the Ararat and Armavir regions were subject to investigation during multiple sampling campaigns during 2010:

(a) The Nubarashen site was established as a pesticide burial site in 1982 or earlier. Four concrete underground cells are believed to contain 512 t of pesticide,

including 192.5 t DDT and 48.4 t of HCHs (Helps 2010). The landslide-affected site has been fenced and safeguarded since early summer 2010. An interior drainage ends at the fence, where it transforms into a ditch. On a hillock down the slope, livestock is occasionally grazed. A bypassing temporal stream runs down the slope to a settlement of summer houses a kilometre away, the 2-km-distant village Mushavan (1,500 permanent residents) and the river Getar, a tributary to the Razdan river that runs through the 20-km-distant capital of Yerevan. Seventeen soil, five sediment, two egg, one cow milk, one cow cream and four passive air samples were taken in Nubarashen and its surroundings.

(b) The Echmiadzin storage is a former local pesticide distribution centre established in the 1970s or 1980s with the majority of obsolete pesticides already brought to the landfill (Ritsema et al. 2006). Two locked storage rooms with a water-tight roof and closed windows contained mostly empty packaging and powders spilled on floors and shelves. Next to the storage rooms are located a residential house and vegetable bed. A fish pond filled with groundwater is located approximately 50 m from the storage rooms. The family (two adults, two adolescents) provides itself with privately grown vegetables, fruits, trout and eggs. Another residential building is situated behind a wall surrounding the farm. Four scratch-off/sweeping, two soil, three water, two sediment and two egg samples were taken at the Echmiadzin storage.

(c) The Jrarat formal governmental storage facility and distribution centre for fertilisers and pesticides (currently referred to as Konstantin and Sisters LTD) was established in the 1970s or 1980s and consists of a small building that contained canisters with methylmercaptophos insecticide (information provided by the owner), rusted metal drums, spilled oils and, in a back room, damaged bags of pesticides. A second larger building contained dozens of damaged bags with a consolidated substance of predominantly white colour and crystalline structure. Both buildings were completely demolished in 1995 or 1996. The spilled pesticides of the outside storage were repacked in March 2011 and transported to a third large locked building approximately 100 m distant, with windows and roof in reasonable condition. According to the owner, this locked building is used for storage of fertilisers and unspecified "biopreparates". Close to the site, a railroad is located that was formerly used for pesticide transport.

Concrete ponds for commercial trout breeding (established in 2000) and a muddy pond are 200 and 50 m distant from the storage buildings, respectively, both supplied with groundwater. The fish are fed with imported Dutch feed. In close proximity to the demolished buildings, women occasionally cut grass and herbs for consumption. Residential buildings and a fruit orchard are nearby. The fish farm workers may freely walk around the site and dogs roam. The closest village is 2 km distant. Thirteen scratch-off/sweeping/plaster, three soil, two water, one sediment, two egg, one trout and three passive air samples were taken in the storage buildings and their surroundings.

(d) The Masis privately owned and permanently safeguarded storage facility is located in a former facility for the processing and distribution of agricultural products, established in the 1970s or 1980s. Pesticides were stored in the past in three rooms. The largest of these, a locked roofless room, has broken windows and is locked. It contains damaged barrels, bottles, bags and a thick layer of spilled powders of various colours on the floor. The two smaller remaining locked storage rooms are preserved and contain a thin powder layer on the floor. The rooms are entered and used occasionally. Nine scratch-off/sweeping/plaster, two soil, one water, one egg and one cow milk samples were taken in the storage rooms and their surroundings.

For a further site description, see Ritsema et al. (2006) and Arnika and AWHHE (2011). The sampling methodology, sampling intervals and a detailed sample overview can be found in the supplementary information (Table S1 and text).

Chemical analysis

Analysed pollutants are listed in Tables S3 and S4 (supplementary information). The chemical analysis of solid samples marked with an upper "*t*" index was conducted as a part of a doctoral thesis focused on the thermodesorption of pesticides. These samples were processed by the standard Environmental Protection Agency (EPA) 3550C ultrasonic extraction methodology (EPA 2007), modified by Hendrych et al. (2009) into a procedure for extracting water-insoluble or slightly soluble semivolatile organic compounds from soils, clays, sediments, sludges and waste solids. Briefly, samples were extracted in hexane in an ultrasound water bath. Extracts of samples N22 and E6 contained a very fine fraction; therefore, they were cleaned up by centrifugation. Extracts were analysed by means of gas chromatography coupled with an electron capture detector (GC–ECD).

Other solid samples were extracted by dichloromethane. Extracts were cleaned by means of gel permeation chromatography. Water samples were extracted by microextraction into isooctane, and no cleanup step was needed. The analysis of OCPs in solid and water samples was conducted by means of GC–ECD. Food item samples were extracted in a

Soxhlet apparatus by a mixture of hexane and dichloromethane (1:1). The extract was purified on a gel permeation chromatogram and analysed on the OCP content by means of gas chromatography coupled with a mass spectrometer (GC–MS; Hrádková et al. 2012). Polyurethane foam discs from passive air samples were extracted with dichloromethane in an automatic Büchi extractor. Extracts were fractionated on a sulphuric acid-modified silica gel column. Samples were analysed by means of GC–MS (Lammel et al. 2009).

In sample N2, the content of polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (PCBs) was expressed in toxic equivalents (TEQs), and the quantification of individual congeners was performed by an isotope dilution method using ^{13}C-labelled quantification standards added before extraction. Samples were analysed by means of high-resolution mass spectrometry (Neugebauer et al. 2011). PCDD/Fs-PCB TEQs in other selected samples were analysed as dioxin receptor–chemically activated luciferase gene expression (DR-CALUX) TEQs. Solid samples were extracted in a Soxhlet apparatus by a hexane/acetone mixture (3:1). Extracts were cleaned in an acid multilayer column with silica and dissolved in dimethylsulfoxide. Food samples were extracted by a mixture of hexane and diethyl ether (97:3) and the extracts cleaned up on an acid silica column. The DR-CALUX activity was determined (24 h exposure) and benchmarked against 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Shyu et al. 2009; Stronkhorst et al. 2002).

Estimates of pesticide emissions into the atmosphere from an open storage site

Between January 1996 and March 2011, the floor in the small demolished building's back room in Jrarat (Fig. S9, supplementary information) was covered by a thick layer of spilled pesticide powders. As only one wall and no roof were preserved, emissions into the atmosphere were investigated. According to the authors' knowledge, no OCP air emission factors for open dumps were previously estimated. Here we present a diffusivity-based approach for estimating emissions of *p*,*p*′-DDT, *p*,*p*′-dichlorodiphenyldichloroethane (*p*,*p*′-DDD), γ-HCH and β-endosulfan as pesticides determined in high concentrations in samples J12 and J13^{t} (Table S3c, supplementary information).

The pesticide air concentration near the surface is based on the ideal gas law (supplementary information). Regression equations based on Mackay et al. (1997) were used to account for the influence of temperature on the saturated vapour pressure of the pesticides studied. Mean daily temperatures were obtained from the 50-km-distant Yerevan meteorological station for 1996–2005 (the compilation method of the relevant meteorological dataset is described in Klein Tank et al. 2002). The unavailable daily temperatures for January 2006–March 2011 were substituted with corresponding median values from the previous years.

The volatilisation flux is defined as diffusion through a thin laminar air layer above the substance surface (supplementary information). Volatilisation is assumed to take place from the entire surface of the contaminated area, i.e. the room of 5× 10 m size with its floor covered by a layer of pesticide powders with a crusted surface. A surface consisting of a pure pesticide is assumed; therefore, the calculation represents the maximum possible amount of the volatilised pesticide. This major simplification is undertaken because the complicated structure of the powder mixture (i.e. consisting of an unknown amount of other than analysed chemicals and inert matter) does not allow for an estimation of the pesticide's actual surface area. Molecular diffusivities were corrected for ambient temperature (supplementary information).

Air mass forwards trajectories

The atmospheric transport pattern of pesticides emitted from the demolished building at Jrarat was studied for 1996–2011. Three-dimensional air mass forwards trajectories were generated at one starting time per day (12 UTC), using the HYSPLIT4 model (Draxler and Rolph 2011). The periods of June 1st–September 15th were selected for atmospheric transport pattern analysis due to the highest daily temperatures (median of 24.5°C, Klein Tank et al. 2002) and hence the highest pesticide emissions. The 120-h-long trajectories were generated at a starting height of 200 m above ground level, checked constantly for remaining within the atmospheric boundary layer. The total of 192,600 hourly segment endpoints (1,605 trajectories×120 endpoints per trajectory) was then sorted onto a 0.5°×0.5° cell grid, using ArcView mapping software.

Human health risk analysis

The risk of food chain contamination by POPs released from landfills and dumps has been brought to attention before (Weber et al. 2011). In this study, human health risks resulting from consumption of food items produced in close proximity to the four investigated POP hot spots were evaluated with respect to the risk of developing cancer.

We applied the dietary exposure model of Renwick et al. (2003), based on the EPA baseline risk assessment approach (EPA 1989; Gaylor et al. 1997). This study assumes human exposure is dependent on the lifetime average chronic daily intake of every single contaminant in a food item. Pollutant specific risks (i.e. an estimate of the probability that an individual will develop cancer during their lifetime) were calculated using the linear low-dose cancer risk equation. The final cumulative carcinogenic health risk related to each

food item at each sampling site was calculated as the sum of partial risks related to individual OCPs. For details about applied equations and parameters, see the supplementary information. The results are compared to the carcinogenic benchmark level, i.e. an exposure posing an upper-bound lifetime excess cancer risk of 1E−6 (i.e. one cancer occurrence over one million people). An exposure for which the risk factor exceeds 1E−6 is scored as significant. Carcinogenic risks above 1E−4 are considered as unacceptable, and addressing such health problems is a high priority (EPA 2011; Xia et al. 2010).

Results and discussion

POP contamination levels

The chemical analysis results are listed in Table S3 (supplementary information). The composition of HCH isomers indicates technical HCH stored in Nubarashen, Echmiadzin and Masis, while lindane is stored in Jrarat.

A detailed review of legal standards and their comparison with determined POP levels is presented in Arnika and AWHHE (2011). In this study, we emphasize the most contaminated sampling spots (Table 1) with regard to the low POP content, as defined by the Basel Convention ratified by the Republic of Armenia in 1999. Wastes consisting of, containing or contaminated with POPs above the low POP content (50 mg kg^{-1} for individual pesticides, Basel Convention 2008) should be disposed of in such a way that the POP content is destroyed or irreversibly transformed. When destruction or irreversible transformation does not represent an environmentally preferable option, it must be otherwise disposed of in an environmentally sound manner (Stockholm Convention 2010).

The strongest soil pollution in Nubarashen is caused by *p*,*p*′-DDT, with 4 gkg^{-1} in surface soil next to the lower fence in the ditch following the interior drainage. A high PCDD/F I-TEQ (2,280 ng kg^{-1}) may be connected with dioxin impurities in pesticides (Weber et al. 2008), e.g. pentachlorophenol (Masunaga et al. 2001) buried in Nubarashen (8.7 t, Helps 2010) and 2,4-dichlorophenoxyacetic acid (Becher et al. 1996) detected in the ditch next to the fence (2.3 mg kg^{-1}; Arnika and AWHHE 2011).

Downslope runoff of contaminated rainwater is indicated by *p*,*p*′-DDT concentrations 1–3 orders of magnitude higher in both the ditch and temporal stream, compared to the hillock in between. Soil core sampling revealed concentrations increasing with depth for many analysed OCPs, which may be a result of contaminated rainwater infiltration into deeper soil layers and/or landslides. In 60 m distance from the lower fence, the concentration of *p*,*p*′-DDT decreases to 0.22 mg kg^{-1}. Air pollution at the burial site is dominated by HCH (2376.8 ng $disc^{-1}$, sum of four isomers). Up to 250 m distant from the burial site, air levels decrease by 1 order of magnitude for most detected substances. At a 2-km distance, OCP concentrations are similar to levels at the Amberd rural background Armenian EMEP station (not published).

The floor of the storage building's inner room in Echmiadzin is highly contaminated by DDT isomers, aldrin, endrin, hexachlorobenzene, chlordane (Table 1) and endosulfan. On the freshly swept outer room floor, a significant DR-CALUX TEQ (869 ng kg^{-1} dry weight) was detected. Contamination release from the storage to its immediate surroundings is suggested by elevated *p*,*p*′-DDT levels (0.22 mg kg^{-1} dry weight) in the adjoining vegetable bed and high levels in eggs ("Human health risks" section). The deposition of dust swept from the storage into its surroundings could particularly be the contamination source. However, this hypothesis was not confirmed by the owners.

DDT levels in orders of grams per kilogram were identified near the barrels stored in the large locked building in Jrarat. The entire building is contaminated, as demonstrated by OCP levels in plaster and sweepings. In the small

Table 1 Sampling spots with pesticide levels determined in at least one solid matrix sample above the low POP content

POP hot spot	Sampling spot	*p*,*p*′-DDT mg kg^{-1}	Aldrin	Endrin	Trans-chlordane
Nubarashen	Ditch	64–4,050	U	U	U
	Temporal stream	100–115	U	U	U
Echmiadzin	Inner room of storage building	117	50	84	109
Jrarat	Large locked building	70–12,485	U	U	U
	Small demolished building	298–515,918	U	U	62
	Area between two demolished buildings	257	U	U	U
	Railway	163	U	U	U
Masis	Outer small room	U	111	U	U

If more than one sample exhibited concentrations above this threshold, the lowest and highest concentrations above are listed

U under low POP content

demolished building's back room, spilled DDT, lindane, chlordane and other OCP powders were identified. This room is a source of nearby contamination due to pesticide powder distribution by wind (256.91 mg kg^{-1} *p*,*p*′-DDT in the nearby soil), a release mechanism suggested to be more significant than volatilisation ("Pesticide emissions into the atmosphere from an open storage site" section). Significant soil contamination by DDTs (sum of all isomers, 258.1 mg kg^{-1}) was also found in the vicinity of the railway formerly used for pesticide transport. Air pollution (i.e. the sum of pollutant concentration in the gaseous phase and in the fraction of atmospheric as well as pesticide particles sampled by passive air samplers) next to the demolished building is dominated by DDTs (sum of all isomers, 4320.4 ng $disc^{-1}$). Up to 200–250 m distant from the storages, OCP air levels decrease by 1–2 orders of magnitude.

The contamination in the large roofless room in Masis is dominated by 12.5 gkg^{-1} HCHs (sum of α, β and γ isomers) detected in scratch-offs sampled next to a damaged barrel containing pink powder and 1.38 mg kg^{-1} HCHs in nearby plaster. Also, high *p*,*p*′-DDE and *o*,*p*′-DDT concentrations were determined here. High aldrin and dieldrin levels (Table 1) dominate the floor contamination in the small outer preserved room. Contamination release from the storage to its immediate surroundings is suggested by elevated *p*,*p*′-DDT levels (0.38 mg kg^{-1} dry weight) in the soil nearby.

Pesticide emissions into the atmosphere from an open storage site

The calculated emissions of pesticides into the atmosphere from the small demolished building's back room in Jrarat during January 1996 and March 2011 were found to be the lowest for *p*,*p*′-DDT and *p*,*p*′-DDD, i.e. 0.27 and 0.57 kg, respectively. This is explained by a low vapour pressure of DDT and its metabolites (Shen and Wania 2005). β-Endosulfan and γ-HCH exhibit a moderately high vapour pressure (Shen and Wania 2005; Willet et al. 1998), which is reflected in significantly higher calculated emissions, i.e. 25 and 155 kg, respectively.

These results are based on the assumption of a surface consisting of a pure pesticide ("Estimates of pesticide emissions into the atmosphere from an open storage site" section), which certainly leads to a significant overestimation in the calculation, given the pesticide concentrations determined in samples J12 and $J13^{t}$ (Table S3c, supplementary information). On the contrary, using a default value and neglecting the influence of wind speed on the laminar layer thickness may have resulted in an underestimation of the calculated volatilisation flux. Further, the surface area subject to volatilisation is undoubtedly larger than 50 m^2, due to the real surface roughness and bumpiness (Fig. S9, supplementary information).

A further question attributes to the prevailing mechanism of air-mediated release of particular pesticides from open dumps into their immediate surroundings. A significant off-site transport mechanism may be wind dispersion of pesticide particles that is assumed to be more significant for DDT than volatilisation, due to its low vapour pressure and determined concentration gradients in Jrarat ("POP contamination levels" section). These mechanisms are interconnected and difficult to quantify by mathematical models. Flux-chamber studies would provide information on air emission rates of pesticides and their mixtures from open dumps. However, they cannot fully replace recalculations based on field measurements due to necessary simplifications. Passive air sampling would be a feasible onsite monitoring tool, although it would benefit from detailed information on sampling of airborne pesticide particles.

If discarded in open piles, these must be considered significant contributors to local air levels of moderately volatile pesticides. However, the potential of HCHs and endosulfan to undergo long-range atmospheric transport (Becker et al. 2011; Willet et al. 1998) has to also be considered. The atmospheric transport pattern of pesticides emitted from the small demolished building in Jrarat is depicted in Fig. 1. The majority of the pesticides emitted during the summer months ("Air mass forwards trajectories" section) are transported westwards, followed by a southwards turn. In a few circumstances, fast moving air masses may transport the pesticides to the north and east of the source. However, a full assessment of the pollutant dispersion on a regional or global scale and identification of potential receptor areas requires the incorporation of wet and dry deposition, scavenging by vegetation and revolatilisation of moderately and highly volatile deposited substances (Bidleman 1999), i.e. multicompartment modelling.

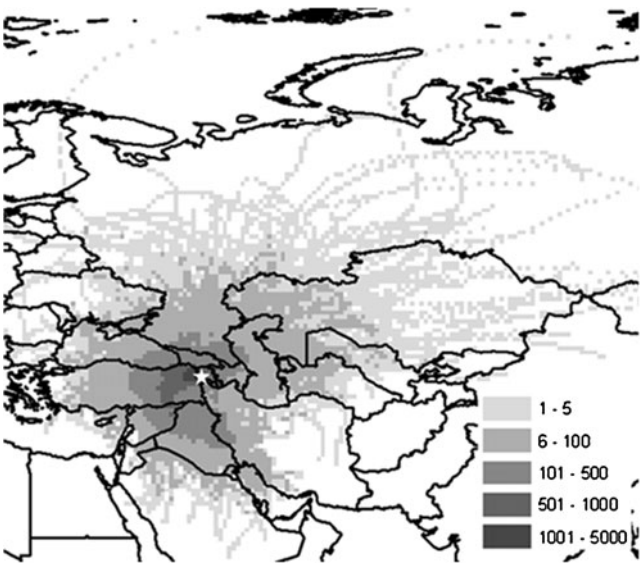

Fig. 1 Air mass forwards trajectories starting at the Jrarat storage (*white star*). Levels of *grey* refer to numbers of trajectory hourly segment endpoints per 0.5°×0.5° cell

Human health risks

POP levels determined in food item samples are listed in Table S3 (supplementary information). A detailed review of legal standards and their comparison with determined POP levels is presented in Arnika and AWHHE (2011). However, the estimate of potential health risks cannot be conducted only by consulting legal standards. A risk analysis is crucial. Non-carcinogenic risks were found to be negligible when compared to carcinogenic; therefore, they are not discussed further. Health risks related to OCPs only were considered, as no PCDD/Fs congener-specific chemical analysis was conducted for any food item sample. A sensitivity analysis for understanding the dependency of risk estimates on the variability in factors contributing to the health risk was conducted.

The calculated cumulative cancer risk probabilities are listed in Table 2. For partial probabilities of individual OCPs, see Table S5 (supplementary information).

The computed dietary exposure risk for all investigated food items is >1E−6, i.e. significant, with *p*,*p*′-DDE contributing the most (Table S5, supplementary information). The highest cancer risk probabilities (>1E−5) were estimated in two Echmiadzin exposure cases, based on composite egg samples taken from households located within a 10–50-m distance from the storage rooms. A cow cream sample from a household within a 2-km distance from the Nubarashen burial site was close to 1E−5. Egg samples taken from free-range hens within 80–100 m from the Jrarat storehouses were slightly above 1E−6. Food item samples taken 300 m from the storage house in Masis were well above 1E−6. When calculating a child exposure scenario, the risk was 69 % of the adult risk, assuming children have the same consumption rate as adults.

Sensitivity analysis identified consumption rate reduction to be most effective in the elimination of potential health risks. A recommended consumption rate (Table 2) suggests a reduction in consumption of each food item with respect to the sampling site to achieve a recommended (acceptable) risk level for long-term dietary exposure. Uncertainties associated with the cancer risk assessment method used here include intake calculations, exposure parameters, toxicity indexes and sample representativeness (samples N30 and M14 were obtained from a single cow). Additionally to food ingestion, occupational, accidental and inhalation exposure roots are especially expected to significantly contribute to human health risks in Jrarat, Masis and Echmiadzin as these partially highly contaminated storages are also used for storing currently used pesticides and other chemicals.

Conclusions

The study revealed a significant contamination of all investigated sites due to inappropriate securing of stored obsolete pesticides, not only in outdated facilities but also in the Nubarashen burial site. At all sites, some building parts and immediate surroundings were contaminated well above the low POP content, suggesting necessary cleanup actions and appropriate disposal of this POP-contaminated waste, along with preventing creation of new POPs, as suggested by the Stockholm Convention. The release of PCDD/Fs from stored obsolete pesticides (Weber et al. 2008) is suggested by samples taken at the storage sites. However, at more distant sites, other sources (e.g. possible waste burning in house yards) may have significantly contributed to determined PCDD/F levels.

Cheap and technically simple passive air sampling revealed significant air concentration gradients in Nubarashen and Jrarat. For a more accurate estimate of volatile pesticide release into the atmosphere from open dumps, further research is recommended to generate air emission factors. Soil concentration gradients and contamination of soil in the immediate

Table 2 Cumulative cancer risk probabilities for adults

Sample number	POP hot spot	Food item	Cancer risk	Recommended consumption amount	Recommended reduction of current consumption rate[a] (%)
N27	Nubarashen	Eggs	1.28E−6	2.3/week	−23
N28		Eggs	1.79E−6	1.1/week	−45
N29		Cow milk	3.06E−6	0.3 l/week	−70
N30		Cow cream	9.54E−6	0.1 l/week	−90
E12	Echmiadzin	Eggs	1.59E−5	1/56 days	−94
E13		Eggs	1.77E−5	1/62 days	−94
J23	Jrarat	Eggs	1.40E−6	1.4/week	−30
J24		Eggs	1.89E−6	1.5/week	−50
J25		Trout	1.87E−6	123 g/day	−49
M13	Masis	Eggs	2.25E−6	1.3/week	−57
M14		Cow milk	4.23E−6	0.35 l/week	−77

[a]Table S2, supplementary information

storage surroundings were confirmed as well. Pesticides leaching into groundwater could not be confirmed nor rejected, as groundwater was sampled from taps. It is not clear whether the DDT levels found originate in the contamination of the groundwater itself or the pipe construction materials.

Pesticide release into nearby soils was also confirmed by significant dietary exposure risks originating from locally produced eggs and milk produced by freely ranging animals. These might be reduced in future by replacing the domestic animals and a cleanup of the particular ranging areas. Further health risks should be avoided by informing employees and nearby residents about possible risks. The wearing of personal protection equipment when entering the sites should be highly recommended.

Due to limitations of the study, a detailed risk analysis supported by additional sampling where necessary should characterise and quantify all risks posed to humans and the environment and define the precise extent of decontamination. Further screening field studies should be conducted at sites supposedly contaminated by POPs, as listed in Ritsema et al. (2006).

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Levels of persistent organic pollutants and polycyclic aromatic hydrocarbons in ambient air of Central and Eastern Europe

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ABSTRACT

The ambient air and soil monitoring network was established in 22 countries of Central and Eastern Europe (CEE) in support of the Global Monitoring Plan under the Stockholm Convention on Persistent Organic Pollutants. Polyurethane foam based passive air samplers were used as a tool for monitoring of POPs in ambient air at remote, rural, suburban, and urban sites with the aim of filling the information gaps identified in this UN region. High atmospheric levels of PCBs, HCHs, DDTs or HCB were observed at the rural as well as urban sites indicating that organochlorines still pose a significant problem in CEE. Pesticide storage, industrial complexes, military zones, and landfills were responsible for the elevated levels of POPs in this survey. The background levels of these compounds, however, were often elevated, too.

Keywords:
Persistent organic pollutants (POPs)
Stockholm Convention
Global Monitoring Plan
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1. Introduction

Central and Eastern Europe (CEE) is a region suffering a general lack of data on concentrations of persistent organic pollutants (POPs) in the environment (UNEP, 2002a; UNEP, 2002b). Many CEE countries (as well as former Soviet Union countries in Asia) have to deal with the same historical burden: heavy industry (coal mining, metallurgy, smelting, steel industry, and power stations), former production of POP chemicals including polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs), extensive agriculture, as well as poor environmental management. Information on production, export, import and application of the POP chemicals was scarce in the past but improved significantly in the last few years with development of the National Inventories (NIs) of POPs in the signatory countries of the Stockholm Convention. In these documents, it has been stated that there is currently no intentional production of POP chemicals in the region. The stocks of obsolete pesticides, POPs containing equipment, and old environmental burdens were identified as the most important sources of the POPs pollution (Ruzickova et al., 2008) together with the industrial technologies and open combustion processes resulting in unintentional releases of polychlorinated dioxins and furans (PCDDs/Fs) and polycyclic aromatic hydrocarbons (PAHs) (Zencak et al., 2007; Kukucka et al., 2009; Gasic et al., 2010; Lammel et al., 2010a; Lammel et al., 2010b; Lammel et al., 2011). Various programs focused on identifying POP pesticides. Re–packing and storing them under the environmentally–sound conditions have been carried out in the CEE countries since 1990s. Still, it has been difficult to estimate a total amount of legally and illegally stored pesticides and other POPs in this region. The situation has been further complicated by recent war conflicts. Burning or damaging of industrial and military targets in the former Republic of Yugoslavia during the Balkan wars and the "Allied Force" operation in the spring of 1999 resulted in a release of the large amounts of POPs (including polychlorinated biphenyls, flame–retardants, and explosives) into the environment (Melas et al., 2000; Kerekes et al., 2001; Picer and Holoubek, 2003; Picer and Picer, 2003). Many damaged PCB filled capacitors remained in service posing further risks, and even when their operation was discontinued, they were stored without proper management (Klanova et al., 2007a; Klanova et al., 2007b; Radonic et al., 2009) . The damaged military facilities presented a problem of their own but no information on their status was available to the public. Even less information was released on the impact of the war conflicts in the Caucasian region (between Armenia and Azerbaijan, in Georgia) or in the Russian Federation (Chechnya).

Availability of data on the environmental levels of POPs is generally limited in the CEE region (Roots and Sweetman, 2007; Skarek et al., 2007; Bartos et al., 2009). The only program monitoring POPs in ambient air on the long–term basis has been carried out in the Czech Republic. This background station of the Czech Hydrometeorological Institute (CHMI) is a part of the European Monitoring and Evaluation Program (EMEP). POPs in ambient air (but also in atmospheric deposition, surface water, sediment, soil and biota) have been monitored in Kosetice since

1988 (Holoubek et al., 2007a; Holoubek et al., 2007b; Dvorska et al., 2008). In 2004, the passive air monitoring network (MOnitoring NETwork) was established in the Czech Republic (Cupr et al., 2006; Klanova et al., 2006), and by 2006, it expanded to the rest of the CEE region. One of the goals was to establish the regional baseline of the POP concentrations in ambient air for the purpose of the Global Monitoring Plan (GMP). A design of this network, however, went beyond the first GMP report. It addressed a more general need for collecting representative regional data on the POP pollution (Mlilukaite et al., 2008; Roots et al., 2010; Stafilov et al., 2011), and improving the knowledge on spatial and temporal variability of POPs in the CEE region. For many countries, this campaign provided the first information on the atmospheric levels of POPs.

2. Materials and Methods

2.1. Sampling sites

Passive air samplers were employed in 22 CEE countries (Armenia, Belarus, Bosnia and Herzegovina, Bulgaria, Estonia, Hungary, Croatia, Czech Republic, Kazakhstan, Kyrgyzstan, Latvia, Lithuania, Macedonia, Moldova, Montenegro, Poland, Romania, Russian Federation, Serbia, Slovakia, Slovenia, Ukraine) between 2006 and 2008. This network consisted of 155 urban, rural and remote sites, many of them affected by industry, traffic, agriculture or residential activities. Out of this number, 55 sites could be classified as remote, rural or urban background, and 10 of them belonged to the EMEP network. Details on the sampling sites are presented in Table S1 and Figure S1 in Supporting Material (SM).

2.2. Sampling

The passive air samplers consisting of the polyurethane foam disks (15 cm diameter, 1.5 cm thick, density 0.030 g cm^{-3}, type N 3038; Gumotex Breclav, Czech Republic) housed in the protective chambers were employed in this study. A theory of the passive air sampling using the similar devices was described elsewhere (Shoeib and Harner, 2002; Harner et al., 2004; Harner et al., 2006). The sampling chambers were prewashed and solvent–rinsed with acetone prior to installation. All PUF disks were prewashed, cleaned (8 hours extraction in acetone and 8 hours in dichloromethane), wrapped in two layers of the aluminum foil, placed in zip–lock polyethylene bags and kept in the freezer prior to their deployment. The PUF disks were exposed for 4 weeks and the field blanks were obtained by installing and removing the PUF disks at all sampling sites. An average sampling rate of such device was estimated to be 3.5–7 m^3 per day (Klanova et al., 2008) based on the co–employment of the active and passive samplers (giving 100–200 m^3 of air in four weeks of deployment). The exposed PUF disks were wrapped in two layers of the aluminum foil, labeled, placed in zip–lock polyethylene bags and transported in a cooler at 5 °C to the laboratory where they were kept in the freezer at –18 °C until the analysis.

2.3. Sample analysis

The surrogate recovery standards (PCB 30 and PCB 185 for PCBs and OCP analysis, *d8*–naphthalene, *d10*–phenanthrene, *d12*–perylene for PAHs analysis) were spiked on each sample prior to extraction. One laboratory blank and one reference material were analyzed with each set of ten samples. All samples were extracted with dichloromethane in a Büchi System B–811 automatic extractor. After extraction, the sample volume was reduced under a gentle stream of nitrogen at ambient temperature. Fractionation was achieved on a silica gel column; a sulfuric acid modified silica gel column was used for PCB/OCP samples. PCB 121 and terphenyl were used as the internal standards for PCB/OCP, and PAH analyses, respectively. Samples were analyzed using a GC–MS instrument (HP 6890 – HP 5975) with a J&W Scientific fused silica column DB–5MS (5% Ph) in electron impact ionization mode for PCBs: PCB 28, PCB 52, PCB 101, PCB 118, PCB 153, PCB 138, PCB 180, OCPs: α–hexachlorocyclohexane (HCH), β–HCH, γ–HCH, δ–HCH, 1,1–dichloro–2,2–bis (p–chlorophenyl) ethylene (*p,p´*–DDE), 1,1–dichloro–2,2–bis (p–chlorophenyl) ethan (*p,p´*–DDD), 1,1,1–trichloro–2,2–bis (p–chlorophenyl) ethan (*p,p´*–DDT), *o,p´*–DDE, *o,p´*–DDD, *o,p´*–DDE, hexachlorobenzene (HCB), and pentachlorobenzene (PeCB), and 16 US EPA polycyclic aromatic hydrocarbons (PAHs) as described earlier (Klanova et al., 2009). The samples (1 μL) were injected at 80 °C oven temperature and 280 °C injector and transfer line temperature. After 2 min, the temperature was raised at 15 °C min^{-1} to 180 °C, then at 5 °C min^{-1} to 310 °C, and the final temperature was held for 20 min. The carrier gas was He at a flow of 0.2 mL min^{-1}. Data were collected in selected ion monitoring mode.

2.4. Quality assurance/quality control

Recoveries were determined for all samples by spiking with the surrogate standards prior to extraction. Recoveries were 76–100%, and 71–98% for all samples, for PCBs/OCPs, and PAHs, respectively. Recovery factors were not applied to any of data. Recovery of native analytes measured for the reference material varied from 88 to 103% for PCBs, from 75 to 98% for OCPs, from 72 to 102% for PAHs. The laboratory blanks were under the detection limits for all compounds. The field blanks consisted of the pre–extracted PUF disks that were taken to each sampling site. They were extracted and analyzed in the same way as the samples, and the levels in field blanks never exceeded 3% of the quantities detected in the samples for PCBs, 1% for OCPs, 3% for PAHs, indicating a minimal contamination during the transport, storage and analysis.

3. Results and Discussion

The following sections summarize data on the ambient air concentrations of POPs in CEE collected between 2006 and 2008. As mentioned above, the temporal trends of atmospheric POPs could only be assessed at Kosetice station in the Czech Republic (Holoubek et al., 2007a), the only CEE site with a long–term air monitoring program. The results of the present study, however, extend the knowledge on the sources of atmospheric POPs in the CEE region, their spatial distribution patterns and concentration ranges. A full range of PCB, HCH and DDT levels measured in background, rural and urban air are shown in Figures 1, 2, and 3. Similar information on HCB and PAH levels is provided in SM, Figures S2 and S4.

In order to establish the regional baseline of the POP concentrations in ambient air, we have to pay special attention to their levels at the background sites. Out of 155 sites assessed in this study, 55 were the background sites (10 EMEP stations, 22 other remote and rural background sites, and 23 urban background sites) (see the SM Table S1 and Figure S1). Descriptive statistics (minimum, maximum, median and mean) of the atmospheric concentrations of POPs at the remote and rural sites (including the EMEP stations in Bosnia and Herzegovina, Estonia, Czech Republic, Kazakhstan, Latvia, Macedonia, Slovakia, and Slovenia) are provided in Tables S2–S6 (see the SM). Median concentrations of POPs in the background air are shown in Figures 4, 5, 6, S2 and S4 for the individual POP groups. All concentrations are reported in the units of nanogram or microgram per sample, each sample corresponding to 100–200 m^3 of air collected in four weeks of deployment (average sampling rate of 3.5 –7 m^3 per day) (Klanova et al., 2008).

3.1. Polychlorinated biphenyls in the CEE atmosphere

Technical mixtures of PCBs were produced in several CEE countries (former Czechoslovakia, Poland, former Soviet Union), and widely applied in the whole region for several decades. It has been identified in several CEE NIs that damaged transformers,

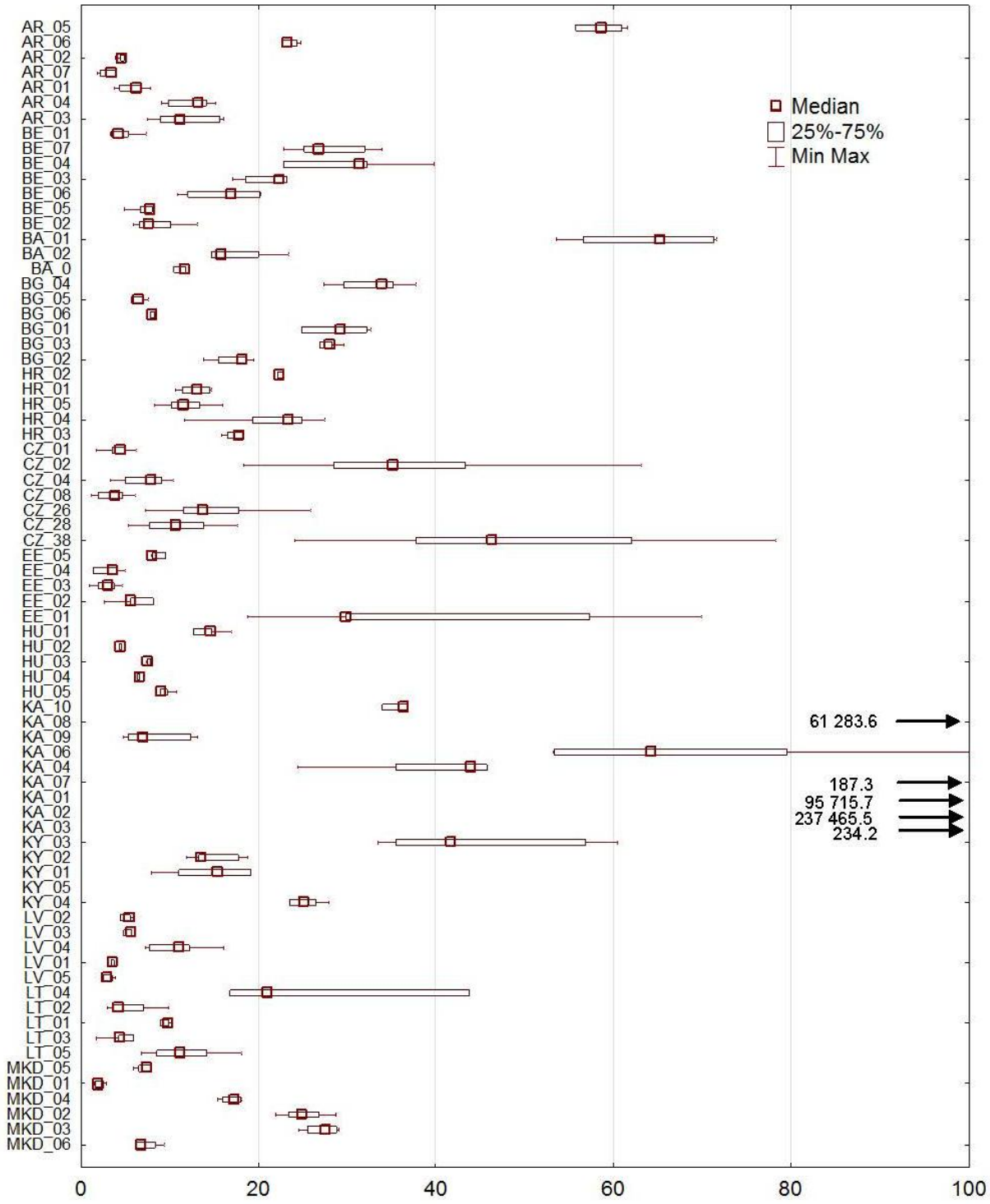


Figure 1a. *PCB levels in ambient air (PAS, ng sample^{-1}) measured at the background, rural and urban sites of the CEE region, 2006-2008.*

condensers, hydraulic systems and other PCB containing devices, their dumping sites, waste incinerators and open systems (paints and wood protecting layers) are the most important sources of the atmospheric PCBs in CEE.

In the present study, the PCB concentrations ranged from 1 to 96 µg sample^{-1} in the ambient air samples collected in CEE between 2006 and 2008 (Figures 1a and 1b). The highest PCB levels (96 µg sample^{-1}) were found in the air samples from Ust–Kamenogorsk, Kazakhstan. These samples were collected outside the impregnation workshop in the industrial complex of the capacitor plant where PCBs were used between 1968 and 1990 as a liquid filling for the capacitors (inside this building, the PCB levels reached 237 µg sample^{-1}). Similar levels of PCBs (61 µg sample^{-1}) were also found at former military base in Balchash, Kazakhstan. It has been estimated that 980 metric tons of PCBs are still contained in transformers and capacitors in Kazakhstan while the total amount of wastes containing PCBs is 250 000 tons. In the Kamenogorsk city centre, the PCB concentrations were three orders of magnitude lower but still up to 234 ng sample^{-1}. PCB

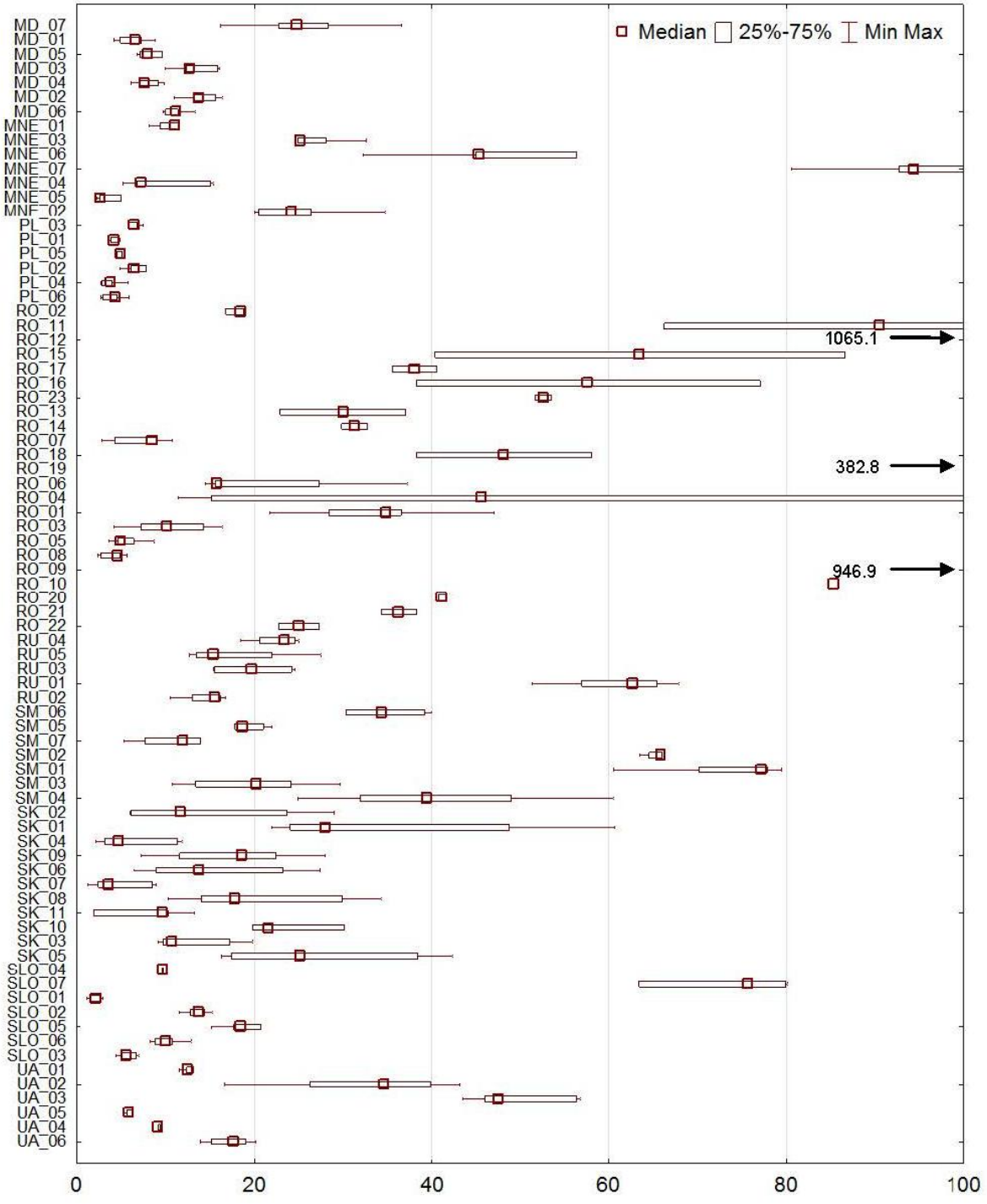


Figure 1b. *PCB levels in ambient air (PAS, ng sample^{-1}) measured at the background, rural and urban sites of the CEE region, 2006-2008.*

levels comparable to Kamenogorsk city were also found at urban/industrial sites in Romania (Braila, Filiasi or Timisoara, levels up to 1 μg sample^{-1}). In the urban sites of many other countries (Armenia, Bosnia, Czech Republic, Kyrgyzstan, Montenegro, Serbia, Slovakia), the PCB concentrations were in the range of tens of nanograms per sample.

As can be seen in Table S2 (see the SM) and in Figure 4, the median PCB concentrations at the CEE background sites ranged between 1 and 124 ng sample^{-1}. Even lower concentrations were found at the EMEP stations (1–23 ng sample^{-1}). In contrast, the highest median concentrations were determined at the background sites in Kazakhstan, Kyrgyzstan, Armenia, but also in the countries of former Yugoslavia (Serbia, Croatia or Montenegro) and Romania. Generally, the PCB contamination of the background air corresponds very well with the overall situation in each country.

3.2. Organochlorine pesticides in the CEE atmosphere

OCPs were produced in many CEE countries during the last century, and applied in all of them. The high atmospheric levels of OCPs are often being found in the countries where they were never produced and their application was banned more than 20 years ago. Pesticide storages and burial sites, along with

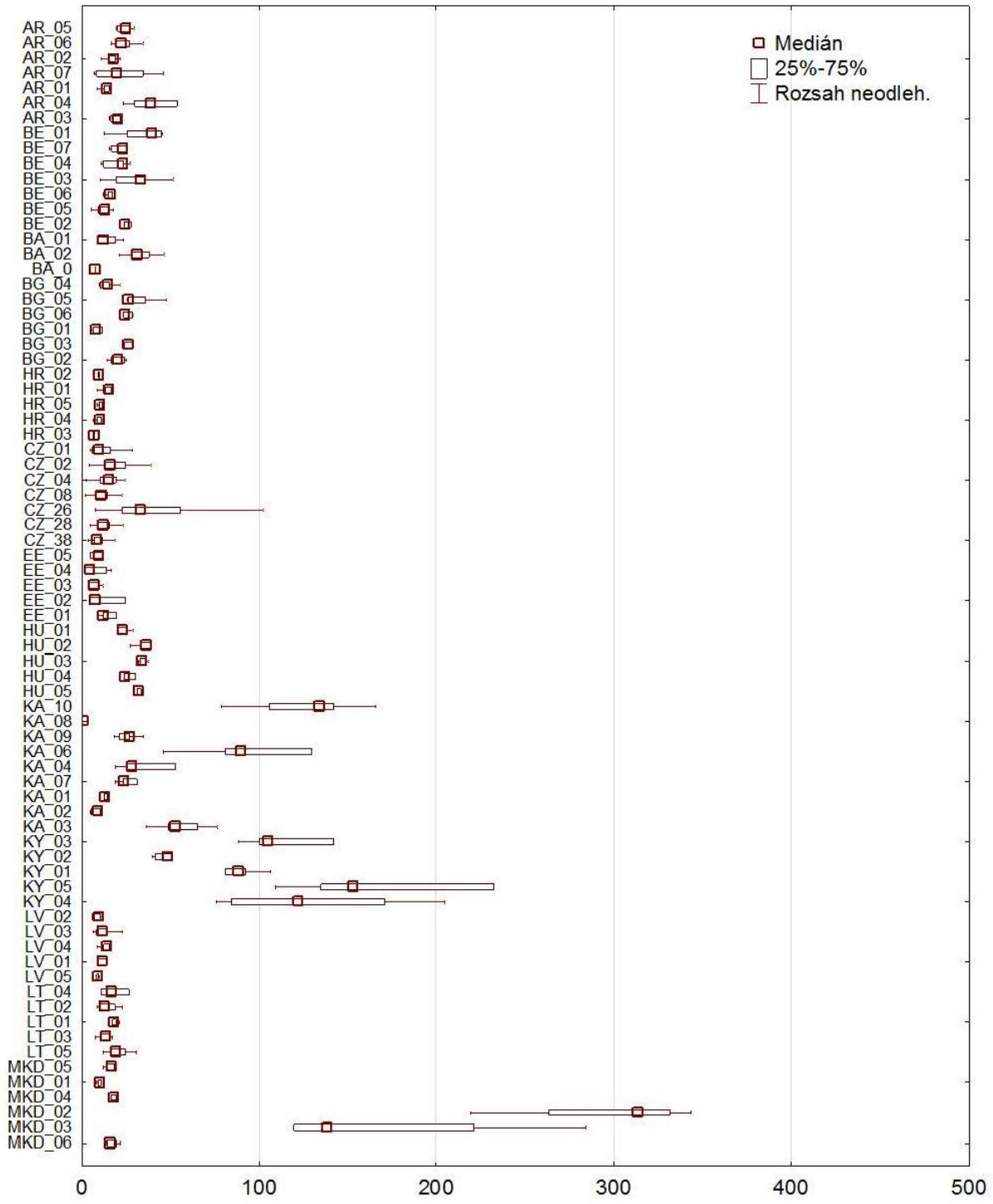


Figure 2a. *HCH levels in ambient air (PAS, ng sample^{-1}) measured at the background, rural and urban sites of the CEE region, 2006-2008.*

contaminated soils, are considered to be the main sources of OCPs. In Kyrgyzstan alone, for instance, almost 5 000 metric tons of pesticides were used annually to control pests, weeds, and pathogens. Almost 2 000 metric tons of pesticides, including more than 1 000 metric tons POP pesticides were buried in this country and high levels of aldrin, HCH, DDT, as well as their degradation products, have been detected in soils in the vicinity these burial grounds. It has been estimated in the preliminary inventory that more than 30 metric tons of DDT is still in the stockpiles. A similar situation exists in Romania where almost 261 metric tons of POP pesticides are known to be deposited in solid or liquid form, often without proper identification and adequate safety procedures. Moldova is another country where the stockpiles of obsolete pesticides have become a significant problem for the country, and a major threat for the environment and population's health. The total stock constituted 3 000 metric tons of pesticides at the time of the campaign, and it has been estimated that the average amount of POP pesticides in the total stock is about 20–30%. The obsolete pesticides are also the main reason for higher atmospheric levels in Ukraine (5 000 store houses in agricultural facilities contained about 22 000 metric tons of obsolete pesticides, including POP pesticides) and other countries. Due to

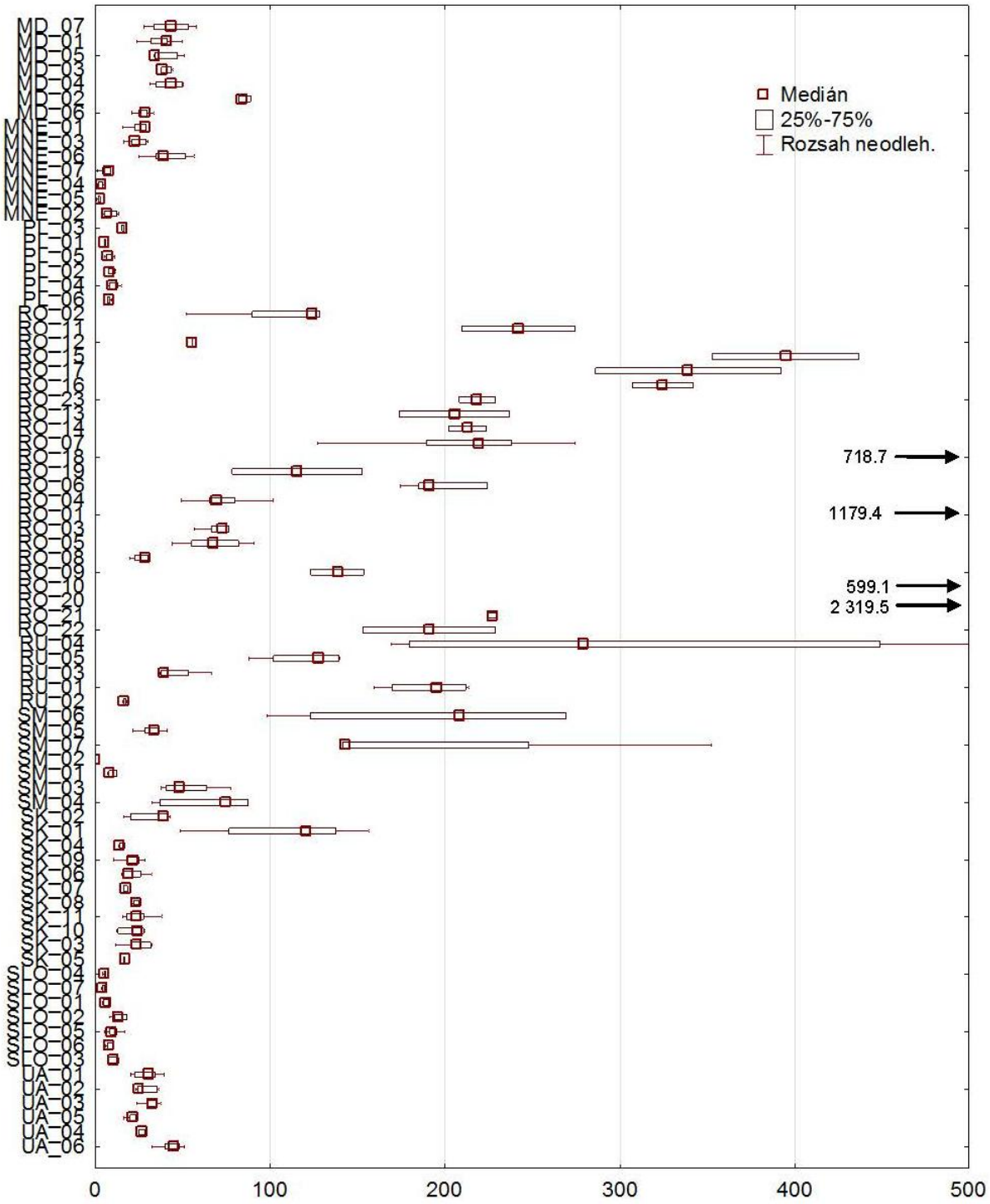


Figure 2b. *HCH levels in ambient air (PAS, ng sample^{-1}) measured at the background, rural and urban sites of the CEE region, 2006-2008.*

the lack of national strategies on obsolete pesticides management, the large quantities of obsolete pesticides have been accumulated in the warehouses often not properly equipped to store such chemicals. Packing materials have deteriorated over time, causing leakages of chemicals into the environment. The pesticides were indiscriminately mixed during repackaging and as a result, the whole stockpile of pesticides is contaminated with POPs.

The atmospheric concentrations of HCHs were not uniform but ranged between 0.1 and 2 320 ng sample^{-1}. The highest HCH levels (up to 2 320 ng sample^{-1}) were found at the industrial sites in Romania (Turda, Onesti or Deva). Kazakhstan, Kyrgyzstan, Macedonia, Serbia, and the Russian Federation were among the countries where the HCH levels reached hundreds of ng per sample (Figures 2a and 2b).

At the background sites, the HCH levels ranged from 0.1 to 148 ng sample^{-1} (0.5–50 ng sample^{-1} at the EMEP sites). As with the impacted sites, the highest concentrations were measured in Kazakhstan, Kyrgyzstan, Romania, Moldova, and Ukraine. The background sites in Romania and Moldova were high in atmospheric levels of both HCHs and DDTs. Interestingly, the lowest levels of HCHs were measured at background sites in the

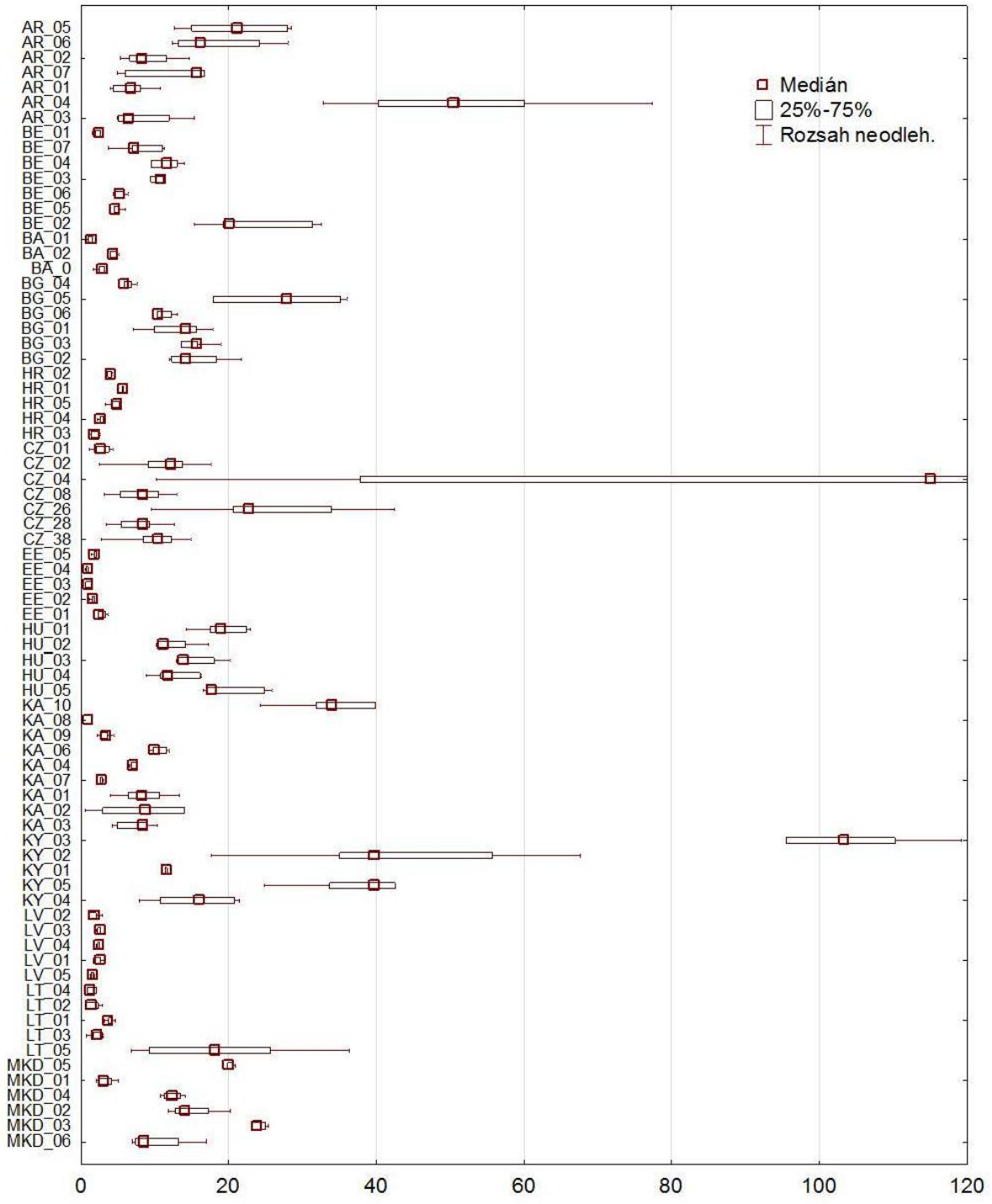


Figure 3a. *DDT levels in ambient air (PAS, ng sample^{-1}) measured at the background, rural and urban sites of the CEE region, 2006-2008.*

Western Balkan countries: Bosnia and Herzegovina, Slovenia, and Croatia (see the SM, Table S3 and Figure 4).

The atmospheric levels of DDTs ranged between 0.1 and 173 ng sample^{-1}. As with HCHs, the industrial sites in Romania showed the highest air concentrations of DDTs (maxima were reached at all Bucuresti sites). Kyrgyzstan, Ukraine, Serbia, or the Czech Republic were also among the highest (Figures 3a and 3b).

A similar situation was observed for DDTs: their levels ranged from 0.1 to 55 ng sample^{-1} (the same for the EMEP stations), and the highest levels, observed at the background sites in Moldova, were order of magnitude higher than those found at the majority of the other sites (see Figure 6 and Table S4 in SM).

HCB was found at much lower concentrations than the other pesticides (0.1–46 ng sample^{-1}). The maxima were detected in the Russian Federation (46 ng sample^{-1}) and Bosnia and Herzegovina (38 ng sample^{-1}). Concentrations of 10–20 ng sample^{-1} were also measured in Ukraine, the Czech Republic, Kazakhstan, Romania, and Serbia (see the SM, Figures S2a and S2b).

The HCB concentrations at the background sites in Bosnia and Herzegovina and Ukraine were an order of magnitude higher (tens

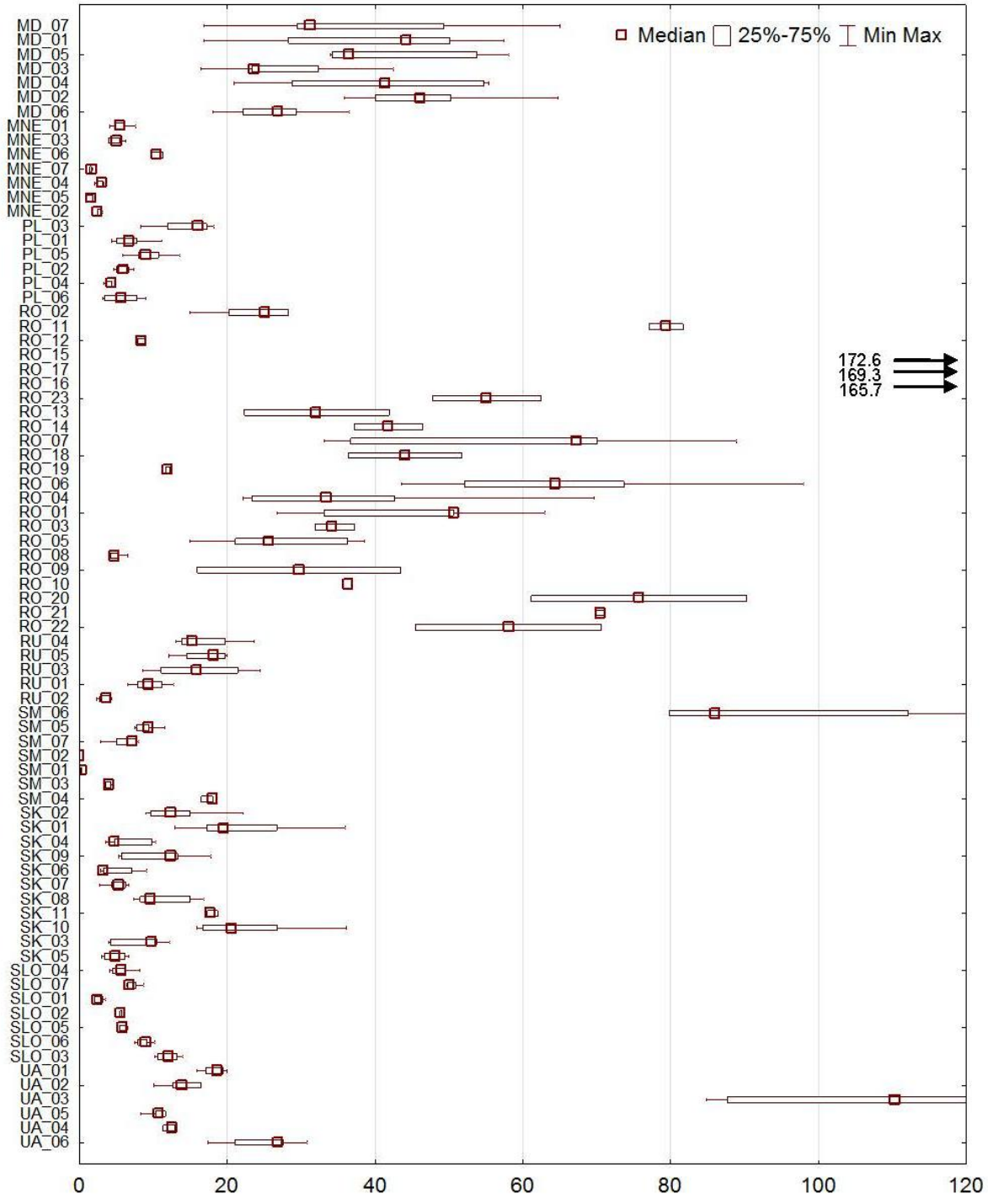


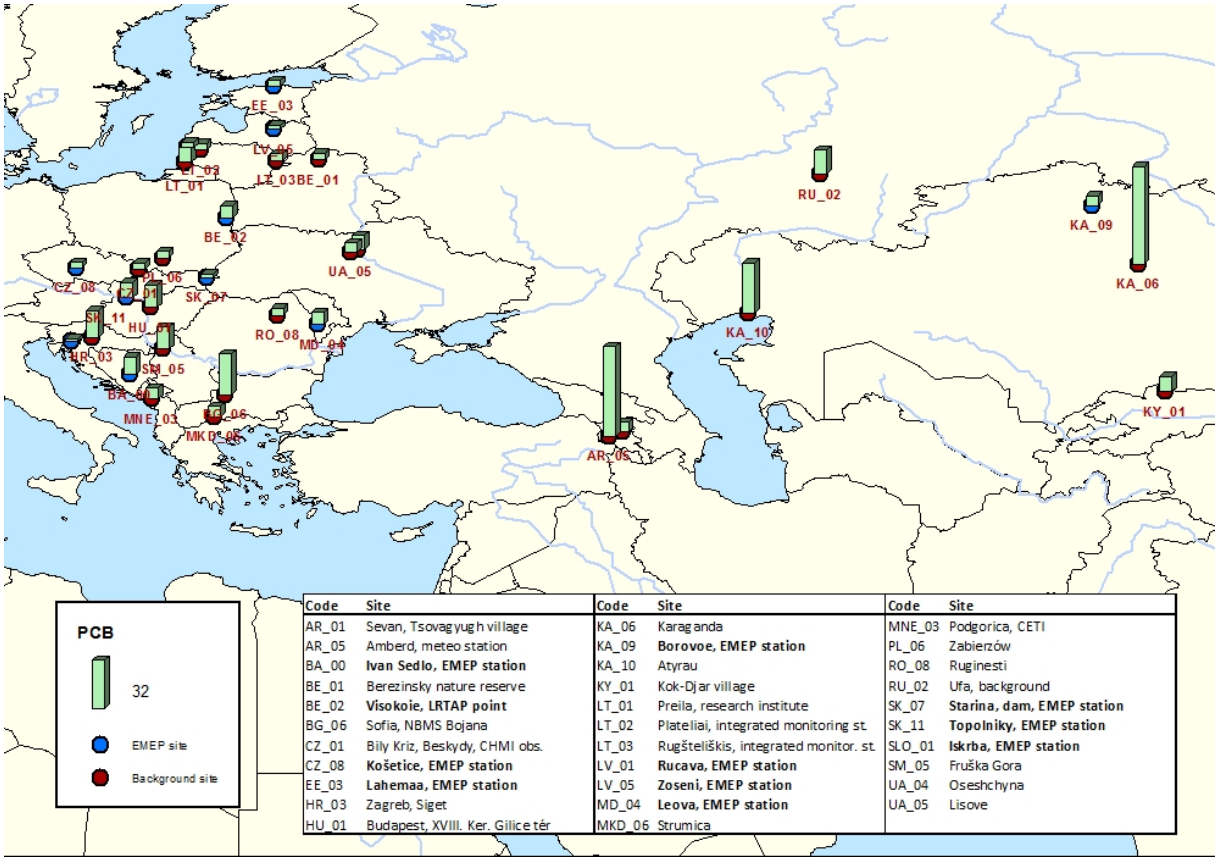
Figure 3b. *DDT levels in ambient air (PAS, ng sample^{-1}) measured at the background, rural and urban sites of the CEE region, 2006-2008.*

of ng sample^{-1}) than those observed in remaining countries (Table S5 and Figure S3 in SM). These levels corresponded with the elevated levels of HCB at residential sites in Bosnia and Herzegovina, and Ukraine.

3.3. Polycyclic aromatic hydrocarbons in the CEE atmosphere

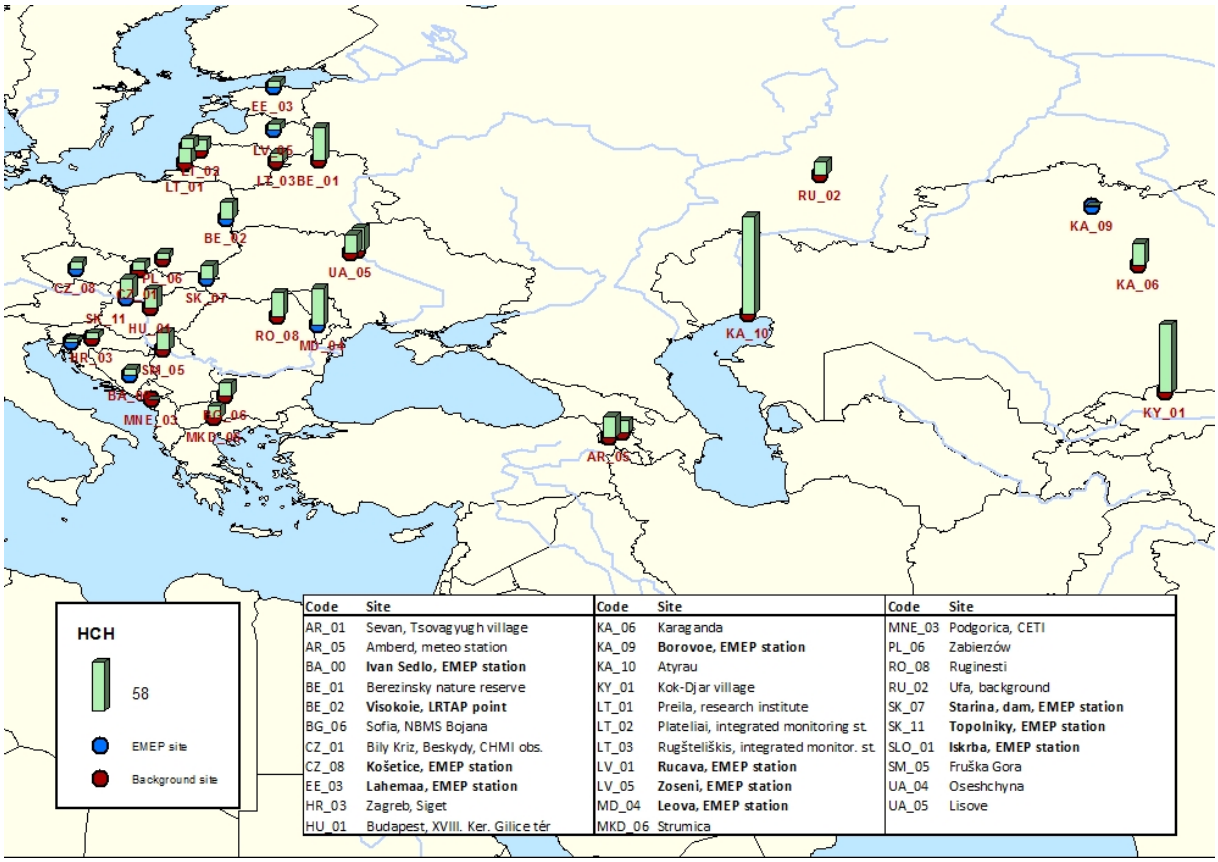
Even though PAHs are not a subject of the international conventions on POPs (their physicochemical properties do not suggest persistence or bioaccumulation potential), they were included in this survey because of their significant health impacts and potential for long–range transport. It has been shown in previous studies that PAHs represent a serious problems in some of the CEE countries (Zencak et al., 2007).

The atmospheric PAHs ranged from 118 ng sample^{-1} to 108 µg sample^{-1} and the highest levels were measured in the big cities and industrial centers of Romania (Deva, 108 µg sample^{-1}; Bucuresti, 54 µg sample^{-1}), Serbia (Kragujevac, 82 µg sample^{-1}), and Kazakhstan (Ust–Kamenogorsk, 30 µg sample^{-1}). Levels between 10–20 µg sample^{-1} were also determined in Bulgaria, the Russian Federation, and Kyrgyzstan (Figures S4a and S4b in SM). As traffic and industrial emissions are the major sources of PAHs at urban and industrial sites, a seasonality of their levels was much less pronounced than at the background sites.



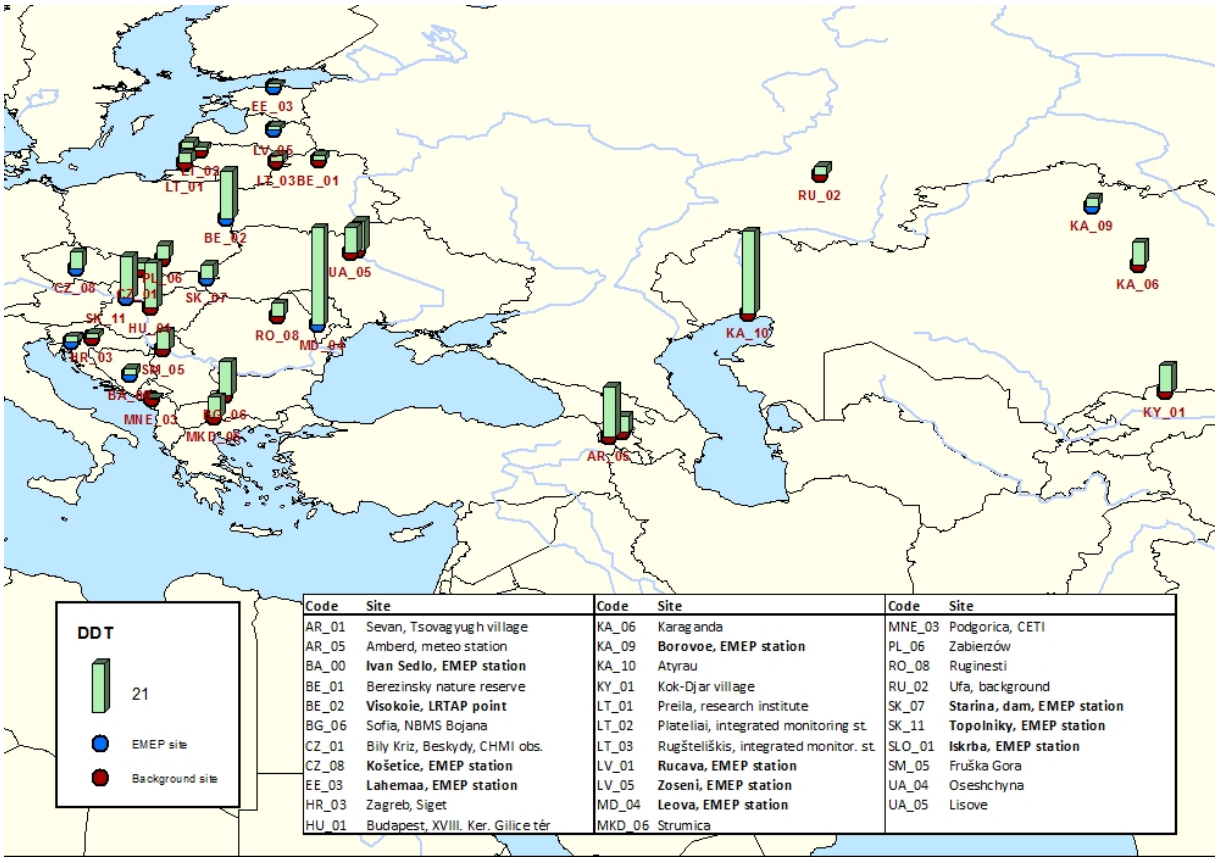
Code	Site	Code	Site	Code	Site
AR_01	Sevan, Tsovagyugh village	KA_06	Karaganda	MNE_03	Podgorica, CETI
AR_05	Amberd, meteo station	KA_09	**Borovoe, EMEP station**	PL_06	Zabierzów
BA_00	**Ivan Sedlo, EMEP station**	KA_10	Atyrau	RO_08	Ruginesti
BE_01	Berezinsky nature reserve	KY_01	Kok-Djar village	RU_02	Ufa, background
BE_02	**Visokoie, LRTAP point**	LT_01	Preila, research institute	SK_07	**Starina, dam, EMEP station**
BG_06	Sofia, NBMS Bojana	LT_02	Plateliai, integrated monitoring st.	SK_11	**Topolniky, EMEP station**
CZ_01	Bily Kriz, Beskydy, CHMI obs.	LT_03	Rugšteliškis, integrated monitor. st.	SLO_01	**Iskrba, EMEP station**
CZ_08	**Košetice, EMEP station**	LV_01	**Rucava, EMEP station**	SM_05	Fruška Gora
EE_03	**Lahemaa, EMEP station**	LV_05	**Zoseni, EMEP station**	UA_04	Oseshchyna
HR_03	Zagreb, Siget	MD_04	**Leova, EMEP station**	UA_05	Lisove
HU_01	Budapest, XVIII. Ker. Gilice tér	MKD_06	Strumica		

Figure 4. *The median levels of PCBs in ambient air (PAS, ng sample^{-1}) measured at the background sites of the CEE region, 2006-2008.*



Code	Site	Code	Site	Code	Site
AR_01	Sevan, Tsovagyugh village	KA_06	Karaganda	MNE_03	Podgorica, CETI
AR_05	Amberd, meteo station	KA_09	**Borovoe, EMEP station**	PL_06	Zabierzów
BA_00	**Ivan Sedlo, EMEP station**	KA_10	Atyrau	RO_08	Ruginesti
BE_01	Berezinsky nature reserve	KY_01	Kok-Djar village	RU_02	Ufa, background
BE_02	**Visokoie, LRTAP point**	LT_01	Preila, research institute	SK_07	**Starina, dam, EMEP station**
BG_06	Sofia, NBMS Bojana	LT_02	Plateliai, integrated monitoring st.	SK_11	**Topolniky, EMEP station**
CZ_01	Bily Kriz, Beskydy, CHMI obs.	LT_03	Rugšteliškis, integrated monitor. st.	SLO_01	**Iskrba, EMEP station**
CZ_08	**Košetice, EMEP station**	LV_01	**Rucava, EMEP station**	SM_05	Fruška Gora
EE_03	**Lahemaa, EMEP station**	LV_05	**Zoseni, EMEP station**	UA_04	Oseshchyna
HR_03	Zagreb, Siget	MD_04	**Leova, EMEP station**	UA_05	Lisove
HU_01	Budapest, XVIII. Ker. Gilice tér	MKD_06	Strumica		

Figure 5. *The median levels of HCHs in ambient air (PAS, ng sample^{-1}) measured at the background sites of the CEE region, 2006-2008.*



Code	Site	Code	Site	Code	Site
AR_01	Sevan, Tsovagyugh village	KA_06	Karaganda	MNE_03	Podgorica, CETI
AR_05	Amberd, meteo station	KA_09	**Borovoe, EMEP station**	PL_06	Zabierzów
BA_00	**Ivan Sedlo, EMEP station**	KA_10	Atyrau	RO_08	Ruginesti
BE_01	Berezinsky nature reserve	KY_01	Kok-Djar village	RU_02	Ufa, background
BE_02	**Visokoie, LRTAP point**	LT_01	Preila, research institute	SK_07	**Starina, dam, EMEP station**
BG_06	Sofia, NBMS Bojana	LT_02	Plateliai, integrated monitoring st.	SK_11	**Topolniky, EMEP station**
CZ_01	Bily Kriz, Beskydy, CHMI obs.	LT_03	Rugšteliškis, integrated monitor. st.	SLO_01	**Iskrba, EMEP station**
CZ_08	**Košetice, EMEP station**	LV_01	**Rucava, EMEP station**	SM_05	Fruška Gora
EE_03	**Lahemaa, EMEP station**	LV_05	**Zoseni, EMEP station**	UA_04	Oseshchyna
HR_03	Zagreb, Siget	MD_04	**Leova, EMEP station**	UA_05	Lisove
HU_01	Budapest, XVIII. Ker. Gilice tér	MKD_06	Strumica		

Figure 6. *The median levels of DDTs in ambient air (PAS, ng sample^{-1}) measured at the background sites of the CEE region, 2006-2008.*

The atmospheric levels of PAHs still ranged over three orders of magnitude (118–99 µg sample^{-1}) at the background sites of the CEE region and their highest concentrations corresponded well with the strongest source areas. The highest PAH levels were again measured in Romania (Ruginesti, 99 µg sample^{-1}) but half of this value (43 µg sample^{-1}) was also measured at the Ivan Sedlo EMEP station in Bosnia and Herzegovina. Unlike the levels of organochlorines, the PAH concentrations at the background sites reached their maxima during the winter sampling periods due to seasonal heating. The higher winter levels of PAHs were also found in Bulgaria, Lithuania, the Czech Republic and Belarus, while the lowest year–round concentrations were measured in Armenia and Slovenia (see the SM, Table S6 and Figure S4).

3.4. A comparison of the results with previously reported studies

Ambient air concentrations of POPs were measured at 55 background sites in the CEE region, ranging from remote to urban. Generally, polychlorinated biphenyls and polycyclic aromatic hydrocarbons were found at the highest levels at the suburban, urban and industrial background sites as their sources are strongly associated with industry, combustion, residential activities, local heating systems and traffic. Organochlorine pesticides such as HCH, DDT, their isomers and metabolites were most frequently detected at higher levels at the rural background sites with a higher influence of the agriculture including pesticide storage and burial sites. The EMEP stations involved in this study showed generally low levels of atmospheric POPs when compared to other background sites with the exception of the HCB contamination. The atmospheric levels of POPs found in this survey can be compared to those of the previous PAS campaign performed at 86 EMEP stations in the summer of 2006 (Halse et al., 2011). However, it should be noted that the previous campaign was carried out during the warmest season, while all seasons were equally covered in the current study. Thus, data from the two studies may not be fully comparable. All concentration data are reported in picograms or nanograms per cubic meter for the purpose of such comparison.

The average atmospheric concentration of the sum of 7 PCBs calculated across the EMEP stations was 21 pg m^{-3} (Halse et al., 2011). In our campaign, the individual PCB levels measured at the EMEP stations ranged between 10 and 293 pg m^{-3} but their long–term median concentrations were between 19 and 117 pg m^{-3}. The range of PCB levels calculated across all background stations, however, was between 10 and 1 200 pg m^{-3}, while the median concentrations at the stations varied from 19 to 187 pg m^{-3}. It shows both a large spatial variability in the PCB levels among the background sites in the CEE region, as well as a large seasonal variability that was not captured in the previous study.

The HCH concentrations at the CEE EMEP stations involved in this campaign varied between 42 and 503 pg m^{-3} (between 60 and 434 pg m^{-3} for the long–term median concentrations) while a range of concentrations between 9 and 311 pg m^{-3} was reported previously (with an average of 64 pg m^{-3} for all EMEP stations) (Halse et al., 2011). These results also showed good agreement between the old and new survey, and the results of two different study designs. The HCH levels at all background sites involved in this study varied from 34 to 1 062 pg m^{-3} (median concentrations from 52 to 883 pg m^{-3}) demonstrating higher contamination of background sites in the countries not involved in previous campaigns.

Halse et al. (2011) reported that the concentration of the sum of 4 DDTs varied between 1 and 356 pg m^{-3} (EMEP average of 32 pg m^{-3}) while the range of concentrations of the sum of 6 DDTs at the CEE EMEP stations measured in this campaign was between 5 and 554 pg m^{-3} (median levels at the individual stations 8–414 pg m^{-3}). This range was identical to the range of median DDT levels determined at all background stations, showing that even some EMEP stations (Rucava in Lithuania) have considerable

atmospheric concentrations of organochlorine pesticides (the full range of measured values was 5–554 pg m^{-3}).

A similar situation was observed for concentrations of HCBs at the EMEP stations: their range was between 1 and 557 pg m^{-3} (the median at the individual stations was between 37 and 336 pg m^{-3}), exactly the same as the range of all CEE background stations. In the previously reported survey, the levels varied between 23 and 115 pg m^{-3} (EMEP average of 49 pg m^{-3}) (Halse et al., 2011).

A greater difference between the EMEP levels and the levels determined at other CEE background sites was observed for PAHs. Previously reported EMEP levels were between 0.2 and 35 ng m^{-3} (the average of all EMEP sites was 6 ng m^{-3}) (Halse et al., 2011) while currently measured atmospheric levels of PAHs at CEE EMEP sites ranged between 2 and 418 ng m^{-3} (median levels between 5–23 ng m^{-3}). The range of PAH concentrations found across all CEE background sites was between 2 and 988 ng m^{-3} (median between 5 and 39 ng m^{-3}). The inclusion of winter sampling periods into the currently reported monitoring exercise is the reason for a greater variability in the PAH levels.

4. Conclusions

As the EMEP stations with ongoing POP monitoring programs are mostly situated in the northern and western parts of Europe (Kosetice station in the Czech Republic is the only active station in CEE), the CEE region suffers from a lack of information on the levels of these compounds in air. First, consistent information on the atmospheric levels of POPs in this region was generated in 2006 when the 3–months passive air sampling campaign was performed at 86 EMEP stations (Halse et al., 2011). In the current study, the atmospheric levels of POPs and PAHs were monitored at 155 sites in Central and Eastern Europe. The passive air samplers have been deployed for 6 consecutive sampling periods of 28 days between 2006 and 2008. This survey provided new information on (i) background atmospheric levels of POPs in the CEE countries, (ii) concentration ranges of atmospheric POPs at urban and rural sites, and (iii) seasonal and temporal variability of the POP levels. Background data collected in this survey were used by the CEE Regional Organizational Group to prepare the first GMP report under the SC in 2009. In order to assess the long–term trends in atmospheric levels of POPs required for the effectiveness evaluation of the SC, selected background sites in the CEE region continue to be monitored under the framework of the MONET passive sampling network.

Acknowledgement

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Supporting Material Available

Tables (PCB, HCH, DDT. HCB, and PAH) and maps (HCB, PAH) showing concentration levels at the background sites as well as box and whisker plots showing HCB and PAH concentrations at all sampling sites. This information is available free of charge via the Internet at http://www.atmospolres.com.

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Mineralogical, chemical and toxicological characterization of urban air particles

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ABSTRACT

Systematic characterization of morphological, mineralogical, chemical and toxicological properties of various size fractions of the atmospheric particulate matter was a main focus of this study together with an assessment of the human health risks they pose. Even though near-ground atmospheric aerosols have been a subject of intensive research in recent years, data integrating chemical composition of particles and health risks are still scarce and the particle size aspect has not been properly addressed yet. Filling this gap, however, is necessary for reliable risk assessment. A high volume ambient air sampler equipped with a multi-stage cascade impactor was used for size specific particle collection, and all 6 fractions were a subject of detailed characterization of chemical (PAHs) and mineralogical composition of the particles, their mass size distribution and genotoxic potential of organic extracts. Finally, the risk level for inhalation exposure associated to the carcinogenic character of the studied PAHs has been assessed. The finest fraction (<0.45 μm) exhibited the highest mass, highest active surface, highest amount of associated PAHs and also highest direct and indirect genotoxic potentials in our model air sample. Risk assessment of inhalation scenario indicates the significant cancer risk values in PM 1.5 size fraction. This presented new approach proved to be a useful tool for human health risk assessment in the areas with significant levels of air dust concentration.

1. Introduction

Polyaromatic hydrocarbons (PAHs) behaving like persistent organic pollutants (POPs) are of a concern for human health (Bartos et al., 2009; Delgado-Saborit et al., 2011; Shah and Balkhair, 2011; WHO, 2003) as well as for the ecosystems of remote areas (such as the polar regions) as they are bioaccumulative, resist degradation and cycle in the environment for a long time. The atmosphere is a main pathway for their long-range transport even though for most POPs the atmospheric burden is only a small fraction of the total environmental load. As POPs are semivolatile (saturation vapor pressures 10^{-6}–10^{-2} Pa) they partition between the phases of the atmosphere according to temperature, particulate matter availability and chemical properties. The distribution among the various particle fractions is important as it controls the atmospheric fate. Gaseous molecules on one hand and molecules associated with aerosol particles on the other hand side undergo different degradation and physical removal processes (dry and wet deposition). Particles of different sizes and compositions have very different atmospheric lifetimes.

The processes on aerosol particles and partitioning between the gaseous and particulate phases are, however, insufficiently known (Lohmann and Lammel, 2004) and are to be better understood in order to describe POPs cycling and assess related impacts to the ecosystems and human health.

The health hazard of ambient air is believed to be determined by conventional gaseous pollutants (ozone, nitrogen oxides) and particulate matter (PM). Numerous scientific studies have linked particle pollution exposure to a variety of health problems, including increased respiratory symptoms, such as irritation of the airways, coughing, difficulty breathing, decreased lung function, aggravated asthma, development of chronic bronchitis, irregular heartbeat, nonfatal heart attacks, and premature death in people with heart or lung disease (Atkinson et al., 2001, 2010; Kan et al., 2007; Li et al., 2011; Pope et al., 2009; Schwartz et al., 1996; Shi et al., 2003). A long-term exposition to elevated levels can cause higher mortality, shorter life, higher incidence of cardiovascular diseases, bronchitis and lung cancers (Parodi et al., 2005). PM is estimated to kill more than 500 000 people worldwide each year (UNEP, 1994). To prevent this loss of life we must understand the characteristics of PM and gain insight into how these characteristics are related to adverse health effects (Nel, 2005).

Particle size and morphology, disregarding its chemical properties, obviously represent one of the hazards. Respirable particles are usually divided into the coarse (diameter more than 2.5 μm), fine (0.1–2.5 μm in diameter) and ultrafine (less than 0.1 μm in diameter) size fractions. Currently, governments and quality monitoring agencies track and regulate 10 μm-diameter (PM_{10}) and 2.5 μm-diameter ($PM_{2.5}$) particles (European Parliament, 2008). Unfortunately, recent studies suggest that the unregulated ultrafine particles are potentially the most dangerous. Growing attention is given to the potential effects of the ultrafine

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dust particles in the human health because they can penetrate deeper into the respiration tract than fine or coarse particles do (Oberdorster et al., 2002), and cause the respiratory problems (Boldo et al., 2011). Pulmonary effects of PM include the triggering of inflammation in the smaller airways, which can lead to the exacerbation of asthma and chronic bronchitis, airway obstruction, and decreased gas exchange. PM can also interfere with the clearance and inactivation of bacteria in lung tissue.

The chemical composition of the particles themselves, as well as the variety and amount of compounds sorbed on their surfaces, are other factors expectedly responsible for the health effects. In the ambient air, coarse particles are mostly derived from soil and sea salt. Fine and ultrafine particles are predominantly derived from combustion of fossil fuels and transformations of biogenic emissions (Manoli et al., 2002; Ruusunen et al., 2011; Valavanidis et al., 2006). Ultrafine particles are a major component emitted from vehicles propelled by fossil fuel combustion engines. The high temperature processes are considered to be the most significant contribution to air pollution in urban areas (Oberdorster et al., 2002). A very large fraction of the total surface area of PM is associated with this size range. Combustion particles commonly have a core of elemental carbon or fly ash coated with a layer of chemicals including organic hydrocarbons and oxygenated hydrocarbons, metal ions, nitrates, and sulfates. It has been estimated for the urban environment in Europe that 42% of these compounds originate from traffic, 22% from industry, 11% from refineries and energetic sources, and 9% from the local incineration, and all these components may play a role in particle toxicity (Donaldson et al., 2002). There are a lot of mineralogy studies (Ebert et al., 2004; Vester et al., 2007) however connection with other particle characterization (such as toxicology) is missed.

In spite of growing attention is devoted to the effects of PM on human health, there are no complex studies linking the toxicological effects of air particles to the PM size dependent chemical composition and the most important chemical compounds sorbed on their surfaces. Filling this gap is vital for future evaluation of the health effects of various fractions of the atmospheric PM. We have to determine whether in addition to monitoring the mass and the number of particles in the air we also need to consider the chemical composition of the particles as well as a number of toxic chemicals associated with their surfaces when evaluating the effects of emission ultrafine particles. This study also characterizes the human health risk for inhalation exposure to main pollutant PAHs sorbed on air particles. The contribution of individual PAHs congeners to total risks is determined.

In our study, a new combination of methods was used to describe the morphology and sorption potential of various size fractions of the PM, to determine the main chemicals associated with their surfaces and to link it to main toxicological effect (genotoxicity) and related human health risks.

2. Materials and methods

2.1. Air particle sampling procedures

For the purpose of this study, samples of airborne particulate matter (<PM10) were collected in Brno, Czech Republic, in August, 2006. The sampling location was in the industrially influenced area affected also by road traffic and domestic emissions in the city of Brno (WGS 84 coordinates: X: 49.17725, Y: 16.573556, Z: 272).

A high volume ambient air sampler PM-10 (Graseby-Andersen, USA, flow 1.13 $m^3\ min^{-1}$, volume 1620 m^3 per 24 h) equipped with a multi-stage cascade impactor (Andersen Instruments Incorporated, USA, series 230, model 235) for particle-size fractionation was used for particle sampling. This impactor fractionates suspended particulates into six size fractions (below 10, 7.2, 3.0, 1.5, 0.95, and 0.45 μm). Particles were sampled at quartz slotted collection substrates (Tisch Environmental, Inc, USA) and quartz filters (Whatman, UK), sampling duration was 7 days.

Each filter has been stabilized for 48 h in a chamber with constant temperature and humidity before weighting. Weighted filters were loaded in to the cartridge, wrapped in two layers of aluminum foil and transported to the sampling site. After exposition the sampling cartridges were wrapped in the new aluminum foil, returned to the laboratory and weighted (Mettler-Toledo GmbH, Switzerland) after another 48 h of stabilization.

2.2. Mineralogical analysis

Scanning electron microscope (SEM) CamScan CS 3200 equipped with microanalytical system Link ISIS 300 (Oxford Instruments) with energy-dispersive SiLi spectrometer (EDS) was applied to characterize morphology and semiquantitative chemical composition. The filters with particulate matter (PM) were coated by carbon and point spectra were measured in spot mode of selected grains, flakes or droplets at magnification 1000×. For bulk composition the 200× magnification was used and an area of 0.2×0.2 mm was measured. The compositional spectra were compared with the EDS library of clay and rock forming minerals (Reed, 1996). After the chemical analysis was done, the samples were coated by gold to reach better morphology resolution. Photomicrographs were taken in secondary electron image (SEI) and backscattered electron image modes (BEI). X-ray diffraction analysis (XRD) was performed using powder diffractometer Philips X'pert MPD system with Bragg–Brentano reflecting geometry and vertical goniometer PW 3020. From each filter a piece ca 10×10 cm was treated by ethanol in an ultrasonic bath and the particles were transferred to a suspension. Ethanol was partly evaporated, the denser suspension was placed on a silicon monocrystal wafer with zero diffraction background and air-dried.

2.3. Chemical analysis

Each particulate fraction was a subject of the chemical analysis with the goal of qualitative and quantitative determination of bound organic compounds with the special focus on PAHs. Our previous experiments indicate that other pollutants like HM or OCPs do not have significant levels on this site. All samples were extracted with 120 ml dichloromethane (DCM) in a Büchi System B-811 automatic extractor (Büchi, Switzerland). Surrogate recovery standards (D8-naphthalene, D10-phenanthrene, D12-perylene) were spiked on each filter prior to extraction. The solvent was evaporated and then condensed back to sample over 60 min. Volume was reduced after extraction under a gentle nitrogen stream at ambient temperature, and fractionation achieved on silica gel column (30 cm length, 1 cm i.d.). Sample was added to activated silica gel in column (activation: 12 hod, 150 °C). Column was rinsed with n-hexane from aliphatic fraction. Dichlormethane was used for elution of PAHs. Terphenyl was used as an internal standard. Samples were analyzed using a GC–MS instrument (HP 6890–HP 5973, Agilent Technologies, Germany) supplied with a J&W Scientific fused silica column DB-5MS (60 m×0.25 mm i.d.×0.25 μm of stacionary phase). Helium was used as an inert carrier gas. Injection of sample was automatic in splitless mode (1 μl, 280 °C). Details of temperature program: 80 °C (1 min), 15 °C min^{-1} to 180 °C, 5 °C min^{-1} to 310 °C (10 min). Temperature of transfer line was 280 °C and of ion source was 200–300 °C. The 29 polyaromatic hydrocarbons (naphthalene, biphenyl, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzonaphtho-thiophene, benzo[b]fluorene, benzo[g,h,i]fluoranthene, cyclopenta[c,d]pyrene, benz[a]anthracene, triphenylene, chrysene, benzo[b]fluoranthene, benzo[j]fluoranthene, benzo[k]fluoranthene, benzo[e]pyrene, benzo[a]pyrene, perylene, indeno[1,2,3-c,d] pyrene, dibenz[a,h]anthracene, dibenz[a,c]anthracene, benzo[g,h,i]perylene, athanthrene, coronene) were analyzed (more details see (Dvorska et al., 2012).

2.4. Quality assurance/quality control

Recoveries were determined for all samples by spiking with the surrogate standards prior to extraction. Amounts were similar to detected quantities of analytes in the samples. Recoveries were higher than 78% for all samples. Recovery factors were not applied to any of the data. Recovery of native analytes measured for the reference material varied from 72 to 102% for PAHs. Field blanks consisted of pre-extracted filters and were taken on each sampling site. They were extracted and analyzed in the same way as the samples, and the levels in field blanks never exceeded 1% of quantities detected in samples indicating minimal contamination during the transport, storage and analysis.

2.5. Genotoxicity test — SOS chromotest

Genetically modified bacteria cells (tester strain *Escherichia coli* PQ 65 harboring a sulA::lacZ fusion) were employed in the study (Quillarded et al., 1982; Quillardet and Hofnung, 1985; Quillardet et al., 1997). DNA is a molecular target and the reporter responds directly to the DNA damage. Cytotoxicity as a result of more general macromolecular damage can be detected in this test as well. The 96-well microtitre plate format was used for slightly modified SOS chromotest (Bartoš et al., 2005; Kubátová et al., 2004; Xu et al., 1989). The tester strain was grown overnight in LB medium containing ampicillin (20 μg ml^{-1}) at 37 °C. After the incubation period, the culture was diluted 50-fold into a fresh LB medium with ampicillin and it was incubated for another 2 h. The optical density (600 nm) of the incubated culture was adjusted to 0.04 and prepared culture was mixed (3:1) with a phosphate buffer (pH = 7.4). In the version with metabolic activation phosphate buffer is replaced by S9 mixture (phosphate buffer, S9 fraction, sterile water, G-6-phosphate and NaDP and KCl + $MgCl_2$).

The stock solution of each DCM organic extract sample was transferred to DMSO. This applied approach simulates the worst case scenario when total extractable fraction of particle bound chemicals are bioavailable. 10 μl of each organic extracts was mixed with 390 μl of the bacterial inoculums in tubes (1.5 ml) to reach the final concentrations of 30 and 15 m^3 in 1 ml of the reaction mixture. 10 μl of DMSO served as a negative control; a solution of 4-nitroquinoline-N-oxide as a positive control. The mixtures were incubated for 2 h at 37 °C. Two microplates were prepared for measurements of enzymatic activities. β-galactosidase activity (genotoxicity assay) was determined after addition of 25 μl of the incubated culture into 100 μl of a B-buffer solution (pH = 7.0) with o-nitrophenyl-β-D-galactopyranoside (2 mg ml^{-1}). Alkaline phosphatase activity (toxicity assay) was determined incubated culture into 100 μl of a P-buffer solution (pH = 8.8) with p-nitrophenyl-phosphate (2 mg ml^{-1}). The microplates were incubated for 45 min at 37 °C and enzymatic activity was determined spectrophotometrically at 420 nm. Toxic effects were quantified as a percentage of the alkaline phosphatase activity in comparison with the negative control. The concentrations showing more than 50% inhibition were excluded (Skarek et al., 2007a, 2007b). The SOS induction factor (IF) was calculated for every tested concentration. The samples with the induction factor higher than 1.5 for after the addition of 25 μl of the any concentration were considered to be significant genotoxins (Quillardet et al., 1997; Skarek et al., 2007a, 2007b).

2.6. Human health risk assessment

Human health risks resulting from inhalation exposure on investigated site were evaluated with respect to the risk of developing cancer (cancer risks). The risk assessment involves predicting the frequency of these negative effects in exposed populations (probabilistic approach). We applied the inhalation exposure model of the EPA baseline risk assessment approach (EPA, 1998, 2012). Pollutant specific risks (i.e. an estimate of the probability that an individual will develop cancer during their lifetime) were calculated using the linear low-dose cancer risk equation.

The chronic daily intake CDI was calculated using the following equation:

$$CDI = C_{air} \cdot IF \quad (1)$$

where C_{air} is a compound concentration (mg m^{-3}) and IF is an Intake Factor (m^{-3} kg^{-1} day^{-1}).

Intake Factor is derived from Eq. (2):

$$IF = \frac{(IR - A \cdot EF \cdot ED \cdot ET)}{BW \cdot AT}, \quad (2)$$

where IR-A (Inhalation Rate) is a breathing rate (m^3 day^{-1}), EF (Exposure Frequency) is a number of exposures per year, ED (Exposure Duration) is a duration of exposure in years, ET (Exposure Time) is a number of hours per exposure, BW (Body Weight) is a default weight of the receptor body (kg), and AT (Averaging Time) is an average exposure extent over a lifetime (35 500 day for carcinogenic exposure). Site specific exposure parameters were obtained from EPA exposure handbook (EPA, 2012) [IR-A = 20 m^3 day^{-1}; EF = 365 days; ED = 70 years; ET = 8 h/day; BW = 70 kg]. CDI for carcinogenic substances is called Life Averaged Daily Dose (LADD).

Human health risk related to contaminated air particles depends on the extent of exposure as well as on the physico-chemical properties of particle-bound chemicals. The chemical-specific risks were calculated from the LADD and slope factor (SF) using the linear low-dose cancer risk equation (Eq. (3)):

$$\text{Cancer Risk} = \text{LADD}.SF \quad (3)$$

Slope factor are a plausible upper-bound estimate of probability of the response per unit chemical intake over the lifetime (EPA, 2012). It is used to estimate an upper-bound probability of the individual developing a cancer as a result of the lifetime exposure to certain level of chemicals (PAHs).

Cancer potency factors for inhalation exposure are expressed as Inhalation Unit Risk (IUR). The IUR values used in this risk assessment are presented in supplementary data Table S1.

The final SF values were calculated according to Eq. (4):

$$SF\left[\frac{mg}{kg.day^{-1}}\right]^{-1} = \frac{IUR\left[\frac{\mu g}{m^3}\right]^{-1}.70[kg] * 1000\left[\frac{\mu g}{mg}\right]}{20\left[\frac{m^3}{day}\right]} \quad (4)$$

The results are compared to the carcinogenic benchmark level, i.e. an exposure posing an upper-bound lifetime excess cancer risk of 1E − 6 (i.e. one cancer occurrence over one million people). An exposure for which the risk factor exceeds 1E − 6 is scored as significant. Carcinogenic risks above 1E − 4 are considered as unacceptable, and addressing such health problems is a high priority (EPA, 2012).

A final cumulative health risk related to each sampling site was calculated as a sum of the partial risks of the individual pollutants.

3. Results and discussion

3.1. Particle size distribution

The total volume of 9272.6 m^3 of air was filtered through the multi-stage cascade impactor and six size fractions of the particulate matter (PM) were collected. Mass of each PM fraction is in Table 1. Histograms of relative particle size distribution on each filter (Fig. 1) are based on SEM/EDS measurements. Some of the PM consists of crystal aggregates cemented by amorphous material. The minerals and soot carbon flakes are distinguished by grayscale. The highest mass of

Table 1
Amount of PM captured on the filters with different pore size and relative proportions of clays, rock forming minerals and soot particles estimated by point counting using SEM–EDS and XRD. Specific surface areas (SSA) are based on published data (Brantley and Mellott, 2000; Dogan et al., 2006; Kandas et al., 2005; Ogata et al., 2006; Rancourt and Dang, 2005; Ruan and Gilkes, 1995).

Filter sample	KI 2 A	KI 2 B	KI 2 C	KI 2 D	KI 2 E	KI 2 F	SSA
Filter pore size (μm)	7.2–10	3–7.2	1.5–3	0.95–1.5	0.45–0.95	0–0.45	(m^2/g)
Yield (mg)	59.3	73.2	28.5	30.4	59.0	133.3	**Average**
Soot carbon	10	9	24	32	51	56	**255**
Quartz	19	22	18	16	12	9	**2E−05**
Illite or muscovite	16	12	5	7	8	4	**106.5**
Illite–smectite (I–S)	12	8	7	1	4	1	**415**
Chlorite/vermiculite	11	12	3	0	0	4	**42**
Kaolinite	2	3	3	1	2	1	**17.5**
Plagioclase	4	4	2	0	0	2	**2E−04**
K-Feldspar	5	3	0	0	4	0	**1E−04**
Amphibol	3	5	0	1	2	0	**5E−05**
Calcite/dolomite	4	12	3	0	0	0	**1E−05**
Gypsum & sulfates	0	4	5	2	6	5	**1E−05**
Goethite/hematite	0	4	0	0	0	0	**217.5**
No. of point counts	86	98	70	60	89	82	

particulate material was collected on the finest filter (<0.45 μm), the lowest on the intermediate fractions (0.95–1.5 and 1.5–3 μm).

3.2. Mineralogical analysis

The mineralogy of the particulate matter is estimated from the SEM morphology (Supplementary data: Figs. S1, S3, S4, S5, S6, S7),

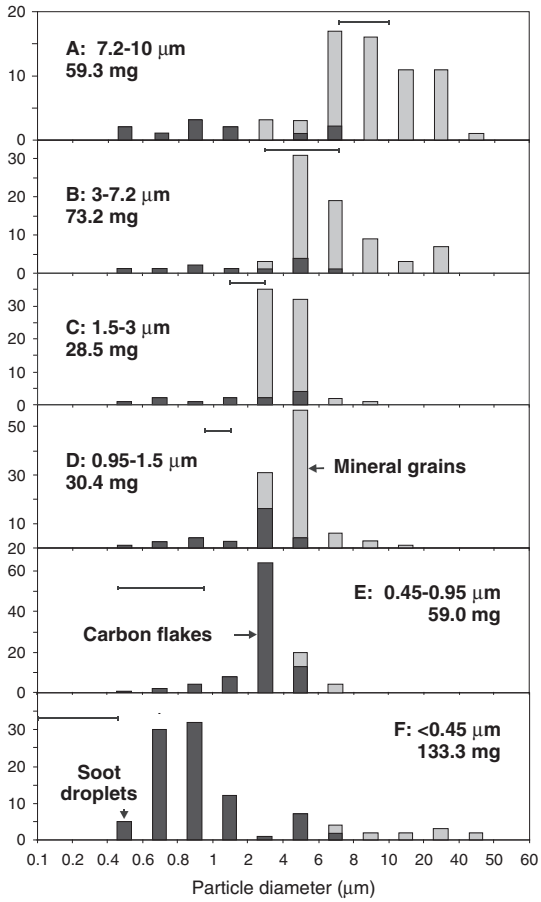

Fig. 1. Particle size distribution measured by SEM on 6 consequent filters (A–F) with decreasing declared pore size in micrometers shown as horizontal bar. Minerals and carbonaceous mater are distinguished by EDS and shown as gray and black columns respectively. Numbers indicate the total yield of PM per fraction in mg.

chemical composition of selected crystals, flakes or irregularly shaped grains (SEM/EDS spectra, Fig. S2) and bulk XRD of the samples (Fig. S2). The measured characteristics are compared with EDS and XRD libraries or published data (Brantley and Mellott, 2000; ICDD, 2002; Meunier, 2005; Welton and Field, 1984) and the frequency of the identified phases is estimated by point counting (Table 1). Diffractogram of the coarse fraction (7.2–10 μm) is presented in Fig. S2, Supplementary data: quartz, calcite, dolomite, gypsum, kaolinite, albite, muscovite and other minerals were identified. Similarly, mineral materials as quartz, muscovite, chlorite, and calcite are main components in the second (3–7.2 μm), quartz, chlorite and muscovite in the third (1.5–3 μm), quartz and muscovite in the fourth (0.95–1.5 μm), and quartz, mascagnite ($(NH_4)2SO_4$), and other sulfates and nitrates in the fifth (0.45–0.95 μm) particulate fractions. On the contrary, the last fraction (<0.95 μm) is amorphous and consist mostly of soot (Fig. 2).

The coarse fraction A includes abundant spores and plant fragments 8–20 μm large along with rock-forming minerals, such as quartz, feldspars, mica, illite–smectite, chlorite and kaolinite. The intermediate fractions consist mainly of clay minerals, e.g. kaolinite, illite–smectite, dolomite, calcite and gypsum. The finest fractions E and F (<0.95 μm) are dominated by carbonaceous PM, most probably soot flakes, droplets and films, poorly crystallized quartz, gypsum, lead and copper sulfates. Carbonaceous particles, however, occur in all size fractions as shown in the histograms in Fig. 1.

The estimated composition of the mineral and carbonaceous phases normalized to the total point counts in each sample was used together with the published average values of the specific surface area (Rancourt and Dang, 2005; Ruan and Gilkes, 1995) of the identified phases to calculate the partial and total surface area of the particulate matter captured on each filter (Table 2). The finest particles fractions have the highest active surface.

3.3. Chemical analysis

Total amount of PAHs (29 compounds and 16 EPA PAHs) found in each particulate fraction is shown in Fig. 4. Particle phase individual PAH concentrations measured in a range of 6 size fractions are presented in supplementary data Table S2. In general, PAHs concentration declined from fine particles to coarse particles. While 43% of PAHs were associated with the fine fraction (<0.45 μm), 20% was found in the fraction between 0.45 and 0.95 μm and around 10% in each of the remaining fractions. This distribution is in very good agreement with estimated specific surface areas coming out from mineralogical analysis (Figs. 3, 9).

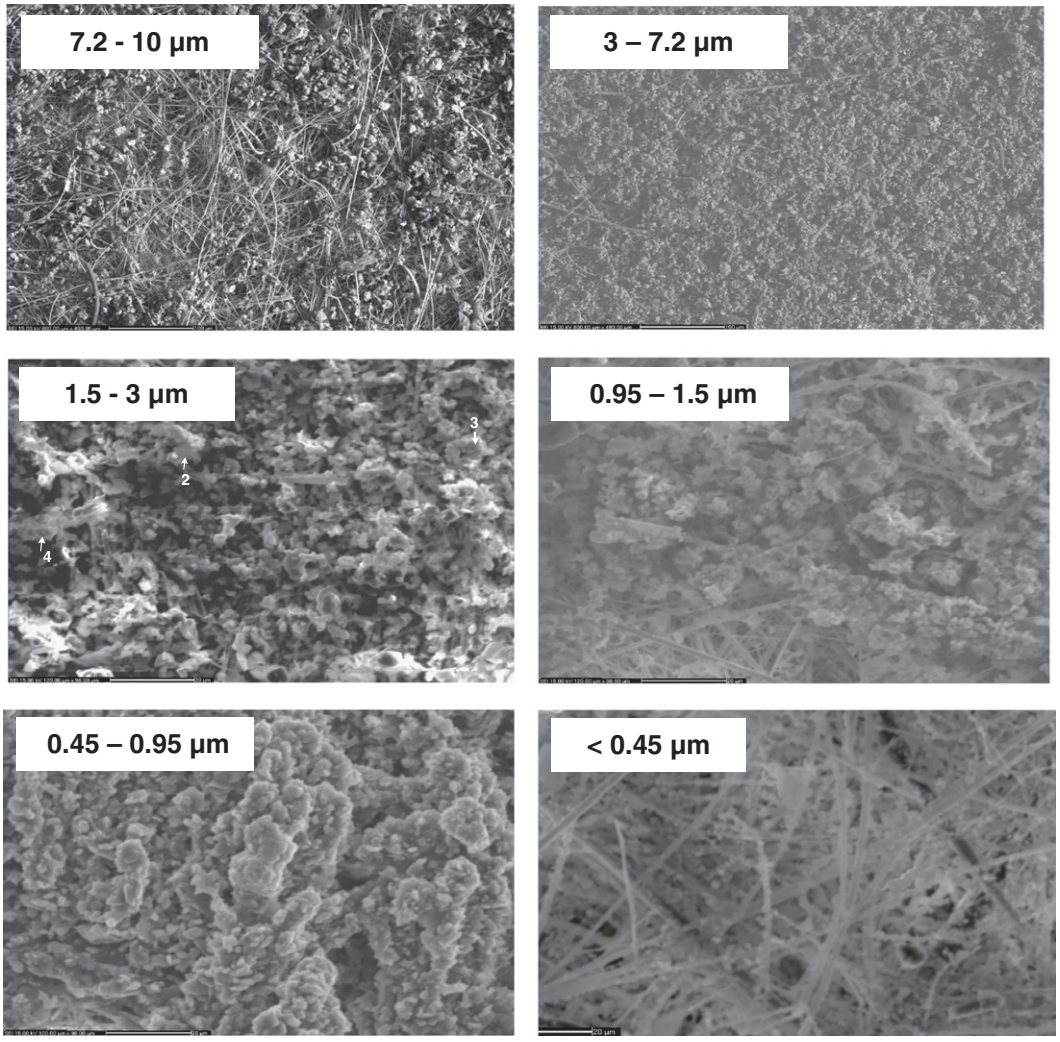

Fig. 2. Morphology of various particular matter fractions (Scanning electron microscopy).

3.4. Toxicological analysis

SOS chromotest was used to study direct and indirect (without and with metabolic activation) toxicological–genotoxic potential of each particular fraction. All samples reached statistical significance of direct genotoxicity (Induction Factor > 1.5), and genotoxicity increased with decreasing particle size (Fig. 5).

Genotoxic potential of all particle fractions with metabolic activation is shown in Fig. 6. Indirect genotoxicity was growing with decreasing particle size again; however, in this case statistically significant genotoxic potential (IF > 1.5) was only detected for the smallest size fraction < 0.45 µm in concentration 15 and 30 m^3 ml^{-1} and for the fraction between 0.95 and 0.4 µm in concentration 30 m^3 ml^{-1}. This result was to be expected since PAHs are known to be non-direct mutagens. Genotoxic potential also corresponds to the amount of PAHs associated with the individual size fractions. Spearmen rank correlation coefficient has been done between PAHs concentration and indirect genotoxic potentials. A significant higher correlation (r > 0.8) was found in case

Table 2
Surface area of the partial mineral and organic phases calculated from the frequency and published specific surface areas and total SA for each filtered size fraction.

Filter sample	KI 2 A	KI 2 B	KI 2 C	KI 2 D	KI 2 E	KI 2 F
Filter pore size (µm)	7.2–10	3–7.2	1.5–3	0.95–1.5	0.45–0.95	0–0.45
Soot carbon	1.76E+00	1.71E+00	2.49E+00	4.13E+00	8.62E+00	2.32E+01
Quartz	3.31E−08	3.03E−08	9.26E−08	1.28E−07	1.03E−07	9.22E−08
Illite or muscovite	3.76E−01	2.87E−01	1.37E−01	1.99E−01	1.15E−01	4.68E−02
Illite–smectite (I–S)	9.27E−01	4.07E−01	2.08E−01	4.84E−02	1.49E−01	2.02E−02
Chlorite/vermiculite	6.45E−02	4.11E−02	1.26E−02	0.00E+00	0.00E+00	2.05E−03
Kaolinite	4.48E−03	6.43E−03	2.25E−03	0.00E+00	0.00E+00	8.54E−04
Plagioclase	1.93E−08	2.55E−08	1.78E−08	0.00E+00	0.00E+00	5.07E−09
K-Feldspar	2.28E−08	1.20E−08	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Amphibol	9.33E−09	8.19E−09	0.00E+00	0.00E+00	4.81E−09	0.00E+00
Calcite/dolomite	1.40E−09	6.12E−09	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Gypsum	0.00E+00	4.90E−09	2.14E−09	0.00E+00	0.00E+00	0.00E+00
Goethite/hematite	0.00E+00	3.55E−02	0.00E+00	0.00E+00	0.00E+00	0.00E+00
Total SA (m^2)	**3.13E+00**	**2.49E+00**	**2.85E+00**	**4.38E+00**	**8.89E+00**	**2.33E+01**

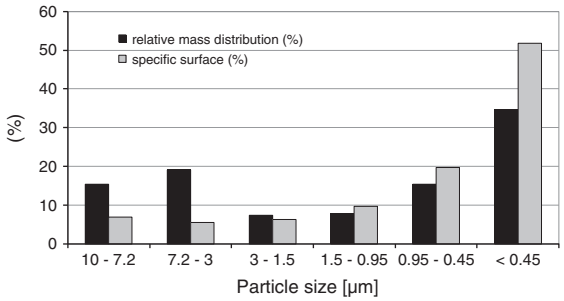

Fig. 3. Relative mass distribution (% of total mass) of airborne particles between the size fractions compared to relative distribution of their specific surface (relative specific surface; %).

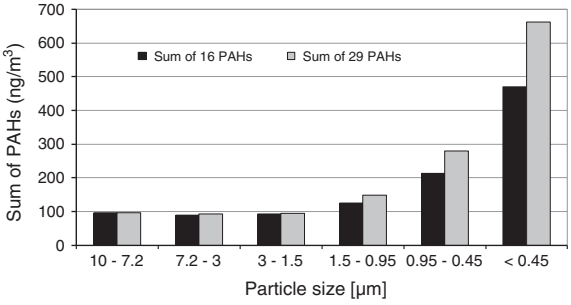

Fig. 4. Concentration of PAHs (ng/m^3) associated with various particle size fractions.

of booth applied doses (30 and 15 m^3 ml^{-1}). However, the correlation was not absolute because the observed genotoxcic activity was also dependent on the presence of other organic pollutants than the PAHs. It concerns predominantly direct genotoxic effect which is not related with parent PAHs, but with their nitro-, hydroxy- and oxy-derivatives and also unknown organic pollutants. However, the concentration of the direct genotoxins apparently correlated with the PAHs contents. Moreover there are also very strong relationship between direct genotoxicity and relative surface area of particles (significant correlation, Fig. 9).

IF can be expressed per one mg of particles and compared to the amount of PAHs associated with one mg of particles. Correlation of these two factors is demonstrated in Figs. 7 and 8 (without and with metabolic activation). In both cases, highest genotoxic potential for mg of particles was found in fractions 3–1.5 μm, 1.5–0.95 μm, and 0.95–0.45 μm.

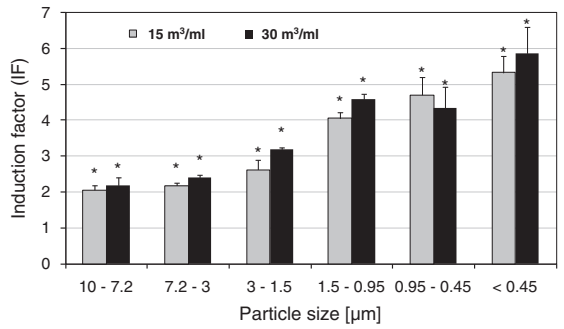

Fig. 5. Genotoxicity without metabolic activation for two highest doses 15 and 30 m^3 ml^{-1} (*statistical significance of direct genotoxicity with Induction Factor >1.5).

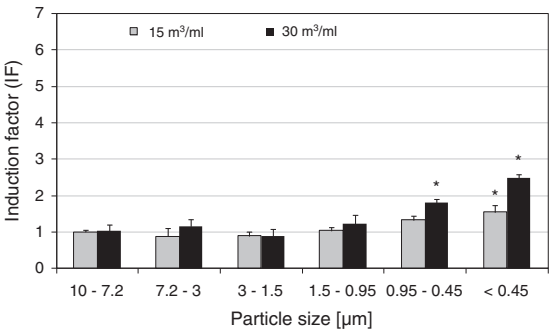

Fig. 6. Genotoxicity with metabolic activation for two highest doses 15 and 30 m^3 ml^{-1} (*statistical significance of indirect genotoxicity with Induction Factor >1.5).

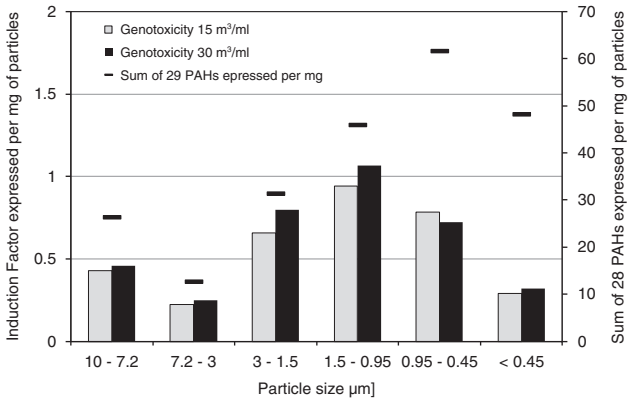

Fig. 7. Genotoxicity without metabolic activation. Induction factor is expressed per mg of particles in selected size fraction.

3.5. Risk characterization and conclusions

Determination of the human health risks was based on the PAHs atmospheric concentrations measured in selected 6 PM size fractions. The individual and cumulative cancer risk values are presented in Table 3. ∑ Cancer risk per fraction 0–0.45 0.45–0.95 0.95–1.5 μm are significant higher than 1E−6 benchmark level (2.5E−5). Risk analysis also indicates that the inhalation exposure for selected site-specific scenario [IR-A = 20 m^3 day^{-1}; EF = 365 days; ED = 70 years; ET = 8 h/day; BW = 70 kg] has significant cancer risk values in PM 1.5 size fraction (0–1.5 μm). Last two probability risk-value for core fraction (1.5–7.5 7.5–10 μm; PM1.5–10) are under this benchmark level. Potential next activity for risk reduction should be focused on fraction PM1.5

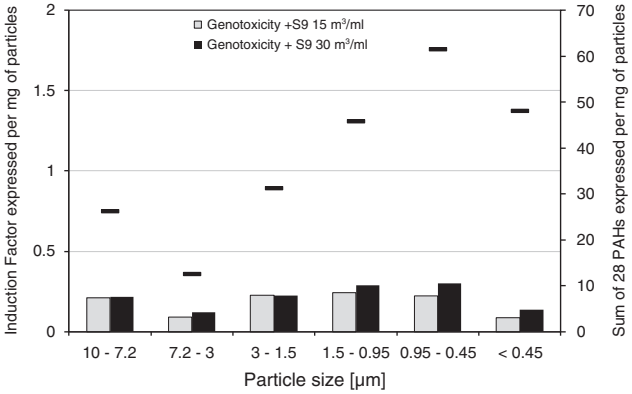

Fig. 8. Genotoxicity with metabolic activation. Induction factor is expressed per mg of particles in selected size fraction.

Table 3
Overall summary of the potential human health cancer risks for the individual PAHs and for the selected particle matter size fractions. (Red values identify all over Risk value > 1E−6; red bars presented quantitative symbology of risk levels). Partial risk levels for the individual chemicals showing also contributions to the total cancer risk/probability.

PM size fraction (μm)	10 - 7,2	7,2 - 3	3 - 1,5	1,5 - 0,95	0,95 - 0,45	0,45 - 0	∑Cancer Risk
Naphthalene	4.76E-07	4.43E-07	5.05E-07	4.21E-07	4.79E-07	5.33E-07	2.86E-06
Acenaphtylene	2.04E-09		1.50E-09	2.57E-09	4.07E-09	7.80E-09	1.80E-08
Acenapthene	6.42E-10			5.13E-10	8.32E-10	1.06E-09	3.05E-09
Fluorene	4.66E-10	8.29E-10	9.20E-10	8.14E-10	1.25E-09	2.72E-09	7.01E-09
Phenanthrene	4.46E-09	4.97E-09	5.20E-09	7.16E-09	9.98E-09	1.77E-08	4.94E-08
Anthracene	1.91E-09	0.00E+00	3.15E-09	4.29E-09	1.33E-08	3.44E-08	5.71E-08
Fluoranthene	6.73E-09	6.67E-09	4.85E-09	7.33E-09	1.07E-08	2.04E-08	5.66E-08
Pyrene	5.07E-09	4.67E-09	3.79E-09	5.86E-09	9.42E-09	2.24E-08	5.12E-08
Benz(a)anthracene		7.44E-08	6.23E-08	9.42E-08	2.29E-07	6.19E-07	1.08E-06
Chrysene		7.55E-09	2.79E-09	1.91E-08	4.65E-08	1.33E-07	2.09E-07
Benzo(b)fluoranthene				2.23E-07	4.61E-07	2.00E-06	2.69E-06
Benzo(k)fluoranthene				1.28E-07	4.38E-07	1.09E-06	1.65E-06
Benzo(a)pyrene				2.90E-07	2.93E-06	8.23E-06	1.14E-05
Indeno(123cd)pyrene					2.09E-07	7.38E-07	9.48E-07
Dibenz(ah)anthracene					5.44E-07	2.96E-07	8.40E-07
Benzo(ghi)perylene				1.00E-08	3.87E-08	1.32E-07	1.80E-07
Biphenyl							0.00E+00
Retene							0.00E+00
Benzo(b)fluorene							0.00E+00
Benzo-Naphtho-Thiophene							0.00E+00
Benzo(ghi)fluoranthene							0.00E+00
Cyclopenta(cd)pyrene							0.00E+00
Triphenylene							0.00E+00
Benzo(j)fluoranthene				1.69E-07	5.42E-07	1.40E-06	2.11E-06
Benzo(e)pyrene							0.00E+00
Perylene							0.00E+00
Dibenz(ac)anthracene					3.20E-07	3.67E-07	6.86E-07
Athanthrene							0.00E+00
Coronene							0.00E+00
∑Cancer Risk per fraction	4.98E-07	5.42E-07	5.90E-07	1.38E-06	6.29E-06	1.56E-05	2.49E-05
							Total ∑Cancer Risk

elimination in this case. Pairwise scatterplots for correlations between main variables and parameters of this study are presented in Fig. 9. This Fig. 9B) indicated that the predicted cancer risks are in significant relationship with the experimental genotoxicity potentials.

We have to be aware of the uncertainties of our results and the possibility of underestimation or overestimation of the risk. Health risks may have been underestimated due to the fact that inhalation of particle-bound chemicals was the only kind of exposure considered in this study. Ingestion exposure via food consumption, for instance, can be also very important exposure scenario.

This project was aimed to fill important gaps in our understanding of the atmospheric fate of POPs, with the special focus on the atmospheric aerosols and related health risks potentials. Properties of individual size fractions of the airborne particles were assessed. The main contribution of this work is to verify the new procedure for the characterization of air particles. Finest fraction (<0.45 μm) of this model air dust sample showed to have the highest mass, highest active surface, highest amount of associated PAHs and also highest direct and indirect genotoxic potentials.

Interestingly, when we expressed the amount of PAHs per mg of particles, we observed highest amount of sorbed PAHs per mg in the fraction between 0.95 and 0.45 μm. Similarly, genotoxic potential (both direct and indirect) per mg of particulate matter was not highest in the finest fraction but in 3–1.5 μm, 1.5–0.95 μm, and 0.95–0.45 μm fractions. This is surprising since it does not correspond with the hypothesis that highest amount of the persistent organic compounds

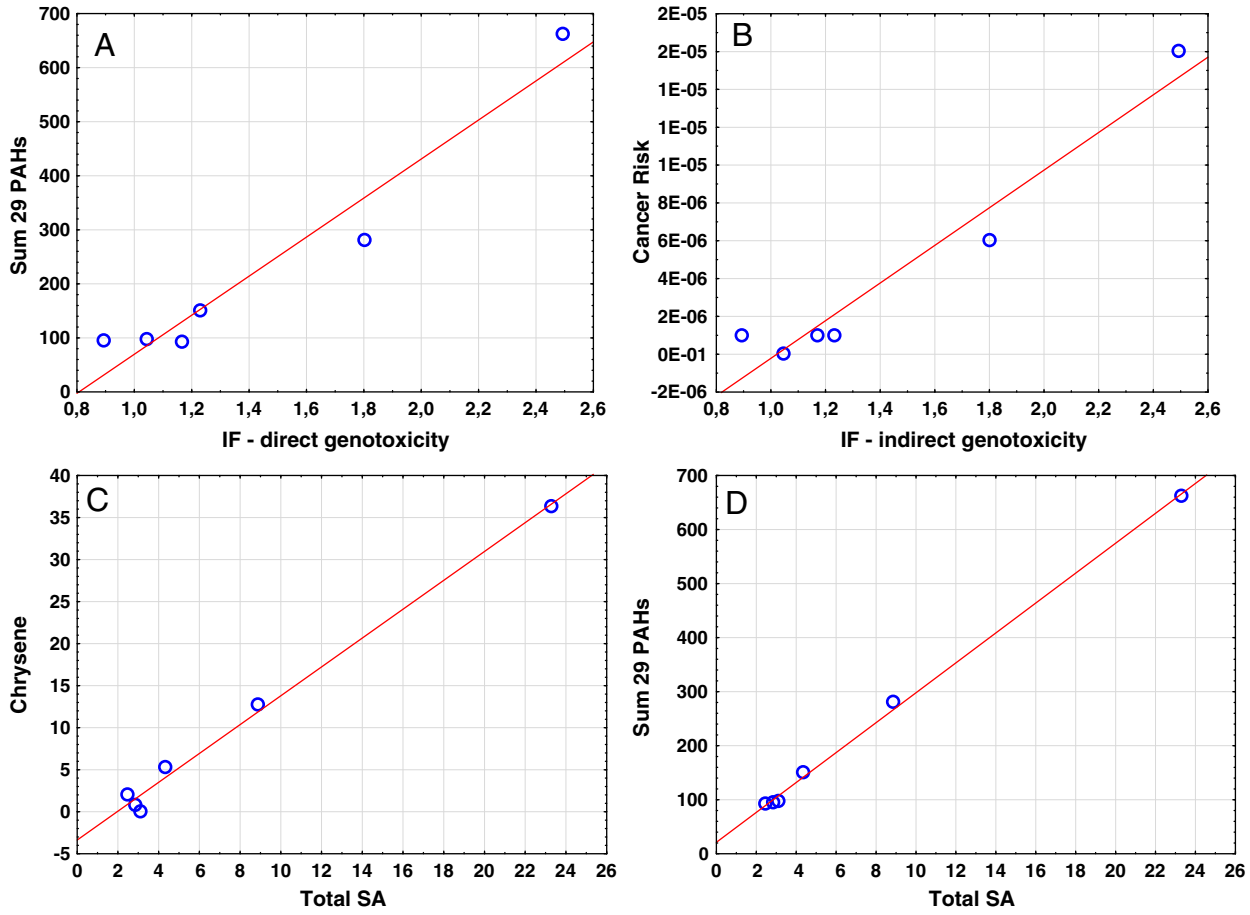

Fig. 9. Scatterplots of main selected significant relationships between parameters of 6 air particle size fractions (significant correlation by Spearmen rank correlation coefficient > 0.8). A) Experimental induction factor of direct genotoxicity vs. ∑ 29 PAH concentrations, B) experimental induction factor of indirect genotoxic effect vs. Cancer risk probability (computed), C) total surface area vs. chrysene concentrations and D) total surface area vs. ∑ 29 PAH concentrations.

will be associated with the finest particulate fraction (Binková et al., 2003; Ruusunen et al., 2011). It is, however, consistent with the results of our morphological analysis (Table 1). As can be seen from this table, material composition of six fractions is comparable — there are significant amounts of amorphous carbon materials with the large active surface in all fractions.

Our results bring new insights into the problem of atmospheric particles. Even though we did not prove that the finest particulate fraction has the highest affinity towards the persistent organic particles, they still carry most significant risks. One reason is the size distribution — majority of the particular mass collected in this study is in the finest fraction (<0.45 μm). Second reason is that fine particles can penetrate deeper into the respiration tract than coarse particles do. All those findings have to be considered when assessing the fate of organic compounds in the atmosphere including long-range transport and sink processes. Deposition (both dry and wet) rates will differ for various particle sizes as will differ also degradation rates of associated compounds.

We are aware that the size distribution as well as the material composition of dust particles vary from site to site. Amount and size of the particles depend on their sources and precursor gas emissions, on the formation mechanism, the distance from source, aerosol chemical and physical transformation processes in the atmosphere and on meteorological parameters. This variability needs to be further investigated as well as sorption mechanisms of various compounds on mineral and amorphous materials. Results obtained from such study could be further used for development of methodology of evaluation of dust pollution, modeling of the sink processes and estimation of deposition fluxes to terrestrial and aquatic environments, identification of risks linked to the inhalatory exposure to atmospheric particles, and new design of preventive and legislative measures. An integrative approach of such study including detailed characterization of the chemical composition and mass size distribution of atmospheric aerosol particles is a key parameter for understanding the transport and sink processes of substances bound to or carried by particles as well as for the assessment of health hazards of the ambient air.

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Synopsis

This project was aimed to fill important gaps in our understanding of the atmospheric fate of POPs in emissions, with the special focus on the atmospheric aerosols and related health risks. The new combination of methods was used to describe the morphology and sorption potential of various size fractions of the PM, to determine the chemicals associated with their surfaces and to link it to main toxicological effects and related human health risks.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2012.12.012.

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Příloha 19

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RESEARCH ARTICLE

Composition and effects of inhalable size fractions of atmospheric aerosols in the polluted atmosphere: Part I. PAHs, PCBs and OCPs and the matrix chemical composition

Linda Landlová · Pavel Čupr · Juraj Franců · Jana Klánová · Gerhard Lammel

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Abstract Atmospheric particulate matter (PM) abundance, mass size distribution (MSD) and chemical composition are parameters relevant for human health effects. The MSD and phase state of semivolatile organic pollutants were determined at various polluted sites in addition to the PM composition and MSD. The distribution pattern of pollutants varied from side to side in correspondence to main particle sources and PM composition. Levels of particle-associated polycyclic aromatic hydrocarbons (PAHs) were 1–30 ng m^{-3} (corresponding to 15–35 % of the total, i.e., gas and particulate phase concentrations), of polychlorinated biphenyls (PCBs) were 2–11 pg m^{-3} (4–26 % of the total) and of DDT compounds were 2–12 pg m^{-3} (4–23 % of the total). The PM associated amounts of other organochlorine pesticides were too low for quantification. The organics were preferentially found associated with particles <0.45 μm of aerodynamic equivalent diameter. The mass fractions associated with sub-micrometer particles ($PM_{0.95}$) were 73–90 %, 34–71 % and 36–81 % for PAHs, PCBs and DDT compounds, respectively. The finest particles fraction had the highest aerosol surface concentration (6.3–29.7)$_{\times}10^{-6}$ cm^{-1} (44–70 % of the surface concentration of all size fractions). The data set was used to test gas-particle partitioning models for semivolatile organics for the first time in terms of the organics' MSD and size-dependent PM composition. The results of this study prove that at the various sites particles with diverse size, matrix composition, amount of contaminants and toxicological effects occur. Legislative regulation based on gravimetric determination of PM mass can clearly be insufficient for assessment.

Keywords Atmospheric particulate matter · Particle size resolved aerosol composition · Persistent organic pollutants

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Introduction

Ambient air particulate matter (PM) is of concern worldwide because of its association with adverse health effects (Heal et al. 2012). The mechanistic understanding is incomplete, but apart from particle size and hygroscopicity (Invernizzi et al. 2006; Monarca et al. 1997) chemical composition matters (Spurny 1998; Brüggemann et al. 2009). The health risk coming with inhalation of ambient PM is a function of both particle size (at least for nanometer particle sizes; Borm et al. 2006) and toxicity of the components. Legislative regulation of PM is based on determination of $PM_{2.5}$ and PM_{10} mass concentration (USEPA 2006; EU 2008) only, which has been questioned as insufficient for assessment. Following epidemiological evidence, particle number concentration matters (and has been considered in regulation of vehicle emissions; EU 2007) and toxic substances have been identified in OM fractions of the ambient aerosol, using apolar (Salmeen et al. 1984; de Kok et al. 2006) or polar extraction (Gundel et al. 1993; Erdinger et al. 2005; de Kok et al. 2006).

Corresponding to the atmospheric lifetime of PM, 1–6 days (global mean for various components; Stier et al. 2006), its

composition is determined by local but at most locations more so by regional sources (Lammel et al. 2003). In consequence, the variation in terms of the chemical composition is limited on a large range of time scales and spatially across the continent and along pollution gradients (Raes et al. 2000; Putaud et al. 2010). However, little is known about the composition of the organic fraction, i.e., particulate organic matter (OM; Jacobson et al. 2000). Apart from primary emissions, PM consists of both inorganic and organic secondary aerosol, formed by various processes of gas-to-particle conversion (e.g., Seinfeld and Pandis 2006; Lammel and Leip 2005; Kroll and Seinfeld 2008). Although connected by various aerosol physical processes the aerosol exhibits pronounced size modes which are corresponding to different lifetimes and composition (Heal et al. 2012). Moreover, PM composition does not only vary between particles (e.g., Covert and Heintzenberg 1984), but also within individual particles, often shell-like.

Persistent organic pollutants (POPs), toxic compounds that are persistent and bioaccumulative in the environment, can be sorbed by atmospheric aerosol particles. Among POPs, polycyclic aromatic hydrocarbons (PAHs; Keyte et al. 2013) account for the largest mass fraction in the particulate OM (≈0.25 % in southern California and 0.3–1.5 % in central Europe in winter, respectively; Schauer et al. 1996; Spindler et al. 2012). The gaseous state is predominant for the lighter molecular weight PAHs, while the substances with more than four rings are preferentially associated with the aerosol particles (Finlayson-Pitts and Pitts 2000). Among atmospheric trace chemical substances, PAHs are considered to pose the highest human health risk (WHO 2003; Bartoš et al. 2009).

The highest mass fraction of particulate PAHs is generally found in fine particles, <0.5 μm size fraction accounting for >50 % or even >90 % of the total PAH content (Čupr et al. 2013; Topinka et al. 2013). Mass median diameters of PAHs in ambient aerosols are in the accumulation range, mostly 0.5–1.4 μm (Schnelle et al. 1995; Kiss et al. 1998; Kawanaka et al. 2009; Lammel et al. 2010b,c; Škrdlíková et al. 2011), but also a second, coarse mode was found (up to 2.4 μm; Chrysikou et al. 2009). Kiss et al. (1998) observed almost identical concentrations of the two- to three-ring PAHs in the samples from all stages of a nine-stage cascade impactor collected in autumn. Distribution over larger parts of the size spectrum can be explained by re-distribution of semivolatile substances in an aerosol (Kiss et al. 1998; Lammel et al. 2010b). However, the factors affecting the size-specific distribution of semivolatile compounds should be assessed in more detail.

Gas-particle partitioning of organic species in aerosols is incompletely understood, but strongly influenced by the PM physical and chemical properties (e.g., Pankow 1987; Zuend and Seinfeld 2012). For lipophilic semivolatile organics, PAHs, organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) predictions largely rely on empirical (Pankow and Bidleman 1992; Finizio et al. 1997; Harner and Bidleman 1998) relationships rather than on descriptions of interactions with the condensed phase on the molecular level. Such descriptions are available (Goss and Schwarzenbach 2001), but require additional parameters to characterize PM, i.e., chemical composition and surface. Phase distributions' temperature trends of PAHs are in accordance with both adsorptive and absorptive mechanisms (Pankow et al. 1994). However, K_{oa} was found to be a better descriptor for PAH gas-particle partitioning than p_L^o (Finizio et al. 1997), and dual models accounting for both adsorptive and absorptive contributions have been suggested (Dachs and Eisenreich 2000; Goss and Schwarzenbach 2001; Lohmann and Lammel 2004). Evidence for the significance of aerosol surface concentration (S), OC, and EC for the partitioning of various substance classes of lipophilic semivolatile organics was found in field campaigns (Pankow 1987; Lohmann and Lammel 2004; Lammel et al. 2011) and supported by modelling (Lammel et al. 2009). However, the various models emphasizing different processes on the molecular level to determine the overall process usually cannot be tested because of lack of input data. Phase distributions of chlorinated organics have been studied less extensive. K_{oa} is usually found to satisfactorily predict gas-particle partitioning (Finizio et al. 1997). While some empirical relationships between the size-specific PM composition and gas-particle partitioning of semivolatile compounds are available (mostly derived from isolated field campaigns at single sites), there was, to our knowledge, no systematic assessment of spatial and seasonal variabilities of these characteristics and relations. Such data are, however, needed to improve our understanding of the atmospheric processes as well as risks associated with atmospheric aerosols. In this study we aim to elucidate the influence of PM chemical composition on gas-particle partitioning of lipophilic semivolatile organics (apolar extraction of PM). So far, the studies aiming for an almost complete characterisation of the aerosol composition have not been linking to the investigation of POPs occurrence in aerosols. Following a pilot study (Čupr et al. 2013), quantitative and qualitative features of six size fractions of PM_{10} collected at six sites with well defined pollution sources were addressed here in order to demonstrate the spatial dissimilarity of particle size resolved fractions. As PM chemical composition is strongly size-dependent, the size-specific partitioning coefficient is introduced. As OM and *S* are included in at least a subset of the represented field data set (here) and mineral surfaces were specified (both to our knowledge for the first time), we make an estimate on BC content and test the whole range of gas-particle partitioning models.

The results of the chemical characterization and partitioning of POPs in aerosols are discussed in this article, while the toxicological characterization is studied in a companion paper (Novák et al. 2013). Seasonal variability is a subject of an ongoing research,

Methodology

Sampling sites and conditions

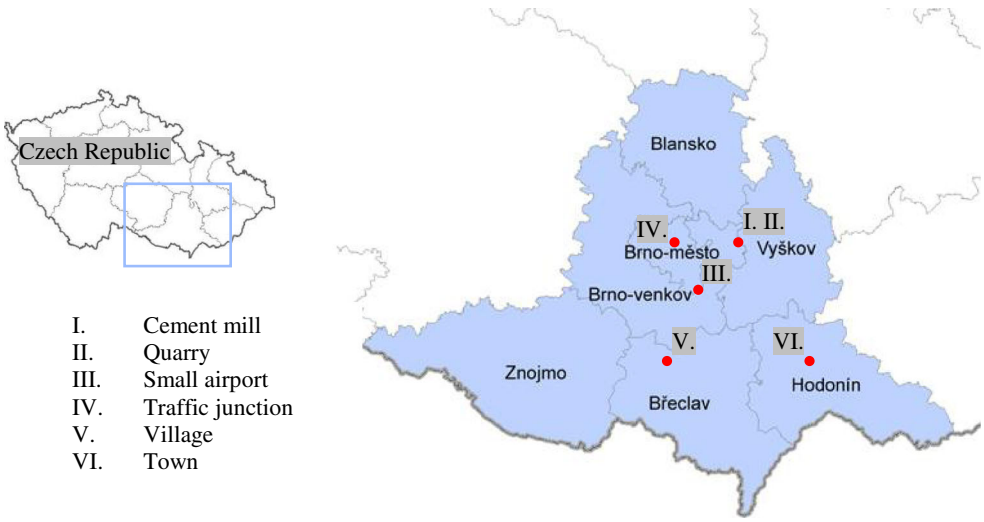
Samplings were carried out at six sites in southern Moravia, Czech Republic (Fig. 1). In order to address variability in the origin (industry, agriculture, combustion, traffic), material composition (mineral, clay, soot), and size distribution of collected particles, a set of the sampling sites covering whole variety of atmospheric pollution sources was selected. Variability was further enhanced by sampling accross the seasons to include extreme conditions. The samples were collected sequentially from August 2007 to February 2008 (Table 1), each site being represented by four samples (each of them collected for 7 days and consisting of six particle size fractions). The industrial sites were sampled during the warm seasons (not to spoil the fingerprint of industrial sources by sources related to residential heating), agricultural sites during the fall (intensive field work), and urban and rural residential areas during the cold seasons (to capture effects of local heating sources). Two locations were near the village of Mokrá (15 km east of Brno, the second biggest Czech city), One of these sites was situated in the industrial area affected by cement production (cement mill), the other in a quarry where limestone mining and transport of the raw material were major sources of particles. Two sites were in Brno city: Tuřany was a small airport in the peripheral part of Brno affected mainly by agricultural activity and air traffic (average of 115 landings and take-offs per week), Kotlářská was a traffic junction situated in the city center. The village of Ivaň was mostly affected by household combustion of solid fuels, while both, industrial and residential activities (glass works, screw-mill and local heating) were major sources of particles in the town of Kyjov.

Air sampling

A high volume ambient air sampler PM10 HVS1 (Umwelttechnik MCZ, Bad Nauheim, Germany, flow 68 m^3 h^{-1}) equipped with a multi-stage cascade impactor (Andersen Instruments Inc., Fultonville, NY, USA; series 230, model 235) was used to sample atmospheric particles. This device has five impactor stages, corresponding to 10–7.2 μm (A), 7.2–3 μm (B), 3–1.5 μm (C), 1.5–0.95 μm (D) and 0.95–0.49 μm (E) of aerodynamic particle size, D (spaced roughly equal $\Delta \log D$), and a backup filter collecting particles <0.49 μm (F). Particles were sampled on slotted glass fiber collection substrates (Tisch Environmental Inc., Cleves, USA; 14.3×13.7 cm) and glass fiber filters (Whatman, 20.3×25.4 cm). Collection substrates in the cascade impactor were exchanged every 7 days.

Each filter has been stabilized for 2×24 h in a chamber with constant temperature and humidity prior to weighting. Weighted filters were loaded into the cartridges, wrapped in two layers of aluminum foil and transported to the sampling sites. The exposed sampling cartridges were again wrapped in two layers of aluminum foil, returned to the laboratory and weighted (after 2×24 h of stabilization). Samples were weighted using a Mettler-Toledo AB204-S balance (Mettler-Toledo, Greifensee, Switzerland) and stored at −18 °C in a freezer prior to further analysis.

The gaseous fractions of target analytes were collected simultaneously using two polyurethane foam (PUF) plugs (Gumotex, Břeclav, Czech Republic; 5×5.5 cm, 0.030 g cm^{-3}) employed in series downstream of a quartz filter in a low volume sampler (Leckel MVS6, F≈2.0 m^3/h, PM_{10} inlet; Sven Leckel Ingenieurbüro, Berlin, Germany). The PUF plugs were pre-cleaned for 8 h in acetone and 8 h in DCM, wrapped in two layers of aluminum foil and a zip-



I. Cement mill
II. Quarry
III. Small airport
IV. Traffic junction
V. Village
VI. Town

Fig. 1 Maps of sampling sites in the South Moravian region, Czech Republic

Table 1 Sampling sites, times and mean meteorological information

Location	Cement mill	Quarry	Small airport	Traffic junction	Village	Town
Site type	Industrial	Industrial	Agricultural	Traffic—residential	Rural—residential	Urban—industrial
Latitude/longitude	49.21°N/16.77°E	49.23°N/16.77°E	49.15°N/16.69°E	49.21°N/16.59°E	48.93°N/16.58°E	49.01°N/17.12°E
Altitude (m a.s.l.)	330	330	237	230	172	192
Sampling time	20.7.07–17.8.07	24.8.07–21.9.07	1.10.07–29.10.07	13.11.07–11.12.07	15.12.07–12.1.08	21.1.08–18.2.08
Temperature (°C)	20	13	8.6	3.5	−1.6	3.4
Relative humidity (%)	64	72	77	84	84	75
Wind speed (m s^{-1})	0.4	1.1	0.9	<0.2	0.3	0.2
Rainfall (mm)	6.3	88.6	28.7	0	1.1	21.9
PM_{10} (μg m^{-3})	155	16.5	19.7	39.4	46.0	13.1

n.a. not available, *SSE* south southeast, *SE* southeast, *N* north

lock plastic bag and stored at −18 °C prior to exposure. The exposed PUF plugs were stored wrapped in aluminum foil and plastic bag at −18 °C prior to analysis.

Mineralogical analysis and derivation of PM specific surface area

Scanning electron microscope (SEM; CamScan CS 3200) equipped with a microanalytical system (Link ISIS 300; Oxford Instruments) with energy-dispersive SiLi spectrometer (EDS) was applied to characterize morphology and chemical composition. The filters with PM were coated by carbon and point spectra were measured in spot mode for selected grains, flakes or droplets at magnification of 1,000×. The spectra were compared with published data on clay and rock forming minerals (Welton and Field 1984; Brantley and Mellott 2000; ICDD 2012; Meunier 2005), and the frequency of occurrence of the identified phases was registered. The SEM quantification method's accuracy is 17 %. X-ray diffraction (XRD) analysis was performed using a powder diffractometer (Philips X'pert MPD system) with Bragg–Brentano reflecting geometry and vertical goniometer (PW 3020). From each filter a piece of 10×10 cm was treated by ethanol in an ultrasonic bath and the particles were transferred to a suspension. Ethanol was partly evaporated; the denser suspension was placed on a silicon monocrystal wafer with zero diffraction background and air-dried. The mineralogical composition of the PM captured on each impactor stage was estimated semiquantitatively from the SEM-EDS and XRD data and the amount of carbonaceous matter was measured as total organic carbon (TOC). The specific surface area (SSA; m^2 g^{-1}) and aerosol surface concentration S (10^{-6} cm^{-1}) of each PM size fraction was calculated as weighted average of partial contributions by each mineral and carbonaceous phase based on its mass fraction and published SSA and/or S data for the identified phases (e.g., Rancourt and Dang 2005; Ruan and Gilkes 1995). The uncertainty corresponds to half of the difference between published upper and lower bounds. For major contributors to the total surface these uncertainties are between 10 % and 50 %. Hence, the uncertainty of S determination is estimated to be 30–40 %. One sample per site was analyzed in this way for composition and S.

Organic analysis

Trace substances

All individual samples were extracted with dichloromethane in a Büchi System B-811 automatic extractor. One laboratory blank and one reference material were analyzed with each set of ten samples. Surrogate recovery standards (D_8-naphthalene, D_{10}-phenanthrene, D_{12}-perylene for PAHs analysis, PCB 30 and PCB 185 for PCBs analysis) were spiked on each filter prior to extraction. Terphenyl and PCB 121 were used as internal standards for polyaromatic hydrocarbon (PAH) and PCB/OCP analysis, respectively. The extract volume was reduced under a gentle nitrogen stream at ambient temperature, and fractionation achieved on a silica gel (PAH analysis) or a sulphuric acid modified silica gel (PCB/OCP analysis) column. Samples were analyzed using GC-MS (HP 6890-HP 5973) supplied with a fused silica column DB-5MS (J&W Scientific) for PCB congeners number 28, 52, 101, 118, 153, 138, and 180, and OCPs, namely, penta- and hexachlorobenzene (PeCB, HCB), α-, β-, γ- and δ-hexachlorocyclohexane (HCH), and the *o,p′*- and *p,p′*-isomers of 1,1-dichloro-2,2-bis(chlorophenyl)ethene (DDE), 1,1-dichloro-2,2-bis(chlorophenyl)ethane (DDD), and 1,1,1-trichloro-2,2-bis(chlorophenyl)ethane (DDT). The 16 US EPA priority PAHs, i.e., naphthalene (NAP), acenaphthylene (ACY), acenaphthene (ACE), fluorene (FLN), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR), benz(*a*)anthracene (BAA), chrysene (CHR), benzo(*b*)-fluoranthene (BBF), benzo(*k*)fluoranthene (BKF), benzo(*a*)-pyrene (BAP), indeno(123*cd*)pyrene (IPY),

dibenz(*ah*)anthracene (DBA), and benzo(*ghi*)perylene (BPE), were determined using GC-MS (HP 6890-HP 5972) supplied with a fused silica column DB-5MS (J&W Scientific).

Recoveries were determined for all samples by spiking with the surrogate standards prior to extraction. The amounts were similar to detected quantities of analytes in the samples. Recoveries were always higher than 76 % for PCBs and OCPs and 71 % for PAHs, respectively. Recovery factors were not applied to any of the data. The recovery of native analytes measured for the reference material varied from 88 % to 103 % for PCBs, from 75 % to 98 % for OCPs, from 72 % to 102 % for PAHs. Laboratory blanks were very low. Field blanks consisted of pre-extracted PUF disks which were taken on each sampling site. They were extracted and analyzed in the same way as the samples; the levels in field blanks never exceeded 3 % of quantities detected in samples for PCBs, 1 % for OCPs, 3 % for PAHs, indicating a minimal contamination during the transport, storage, and analysis (Klánová et al. 2007).

The resulting LOQs were 0.1 pg m^{-3} (per impactor stage) for PCBs and OCPs and 1.0 pg m^{-3} for PAHs in the particulate phase (impactor) and 0.5 pg m^{-3} for PCBs and OCPs and 2.5 pg m^{-3} for PAHs in the gas phase (low volume sampler).

Carbon analysis

TOC was measured using an elemental analyzer (ELTRA METALYT CS 100/1000S, ELTRA GmbH, Germany) according to European Standard EN 15936 (ECS 2012). Following removal of carbonates by HCl, the sample was burned at 1250 °C in oxygen flow and the released CO_2 (originating from both, elemental and organic matter carbon) was measured by infrared detector. The uncertainty (2 sigma) of the method is 9 %. The amount of OM/soot in PM was calculated stoichiometrically from the measured TOC value multiplied by factor 1.2 commonly used in the coal and organic geochemistry (Taylor et al. 1998).

Models of trace organics' gas particle partitioning

The data set was used to test gas-particle partitioning models for semivolatile organics in terms of the organics' mass size distribution (MSD) and size-dependent PM composition. We used models which assume different processes to determine gas-particle partitioning, i.e., an adsorption model (Junge–Pankow; Pankow 1987), an absorption model (i.e., K_{oa} model; Harner and Bidleman 1998) and two dual adsorption and absorption models, i.e., Lohmann and Lammel (2004), and a poly-parameter linear free energy relationship (ppLFER; Goss and Schwarzenbach 2001; Roth et al. 2005; see Supplemental Material [SM] for details). Absorption into the OM phase of the aerosol was expected in all models which consider absorption. Adsorption of PAHs was considered to occur only on black carbon (BC) surface in the Lohmann and Lammel (2004) model. In contrast the empirical Junge–Pankow model describes non-specified and the ppLFER model specified molecular interactions (i.e., van der Waals interaction, electron donor and acceptor interactions) to determine adsorption to all aerosol surfaces. Both dual models treat absorptive and adsorptive contributions additive.

Sorption to soot (BC) is relevant for PAHs (Lohmann and Lammel 2004), but BC was not analyzed in the samples. Instead, BC is part of OM in our data set. Therefore, the BC content was estimated, based on the long-term mean EC/OM ratio in various seasons and depending on air mass origin in the region (Spindler et al. 2012). The details are described in the SM.

Particulate mass fraction, θ, and the partitioning coefficient, K^*_p, are defined by the concentrations in the two phases:

$$\theta = c_p/(c_p + c_a) \quad (c_p \text{ and } c_a \text{ are expressed in ng/m}^3;\ c_p \text{ represents the whole particle size spectrum})$$

$$\begin{aligned} K^*_p(\quad) &= c_{TSP}(\mu g/m^3)K_p(m^3/\mu g) \\ &= c_{TSP}(\mu g/m^3)c_p(mol/\mu g)/c_a(mol/m^3) \\ &= c_p(ng/m^3)/c_a(ng/m^3) = (1/\theta - 1)^{-1} \end{aligned}$$

We introduce θ and K^*_p in terms of the size segregated c_{pi}, with $c_{TSP}=\Sigma c_{pi}$, where i denotes one particle size fraction.

(a) Adsorption

(1) Junge–Pankow: $\theta=c_J S/(c_J S+p_L)=1/(1+p_L/(c_J S))$. Unspecific for compounds and aerosol surfaces c_J= 17.2 Pa cm had been suggested (Junge 1977). For PAHs, c_J=171 Pa cm was suggested based on theoretical predictions (Pankow 1987), and a wide range of c_J=43–1,740 Pa cm was suggested based on a few measurements in urban aerosol (Lammel et al. 2010b). Here, we adopted the value of c_J=17.2 Pa cm.

As:

$$K^*_p = c_J S/p_L = c_J \Sigma S_i/p_L, \quad (1)$$

we define the related particle size fraction specific partitioning coefficients:

$$K^*_{pi} := c_J S_i/p_L, \text{holding } K^*_p = \Sigma K^*_{pi} \quad (2)$$

(2) Adsorption contribution to the Lohmann and Lammel (2004) model:

$$K^*_{\mathrm{p}} = c_{\mathrm{TSP}} 10^{-12} K_{\mathrm{sa}} f_{\mathrm{BC}} \quad (3)$$

As:

$$f_{\mathrm{EC}} = \Sigma c_{\mathrm{BC}i}/c_{\mathrm{TSP}}, \text{ and } c_{\mathrm{BCi}} = f_{\mathrm{BCi}}\, \mathrm{c}_{\mathrm{p}i}, \quad (4)$$

we define the related particle size fraction specific partitioning coefficients:

$$K^*_{\mathrm{p}i} := 10^{-12} K_{\mathrm{sa}} f_{\mathrm{BC}i} c_{\mathrm{pi}}; \text{ hence, again, } K^*_{\mathrm{p}} = \Sigma K^*_{\mathrm{p}i} \quad (5)$$

(3) Adsorption contribution to the ppLFER model:

$$K^*_{\mathrm{p}} = 10^{-6} K_{\mathrm{surf/air}}\, c_{\mathrm{TSP}} S = 10^{-6} K_{\mathrm{surf/air}} \Sigma c_{\mathrm{p}i} \Sigma S_i, \quad (6)$$

where $K_{\mathrm{surf/air}}$ is a regressed sum of products of substance and surface parameters (for descriptors, see SM).

In order to hold $K^*_{\mathrm{p}}=\Sigma K^*_{\mathrm{p}i}$, we define $K^*_{\mathrm{p}i}=10^{-6} K_{\mathrm{surf/air}}\, c_{\mathrm{TSP}}\, S_i$, i.e., weighing S_i with $\mathrm{c}_{\mathrm{TSP}}$.

(b) Absorption

(1) In the K_{oa} model (Harner and Bidleman 1998), from

$$K^*_{\mathrm{p}} = c_{\mathrm{TSP}} 10^{-9} K_{\mathrm{oa}} f_{\mathrm{OM}}/\rho_{\mathrm{oct}}, \quad (7)$$

we obtain

$$K^*_{\mathrm{p}} = \Sigma c_{\mathrm{p}i} 10^{-9} K_{\mathrm{oa}} \left[\Sigma\left(f_{\mathrm{OM}i} c_{\mathrm{p}i}\right)/\Sigma c_{\mathrm{p}i}\right]/\rho_{\mathrm{oct}} = 10^{-9} K_{\mathrm{oa}} \Sigma f_{\mathrm{OM}i} c_{\mathrm{p}i}/\rho_{\mathrm{oct}} \quad (8)$$

As:

$$f_{\mathrm{OM}} = \Sigma c_{\mathrm{OM}i}/c_{\mathrm{TSP}}, \text{ and } c_{\mathrm{OM}i} = f_{\mathrm{OM}i}\, c_{\mathrm{p}i}, \quad (9)$$

we obtain particle size fraction specific partitioning coefficients

$$K^*_{\mathrm{p}i} := 10^{-9} K_{\mathrm{oa}} f_{\mathrm{OMi}} c_{\mathrm{p}i}/\rho_{\mathrm{oct}}, \quad (10)$$

hence, again $K^*_{\mathrm{p}}=\Sigma K^*_{\mathrm{p}i}$.

(2) From the absorption contribution to the Lohmann and Lammel (2004) model:

$$K^*_{\mathrm{p}} = c_{\mathrm{TSP}} 10^{-12} K_{\mathrm{oa}} f_{\mathrm{OM}} \quad (11)$$

As:

$$f_{\mathrm{OM}} = \Sigma c_{\mathrm{OM}i}/c_{\mathrm{TSP}}, \text{ and } c_{\mathrm{OM}i} = f_{\mathrm{OM}i} c_{\mathrm{p}i}, \quad (12)$$

we obtain

$$K^*_{\mathrm{p}i} := 10^{-12} K_{\mathrm{oa}} f_{\mathrm{OMi}} c_{\mathrm{p}i}, \quad (13)$$

hence, again, $K^*_{\mathrm{p}}=\Sigma K^*_{\mathrm{p}i}$.

(3) The absorption contribution to the ppLFER model is given as (Goss and Schwarzenbach 2001):

$$K^*_{\mathrm{p}} = 10^{-6} K_{\mathrm{bulk/air}} c_{\mathrm{TSP}} = 10^{-6} K_{\mathrm{bulk/air}} \Sigma c_{\mathrm{p}i}, \quad (14)$$

where $K_{\mathrm{bulk/air}}$ is a regressed sum of products of substance and surface parameters (for descriptors, see SM).

We define particle size fraction specific partitioning coefficients:

$$K^*_{\mathrm{p}i} = 10^{-6} K_{\mathrm{bulk/air}}\, c_{\mathrm{p}i}, \quad (15)$$

hence, again, holding $K^*_{\mathrm{p}}=\Sigma K^*_{\mathrm{p}i}$.

Results and discussion

Atmospheric PM mass size distributions

The monthly mean MSDs varied across sites (Fig. 2). The differences reflected the dominance of different sources: PM_{10} was 17–40 μg m^{-3}. At the quarry site, PM_{10} was 17 μg m^{-3} (variability of 23 % for four independent weekly samples), at the small airport 21 μg m^{-3} (13 %), at the traffic junction 33 μg m^{-3} (13 %), at the village site 40 μg m^{-3} (10 %), at the

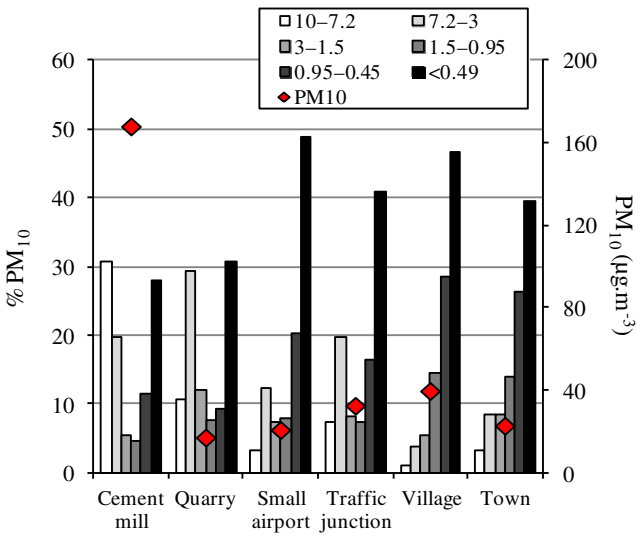


Fig 2 Total PM_{10} concentrations (*diamonds*) and relative mass contributions of the individual particle fractions (*bars*) at six sampling sites. Arithmetic mean of four samples per site

town site 23 μg m^{-3} (29 %) and at the cement mill 185 μg m^{-3} (61 % variability). Such a high concentration was expected, because this sampling site was located in a production plant area where the powder material is produced. At this site, the high concentration of measured coarse particles originated in the milling of raw material to raw powder. Fine particles ($PM_{0.95}$) were emitted during sintering in a rotary furnace. This was reflected by the occurrence of soot in the two finest size fractions. This could be due to milling sintered clay to cement. Fraction of the coarsest particles was 31 % (weekly variability 33 %), the finest fraction was 28 % (22 % variability). At the quarry site, the dominant coarse particle source was mining of raw material. The relative mass contribution (RMC) of two coarsest fractions was 11 % and 29 % with a variability of 35 % and 29 %. The finest particles originated in material conveying (trucks) and milling, and their RMC was 31 % (variability 35 %). The MSD was more shifted towards smaller particles at the airport site (RMC 48 % and variability 8 % for $PM_{0.49}$) but the coarse fraction was still significant (RMC 4 % and variability 19 % for the coarsest fraction, and RMC 12 % and variability 22 % for the second coarsest fraction). Similarly, at the traffic junction site the influence of the dominant source explains a much higher concentration of fine particles (RMC 41 % and variability 8 % for $PM_{0.49}$). Particle re-suspension is expectedly a significant source of coarse particles (RMC 8 % and variability 16 %, RMC 20 % and variability 16 %, for the coarsest and the second coarsest fraction). Two maxima, in the 3–7.2 and <0.45 μm size ranges, were observed (Fig. 2), similar to size distributions at similar sites (e.g., Horvath et al. 1996). Maximum in the range of 3–7.2 μm was also observed at other sites of this study affected by traffic (quarry and airport). In the village, reflecting the influence of domestic heating, one mode peaking <0.49 μm was measured (RMC 47 % and variability 6 %, RMC 28 % and variability 4 % for two finest fractions, respectively). Also at the town site, similar to the village, the fine particles were highest concentrated (RMC 39 % and variability 12 %, RMC 26 % and variability 15 % for two finest fractions, respectively). Presumably, high temperature processes and domestic heating contributed substantially to this size distribution.

Expressed in characteristic PM mass fractions, it is found that at the cement mill, PM_3 and $PM_{0.95}$ were 44 % and 35 %, respectively; at the quarry, 56 % and 35 %, at the small airport, 85 % and 69 %, at the urban traffic junction, 73 % and 57 %, respectively; in the village, 95 % and 75 %, and in the town, 88 % and 66 %, respectively. The highest fraction in PM_3 (95 %) measured in the village in the winter time was obviously related to solid fuel combustion. The fractions found at the town and village sites are similar to MSD measured in Brescia, Italy, in spring (heating period, urban), where PM_3 was 79–96 %, $PM_{0.95}$ was 63–82 %, and PM_{10} was 35–128 μg m^{-3}.

PM composition

The PM composition at various sites was also clearly influenced by the local sources. The finest particle fractions had the largest surface (except for town) and the highest OM mass concentration (except for cement mill). The mineral composition (nine groups) is shown in Table 2.

Particles originated from cement mill composed of particularly inert materials. Super-micrometer particles were almost purely inorganic. Every fraction contained quartz as the main component of cement (20 %), calcite as a dominant part of limestone (20 %) and gypsum as a regulator of setting process (20 %). Minor components were clay, zeolitic and cement minerals.

At the quarry, most particulate mass comprised inert material analogous to the cement mill. Quartz and calcite were included in every fraction, whereby content of quartz increased along with calcite (≈40 %). Another important component was gypsum. From limestone and other rocks, there were zeolites, feldspars, micas and clays in particles. OM content increased with decreasing particle size (7–20 %).

Quartz and calcite were not the main components of all fractions at the airport. $NaNO_3$ (Chile saltpetre) was found to peak in fine fractions (25 % in 0.95–1.5 μm particles and 9 % in particles <0.45 μm). Levandulan (arsenite) was measured in fraction *D* (25 %). OM content (A–F) ranged from 9 % to 28 % with the highest level in the finest fraction.

Higher levels of TOC were also found in the finest fractions at the traffic, village and town sites (46 %, 34 % and 36 %, respectively). However, different patterns were observed at these sites. TOC concentrations as well as soot content were at least factor of 2 higher in the fine fractions when compared to the coarse fractions at the town and traffic (both urban) sites, while they were more uniform at the airport and village (both rural) sites. At these rural sites, there were also found the highest levels of the clay materials. Significantly higher levels of sulphates were found at the traffic as well as both rural (village and airport) sites when compared to town, carbonates were most abundant at the town site, especially in the coarse fractions, Nitrates contributed more at the airport and traffic sites, evaporites were found in all particle fractions of the town site while at the traffic site, they were only abundant in the coarser fractions.

Figure 3 presents the variability of SSA of PM collected from six sites. The highest surface area was observed at combustion affected sites, a contribution of the finest fraction was most significant at the traffic site.

Organic contaminants particulate levels and mass size distributions

PAHs

Levels and MSD of PM are shown in Fig. 4. Levels of particle-associated PAHs were 1–30 ng m^{-3}. This

Table 2 Organic and mineral composition (%), specific surface area SSA ($m^2\ g^{-1}$) and aerosol surface concentration S ($10^{-6}\ cm^{-1}$, in brackets) of atmospheric PM size fractions

	Fractions (μm)	TOC	Organic matter/soot	Boehmit goethite/hematite Fe,Al	Illite–smectite $SiAlO_x$	Chlorite–kaolinite–allophane $SiAlO_x$	Cement minerals $AlSiO_x$	Rock forming minerals SiO_2; Al_2O_3	Carbonates (CO_3)	Sulphates, etc. (SO_4)	Evaporites Cl F	SSA (S) $m^2\ g^{-1}$ ($10^{-6}\ cm^{-1}$)
Cement mill	7.2–10	0.58	0.70	<5.0	<2.0	<5.0	35.2	21.1	21.1	21.83	<5.0	1.80 (1.2)
	3–7.2	1.00	1.20	<5.0	<2.0	<5.0	30.2	22.6	22.6	23.38	<5.0	3.06 (1.1)
	1.5–3	2.29	2.75	6.90	<2.0	<5.0	27.6	20.7	20.7	21.38	<5.0	22.0 (1.7)
	0.95–1.5	3.19	3.82	<5.0	<2.0	6.37	31.8	19.1	19.1	19.75	<5.0	11.7 (0.8)
	0.49–0.95	11.8	14.1	<5.0	<2.0	<5.0	26.2	19.7	19.7	20.32	<5.0	36.1 (3.2)
	<0.49	7.94	9.53	5.62	<2.0	<5.0	33.7	16.9	16.9	17.42	<5.0	36.5 (18.1)
	Total	26.8	32.1	12.5	0.0	6.4	184.7	120.1	120.1	124.1	<30.0	(26.2)
Quarry	7.2–10	7.28	8.74	10.7	16.0	10.7	2.5	32.0	16.5	5.34	<5.0	64.7 (1.2)
	3–7.2	6.02	7.22	<5.0	25.6	14.6	2.5	29.9	22.6	<5.0	<5.0	48.4 (2.6)
	1.5–3	8.46	10.1	9.17	16.0	9.17	2.5	27.5	14.2	13.7	<5.0	64.6 (1.1)
	0.95–1.5	8.64	10.4	<5.0	21.5	<5.0	2.5	30.7	19.0	18.4	<5.0	47.9 (0.7)
	0.49–0.95	9.88	11.9	<5.0	<2.0	14.6	7.28	22.6	21.8	21.8	<5.0	34.6 (0.6)
	<0.49	23.6	28.3	10.2	15.2	<5.0	2.5	15.8	15.2	15.2	<5.0	109.6 (6.3)
	Total	63.9	76.7	30.1	94.3	49.1	19.8	158.5	109.3	74.4	<30.0	(12.5)
Small airport	7.2–10	22.2	26.7	21.8	<2.0	<5.0	<2.5	22.5	21.8	<5.0	7.26	115.4 (0.6)
	3–7.2	14.1	17.0	<5.0	8.2	<5.0	16.4	25.5	8.22	24.7	<5.0	51.5 (2.0)
	1.5–3	21.8	26.2	<5.0	4.9	<5.0	<5.0	30.1	29.1	<5.0	9.71	71.6 (1.6)
	0.95–1.5	12.03	14.4	<5.0	<2.0	<5.0	<5.0	36.15	<5.0	<5.0	49.4	36.8 (0.6)
	0.49–0.95	9.60	11.5	<5.0	<2.0	44.24	<5.0	<5.0	<5.0	44.2	<5.0	42.7 (1.5)
	<0.49	27.67	33.2	22.3	7.4	<5.0	<5.0	<5.0	14.8	<5.0	22.3	140.5 (14.7)
	Total	107.4	129.0	44.1	20.5	44.2	16.4	114.2	73.9	68.9	88.7	(21.0)
Traffic junction	7.2–10	9.56	11.5	<5	<2.0	<5.0	<5.0	25.3	<5.0	25.3	37.9	29.3 (1.0)
	3–7.2	10.5	12.6	10.9	<2.0	<5.0	<5.0	21.8	<5.0	21.8	32.8	56.0 (4.8)
	1.5–3	15.3	18.4	11.6	<2.0	<5.0	<5.0	23.3	<5.0	11.6	35.0	72.3 (2.1)
	0.95–1.5	23.0	27.6	14.5	<2.0	<5.0	<5.0	21.7	<5.0	21.7	14.5	101.8 (2.6)
	0.49–0.95	23.6	28.3	<5.0	17.9	<5.0	<5.0	<5.0	<5.0	53.7	<5.0	90.2 (5.5)
	<0.49	45.7	54.9	11.3	<2.0	<5.0	<5.0	<5.0	<5.0	33.8	<5.0	164.5 (26.3)
	Total	127.7	153.3	48.3	17.9	<30.0	<30.0	92.1	<30.0	167.9	120.2	(42.3)
Village	7.2–10	24.5	29.4	<5.0	<2.0	20.2	<5.0	20.2	<5.0	30.3	<5.0	80.9 (0.5)
	3–7.2	18.4	22.1	17.3	<2.0	<5.0	<5.0	26.0	8.6	26.0	<5.0	94.1 (1.7)
	1.5–3	20.2	24.3	<5.0	<2.0	50.5	<5.0	12.6	<5.0	12.6	<5.0	77.1 (1.1)
	0.95–1.5	20.5	24.6	<5.0	15.1	<5.0	<5.0	<5.0	<5.0	60.3	<5.0	77.8 (4.4)
	0.49–0.95	24.2	29.1	<5.0	<2.0	<5.0	<5.0	<5.0	<5.0	70.9	<5.0	74.2 (9.6)

Table 2 (continued)

	Fractions (μm)	TOC	Organic matter/soot	Boehmit goethite/hematite Fe,Al	Illite–smectite $SiAlO_x$	Chlorite–kaolinite–allophane $SiAlO_x$	Cement minerals $AlSiO_x$	Rock forming minerals SiO_2; Al_2O_3	Carbonates (CO_3)	Sulphates, etc. (SO_4)	Evaporites Cl F	SSA (S) $m^2\ g^{-1}$ (10^{-6} cm^{-1})
	<0.49	33.9	40.7	9.89	<2.0	<5.0	<5.0	<5.0	29.7	19.8	<5.0	125.2 (29.7)
	Total	141.7	170.2	27.19	15.1	70.7	<30.0	58.8	38.3	219.9	141.7	(46.9)
Town	7.2–10	14.2	17.0	<5.0	<2.0	<5.0	<5.0	27.6	36.9	18.4	<5.0	43.4 (0.3)
	3–7.2	13.9	16.7	<5.0	<2.0	<5.0	<5.0	27.8	27.8	<5.0	27.8	42.5 (0.9)
	1.5–3	19.4	23.3	20.9	<2.0	<5.0	<5.0	20.9	13.9	<5.0	20.9	104.9 (1.0)
	0.95–1.5	29.2	35.0	24.4	<2.0	<5.0	<5.0	8.12	<5.0	8.12	24.4	142.3 (1.5)
	0.49–0.95	30.1	36.1	38.4	<2.0	<5.0	<5.0	<5.0	<5.0	<5.0	25.6	175.4 (4.4)
	<0.49	36.5	43.7	<5.0	<2.0	<5.0	<5.0	<5.0	<5.0	22.5	33.7	111.6 (6.4)
	Total	143.3	171.8	83.7	0	0	0	84.4	78.6	49.0	132.4	(14.5)

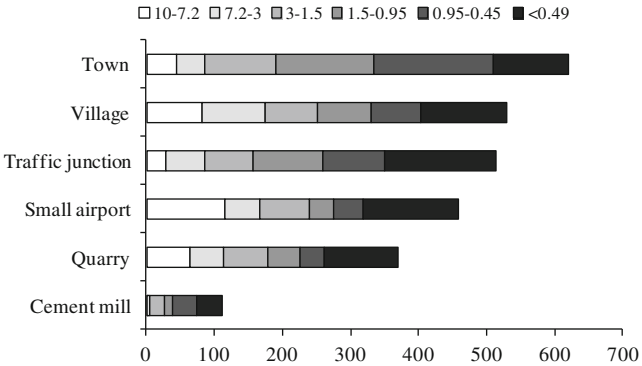


Fig. 3 Particulate size-specific surface area, SSA ($m^2\ g^{-1}$). One sample per site. Size fractions given in μm a.e.d

corresponded to 15–35 % of the total, i.e., gas and particulate concentration. The variation among samples at the individual sites is quantified in Table S1. The highest total concentration (c_g+c_p) of PAHs was observed in the village air, ≈55 % higher than at the town site, ≈80 % higher than at the traffic junction, and almost four times higher than at the small airport site, which is the closest to what could be representing the central European background (Table 2). This may reflect the local source strengths, seasonally variable, while mixing seemingly did not influence this ranking (mean wind velocity ≲1 m s^{-1} at these three sites; Table 1).

Most mass was contributed by the five-ring PAHs followed by the four-ring PAHs at all sites, with the exception of the village site (reverse order).

In MSDs the concentration of PAHs (16 EPA priority PAHs) decreased from fine to coarse particles (expressed as $\Delta c/\Delta \log D$; Fig. 3a) at all sites implying that the majority, 73–90 % of the PAH mass was concentrated in $PM_{0.95}$ and 85–99 % was found in PM_3. Lowest shares are found at the cement mill and at the quarry (Fig. 4, Table 2 and Table S2). Many prior studies have shown the maximum of MSD of PAHs in sub-micrometer particles or slightly larger (Schnelle et al. 1995; Kiss et al. 1998; Kawanaka et al. 2009; Lammel et al. 2010b, c; Škrdlíková et al. 2011; Spindler et al. 2012).

The mass mixing ratio size distributions (ng (g PM_{10})$^{-1}$; Fig. 3b) reveal that the 0.49–0.95 μm size fraction (main fraction of the accumulation mode) had similar potential for adsorption of PAHs as the finest fraction (<0.49) in the localities with contribution of traffic and combustion and higher than the finest fraction in the other localities. The mass fraction found in particles ($PM_{0.95}$) was higher for heavier PAHs, and lowest for NAP which was mostly in the gas phase, NAP mass fraction in $PM_{0.95}$ only exceeded 50 % at the traffic site. This could be related to high S ($S_{0.95}/S_{total}$=14 %, but 5–12 % at the other sites, 17 % at the quarry) or BC typically high at traffic sites (not determined else than as the sum of OC and BC) or both.

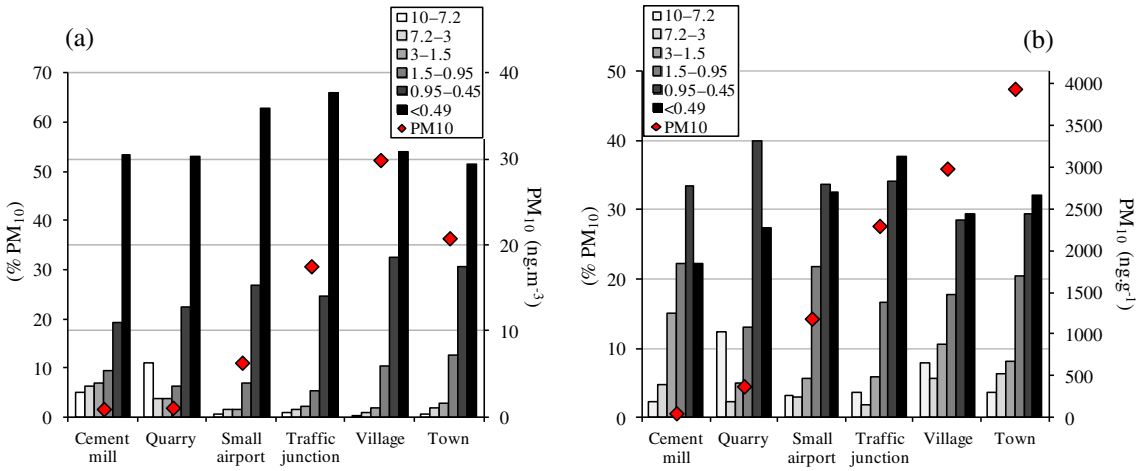


Fig. 4 Total PM_{10}-associated concentrations of PAHs (*diamonds*) and relative contributions of the individual size fractions (*bars*) at six sampling sites: **a** PAH concentration (ng m^{-3}) and **b** PAH PM mass mixing ratio (ng g^{-1}). Arithmetic mean of four samples per site

PCBs and DDT compounds

Levels of particle-associated PCBs were 2–11 pg m^{-3} (4–26 % of the total) and of DDT compounds were 2–12 pg m^{-3} (4–23 % of the total) (Table 3). The PM associated amounts of other OCPs were too low for quantification. The variation among samples at the individual sites is quantified in Table S1.

For PCBs and OCPs, similar size distribution trends were found as for PAH (Figs. 5 and 6, Table S2b in the SM). The concentrations of OCPs were often below the limit of quantifications (LOQ). Extremely low values were found for HCHs, HCB and PeCB, i.e., <LOQ (=0.05 pg m^{-3}) almost throughout for HCB, α-, β-, and δ-HCH, ≤1.3 pg m^{-3} for PeCB (≤0.3 pg m^{-3} per impactor stage) and ≤2.3 pg m^{-3} for γ-HCH (≤0.4 pg m^{-3} per impactor stage). This reflects the combined effect of a pronounced partitioning to the gas phase (e.g., Bidleman 1988), a reduced strength of OCP's dominant source, i.e., volatilisation from ground surfaces (e.g., Holoubek et al. 2007) in the cold season (for all sites except for the cement mill and the quarry), and declining long-term trends in Europe (Dvorská et al. 2009).

Organic contaminants gas-particle partitioning

The gas-particle partitioning trends across sites (Fig. 5) reflect the combined influences of temperature (Table 1) and aerosol surface and composition (Table 3): with decreasing temperatures (from +13 °C to −1.6 °C) and increasing aerosol surface concentration ($S=\Sigma(c_{p\,i\times}SSA_i)$, from 12.5×10^{-6} to $46.9_{\times}10^{-6}$ cm^{-1}) from the quarry and small airport to the traffic junction and village sites, the particulate mass fraction, $\theta=c_p/(c_p+c_a)$, is slightly increasing from $\phi_{PAH}=0.23$ to $\phi_{PAH}=0.27$. Expectedly, this trend is dominated by the semivolatile four-ring PAHs whose particulate mass fraction shift is from $\theta_{4rPAH}=0.26$ to $\theta_{4rPAH}=0.38$ (Fig. 4c; Table S2a). ϕ_{PAH} and ϕ_{4rPAH} are lowest at the cement mill (Fig. 4c, Table S2a). There, both the aerosol surface and the OM are similar (differences≈20 %) to the small airport site, but the temperature was 11.4° higher. This difference in mean temperature corresponds with a doubling of θ_{4rPAH} (0.13 and 0.27, respectively). This trend is very similar to the observation at an urban Mediterranean site, where doubling of θ of the semivolatile PAHs corresponding to a temperature decrease of 13°; Lammel et al. 2010b). ϕ_{PAH} and ϕ_{4rPAH} are highest at the town site, 0.35 and 0.42, respectively. This trend of θ is unexplained: It is not due to temperature (same as at the traffic junction) nor due to aerosol surface ($S=14.5\times10^{-6}$ cm^{-1} at the town site, but much higher, 42.3×10^{-6} cm^{-1}, at the traffic junction), nor to OM (TOC concentration was≈35 % higher at the traffic junction site). It could be related to other chemical properties of the aerosol, e.g., a more hydrophobic nature: precipitation at the town site (21.9 mm but none at the traffic junction site; Table 1) had presumably reduced the hygroscopic aerosol material and led to an enrichment of more hydrophobic particles.

Model-predicted organics' phase distributions

The analysis was limited to the lowermost three stages, <1.5 μm a.e.d., as the PAH concentrations in stages corresponding to the coarse mode were too low for a meaningful analysis of gas-particle partitioning.

The partitioning expressed as θ (Table 4a, Fig. 7) or K_p* (Table S3a, Fig. S1) of most semivolatile PAHs is quite well predicted by the K_{oa} and LL models and overpredicted by the JP and the ppLFER models. However, the ppLFER model predicts well the partitioning of FLN (Table S3a). The uncertainty of the aerosol surface concentration influences the results of the JP and ppLFER models. Furthermore, the PM bulk chemical composition is not necessarily representative for the composition at the particle surface, but this is an implicit

Table 3 PAH, PCB and DDT concentrations in the atmospheric gaseous and particulate phases (PM_{10}) (ng m^{-3})

Location		Cement mill	Quarry	Small airport	Traffic junction	Village	Town
Sum of PAHs	Gaseous	5.57	3.91	21.7	47.5	81.7	38.5
	Particulate	1.01	1.16	6.39	17.6	30.0	20.9
2rPAHs	Gaseous	0.194	0.503	1.28	4.85	2.53	3.68
	Particulate	0.124	0.066	0.055	0.10	0.080	0.059
3rPAHs	Gaseous	3.56	2.39	14.5	31.1	57.5	23.4
	Particulate	0.073	0.057	0.258	0.867	1.64	0.913
4rPAHs	Gaseous	1.66	0.813	5.09	10.34	19.65	9.76
	Particulate	0.254	0.284	1.85	5.89	12.1	7.21
5rPAHs	Gaseous	0.094	0.080	0.219	0.344	0.322	0.43
	Particulate	0.382	0.516	2.85	7.21	11.7	9.00
6rPAHs	Gaseous	0.004	0.005	0.004	0.027	0.004	0.047
	Particulate	0.151	0.201	1.18	2.80	3.77	3.06
7rPAHs	Gaseous	<0.0025	<0.0025	<0.0025	0.003	<0.0025	0.008
	Particulate	0.019	0.028	0.190	0.715	0.722	0.616
Total PCBs	Gaseous	0.047	0.033	0.041	0.085	0.017	0.030
	Particulate	0.002	0.002	0.004	0.011	0.006	0.004
Cl_3PCBs	Gaseous	0.013	0.012	0.016	0.054	0.006	0.013
	Particulate	<0.0006	<0.0006	<0.0006	0.0004	<0.0006	<0.0006
Cl_4PCBs	Gaseous	0.008	0.008	0.009	0.015	0.003	0.007
	Particulate	0.0004	<0.0006	<0.0006	0.0007	0.0007	0.0005
Cl_5PCBs	Gaseous	0.010	0.004	0.006	0.005	0.003	0.004
	Particulate	0.0006	0.0006	0.0007	0.0011	0.0009	0.0007
Cl_6PCBs	Gaseous	0.013	0.007	0.009	0.010	0.004	0.005
	Particulate	0.001	0.001	0.001	0.004	0.002	0.001
Cl_7PCBs	Gaseous	0.003	0.001	0.002	0.002	0.001	0.001
	Particulate	0.0004	<0.0006	0.0010	0.0045	0.0016	0.0012
Sum of DDT compounds	Gaseous	0.054	0.036	0.052	0.037	0.039	0.069
	Particulate	0.002	0.002	0.003	0.010	0.012	0.009
DDE	Gaseous	0.038	0.029	0.045	0.029	0.035	0.053
	Particulate	0.0009	0.0009	0.0016	0.0033	0.0055	0.0033
DDD	Gaseous	0.008	0.003	0.004	0.003	0.002	0.003
	Particulate	0.0006	0.0006	0.0008	0.0012	0.0012	0.0009
DDT	Gaseous	0.008	0.003	0.003	0.005	0.002	0.013
	Particulate	0.0007	0.0006	0.0009	0.0054	0.0057	0.0048

Arithmetic mean of four samples per site. Concentrations of individual samples < LOQ were replaced by LOQ/2 unless all individual samples were < LOQ, where LOQs for particulate species (ng m^{-3} per impactor stage) were 0.0001 for PCBs and OCPs and 0.001 for PAHs. LOQs for gaseous species (ng m^{-3}) were 0.0005 for PCBs and OCPs and 0.0025 for PAHs

assumption of the ppLFER model. The ratio of sensitivity of predicted θ to the uncertainty of individual components' SSA, $\Delta\theta/\Delta SSA_{OM}$ is <0.06 (e.g., SSA_{OM}=150–360 m^2 g^{-1} according to the literature, adoption of an extreme or mean value, i.e., SSA_{OM}=255 m^2 g^{-1}). For PHE, the particulate mass fraction, θ, cannot be calculated using the ppLFER model, because of lack of input data. The agreement looks different for size-specific particulate mass fractions, $\theta_i=c_{pi}/(c_{pi}+c_a)$, which were tested for three size classes <1.5 μm of aerodynamic size: PYR, BAA and CHR are quite well predicted by the LL and ppLFER models, underpredicted by the K_{oa} model and, again, overpredicted by the JP model (Fig. 7). Sensitivity studies varying the values propagated for c_p<LOQ show underestimation also for the cases with low particulate mass fraction, θ_i <0.1.

In this study, S was in the range (12–47)×10^{-6} cm^{-1}, and hence, significantly higher than default values used to represent 'climatological' means ((3.5–11)×10^{-6} cm^{-1}; Jaenicke 1988) but within the range found in a Mediterranean urban environment in a previous measurement ((9–280)×10^{-6} cm^{-1}; Lammel et al. 2010b). In previous studies using not measured values but estimates for both c_J

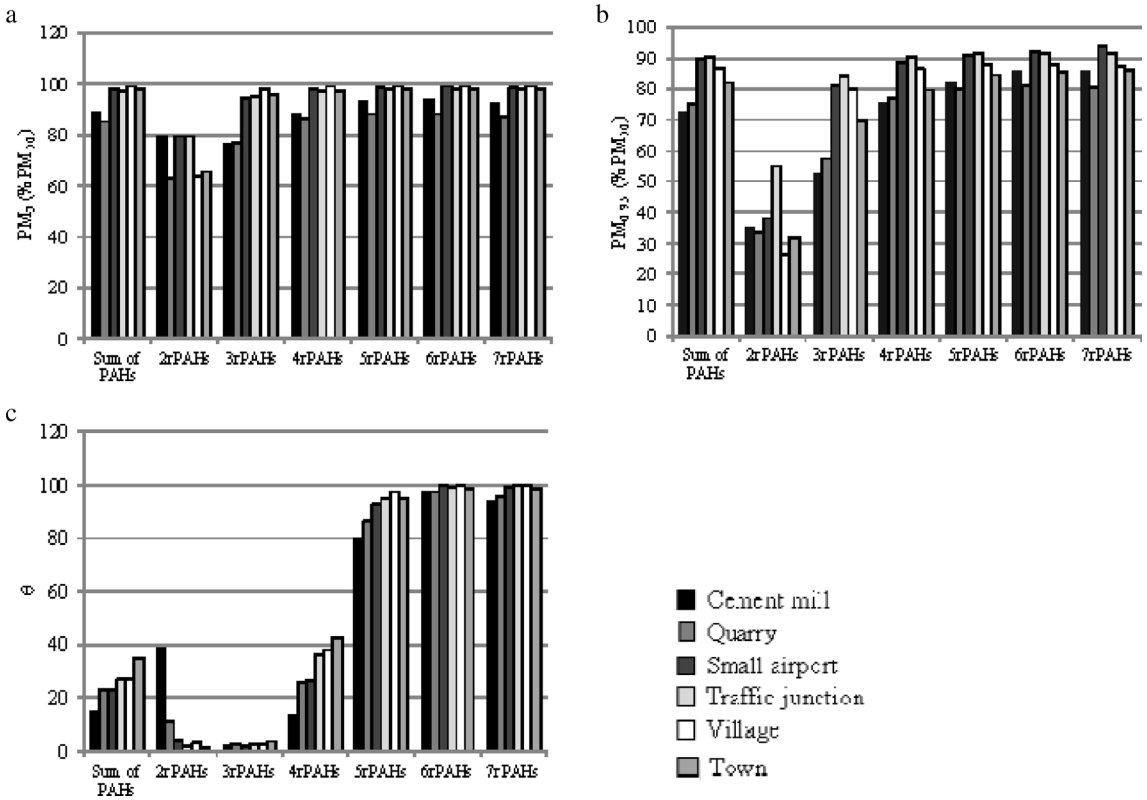


Fig. 5 PAH mass fractions associated with **a** particles <3 μm (PM_3) and **b** <0.95 μm ($PM_{0.95}$) and **c** particulate mass fraction $\theta=c_p/(c_p+c_a)$ in PM_{10} (%). Arithmetic mean of four samples per site

(17.2 Pa cm) and *S*, both underprediction (e.g., Bidleman 1988; Finizio et al. 1997) and overprediction (e.g., Radonić et al. 2009; Balasubramanian and He 2010) of K_p* by the JP model were found. In the only previous study where *S* was actually determined (based on particle number size distribution and assumed particle sphericity; Lammel et al. 2010b), could the JP model predict. However, the apparent c_J varied largely among PAHs (c_J=43–1,740 Pa cm), which should not be the case were unspecific adsorption, the process tested by this model, dominating gas-particle partitioning. Therefore, results presented here cannot be conclusive with regard to improving partitioning prediction using the JP or any other adsorption model.

We inspect correlations between predicted and observed K^*_{pi} (Table S3a, Fig. S1). These suggest that all models perform similar in predicting the partitioning of PHE, ANT and FLT, while ppLFER predicts better the partitioning of FLN. Note that the variation of K^*_{pi} of none of these PAHs were well captured by the models (R^2<0.5). The variations of PYR, BAA, and CHR is captured satisfactorily (R^2>0.6) by the JP, K_{oa} and LL models, but not by the ppLFER model, which clearly falls short (R^2<0.5), obviously related to few outliers (underpredictions; Fig. S1). The LL model results are found insensitive (R^2 changes by <0.03) to variation of BC by a factor of 2 (not measured, but scaled as EC/OM depending on season and air mass origin in the region). The ppLFER model predicts K^*_{pi} better (0.1–0.2 higher R^2 values) if the surface properties of diesel soot (Roth et al. 2005) are adopted for 'EC'. With regard to adsorption to particulate OM this phase was represented as 1-octanol in the ppLFER model. If cellulose, a less lipophilic, high molecular weight organic substance, is adopted as surrogate surface material for particulate OM rather than octanol the results are found insensitive (R^2 changes by <0.03). However, with regard to absorption into OM descriptors for other material than SRM 'Washington urban aerosol' should be derived and tested. The ppLFER model results are found insensitive (R^2 changes by <0.02), too, to variation of BC by a factor of 2. Lack of correlation results from failing to capture the wide range of K^*_{pi} values (e.g., spanning >3 orders of magnitude in the case of BAA and CHR, while the model predictions span only≈1 order of magnitude). This indicates that the processes described by the model, i.e., various types of molecular interactions between these more lipophilic PAHs and aerosol surfaces and the OM phase, are not well parameterized. This could be related to the bulk approach used, not capturing eventual shell-like structure of PM with lipophilic fraction being enriched at the surface. As the entire range of K^*_{pi} values is captured in the case of the less lipophilic semivolatile PAHs,

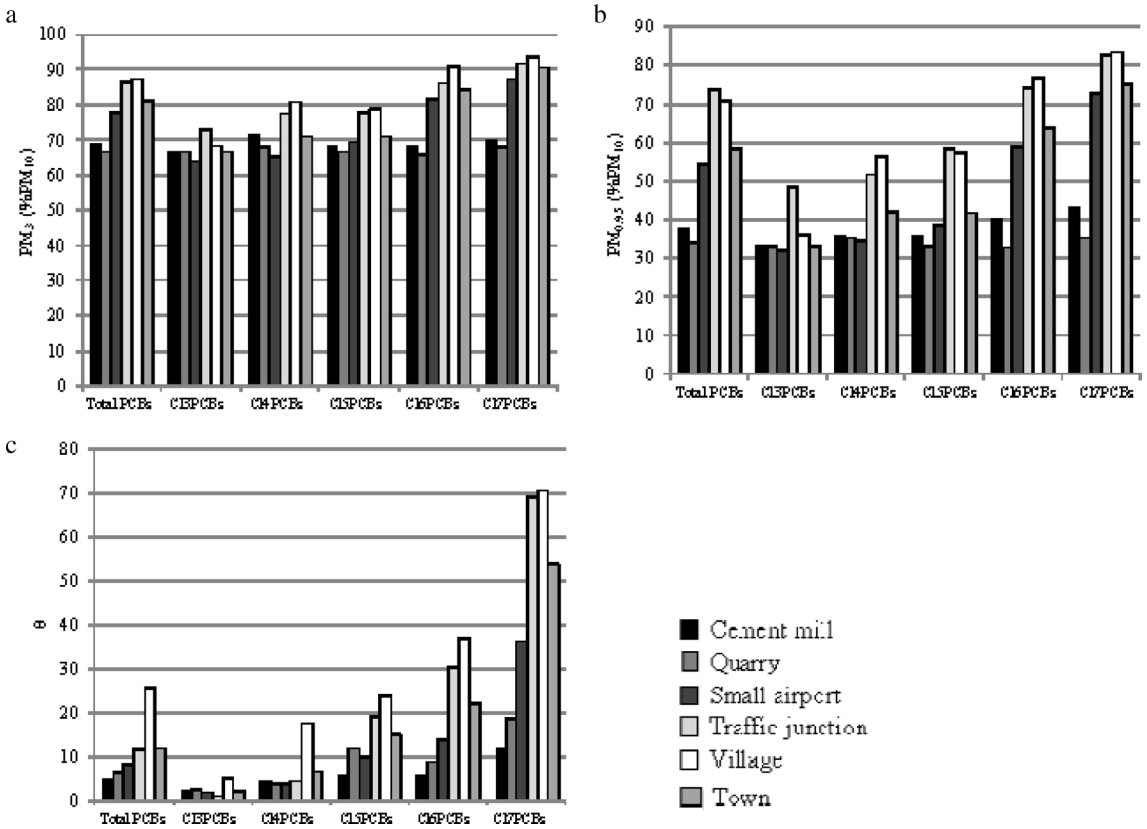


Fig. 6 PCB mass fractions associated with **a** particles <3 μm (PM_3) and **b** <0.95 μm ($PM_{0.95}$) and **c** particulate mass fraction $\theta = c_p/(c_p+c_a)$ in PM_{10} (%). Arithmetic mean of four samples per site

experimentally insufficient characterisation of the surfaces or analytical inaccuracy cannot explain the discrepancies.

Table 4 Mean predicted and observed total particulate mass fractions, θ, of (a) PAHs and (b) PCBs and OCPs

	Observed	Predicted			
Model		JP	K_{oa}	LL	ppLFER
FLN	0.04	0.23	0.00	0.00	0.02
PHE	0.10	0.61	0.01	0.03	n.d.[a]
ANT	0.03	0.63	0.01	0.04	0.15
FLT	0.30	0.95	0.15	0.30	0.72
PYR	0.22	0.85	0.17	0.34	0.81
BAA	0.85	0.99	0.79	0.76	1.00
CHR	0.80	1.00	0.80	0.92	1.00
PCB138	0.39	0.99	0.49	n.a.	0.97
PCB153	0.25	0.99	0.46	n.a.	0.97
DDE	0.17	0.97	0.61	n.a.	0.95

Five sites, one impactor sample each

JP Junge–Pankow (Pankow 1987), K_{oa} Harner and Bidleman (1998), *LL* Lohmann and Lammel (2004), *ppLFER* Goss and Schwarzenbach (2001), *n.a.* not applicable

[a] Lack of input data

The conclusiveness of these model tests is certainly limited by the size of the data set with S measured (five samples with each three particle size classes) and the uncertainty of some of the input data (determination of S, estimation of BC, particle sphericity). The ppLFER and LL model results were found insensitive to variation of BC (above). To explore the related sensitivity of the results these model tests were repeated for an extended data set encompassing eight samples with each three particle size classes, by including three more samples from one site, the airport, with S not measured but linearly scaled from c_{pi}. This extrapolation of S across samples was justified as PM composition was almost invariable. The results with regard to models' performance, however, were insensitive to this extension of the sample size.

Only few chlorinated organics could be studied due to low levels in the particulate phase (many data < LOQ). For two PCBs and DDE, the partitioning expressed as θ (Table 4b, Fig. 7) or K_p* (Table S3b, Fig. S1) is quite well predicted by the K_{oa} model and clearly overpredicted by the JP and the ppLFER models. The correlations between predicted and observed K^*_{pi} and θ (Table S3b, Fig. S1) reveal that the JP and K_{oa} models perform well with regard to K^*_{pi}, while the ppLFER model shows low correlations. Negative correlations with regard to θ are related to predicted values close to 1. The

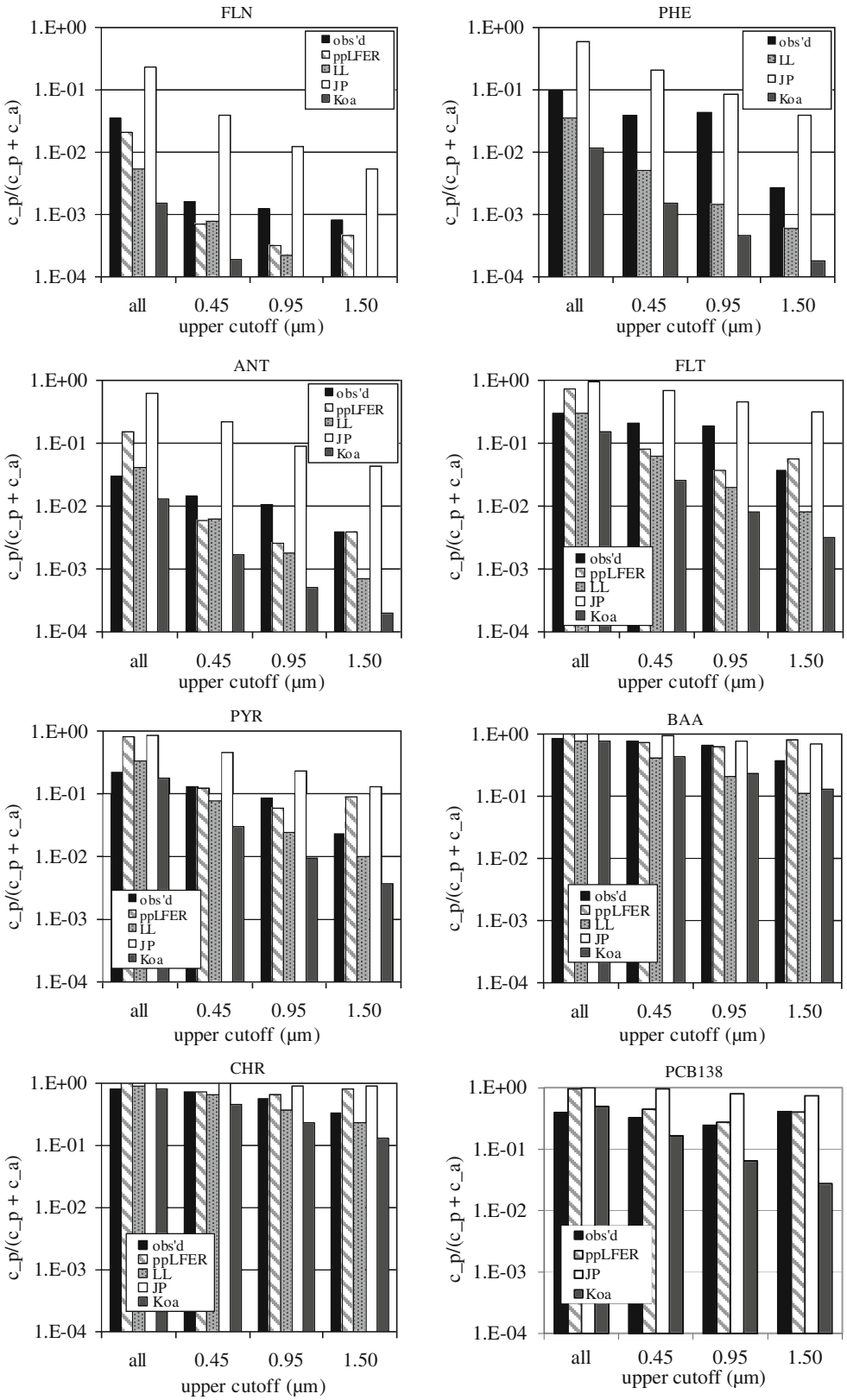


Fig. 7 Performance of various models of gas-particle partitioning in predicting the total ('all', $\theta=c_p/(c_p+c_a)$) and size-specific ($\theta_i=c_{pi}/(c_{pi}+c_a)$; sizes <1.5 μm a.e.d., $i=4, \ldots, 6$) particulate mass fractions of seven semivolatile PAHs, two PCBs and DDE. Five sites, one impactor sample each

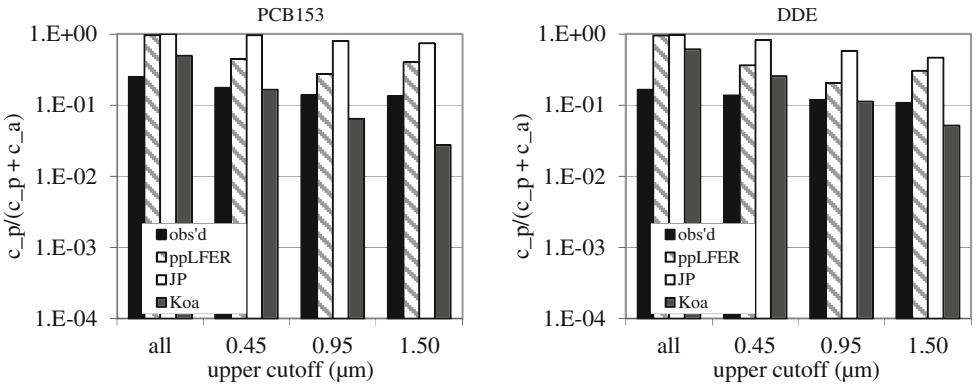


Fig. 7 (continued)

low performance of the ppLFER model could be due to outliers (Fig. S1).

Conclusion

For the first time the phase and MSD of several substance classes of lipophilic semivolatile organics was determined at a wide range of type of sampling site together with the PM matrix composition and MSD being characterized. The results prove that at the various sites particles with diverse size, matrix composition, amount of pollutants and toxicological effects occur. More parameters are needed in order to achieve an almost complete chemical characterisation of the PM matrix. Major components covered only partly or not explicitly in this study are secondary inorganic aerosols (sulphates, nitrates), BC and water. PM water could be estimated based on a more complete ionic composition (e.g., Nenes et al. 1998). The variability of MSD seems to be explainable with the MSD upon particle emission and redistribution. The gas-particle partitioning of PAHs, however, is not well explained by present knowledge, while the gas-particle partitioning of two PCBs and DDE is satisfactorily predicted by assuming absorption into OM to rule the process (K_{oa} model). The ppLFER gas-particle partitioning model (Roth et al. 2005; Arp et al. 2008) which represents the current understanding of the underlying molecular processes, therefore, is most promising. In this model in lack of complete data substance parameters of $(NH_4)_2SO_4$, quartz and 1-octanol (Goss and Schwarzenbach 2001; Schwarzenbach et al. 2003) were taken as surrogate parameters for ammonium salts, minerals, and particulate OM (and a highly polar, polymer, cellulose, was tested, too). However, particulate OM phases are very diverse with regard to molecularity, viscosity and polarity (Roth et al. 2005; Mikhailov et al. 2009). Data describing the diversity of such matrices are lacking. Furthermore, this and other gas-particle partitioning models should be extended to account for the shell-like structure of particles (rather than bulk approach).

Air pollution is usually assessed based on the PM_{10} and $PM_{2.5}$ size fractions. The results of this study suggest that a better size resolution is needed to characterize the MSDs of PAHs and chlorinated organics, which are concentrated in the PM_1 size fraction. PAHs are indicative for the presence of the even more toxic nitro- and oxy-PAHs (same size fraction; Grosjean et al. 1983; Finlayson-Pitts and Pitts 2000; Lammel et al. 2010c; Ringuet et al. 2012), substance classes less studied so far.

Because of semivolatility of most of the contaminants addressed here, re-distribution within the aerosol may occur during transport. No immediate evidence for this process was found in this study. Inhomogeneous PM composition in the same size range was not addressed in this study, but the chemical composition was assumed to be uniformly distributed within particles and across particles of same size class (concept of mixing state; Covert and Heintzenberg 1984). However, particles often have shell-like structure with a water insoluble core (oxides, etc.) and enrichment of salts and, eventually, an organic film at the surface (Gill et al. 1983). The sorption to minerals, therefore, might be overestimated by this study. Good performance of gas-particle partitioning models can only be expected when based on particle surface composition, or as far as bulk chemical composition does not deviate too much from surface composition. To the end of identifying individual aerosol parameters influencing gas-particle partitioning an experimental design with fixed or almost constant key parameters temperature and aerosol surface would be preferential.

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Composition and effects of inhalable size fractions of atmospheric aerosols in the polluted atmosphere. Part II. *In vitro* biological potencies

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ABSTRACT

Exposure to particulate matter (PM) in ambient air has been shown to lead to adverse health consequences. Six size fractions of PM with aerodynamic diameter smaller than 10 μm (PM_{10}) and gas phase were collected at six localities with different major pollution sources. Extracts of samples were assessed for AhR-mediated toxicity, (anti-)estrogenicity, (anti-)androgenicity and genotoxicity. The biological responses were interpreted relative to chemical characterization. Historically, for regulatory purposes, evaluation of air pollution was based mainly on assessment of the sum of PM_{10}. In the case of AhR-mediated activity, PM_1 was responsible for more than 75% of the activity of the particulate fraction from all localities. The assessed effects were correlated with concentrations of polycyclic aromatic hydrocarbons (PAH), organic carbon content and specific surface area of the PM. A significant proportion of biologically active chemicals seems to be present in the gas phase of air. The results suggest that an average daily exposure based just on the concentrations of contaminants contained in PM_{10}, as regulated in EU legislation so far, is not a sufficient indicator of contaminants in air particulates and adoption of standards more similar to other countries and inclusion of other parameters besides mass should be considered.

1. Introduction

Exposure to air pollutants is associated with various diseases such as bronchitis, asthma, lung cancer, respiratory problems or arteriosclerosis — for review see Bernstein et al. (2004). Ambient air can be polluted by a complex mixture of pollutants that are either associated with particulate matter (PM) or present in the gas phase. PM can be subdivided into different fractions according to its aerodynamic diameter. These fractions are coarse (2.5–10 μm), fine $PM_{2.5}$ (<2.5 μm), and ultrafine $PM_{0.1}$ (<0.1 μm; De Kok et al., 2006). While the coarse particles, formed mainly by mechanical processes (Bernstein et al., 2004), are deposited mostly in upper respiratory airways and are eventually expelled by mucociliary clearance, fine and ultrafine particles, originating mainly from combustion sources, pass into the alveoli where they can persist (Lippmann et al., 1980). Moreover, ultrafine particles have been determined to be able to penetrate into the circulatory system and to even produce toxic effects in organs other than the lung (Polichetti et al., 2009). Thus, the toxicological significance of chemicals associated with PM seems to be inversely proportional to particle diameter (de Kok et al., 2006; Englert, 2004; Kampa and Castanas, 2008).

Organic extracts of PM contain substances with the potential to elicit genotoxic, dioxin-like or estrogenic and antiestrogenic activities (Claxton et al., 2004; Clemons et al., 1998; Novák et al., 2009). Previous studies of the toxic potency of ambient air have focused mostly on pollutants associated with a single size fraction of air particles omitting the distribution of the toxic compounds within different size fractions of PM. Some contaminants are present also in the gas phase (Castro-Jiménez et al., 2009; Fernández et al., 2002; Lammel et al., 2009) and thus extracts of the gaseous phase of ambient air can produce specific effects such as dioxin-like activity or modulate estrogen- or androgen-dependent signaling pathways (Klein et al., 2006; Novák et al., 2009).

In this study the biological potency of six subfractions of PM_{10} from six localities has been studied. Samples were extracted with organic solvents and selected biological responses measured and concentrations of several classes of chemicals determined. The sampling sites were chosen to provide samples with broad range of diverse physical–chemical characteristics. A summary of gravimetric, geological and chemical analyses of the PM samples are given elsewhere (Landlová et al., part I) while toxicological characterization of organic extracts of PM and gas phase fractions of the air samples are given here. The *in vitro*

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bioanalytical characterization included dioxin-like activity, (anti-) estrogenicity, (anti-)androgenicity and genotoxicity.

2. Material and methods

2.1. Air sampling

Particulates were collected by using a high volume air sampler PM10 HVS1 (Umwelttechnik MCZ GmbH, Germany), which was equipped with a multi-stage cascade impactor (Andersen Instruments Incorporated, USA, series 230, model 235), which collected six size fractions of particulates. Particles were sampled on slotted glass fiber collection substrata and glass fiber Hi-Vol filters. The collected fractions represent particulates with aerodynamic diameters of 7.2–10 μm (A); 3–7.2 μm (B); 1.5–3 μm (C); 0.95–1.5 μm (D); 0.49–0.95 μm (E) and <0.49 μm (F). In parallel with the cascade impactor, chemicals in the gaseous phase were collected by using a medium volume sampler Leckel MVS6 (Sven Leckel Ingenierbüro, DEU) equipped with quartz and polyurethane foam filters in tandem (fraction G).

Halves of the filters from the cascade impactor were used for gravimetric, mineralogical and heavy metal assessments (Landlová et al., part I). The other filter halves, as well as polyurethane foam filters from the Leckel sampler, were extracted with dichloromethane in a Büchi System B-811 automatic extractor. These extracts were used for both instrumental and bioanalytical characterizations. Composites of four one-week subsamples were used for bioanalytical characterization.

Samples were collected over a 28 day period at six localities in the south-east of the Czech Republic differing mainly in the type of dominant pollution sources from July 2007 to February 2008 (Table 1). Locality I was a cement mill near Brno. This facility was located in a relatively clean locality so the main source of pollution was cement production. Locality II was a stone quarry that is located near locality I and served as a source of limestone for the cement mill. The quarry is located in woods not far from locality I, so the main source of air pollution was the quarrying and transportation of the limestone. Locality III was a small airport in rural countryside about 2 km south-east from the Brno metropolitan area. The air traffic at the airport was not intense so the main source of air contamination was probably agriculture and pollution-transported from the Brno metropolitan area. Locality IV was an important traffic junction in the center of Brno. The main source of pollution was probably vehicle traffic. Site V was located in a small village 25 km south of Brno. The village is in a rural area relatively far from larger urban areas. Households in this village mainly heat with coal that probably represented the main source of pollution. Sampling site VI was located in the industrial zone of a small town 50 km south-east of Brno. The locality was in the vicinity of glassworks, machine works and a tile factory. More detailed descriptions of the localities and sampling procedures and conditions including methodology and QA/QC and results of chemical and mineralogical analyses are published elsewhere (Landlová et al., part I).

2.2. Bioassay procedures

Four individual bioassays were used to assess biological effects of air samples. The H4IIE-*luc*, rat hepatocarcinoma cells stably transfected with the luciferase gene under control of the AhR were used to quantify the dioxin-like activity of the samples. This bioassay is a well-established model for evaluation of AhR-mediated activities of pure substances as well as environmental samples and the activity is reported as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin equivalents (bioTEQ; Villeneuve et al., 2000). popTEQ, a portion of the overall bioTEQ produced by persistent organic pollutants (POPs), was assessed by removing non-persistent chemicals such as PAHs by treating samples with sulfuric acid using method described previously (Novák et al., 2007). Estrogenic effects were examined using a cell model MVLN; human breast carcinoma cells transfected with a luciferase gene under control of estrogen receptor activation (Demirpence et al., 1993; Freyberger and Schmuck, 2005; Villeneuve et al., 2002). Effects of extracts on MVLN cells were assessed in the presence or absence of competing endogenous ligand. Antiestrogenicity was assessed by simultaneous exposure of the sample extract and 17β-estradiol (11 pM). The bioluminescent yeast assay was used for detecting anti/androgenic activity. The assay was based on a yeast (*Saccharomyces cerevisiae*) strain stably transfected with genes for human androgen receptor along with firefly luciferase under transcriptional control of androgen-responsive element. Another yeast strain constitutively expressing luciferase served for assessment of cytotoxicity (Leskinen et al., 2005). Yeast cells were exposed to the samples alone or in combination with testosterone (10 nM) to assess the effect in interaction with physiological ligand of the AR. Genotoxic potency of extracts was determined by use of the microplate version of the SOS chromotest with *Escherichia coli* PQ 37 as a bacterial test strain (Quillardet and Hofnung, 1985), which has been described previously (Škarek et al., 2007a). More detailed information on cultivation and experimental conditions of the bioassays is included in online Supporting Information.

Table 1
Sampling sites and their main pollution sources.

Label	Locality	Pollution source
I	Cement mill	Cement production, waste incineration
II	Stone quarry	Quarrying, stone transport
III	Small airport	Agriculture, air traffic
IV	Traffic junction	Traffic, local heating
V	Village	Local heating
VI	Town	Industry, local heating

2.3. Data analysis

Correlations were calculated using the nonparametric Kendall tau procedure because there was insufficient data to confirm if it was normally distributed, which is one of the assumptions of parametric testing. Kendall tau was chosen because it is more robust than the Spearman correlation based on ranks. Statistical significance was defined as Type I (p) errors of less than 0.05. Results of the H4IIE-*luc* assay were reported as toxic equivalents (bioTEQ) expressed as fg of tetrachlorodibenzo-*p*-dioxin per m^3 of air or ng per g of particulate matter in the respective fraction. The calculation was based on EC_{25} values as described previously (Villeneuve et al., 2000). The calculated TEQ (pahTEQ) values were derived from analytical data on 12 PAHs with available relative potency values, which were assessed with the same model cell line as in our study (Machala et al., 2001), using toxic equivalency factor approach described previously (Safe, 1998).

IC_{25} values (m^3 of air or μg of particulate matter per ml of exposure medium) for antiestrogenicity and antiandrogenicity, were calculated from dose–response curves compared to signal of competitive concentration of added natural ligand 11 pM estradiol and 10 nM testosterone, respectively, which was considered to be the maximum (100%) response. The values in graphs and statistical analyses are expressed as an index of antiestrogenicity (AE) or antiandrogenicity (AA), respectively, which correspond to reciprocal value of IC_{25}. Thus greater antiestrogenicity and antiandrogenicity are expressed as the decrease in activity of the signal given by a specified amount of competing estrogen or testosterone in the medium, respectively. In the case of the yeast model, results from the AR-specific yeast strain were normalized to the results from a constitutively luminescent strain to take into account the effects of the samples on yeast propagation (Leskinen et al., 2005). Genotoxicity is expressed as relative genotoxic units (RGTU), a reciprocal value of minimum genotoxic concentration × 100 (Čupr et al., 2006).

3. Results and discussion

The study was designed to examine diverse samples of air particulate matter (PM) to address a variety of possible scenarios that can occur in the environment (Table 1). To at least partly cover the factor of season variation and because it was not possible to sample all localities simultaneously, sampling started in summer at localities with sources of pollution that do not change seasonally (cement mill, quarry) and ended at sites that were presumed to be most polluted in cold season due to local heating (village and industrial town) in winter. The diversity of PM types has been demonstrated based on gravimetric, mineralogical and chemical characterization (Landlová et al., part I). In order to determine relative abundance and the toxic potential of various PM size fractions, inhalable PM_{10} was aerodynamically classified to subfractions of coarse PM (A, B), accumulation size PM (C, D, E) and mostly ultrafine PM (F) (see Supporting Information Table S3). This relatively high resolution PM classification could provide an insight into size-specific distribution of chemicals with toxic potential within inhalable PM. At localities I and II, PM contained a relatively greater proportion of coarse PM (fractions A, B) when compared with the other sites. This was caused by the characteristics of the main pollution sources at these sites, which was cement production at locality I (cement mill) and quarrying and transport of limestone in case of locality II (stone quarry). At the other sites, the ratio was shifted toward fine and ultrafine particulate fractions (Landlová et al., part I). Particulates from localities III (airport-rural countryside) and IV (traffic junction) contained, beside maximum in the finest fraction of air particulates, relatively high proportions of the B fraction (3–7.2 μm) of PM that probably came from the traffic (dust whirling; Bernstein et al., 2004). At the other two localities, there were relatively small amounts of particle fractions A, B, C (>1.5 μm) and the main portion of particulate matter consisted of ultra-fine particles that probably originated from combustion of coal for local heating in case of locality V (village) and industry such as glass works, machinery plant and tilery at locality VI (small town-industrial locality). More discussion of non-toxicological characterization of the PM samples can be found in Landlová et al. (part I). Toxic potencies are based on organic extracts of the filters to describe the worst-case scenario of organic compound exposure but it does not take into account effects of particles *per se* as it is not clear how to use them directly in the submerged cell exposure scenario in a well-defined way (Paur et al., 2011). Toxic potencies of PM extracts, were expressed per weight of PM (Fig 1A, C, E), to describe differences among particulate matter in the different size fractions, as well as per volume of air (Fig 1B, D, F) to account for amounts of PM fractions in the samples of air and to compare the toxic potencies of PM fractions with the toxicity of compounds present in the gas phase fraction (G).

3.1. Dioxin-like activity of PM extracts

The H4IIE-*luc* assay is useful for detecting the potency of mixtures of chemicals that activate the aryl hydrocarbon receptor (AhR). These compounds have been shown to be involved in numerous health effects such as impairment of the reproduction, immune and nervous systems (Mukerjee, 1998). Moreover, the AhR interacts with hormonal signaling pathways and thus compounds activating AhR might indirectly act as endocrine disruptors as has been shown in the case of estrogen receptors (Safe and Wormke, 2003). There have been several studies describing AhR-mediated effects of PM_{10} (Brown et al., 2005; Cigánek et al., 2004; Clemons et al., 1998), PM_1 (Wenger et al., 2009a) or total particulate matter (Cigánek et al., 2004; Hamers et al., 2000; Klein et al., 2006).

AhR-mediated activity was observed in samples from all localities. However, in some fractions, the potency was insufficient to allow calculation of an EC_{25}-derived bioTEQ (Fig 1A, B). The potency of air sample extracts exhibited a spatial gradient gradually increasing from locality

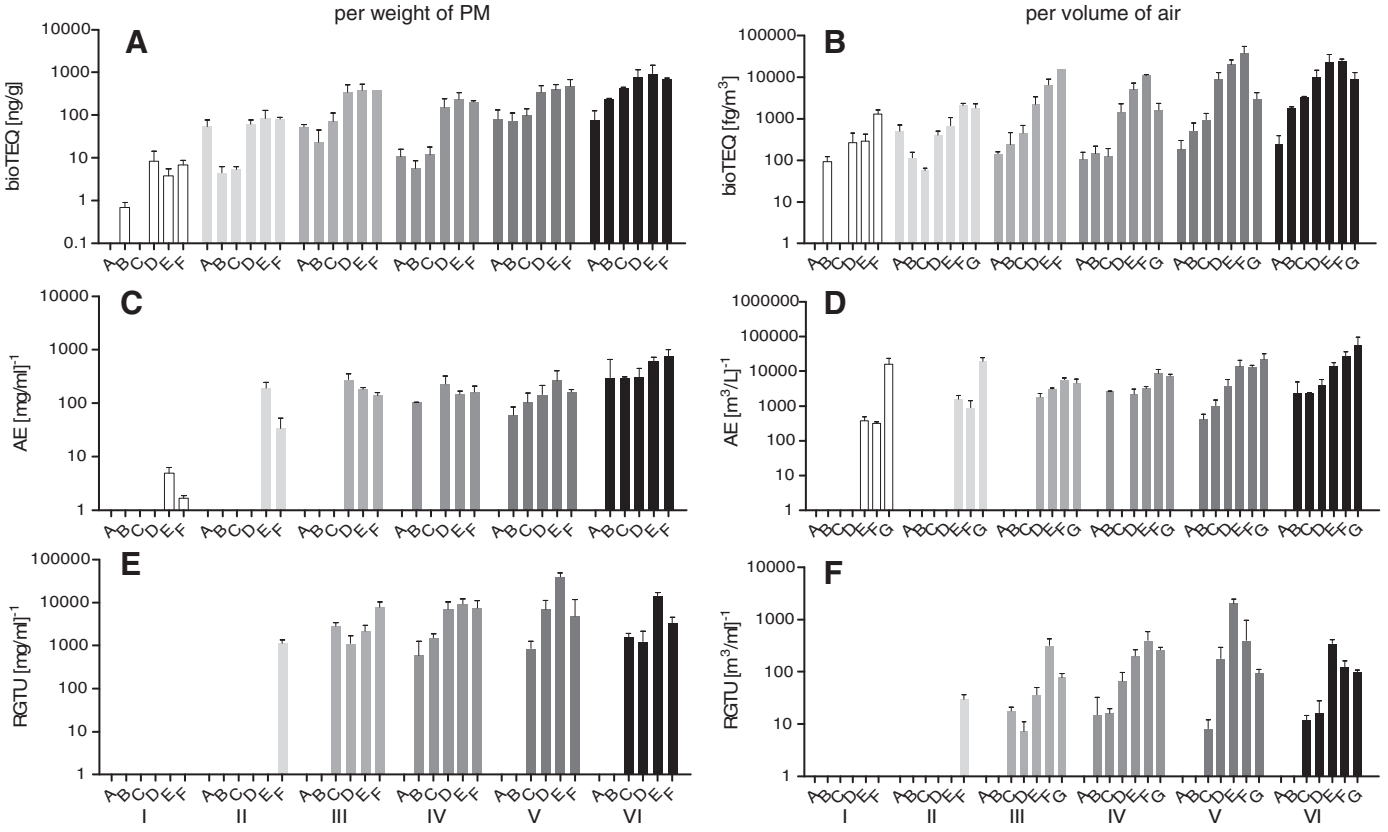

Fig. 1. Toxicological characterization of PM size fraction extracts (mean ± SD); bioTEQ — AhR mediated activity; AE — antiestrogenic index (reciprocal value of IC_{25}); RGTU — relative genotoxic units (reciprocal value of minimal genotoxicity concentration × 100); A–F: size fractions of PM (A:7.2–10; B: 3–7.2; C 1.5–3; D: 0.95–1.5; E: 0.49–0.95 and F: <0.49 μm); G: gas phase fraction; I–VI: sampling sites.

I to locality VI with a potency inversely proportional to the aerodynamic diameter of the particles. However, in the case of locality II, a significant portion of the dioxin-like compounds were present in the coarsest fractions of PM (A). The concentration of bioTEQ in this fraction was 10-fold greater than those of the successive PM fractions (B, C) from the same locality. The trend was similar when the concentrations of bioTEQ were expressed per cubic meter of air. The B fraction accounted for more than 30% of PM_{10} at this locality (Landlová et al., part I). This is consistent with the concentrations of PAHs (Landlová et al., part I). PAHs can also be strongly associated with organic matter in PM (Fernández et al., 2002). However, the amount of organic matter in fraction A was not significantly greater than that in the other PM fractions from the same locality (Landlová et al., part I). Thus, the A fraction from the locality near the quarry has unique characteristics that were not described by the mineralogical characteristics measured.

AhR-mediated activity of persistent compounds (popTEQ) was responsible for 3–8% of the concentration of bioTEQ at localities II, III and V; 18 and 12% at sites I and VI, respectively, and 32% at locality IV. For more details see the Supporting Information. The greater proportion of popTEQ in samples from locality I might be explained by the fact that the cement kiln incinerated also fuel containing chlorinated compounds that could contribute to bioTEQ at this locality. However, the absolute values are less than in the case of samples from localities IV, V and VI. At locality VI, the mean contribution of POPs to bioTEQ was elevated only in the A size fraction, which had a relatively great popTEQ concentration that was responsible for 71% of bioTEQ. The rest of the PM fractions had TEQ concentrations that were similar to those of other localities where the popTEQ contribution was less (see Supporting Info). The relatively great popTEQ concentration in the PM fraction at this locality probably originated from industrial sources. The primary presumptive source of pollution at locality IV was traffic but it is not likely that traffic could produce such large amounts of dioxin-like POPs. The absolute concentrations of popTEQ at locality IV were similar to those at locality V but their relative contribution was more significant due to the fact that the overall bioTEQ concentrations were about four times less at locality IV. It is likely, at least at locality V, that persistent compounds originated from local heating because this locality is situated in a rural area without significant sources of pollution besides heating with coal. Moreover, occasional burning of household wastes along with coal occurred in the village and thus a significant amount of dioxins and other dioxin-like compounds could be generated (Gullett et al., 2001). Although locality IV is situated in the city and most households have gas heating, it might be possible that waste burning could contribute to the type of pollution as in the case of locality V.

AhR-mediated responses in the trans-activation assay were correlated with concentrations of PAHs (both sum of 16 PAHs and sum of 8 genotoxic PAHs), total organic carbon content in PM and less distinctively with calculated specific surface area of PM (Fig 2). However, the toxic equivalents calculated from PAHs levels (pahTEQ; see Supporting Info Table S1) contributed on average 17% and 3−6% of overall bioTEQ in PM extracts at site IV and the rest of the localities, respectively (Table S2). The relative contributions of PAHs are generally similar to those in the study of Wenger et al. (2009a) or less than those observed in other studies (Brown et al., 2005; Cavanagh et al., 2009; Novák et al., 2013). The rest of the TEQ concentrations could be attributed to both non-assessed PAH representatives or, more likely, PAH derivatives such as polycyclic aromatic ketones and quinones present in ambient air that have been shown to possess dioxin-like potency (Bekki et al., 2009; Misaki et al., 2007).

3.2. Antiestrogenic activity assessment of PM extracts

Xenoestrogenicity, i.e. affecting of estrogen receptor by xenobiotics, is one of the best described modes of action of endocrine disruptors and

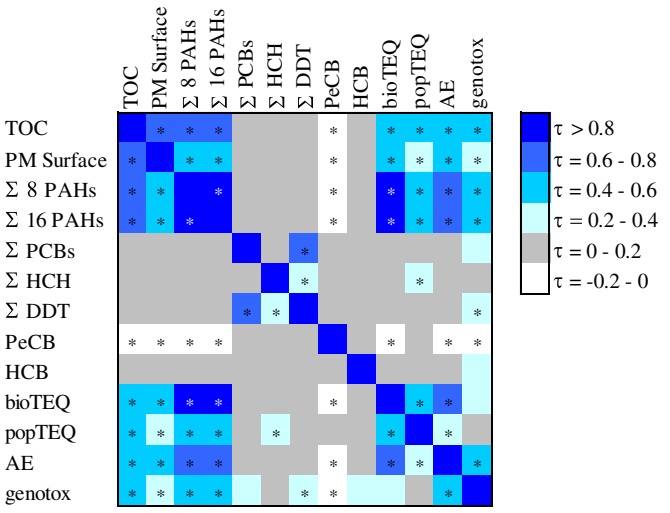

Fig. 2. Correlation of assessed parameters in PM (Kendall tau; significant values are labeled with asterisk); TOC — total organic carbon, PM surface — calculated PM surface area, 8 PAHs — Σ 8 carcinogenic PAHs, 16 PAHs — Σ US EPA indicator PAHs, Σ PCBs — polychlorinated biphenyls, Σ HCH — hexachlorcyclohexan, PeCB — pentachlorbenzen, HCB — hexachlorbenzen, bioTEQ — total AhR-mediated activity, popTEQ — AhR-mediated activity of persistent compounds, AE — antiestrogenic index, genotox — genotoxic potential.

there is general agreement that defects in estrogenic signaling are important in mediating reproductive and developmental toxicity and carcinogenesis (Borgert et al., 2003; Combes, 2000). While weak estrogenic response was observed in three fractions of the largest air particulates (A, B, C) from locality I (see Fig S1 in the Supplementary Information) the other extracts of air particulates displayed antiestrogenic or no effect (Fig 1B, C). Some previous studies reported estrogenic effects associated with air PM (Clemons et al., 1998; Wang et al., 2004; Klein et al., 2006; Wenger et al., 2009b) and the gas phase of ambient air (Klein et al., 2006). On the other hand, antiestrogenic properties of PM and gas phase air extracts were observed in two different regions in our previous study (Novák et al., 2009). This discrepancy was probably caused by the fact that, due to different characteristics of pollution sources, the composition of environmental pollutant mixtures is site-specific. Some air pollutants such as some PAHs are estrogenic (Villeneuve et al., 2002), while other PAHs or compounds in diesel exhausts, are reported to be antiestrogenic (Arcaro et al., 1999; Okamura et al., 2002). The overall activity of the mixture depends on ratios of the constituents. Thus, although there might be some differences in the bioassay conditions in our study and those of previous studies (Novák et al., 2009), the difference in observed effects is probably due to the specific composition of air pollutant mixtures in the region studied here. This hypothesis could be supported by the fact that PM extracts from locality I was ambiguous. While extracts of the coarser PM fraction elicited estrogenic effects, extracts of the finer fractions were antiestrogenic. This result suggests that PM size fractions from the same locality could contain mixtures of organic compounds that possess different toxicological characteristics. Effects of samples III, IV, V, VI, when expressed as an antiestrogenicity index (AE, inverse value of IC_{25}) in gravimetric mode, were significantly correlated with bioTEQ, sum of PAHs, total organic content and specific surface of the PM (Fig 2). These correlations are consistent with PAHs, and dioxin-like and antiestrogenic compounds concur in PM samples. This result is also consistent with specific interactions i.e. antiestrogenic effect of dioxin-like compounds via AhR-dependent mechanism as has been described previously (Okamura et al., 2002; Ueng et al., 2004). The link between dioxin-like activity and antiestrogenicity has been thoroughly described by mechanistic studies reviewed by Safe and Wormke (2003).

3.3. Genotoxicity assessment of PM extracts

Genotoxicity is a well-documented effect of air pollutants both *in vivo* and *in vitro* (Lewtas, 2007). It is known to be closely related to oxidative properties of PM that are often associated with metal content (Wessels et al., 2010). Thus the organic air sample extracts used in our study do not describe overall genotoxic potential of the ambient air and they indicate mainly genotoxic potential of organic pollutants within the samples. To minimize consumption of samples microplate modification of SOS chromotest has been used. The study focused only on direct genotoxicity (without metabolic activation). Genotoxic effects were produced by PM extracts from localities II–VI (Fig 1E, F). The most genotoxic samples came from locality IV. Again, the greatest effects were produced mainly by compounds present in the small size fractions of air particulates. This is consistent with the studies on genotoxicity of size fractions of PM_{10} (Buschini et al., 2001; Čupr et al., 2013; Funasaka et al., 2003). On the other hand, in total suspended particles, Škarek et al. (2007b) observed greater genotoxic potency associated with fractions with particle diameters greater than $PM_{2.5}$. Interestingly, there was a decrease of genotoxicity of F fraction at localities V and VI. A similar trend has been described previously and it has been proposed that it is caused by lower levels of POPs in the finest PM fraction (Čupr et al., 2013; Topinka et al., 2013). However, the dioxin-like potential profile does not support this hypothesis with our samples (Fig 1). Genotoxic potency was correlated with antiestrogenicity, the concentration of the sum of PAHs and DDT as well as total organic carbon (Fig 2). This is in agreement with data from the literature because many PAHs are known for their genotoxic properties (Topinka et al., 2013) and it has been described that exposure to DDT, which interferes with estrogenic signaling, is associated also with genotoxic effects in mussels as well as in mammals *in vivo* (Binelli et al., 2008; Canales-Aguirre et al., 2011).

3.4. Anti-androgenicity of PM extracts

Anti-androgenicity has been shown to play a role in mediating sexual disorders caused by xenobiotics in males (Sultan et al., 2001). Previously, antiandrogenic effects had been associated with some mixtures of air pollutants such as diesel exhausts (Okamura et al., 2004; Taneda et al., 2004). In our previous work, we have shown anti-androgenic properties of extracts from PM_{10} samples (Novák et al., 2009, 2013). In the present study, PM-associated antiandrogenic effects were produced only by extracts from the largest diameter particulates (A) and finest (F) fractions from locality I with 0.14 and 0.12 of volumetric antiandrogenic index $[(m^3/ml)^{-1}]$, respectively. Some compounds in diesel emissions, such as 3-methyl-4-nitrophenol, have been described as antiandrogenic (Li et al., 2006; Owens et al., 2006) and the cement kiln close to locality I also burns fuel oils and other low quality fuels, so it might be possible that the antiandrogenic activity could be due to compounds from the kiln emissions. Alternatively, the burning processes in the kiln are carefully controlled so there should be a minimum of un-burned compounds in the exhaust.

3.5. Gas fraction effects

Most previous studies concerned with *in vitro* effects have focused on compounds associated with PM in air. However, there have been some studies in which a considerable portion of the observed biologically active compounds can be present also in the gas phase (Klein et al., 2006; Novák et al., 2013, 2009). In this study similar results were observed for all four bioassays. Significant activity was observed in the extracts of the gaseous fraction (G) from most localities.

AhR-active chemicals were detected in extracts of the gas fraction from all localities, however only extracts from localities II, IV, V and VI produced enough activity to describe an EC_{25} and quantify the concentrations of bioTEQ (Fig 1B). AhR-mediated activity was produced mainly by non-persistent compounds because popTEQ concentrations accounted for less than 4% of the concentration of bioTEQ at localities II, V, VI. The greatest contribution from POPs was 17% at locality IV which is equivalent to concentrations observed in the PM fractions. Alternatively, when comparing the calculated contribution of PAHs (pahTEQ) to the total concentration of bioTEQ, the situation is not significantly different from that in PM fractions and the assessed PAHs did not account for more than 4% of bioTEQ (Supporting Info). Similar results have been reported previously (Novák et al., 2013). This indicates that also in the gas phase routinely assessed PAHs do not account for most of the AhR-mediated activity and other non-persistent chemicals such as their derivatives are involved.

Anti-estrogenic effects have been produced by gas fraction extracts from all localities and the activities were comparable to or greater than the effects of the PM fraction extracts. The data are comparable with antiestrogenicity of the gas phase extracts from the industrial region in our previous study (Novák et al., 2009). Greater effects were observed in the samples from localities V and VI where they could be increased by compounds from combustion sources that were probably less significant in the studied regions in the previous work.

Significant genotoxic effects were produced by the gas phase fraction extracts from localities III, IV, V and VI (Fig 1E, F). The greatest genotoxic potential was observed in the air from locality IV while the others contained less than half as much genotoxic activity. This was probably due to the intensive traffic at locality IV, which was presumably the principal source of pollutants there and traffic has been suggested as an important source of genotoxic compounds before (De Kok et al., 2006; Škarek et al., 2007a). However, direct comparison of the data is not possible because the previous studies do not provide any absolute genotoxicity units (Du Four et al., 2005; Škarek et al., 2007a,b).

Antiandrogenicity was almost exclusively associated with gas phase fractions of samples and it was detected in samples from all six localities (0.28; 0.58; 2.3; 0.19; 0.49 and 1.8 IC_{25}^{-1} $[m^3/ml]^{-1}$, respectively). The detected potentials seem to be less than those reported in previously studied industrial regions (Novák et al., 2009) but similar to potentials of air from Banja Luka that were assessed with mammalian cell-based bioassay (Novák et al., 2013).

3.6. Implications for air quality assessment

The present data describe toxic potential of ambient air pollutants and so they cannot be directly applied to a situation *in vivo*, because they do not cover important processes such as metabolisation or differences in bioavailability among the fractions in the study (Elad et al., 2008), which would play important roles *in vivo*. However, the results indicate that there can be significant differences in toxicological characteristics of PM from different pollution sources (Grahame and Schlesinger, 2007) and also in different size fractions of PM. The observed biological effects were mostly in the fine and ultrafine fractions. Although it does not have to be a common rule for other toxic effects such as endotoxins that were described to be associated mostly with the coarse fractions of PM (Traversi et al., 2011) or oxidant capacity and toxicity that was shown to be connected to the pollution source rather than to the PM size (Wessels et al., 2010). While in the US ambient air quality standards focus mainly on $PM_{2.5}$ levels assessment (US-EPA, 2007), EU guidelines for assessment of air pollution focus so far on evaluation of daily average of PM_{10} exposure (EU-Commission, 1999) without considering the distribution among size-spectra of PM including ultrafine fraction of particles that seems to be the most harmful (Araujo and Nel, 2009; Sioutas et al., 2005). In this study, PM_1 accounted for more than 90% of the dioxin-like equivalents of PM_{10} at all localities besides the stone quarry (II) and the industrial locality (VI) and even there PM_1 accounted for more than 75% of the bioTEQ of all PM extracts (Fig 3). Greater contribution of the finer fractions was also observed in the results of the other bioassays. Moreover, the fine and ultrafine PM are the most effective in penetrating into the respiratory and circulatory tracts (Lippmann et al.,

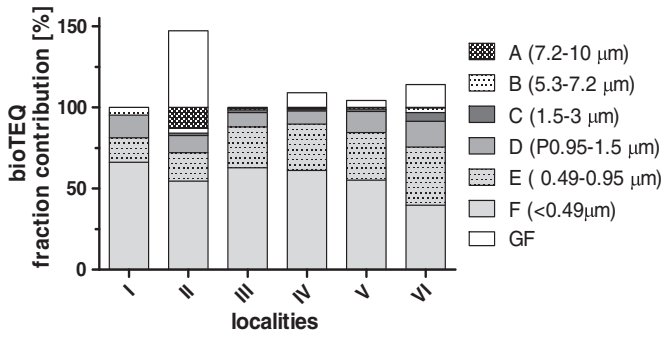

Fig. 3. Percentage contribution of PM size fractions to overall assessed toxicity equivalent of TCDD (bioTEQ) of PM_{10} from localities I–VI with bioTEQ of gas phase; PM — particulate matter size fractions [μm]; GF — gas phase fraction.

1980; Polichetti et al., 2009). Thus, evaluation of air pollution should not be limited just to gravimetric assessment of PM_{10} but should consider also the size spectrum of more dangerous fine and ultrafine PM fractions as well as results from bio-analytical methods to integrate toxic potencies of samples including unidentified as well as identified chemicals. Since a significant portion of toxic pollutants was present in the gas phase (Fig 1B, D, F) with 50% of bioTEQ of fraction G approximately half as great as that of PM_{10} at locality II (Fig 3) more attention should be given to the gaseous phase. Thus, fraction G could be also involved in mediating toxic effects of ambient air pollutants and should be also taken into account in ambient air contamination evaluation.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2013.10.013.

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Příloha 21

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Dispersion modeling of selected PAHs in urban air: A new approach combining dispersion model with GIS and passive air sampling

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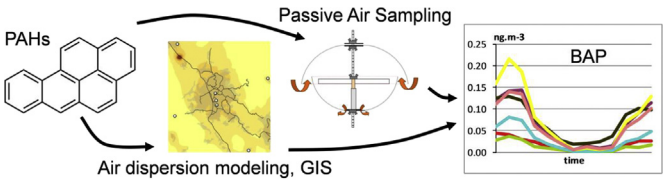
HIGHLIGHTS

- Passive air sampling data and dispersion modeling gives insight into PAH sources.
- The study identified probable presence of unquantified sources of PAHs.
- Combined GIS and dispersion modeling is promising for future air quality assessment.

GRAPHICAL ABSTRACT



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ABSTRACT

This study introduces a new combined air concentration measurement and modeling approach that we propose can be useful in medium and long term air quality assessment. A dispersion study was carried out for four high molecular weight polycyclic aromatic hydrocarbons (PAHs) in an urban area with industrial, traffic and domestic heating sources. A geographic information system (GIS) was used both for processing of input data as well as visualization of the modeling results. The outcomes of the dispersion model were compared to the results of passive air sampling (PAS). Despite discrepancies between measured and modeled concentrations, an approach combining the two techniques is promising for future air quality assessment. Differences between measured and modeled concentrations, in particular when measured values exceed the modeled concentrations, are indicative of undocumented, sporadic pollutant sources. Thus, these differences can also be useful for assessing and refining emission inventories.

1. Introduction

Methods for obtaining spatially-distributed air concentration information for persistent organic pollutants (POPs)/semi-volatile organic compounds (SVOCs) remain a key challenge within the field of environmental science, despite their ongoing development. However, knowledge of air concentrations of these compounds is important both for regulatory treaties and human health risk assessment. Both measurement and modeling are commonly used techniques, and have specific advantages and disadvantages depending on sources, compounds and study application.

Various passive air sampling (PAS) devices have been introduced and applied since late 1990s (Choi et al., 2007; Klanova et al., 2006; Santiago and Cayetano, 2007) as they are capable of sampling various SVOCs in a cost-effective and logistically easy manner allowing for a dense spatial sampling coverage. Interpretation of PAS results continuous to be, however, a major challenge. Theoretical sampling volumes enabling derivation of atmospheric concentrations of selected chemicals from their mass in PAS are usually based on the field intercalibration of active and passive air sampling devices (Harner et al., 2013; Hazrati and Harrad, 2007; Chaemfa et al., 2008; Melymuk et al., 2011). For the gas phase-associated chemicals, they can be further refined using depuration compounds (Persoon and Hornbuckle, 2009), which also account for

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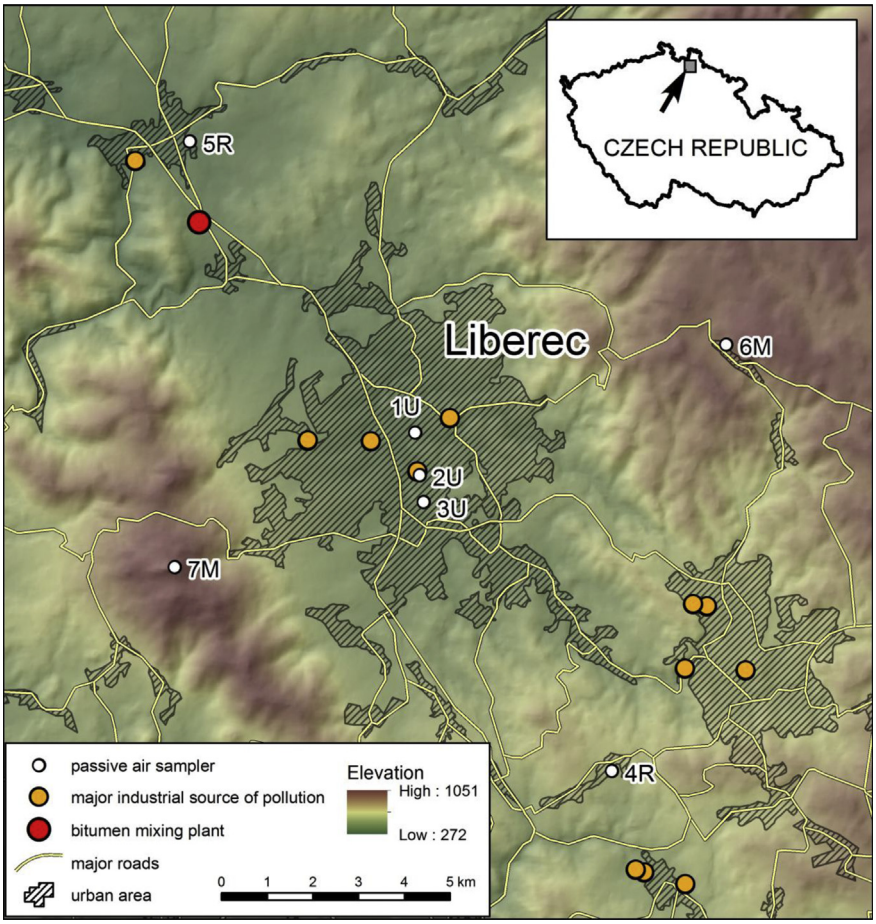

Fig. 1. Location of the PAS sites and major industrial sources in the study area.

the effects of meteorological conditions. However, results for chemical compounds associated with atmospheric particles are more difficult to interpret. Their gas-particle partitioning is a temperature-dependent process affecting their distribution among the atmospheric phases and thus, also the efficiency of their sampling (Klanova et al., 2008). Sampling of particulate matter cannot be described easily nor quantified by depuration compounds, and thus we rely on experimental data to estimate the efficiency of particle sampling.

Modeling tools are also frequently used in characterizing concentrations of SVOCs, particularly fugacity-based multimedia models (Csiszar et al., 2013; Mackay, 2001) and atmospheric dispersion models (Hsu et al., 2003; Morselli et al., 2012; Tao et al., 2006). A recent development is the use of PAS data in the evaluation of modeling results (Csiszar et al., 2014, 2013; Estellano et al., 2014; Halse et al., 2011), as in this study. However, the majority of current models do not include a spatial analysis component or ability for adequate visualization and spatial interpretation of results. Employing spatial tools can improve confidence and clarity of modeling results. This can be either in the form of built-in spatial functions or through incorporation with geographic information systems (GIS).

The use of GIS in air pollution modeling and assessment has seen significant growth in last decade. GIS alone can be useful in air quality assessment as studies by e.g. (Guo et al., 2007; Shad et al., 2009) show. It has also been used to make dispersion modeling easier and more precise (Briggs et al., 2010; Gulliver and Briggs, 2011). Nevertheless, despite its utility, its application is still seeing limited use because of lack of compatibility between these two types of software. However, use of GIS functions is becoming more common in the last decade, as popular dispersion models incorporate GIS extensions to ease both data input and results visualization, or offer additional software tools enabling users to take advantage of GIS functions.

This paper reports on a novel application of a dispersion model supported by GIS analysis to atmospheric polycyclic aromatic hydrocarbons (PAHs). Four PAHs, benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and indeno(123cd)pyrene (BAP, BBF, BKF and IPY, respectively) were selected as the compounds of interest as they are associated with significant negative health effects, particularly carcinogenicity; BAP is identified as a known carcinogen, while BBF, BKF, and IPY are classified as possible carcinogens (IARC, 2013), and all are largely particle-bound at ambient temperatures, which can lead to more complex environmental behavior. Additionally, these four compounds had both measured concentrations and emission inventories available, allowing for comparison of model results with measured data. The dispersion model was used to predict the atmospheric concentrations of these PAHs, and GIS were used to process source and elevation input data, create a receptor grid, and cartographically visualize model results. These results were then verified using data from a passive air sampling network (Dušek et al., 2010).

2. Methods

2.1. Study area

This case study was carried out in the area of Liberec (in northern Czech Republic, close to the German and Polish borders)

(Fig. 1). The city of approximately 100,000 inhabitants is situated in a basin elevated between 330 and 500 m ASL, protected by two mountain ranges reaching 1000 m from southwest and northeast. Lower natural barriers enclose the city from southeast and northwest. These orographic conditions suggest that local pollution sources (including a waste incineration plant) are expected to have a significant impact in this area.

2.2. Dispersion model

The applied SYMOS'97 (Stationary Sources Modeling System) model is a regional Gaussian dispersion model predicting atmospheric concentrations of pollutants to a distance of up to 100 km from sources (Bubnik et al., 1998). As Czech law requires the use of this model for air quality assessment, it is used by state institutions, environmental agencies, and private companies. However, the model has some drawbacks, such as an inability to predict dispersion under inversions and calm wind conditions, and poor incorporation of the physicochemical properties of modeled substances. On the other hand, the model does not limit the number of input emission sources or receptors. Four types of input data are needed in order to perform a model run: (a) terrain elevation, (b) meteorological data, (c) emission data, and (d) receptors; all are discussed further below.

2.2.1. Terrain elevation data

Terrain elevation data are important for modeling the influence of natural topography and modifying the emission dispersion direction.

The SYMOS'97 dispersion model only allows for insertion of a regular rectangular grid of terrain elevation data. In this study the spatial resolution of the terrain elevation grid was 150 m, which was sufficient to prevent smoothing of the terrain; a denser grid would have negatively affected the computation duration.

2.2.2. Meteorological data

The only meteorological data required for SYMOS'97 are wind speed and direction. These have to be entered in a specific form, classified according to five stability classes according to the classification of Bubnik and Koldovsky (1974). Each stability class is divided into three wind speed classes with average wind speeds of 1.7, 5.0 and 11 m s^{-1}. For this study, meteorological data were provided by the Czech Hydrometeorological Institute (CHMI) in a form of 13 wind roses for an elevation of 10 m above ground and covering the sampling periods of the passive air samplers (Table S1).

2.2.3. Emission data

Emission data (year 2006) for industrial and local heating sources were provided by CHMI, Department of Air Quality Protection. Their *Registry of Emissions and Air Pollution Sources* (CHMI, 1996) provides emission data of selected pollutants for the following air pollution source categories in the Czech Republic: small, medium and large stationary sources and mobile sources. For medium (e.g. small enterprises) and large (industry) sources, additional parameters such as smokestack height, diameter and emission speed are included. All pollution sources were localized using geographic coordinates (Fig. S1). Emission data were further processed to obtain emission totals coinciding with the PAS sampling intervals (Table S1). Emission data from sources outside of the Czech Republic were not available.

Emissions from domestic heating sources (i.e. small sources in the Registry) were provided in the form of individual fuel type use per village/city per year. Emission factors (CHMI, 1996) were then used to convert the amounts of fuel to emitted pollutant quantities. The annual fuel use totals were then apportioned to individual time periods based on the daily mean air temperatures (using temperature records obtained from the CHMI) thus accounting for the heating season. The Corine Land cover database (European Environmental Agency, 2010) was used to map urban areas, and small villages were added by manual vectorization. As the model requires data on non-point sources, such as settlement areas, in the form of a grid, all polygons were converted to grids with a resolution of 250 m (Fig. S2). The total emissions for each settlement were divided by the number of corresponding grid cells in order to spread the emissions evenly over the entire area of each city/village.

As the emission data for mobile (linear) sources from the Czech *Registry of Emissions and Air Pollution Sources* are not of sufficient resolution, an alternative approach was developed using various other data sources. As a first step, all roads were vectorized using the GIS (Fig. S3). Traffic volumes were obtained from the 2005 major road traffic census of the Road and Motorway Directorate of the Czech Republic (RSD, 2006). Using these figures, fuel consumption was calculated for each type of road and vehicle (Pisa et al., 2001). Fuel-specific emission factors (Ntziachristos and Samaras, 2000) were used to convert the amounts of fuel consumed to emissions per length of each road. Similar to the non-point pollution sources, the linear/mobile sources were input into the model in the form of points placed in a line (Fig. S4). The distance between two adjacent points was set to 100 m, where each point represented a specific emission flux equivalent to the amount of fuel burned on that road segment. Use of GIS was crucial for all these steps.

2.2.4. Receptors

The last crucial input for the SYMOS'97 dispersion model was a receptor point grid. These points did not affect the results of calculation, but rather served to store the results. Sufficient density of the grid and appropriate point placement was essential for proper visualization of the model results. Unlike the terrain elevation grid, receptor points can also be embedded into the model as an irregular grid. This option was used in cases where higher spatial resolution was required, such as in heavily populated areas, near major PAH sources, or in zones parallel to roads (Fig. S5). Also, to avoid relying on spatial interpolation when model results were compared to field monitoring data, receptors were also placed directly on the seven passive air sampling sites located in the study area (Fig. 1).

As traffic emissions are released close to ground level, reduced dispersion conditions and greater harmful effects are to be expected (Tomlin et al., 2009). Thus, a simple model enabling generation of receptor points along roads and at a constant distance from roads was created using the ArcGIS Model builder. This is in contrast to simply generating the receptor points by converting the boundaries of buffers created along linear features into points. This approach eliminates variations in distances between receptors and linear sources. A similar approach to modeling atmospheric concentrations along linear sources has been incorporated in some other models (Banerjee et al., 2011; Ganguly et al., 2008). A model schematic is shown in Fig. S6 and examples of both approaches are given in Fig. S7.

2.3. Modeled data visualization

The output of each dispersion model run was an MS Excel file containing almost 12,000 receptors, which were then processed using ArcGIS software. Ordinary kriging (Krige, 1951) spatial interpolation was used to map modeled values, using different color shades to represent predicted pollutant concentrations (Fig. 3).

2.4. Passive air sampling data

Passive air samplers were deployed for 13 consecutive 28-day periods at seven sampling sites in the centre and vicinity of Liberec, Czech Republic. Samplers were deployed from December 2005 to December 2006. Three of them were situated in the inner city (1U, 2U, 3U), two in rural areas (4R, 5R), and two in the neighboring mountains (6M, 7M) (Fig. 1 and Fig. S1). All sampling sites belong to the RECETOX long-term MONET air quality monitoring network (Dušek et al., 2010). The atmospheric concentrations of the selected PAHs (BAP, BBF, BKF and IPY) were calculated by adapting the experimental values of the sampling rates of gas-phase compounds. For this type of PAS, the gas-phase sampling rate is 7 m^3/day over a 28 day sampling periods, and the sampling efficiency of particle-associated chemicals is estimated to be an order of magnitude lower (Klanova et al., 2008). Thus, assuming that the selected high-molecular weight PAHs were mostly sorbed to particles during the whole experimental period, we used a PAS sampling rate of 0.7 m^3/day to estimate their atmospheric concentrations. However, it should be noted that gas-particle partitioning is strongly temperature dependent, and the possible implications on results of the study are further discussed in section 3.2.

3. Results and discussion

3.1. Modeled concentrations

The results generated by the SYMOS'97 dispersion model show smooth seasonal trends, with higher values during winter and low values during summer (Fig. 2, Figs. S8 and S9) caused by the absence of domestic heating sources in summer and better conditions for pollutant dispersion (Vecchi et al., 2004). The highest concentrations of all PAHs were predicted at the urban sites near the Liberec city centre (U1, U2, U3), likely as a result of higher density of local heating as well as traffic sources. High concentrations of BAP, BBF and BKF were also predicted at the 5R sampling site, near the largest regional industrial source of PAHs (a bitumen mixing plant near Liberec, at 1850 m from the 5R site, Fig. S10). IPY did not have elevated concentrations at 5R because the plant is not a significant source of this compound.

For BAP, the only PAH addressed in air quality regulations, the modeled concentrations were always less than 0.25 ng m^{-3}, and well below the Czech and European regulatory limit of 1 ng m^{-3} (European Union, 2014; Chamber of Deputies of the Parliament of The Czech Republic (2012)) at all sampling sites and over the whole study duration.

The modeled concentrations had a strong negative relationship with both elevation and settlement size, as can be observed at the rural (4R) and mountain (6M and 7M) sampling sites (7M is at the peak of Jested Mountain, 1000 m ASL). The lowest elevation site (4R) is at 520 m ASL and is situated in a large village, 6M is at 740 m ASL and in a small village, and there is almost no settlement on the peak (7M). All three sites have almost the same distance to the Liberec city centre (6 km) and a similar share of the winds blowing from the city centre (5–7%) during the winter season. However, concentrations of BAP at 7M are on average 1.67 times lower than at 6M and 2.32 times lower than at 4R, and distributions are similar for the other three PAHs. As there are no industrial sources that

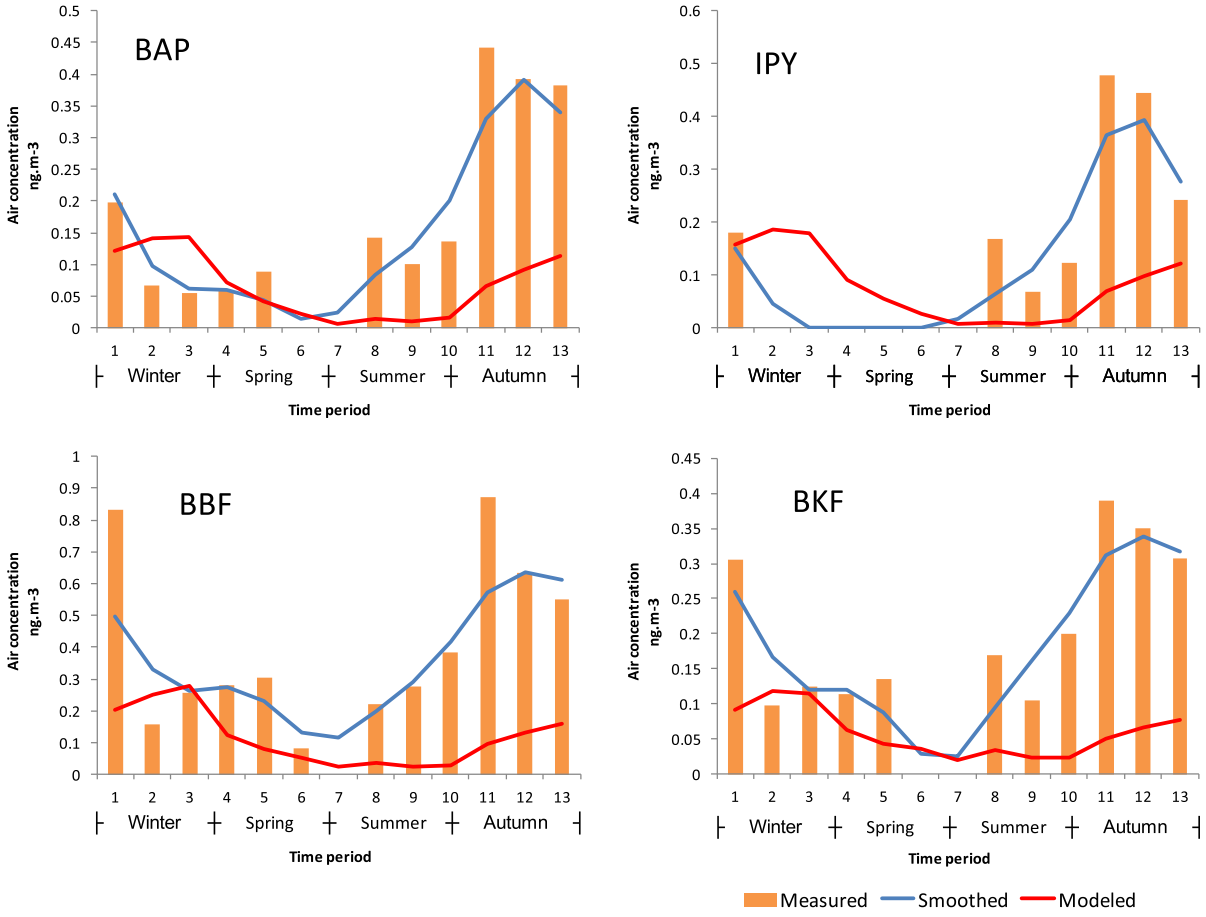

Fig. 2. Measured, smoothed measured (smoothed by weighted moving means of medians of adjacent values) and modeled concentrations of selected PAHs at one urban sampling site (1U).

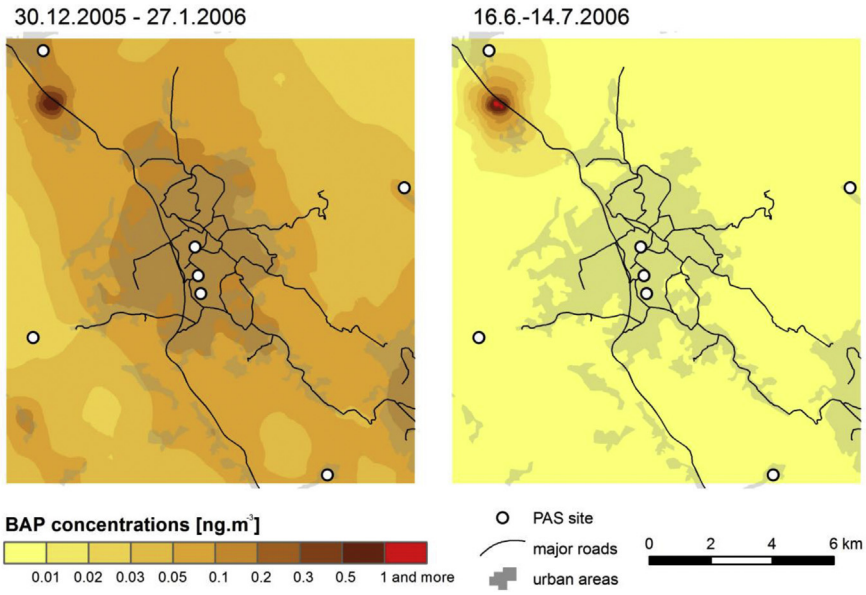

Fig. 3. Spatial distribution development of BAP concentrations during 1st (winter) and 7th (summer) sampling period, obtained by air dispersion modeling. Note the emissions from the bitumen mixing plant in northwest corner of the study area.

could be responsible for such differences in concentrations, it suggests that the differences are attributed to the combined effects of population density and elevation. Higher population density in an area is associated with increased local emissions (Hafner et al., 2005), and higher elevation is associated with lower concentrations of atmospheric particulates and higher dispersion (Whiteman, 2000), and thus, by extension, lower concentrations of particulate-bound PAHs. However, due to the correlation of settlement size and elevation we are not able to identify the relative significance of each contribution.

Based on the model results, more than 95% of the winter BAP emissions at all sampling sites except 5R originated from domestic heating. The remainder was from traffic and industrial emissions. However, at the site closest to the bitumen mixing plant (site 5R), 25% of winter emissions are attributed to the bitumen plant (Fig. S11), and an even higher fraction of the summer emissions. This is supported by the fact that at the 5R site almost 30% of winds are blowing from the direction of the plant both in winter and summer.

Modeled concentrations of BAP were very low during summer due to the lack of domestic heating sources. The highest summer levels were predicted at the 5R site due to the presence of the bitumen mixing plant. A stronger contribution of traffic emissions was predicted at the urban sites (1U, 2U and 3U). At the rural (4R) and the mountain sites (6M and 7M), predicted BAP levels were close to zero. This also confirms the hypothesis that local heating sources are the major factor controlling the atmospheric levels of BAP.

While none of the individual sampling sites had modeled concentrations exceeding 1 ng m^{-3}, the model did predict a concentration hot spot at the bitumen mixing plant where BAP levels exceeded the Czech regulatory air quality limit. The dispersion model predicted BAP concentrations exceeding this regulatory limit at this site in all time periods, with an annual average of 1.38 ng m^{-3} and a maximum predicted concentration of 2.05 ng m^{-3}. As demonstrated in Fig. 3 and S12, the impact of the bitumen mixing plant was constant throughout the year while local heating sources only contributed in winter. The estimated contribution of traffic sources was surprisingly small, between 0.6 and 2.8% in winter season and 4.0–84% in summer season due to absence of local heating sources. Very low summer BAP levels were predicted at the urban sites where traffic was the only summer source (Figs. S11, S13).

3.2. Measured data

Measured PAHs air concentrations showed expected seasonal trends; winter concentrations were highest, while summer concentrations were often below detection limits (Fig. 2 and S9, S14). However, overlying the general seasonal trend, there was also significant variability between sequential samples. For example, in the eighth sampling interval (July 14–August 11) at the 4R site concentrations of all studied pollutants were 18 times higher than in seventh and ninth sampling periods, while in the eleventh sampling interval (October 6–November 3) at the 2U site concentrations were three times higher than in tenth and twelfth sampling periods.

The causes of the elevated concentrations in individual months are uncertain. Concentrations in the eighth sampling period were elevated at all sites, but the highest levels were measured at the rural site (4R), situated in the southeastern part of the region. The wind conditions at this site and in this sampling period were similar to other sampling periods, and thus do not provide an adequate explanation for the elevated concentrations; therefore the most probable causes are incidental sources of pollution (e.g. biomass burning, barbecuing, forest fires) (Lemieux et al., 2004) which are not included in the emission inventory. In cases such as the eighth sampling period, when elevated concentrations were observed across the whole study region (Fig. S14), this is suggestive of a larger regional unquantified source, such as forest fire; in fact, Liberec and surrounding regions of Czech Republic, Poland and Germany had 440 ha burned by forest fire in 2006 (JRC, 2012). Furthermore, in Czech Republic, Poland and Germany, the peak of the 2006 fire season was in July (JRC-IES, 2008, 2007). Elevated levels of PAHs in individual months could also be related to temperature and precipitation conditions. The elevated PAH concentrations measured during the eighth sampling period coincided with very hot and dry weather compared to the long-term summer

average, increasing volatilization and limiting wash-out/rain-out, while the following sampling period was relatively cold and very rainy. Additionally, the PAS themselves can be biased by meteorological conditions: PAHs shift towards the gas phase at higher temperatures, and thereby experience higher PAS sampling rates during the hot seasons (Klanova et al., 2008). Therefore, derivation of PAS air concentrations from a simple flat rate model could result in overestimation of the atmospheric concentrations of these compounds in warm periods. The likely explanation for the anomalous high concentrations in the eighth sampling period is a combination of sampling artifacts, meteorology and unquantified regional sources.

The autumn extreme was most significant at the Liberec city centre site (2U), close to the waste incineration plant (Fig. S14). Interestingly, elevated PAH concentrations were not observed at the other two urban sites (1U and 3U), despite these sites being in close proximity (940 m north and 590 m south of 2U, respectively), suggesting a relatively localized source. Although a change in prevailing wind direction observed during this sampling interval (34% coming from south and only 6% coming from northwest) can partially explain some of the concentration variability, it cannot explain very different levels measured at sites in close proximity. The spatial variability is most likely the result of unregistered local sources of PAHs, such as domestic biomass burning.

3.3. Comparison between modeled and measured data

Measured PAH concentrations were generally higher and more variable than those predicted by dispersion model (Fig. 2 and S9). Correlations between measured and modeled data (Table 1) demonstrated that for the full yearly data, the relationships were weak, with a maximum of $R = 0.326$ ($p = 0.278$). This is attributed to presence of too many values below the detection limit during winter and spring. To exclude this influence, we examined the correlation for only summer and autumn. This gave much higher values, up to $R = 0.980$ at maximum. However, the lowest correlations were for sites 2U and mainly 4R, due to occurrence of elevated concentrations in individual months (as discussed in Section 3.2). If excluding these two sites, the mean correlation coefficient between measured and modeled data for all studied substances in summer and autumn season is 0.772, p value is 0.104.

Discrepancies between measured and modeled data are attributed to (1) weaknesses in the dispersion model, (2) uncertainties in quantification of the air concentrations, and (3) the impact of local sources not included in the emission inventory.

SYMOS'97, as a simple Gaussian model, cannot reflect the impact of complex meteorological conditions that affect the dispersion of pollutants. In addition, it is not very sensitive to the various physicochemical properties of chemical compounds. This model is also not recommended for modeling concentrations in street canyons and in complex terrain during inversions and calm periods (Bubnik et al., 1998). More advanced Gaussian models have been improved with tools enabling prediction of chemical transformations of pollutants and their atmospheric scavenging, as well as modeling the air flow over complex terrains. Nevertheless, the use of Lagrangian and Eulerian models appears to be better. These models often enable modeling of multiple substances with different physicochemical properties and perform more successfully with respect to simulation of atmospheric conditions and air flow affecting pollutant dispersion. On the other hand, the use of more complex models usually requires higher computing capacity as well as detailed meteorological data, which is not always available. Thus, appropriate choice of a model requires consideration of available data, properties of the compounds to be modeled, and the requirements of the intended output in terms of temporal and spatial resolution. In this study, the large discrepancy between the measured and modeled data suggests SYMOS'97 may not be appropriate to model particle-bound PAHs. While SYMOS'97 has been shown to effectively model contaminant plumes and volatile air contaminants on small scales (Keder, 2008; Keder et al., 2005), the discrepancies observed here suggest that more information is needed to determine to model contaminants with multiple diffuse non-point sources and complex partitioning behavior.

Therefore further research is recommended to apply more sophisticated air quality models on this type of data.

The second potential source of discrepancies between modeled and measured values is the estimation of air concentrations using passive air samplers, as described in Section 3.2. In recent years, several methodologies for estimation of the sampling rates have been used, and work on improving the characterization of PUF-PAS (especially for particle-bound compounds) is continuing. Incorporation of the influence of meteorological parameters (temperature and wind speed) is crucial when developing such models as gas-particle partitioning is the most important process driving the sampling efficiency: for example, BAP is entirely particle bound at −6 °C but shifts to 10–20% gas phase at 22 °C, leading to higher PAS sampling rates (Klanova et al., 2008).

When interpreted with care, passive air sampling data can be extremely valuable in identifying sources not included in emission inventories (biomass burning, open-fire cooking), and assessing

Table 1
Correlation coefficients and p-values for comparison of measured and modeled data for whole year and summer + autumn (S + A) only for each site. Correlations that are statistically significant ($p < 0.05$) are in bold italics.

Site	Period	BAP		IPY		BBF		BKF	
		R	p	R	p	R	p	R	p
1U	Whole year	0.276	0.361	0.026	0.932	0.247	0.415	0.230	0.450
	S + A	***0.889***	***0.018***	0.670	0.146	0.665	0.150	0.769	0.074
2U	Whole year	−0.026	0.933	−0.065	0.833	0.125	0.685	0.013	0.965
	S + A	0.357	0.487	0.368	0.473	0.356	0.488	0.403	0.428
3U	Whole year	0.111	0.718	0.018	0.953	0.236	0.438	0.236	0.437
	S + A	0.582	0.226	0.400	0.432	0.398	0.434	0.604	0.204
4R	Whole year	−0.322	0.283	−0.288	0.340	−0.329	0.272	−0.331	0.269
	S + A	−0.220	0.675	−0.156	0.768	−0.260	0.619	−0.155	0.769
5R	Whole year	0.021	0.946	−0.007	0.981	0.082	0.789	0.247	0.415
	S + A	***0.836***	***0.038***	***0.898***	***0.015***	***0.931***	***0.007***	0.661	0.153
6M	Whole year	0.161	0.599	0.284	0.347	0.326	0.278	0.111	0.717
	S + A	***0.970***	***0.001***	***0.938***	***0.006***	***0.910***	***0.012***	***0.908***	***0.012***
7M	Whole year	−0.075	0.807	0.110	0.720	0.062	0.842	−0.014	0.964
	S + A	0.769	0.074	***0.913***	***0.011***	***0.944***	***0.005***	0.785	0.065

their impact on local and regional air quality. These sources include not only incidental combustion sources (other than domestic heating sources) but also various secondary pollution sources, including releases from historically contaminated areas. It is, however, very important to select an appropriate time resolution of passive sampling for the specific source types. Passive air sampling periods vary between sampling networks. XAD samplers have a high capacity and can be deployed over a period of one year (Barthel et al., 2012; Hayward et al., 2010), providing annually-averaged data which are useful for assessment of long-term time trends. Conversely, PUF-based PAS are typically deployed in 3-month intervals, which can provide information on the seasonality of data, and winter and summer extremes (Gouin et al., 2005; Melymuk et al., 2012; Motelay-Massei et al., 2005) It is still rather difficult to interpret seasonally-resolved data as a function of meteorological parameters. Monthly deployment is an option when assessing chemical compounds with variable but sufficiently high levels in the atmosphere. Additionally, combinations of passive sampling devices can be used for short-term surveys. Passive samplers provide a strong advantage in areas with high spatial heterogeneity in air concentrations, e.g., urban and industrial areas, because they allow dense spatial coverage which can strengthen the application of air quality models in such challenging locations.

The use of atmospheric modeling techniques can provide additional insight into the specific fate and transport processes governing atmospheric release and distribution of pollutants. Well-designed case studies in combination with advanced dispersion models can generate new knowledge, filling the gaps in our current understanding of the fate and impact of chemicals released from various primary and secondary sources. Modeling techniques can also provide greater insight into emission inventories, by identifying locations influenced by undocumented sources, e.g., forest fires in the current study. Additionally, the incorporation of GIS in data pre-processing and characterization of spatial source distributions improves the accuracy of model inputs and speeds analysis and interpretation, as was used in this study. GIS tools can also be helpful in sampling network design. When incorporating measured data into modeling frameworks, appropriate sampling design is especially important; for example, radial sampler placement is well suited to modeling large point sources and dispersion plumes, while regional assessment of non-point sources is better treated with gridded sampling. The use of GIS tools also brings greater information on factors such as orography, wind and land use, and provides opportunity to assess model performance relative to measured data under the influence of these conditions. This can be further useful in identifying regionally representative sampling locations.

4. Conclusions

A new approach to air quality monitoring and assessment was introduced in this study. A combination of air dispersion modeling, GIS and passive air sampling was applied on a one-year-period, where high molecular PAHs (benzo(a)pyrene, benzo(b)fluoranthene, benzo(k)fluoranthene and indeno(123cd)pyrene concentrations were addressed in the area of Liberec, Czech Republic. GIS was used to pre-process dispersion model input data, to process modeling outcomes and visualize them in a map. GIS was shown to be a useful tool, capable of improving the quality of pollution data by connecting them to the external data files such as the CORINE Land Cover database.

A combination of passive air sampling techniques with air dispersion modeling was shown to be a useful tool for addressing spatial and seasonal variability of atmospheric concentrations.

Certain discrepancies exist between the measured and modeled data. Gaps between modeled and measured concentrations are attributed to undocumented sources and model limitations. This, however, requires further investigation in follow-up studies focused on reduction of both measurement and modeling uncertainties. However, overall the results suggest that the combination of air dispersion modeling, GIS and passive air sampling has a great potential in the future of air pollution modeling and monitoring, and further, can be useful in human risk assessment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atmosenv.2014.07.002.

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Evaluation and guidelines for using polyurethane foam (PUF) passive air samplers in double-dome chambers to assess semi-volatile organic compounds (SVOCs) in non-industrial indoor environments†

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Indoor air pollution has been recognized as an important risk factor for human health, especially in areas where people tend to spend most of their time indoors. Many semi-volatile organic compounds (SVOCs) have primarily indoor sources and are present in orders of magnitude higher concentrations indoors than outdoors. Despite this, awareness of SVOCs in indoor air and assessment of the link between indoor concentrations and human health have lagged behind those of outdoor air. This is partially related to challenges associated with indoor sampling of SVOCs. Passive air samplers (PASs), which are widely accepted in established outdoor air monitoring networks, have been used to fill the knowledge gaps on indoor SVOCs distribution. However, their applicability for indoor environments and the assessment of human health risks lack sufficient experimental data. To address this issue, we performed an indoor calibration study of polyurethane foam (PUF) PAS deployed in a double-dome chamber, covering both legacy and new SVOC classes. PUF–PAS and a continuous low-volume active air sampler (AAS) were co-deployed for a calibration period of twelve weeks. Based on the results from this evaluation, PUF–PAS in a double-bowl chamber is recommended for indoor sampling and health risk assessment of gas phase SVOCs, including novel brominated flame retardants (nBFR) providing sufficient exposure time is applied. Data for particle associated SVOCs suffered from significant uncertainties caused by low level of detection and low precision in this study. A more open chamber design for indoor studies may allow for higher sampling rates (R_S) and better performance for the particle associated SVOCs.

Environmental impact

This study presents recommendations and guidelines for using polyurethane foam (PUF) passive samplers (PAS) for monitoring semivolatile organic compounds (SVOCs) in non-industrial indoor environments. The results provide an in-depth evaluation of PUF–PAS performance for seven SVOC classes including, for the first time in a non-industrial indoor environment, novel brominated flame retardants (nBFRs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs). Potential users will find guidance for the choice of compounds, relevant exposure times, and sampling rates which can help to achieve more accurate application.

Introduction

Semi-volatile organic compounds (SVOCs) include a wide range of compounds with potential or proven negative impacts on human health. They are present in non-industrial indoor environments (*e.g.* residential and public buildings) either in active primary sources (including building materials and house appliances) or in temporary reservoirs acting as secondary sources.[1–3] The latter include indoor materials and appliances, originally not containing SVOCs, which over time adsorb SVOCs from the indoor air and re-emit them under certain conditions in the indoor environment. As a result, many SVOCs are found at higher concentrations indoors than outdoors.[4–6] This in combination with the indoor lifestyle of most urban citizens make inhalation of indoor air a relevant human exposure pathway for some SVOCs.[7,8]

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Indoor air, as a crucial medium for human risk assessment of SVOCs, has recently attracted growing attention of the scientists. However, there are still important knowledge gaps regarding the pattern of exposure, and contaminant fate and distribution indoors. Addressing those gaps requires overcoming inherent challenges associated with the sampling of SVOCs: (i) low concentrations (generally 1–3 orders of magnitude lower than many VOCs of regulatory interest),[2,5,9–11] (ii) partitioning behaviour (significant association with the atmospheric particles), and (iii) difficulties in performing appropriate calibration experiments under controlled conditions.

Active air sampling (AAS) techniques separating the gas and particle phases are recommended for quantification of human exposure since the air quality guidelines for some SVOC classes (*e.g.* PAHs) are based on particle associated compounds.[8] However, deployment of AAS indoors is associated with major limitations since they (i) are intrusive; (ii) are logistically demanding; (iii) can cause sampling artefacts (*e.g.* depletion of air concentrations), and (iv) can only provide data for short-term monitoring. These limitations have hampered the development of broad indoor monitoring programmes. On the other hand, passive air samplers (PAS) overtake many of these limitations by being cheap, easy to handle, and tolerable. They therefore have the potential for enabling large-scale indoor sampling campaigns.

PAS were initially developed to collect SVOCs from the gas phase only[12,13] but during the last few years, they have been increasingly used to report levels of particle associated SVOCs as well.[14,15] Whether they are capable of providing reliable and reproducible data on particle associated compounds remains a question,[16–19] since their effectiveness in collecting particles can be affected by many factors that have not yet been fully characterized. These include particle size, material composition, wind velocity, air humidity, and others. In fact, the uptake efficiency of particle associated compounds in non-industrial indoor environments is expected to be even lower than in outdoor environments due to lower air flows, lower sampling rates of PAS, and in some environments, lower concentrations of particulate matter (PM).[41] The most common PAS design for sampling of SVOCs, the stationary polyurethane foam (PUF) disk, has been proven to be suitable to assess spatial and temporal variability of SVOCs in outdoor environments.[14,20] It is used in global monitoring networks as well as in local and regional case studies. It has been increasingly used also for indoor monitoring of SVOCs[4,6,21,22] even though this application is critical due to a limited number of calibration studies as well as a limited number of SVOC classes included in previous calibration exercises. The indoor application is further complicated by the use of different types of chamber designs. Originally, an open chamber design was suggested for indoor environments in order to minimize restriction of low indoor air flows[6] while a more closed chamber design (*i.e.* the double-dome) was suggested for outdoor environments in order to reduce effects of high air flows, *etc.* Despite this, the closed chamber design has also been used in many indoor measurements.[3,21,23,25,42,43] There is therefore a clear demand for an in-depth evaluation of this kind of PUF–PAS as a valid tool for indoor monitoring and human exposure assessments of SVOCs.

In this study, we evaluated in parallel the indoor and outdoor[16] performance of PUF–PAS in the closed double-dome chamber design for both legacy and new SVOCs. For the first time in a non-industrial indoor environment, the evaluation included novel brominated flame retardants (nBFRs), polycyclic aromatic hydrocarbons (PAHs), and polychlorinated dibenzo-*p*-dioxins and furans (PCDDs/Fs). The evaluation of PUF–PAS was based on long-term comparison with co-deployed AAS where detection limits, precision, fingerprinting (their ability to reflect true composition of the contaminant mixture in air), and sampling rate (R_S) were considered. The aim was to provide guidance for the use of PUF–PAS for indoor sampling, *i.e.* for which SVOCs can PUF–PAS be applied indoors, which exposure time is appropriate for these compounds, and which R_S should be used when calculating the air concentrations.

Materials and methods

Sampling site

Passive and active air samplers were concurrently deployed indoors in a lecture room of the Research Centre for Toxic Compounds in the Environment (RECETOX), Masaryk University in Brno, Czech Republic. The room had a total volume of 150 m^3; was fully carpeted and contained chairs, tables, whiteboards, computers, and bookcases. Heating was provided by purely diffusive radiators, and air was circulated by natural ventilation. Temperature was constant around 20 °C throughout the experiment, and the air velocity was negligible. Overall, the environmental conditions in the room were constant.

Passive samplers

PUF disks; 15 cm diameter, 1.5 cm thickness, 424 cm^2 total surface area (A_{PUF}), 0.030 g cm^{-3} density (type T-3037 Molitan, a.s., Czech Republic), were used as stationary PAS. The PUF–PAS disks were deployed in protective chambers consisting of two stainless steel bowls (upper 30 cm diameter and lower 24 cm diameter). Depuration compounds (DCs)/performance reference compounds (PRCs)[20] were not used in the PUF–PAS in order to avoid the release of pollutants to the indoor environment.

Reference active air sampler

A low volume AAS (LVS3, Sven Leckel Ingenieurbüro GmbH, Germany) was continuously operated as a reference sampler to provide weekly time integrated concentrations of the targeted SVOCs. The low volume AAS consisted of a sampling head connected to a pump with a flow of 2.3 m^3 h^{-1}. SVOCs in the particulate phase were collected by a 47 mm quartz filter (QFF, Whatman) housed in an inlet equipped with PM10 jet tubes (CEN standard EN 12341, the EU Council Directive 1999/30/EG). Two PUF plugs (55 mm diameter, 50 mm length, 0.030 g cm^{-3} density, type T-3037 Molitan, a.s., Czech Republic) were used as sorbents for SVOCs in the gas phase.

Sample preparation

Preparation and storage of the PUF–PAS disks, active PUF plugs, and QFFs followed previously published procedures[19] and is described in ESI.†

Sample cleanup and analysis

Samples were analyzed for polychlorinated biphenyls (PCBs, $n = 7 + 11$), organochlorine pesticides (OCPs, $n = 12$), polycyclic aromatic hydrocarbons (PAHs, $n = 16$), polybrominated diphenyl ethers (PBDEs, $n = 10$), novel brominated flame retardants (nBFRs, $n = 17$) (also called "novel" halogenated flame retardants (NFRs)), polychlorinated dibenzo-*p*-dioxins (PCDDs, $n = 7$), and polychlorinated dibenzofurans (PCDFs, $n = 10$). See Table 1 for full names and abbreviations of compounds within each class.

Cleanup and analysis were performed at the RECETOX laboratories according to previously published procedures.[19] Details can be found in ESI.†

Experimental design

The calibration was carried out for 12 weeks, from September to December 2010. PUF–PAS ($n = 36$) and the reference AAS ($n = 1$) were deployed side by side (~200 cm height) and sampling was conducted concurrently with the two sampler types. One set of triplicate PUF–PAS was harvested every seventh day throughout the 12-week calibration period. This generated 12 sets of triplicate PUF–PAS, each corresponding to a specific exposure time ranging from one to 12 weeks. The filter and PUF plugs of the reference AAS were simultaneously replaced every seventh day, generating 12 sets of reference samples, each with an exposure time of one week.

The size of the room and natural air ventilation was considered large enough to avoid depletion of SVOCs when sampled with many PUF–PAS and an AAS simultaneously. This was confirmed by the values of average weekly concentrations derived from the reference AAS (see following sections).

Evaluation of sampling performance

(i) Detection and minimum exposure times. Detection of target compounds after reasonable exposure times is a basic requirement when considering the employment of a sampler and especially important when evaluating PAS performance indoors where the conditions (*e.g.* low air velocity, and low concentration of total suspended particles) may inhibit the uptake dynamics and restrict the window of applicability.

Three parameters were analysed to evaluate the PUF–PAS capability of detecting SVOCs indoors: (A) compound specific method detection limits (MDLs) based on instrumental detection limits (IDL) and field blanks, (B) lowest detectable concentrations (LDCs) estimated from MDLs and specific R_S for various exposure times, and (C) detection frequencies based on accumulated amounts above MDLs in the PUF–PAS and the reference AAS, respectively. Information from the three parameters was further used to assess the minimum exposure time for each compound.

(ii) Precision. A comprehensive precision of parallel sampling and chemical analysis was determined based on the variance (expressed as relative standard deviation RSD in %) of accumulated amounts ($n_{PUF\text{-}PAS,t}$, pg per sample) of individual compounds in triplicate PUF–PAS samples. The analysis was repeated for each set of triplicates collected at various exposure times (*i.e.* 1–12 weeks).

(iii) Fingerprinting. The evaluation of compound profiles or the fingerprint of compounds is an important diagnostic parameter defined as the ability of PUF–PAS to provide consistent information on the relative abundance of different compounds within a given SVOC class compared with that obtained from the AAS.

Each compound's relative contribution to the total mass or concentration of its class (expressed as a percentage) was calculated for PUF–PAS (based on accumulated amounts in the PUF–PAS) as well as for gas phase, particle phase and bulk (gas + particle) phase of the reference AAS (based on concentrations). The level of agreement was assessed by linear regression analysis.

(iv) Sampling rates (R_S). R_S were calculated using two different methods commonly and interchangeably used in the literature for PUF–PAS; Method 1 and 2. Both methods are described in detail by Bohlin *et al.* (ref. 16).

Method 1. Linear regression analysis of the equivalent air volume ($V_{eq.,t}$) sampled by each PUF–PAS plotted against the corresponding exposure time (t) in days (Fig. S1†).[23–25] The slope of the regression line provided information of the length of the linear uptake phase as well as the PUF–PAS R_S expressed in volume per time unit (*i.e.* m^3 per day). This method gives one overall R_S for the time frame of the linear uptake phase (*i.e.* time-integrated R_S).

Method 2. Comparison of the $n_{PUF-PAS,t}$ at each exposure time and the concentration from the active air sampler ($C_{act,t}$, pg m^{-3}) over the same exposure time.[26,27] This method provides one R_S per individual set of triplicate and exposure time (*i.e.* exposure time specific R_S). These R_S should be constant with the exposure time if the uptake is within the linear phase.

(v) Sampling of particle associated compounds. The sampling performance for particle associated compounds was assessed by comparing results from all previous evaluation endpoints between: (A) compounds mainly found in gas phase (*i.e.* more than 60% of their total concentration found in PUF plugs), and (B) compounds mainly associated with particles (*i.e.* less than 60% of their total concentration found in PUF plugs). The two categories were defined based on the results of the reference AAS.

Applicability for human health risk assessment

Human health risk resulting from lifetime indoor inhalation exposure of the targeted SVOCs was evaluated with respect to the risk of developing cancer. Quantification of the human health risk was based on results from the PUF–PAS, following a previously published methodology.[28] The exposure scenario was selected based on the goal of this paper: to compare human health risks derived from active and passive sampling

Table 1 Overview of results for PUF–PAS deployed in double-dome chambers: suggested indoor sampling rates (R_S, average ± 95% CI); exposure times within the linear uptake phase; detection frequencies in PUF–PAS; average precision of triplicate for 1 to 12 weeks exposure times, and previously published R_S[b]

	Sampling rate (R_S, m^3 per day) ± 95% CI	Linear phase (weeks)	Detection frequency (%) in PUF–PAS	Variability (%RSD) of PUF–PAS replicates	Sampling rate (R_S, m^3 per day) previously published[6,23,25,26]
Polychlorinated biphenyls (PCBs)					
PCB 28	1.0 ± 0.2	1–12	100	10	0.75, 2.8
PCB 52	1.3 ± 0.1	1–12	100	9	0.67, 2.3
PCB 101	1.7 ± 0.2	1–12	100	9	0.8, 3.2
PCB 118	1.2 ± 0.2	1–12	100	10	3.2
PCB 153	1.7 ± 0.2	1–12	100	7	1.03, 2.4
PCB 138	1.7 ± 0.2	1–12	100	7	1.18, 2.4
PCB 180	1.5 ± 0.2	1–12	100	10	1.55, 2.2
PCB77	1.0 ± 0.2	1–12	100	13	2.3
PCB81[a]	1.1 ± 0.3[a]	—	36	25	2.3
PCB105	1.0 ± 0.2	1–12	100	14	0.99, 3.2
PCB114	1.1 ± 0.1[a]	4–12	59	22	3.2
PCB123	1.4 ± 0.4	2–12	89	23	3.2
PCB156	1.1 ± 0.2	1–12	100	15	2.4
PCB157	0.9 ± 0.2[a]	4–12	50	25	2.4
PCB167	1.1 ± 0.2	1–12	100	12	2.4
PCB189	1.1 ± 0.4	4–12	71	23	2.2
Organochlorine pesticides (OCPs)					
PeCB	3.4 ± 0.8	1–10	100	13	
HCB	2.5 ± 0.4	1–12	100	16	
o,p′-DDE	1.3 ± 0.2	1–12	100	10	
p,p′-DDE	1.3 ± 0.5	5–12	61	24	
o,p′-DDD	1.4 ± 0.3	5–12	54	31	
p,p′-DDD	1.2 ± 0.4	2–12	100	16	
o,p′-DDT	1.2 ± 0.3	1–12	100	15	
p,p′-DDT	1.1 ± 0.5	1–12	100	21	
Polybrominated diphenyl ethers (PBDEs)					
BDE 28	1.2 ± 0.2	1–12	100	19	1.74, 2.5
BDE 47	1.1 ± 0.2	1–12	100	11	1.95, 2.5
BDE 99	0.9 ± 0.3	2–12	67	45	1.12, 2.5
BDE 100	2.9 ± 1.3	7–12	36	93	1.34, 2.5
Novel brominated flame retardants (nBFRs)					
2,4,6-Tribromophenylallyl ether (ATE)	1.4 ± 0.5	1–8	83	48	
α,β,γ,δ-Tetrabromoethylcyclohexane (TBECH)	1.4 ± 0.1	1–11	100	25	
2-Bromoallyl-2,4,6-tribromo-phenyl ether (BATE)	1.5 ± 0.6	1–8	90	57	
1,2,5,6-Tetrabromocyclooctane (TBCO)	1.9 ± 0.2	1–9	94	29	
2,3,5,6-Tetrabromo-*p*-xylene (*p*-TBX)	4.6 ± 1.3	1–10	100	45	
Pentabromoethylbenzene (PBEB)	2.0 ± 0.3	1–9	100	32	
2,3,4,5,6-Pentabromotoluene (PBT)	1.7 ± 0.4	1–10	100	25	
2,3-Dibromopropyl-2,4,6-tribromophenyl ether (DPTE)	2.1 ± 0.6	1–8	94	34	
Hexabromobenzene (HBB)	1.2 ± 0.3	1–9	100	21	
2-Ethylhexyl-2,3,4,5-tetrabromobenzoate (EHTBB)	1.7 ± 0.7[a]	1–10	86	55	
Polycyclic aromatic hydrocarbons (PAHs)					
Fluorene	5.5 ± 0.5	1–9	100	14	1.9
Phenanthrene	1.7 ± 0.1	1–9	100	12	1.9
Fluoranthene	0.9 ± 0.1	2–9	83	17	4.2
Pyrene	0.8 ± 0.1	2–9	83	10	7.8
Benz(*a*)anthracene	0.2 ± 0.0	3–9	81	33	12.5
Chrysene	0.2 ± 0.0	2–9	86	11	4.5
Benzo(*b*)fluoranthene	0.04 ± 0.0[a]	—	100	41	3.5
Benzo(*k*)fluoranthene	0.03 ± 0.0[a]	—	100	15	3.3

Table 1 (*Contd.*)

	Sampling rate (R_S, m^3 per day) ± 95% CI	Linear phase (weeks)	Detection frequency (%) in PUF–PAS	Variability (%RSD) of PUF–PAS replicates	Sampling rate (R_S, m^3 per day) previously published[6,23,25,26]
Polychlorinated dibenzo-*p*-dioxins (PCDDs)					
1234678-HpCDD	0.7 ± 0.4[a]	—	61	82	
OCDD	0.4 ± 0.1[a]	—	58	78	
Polychlorinated dibenzofurans (PCDFs)					
2378-TCDF	0.9 ± 0.1[a]	—	64	72	
12378-PeCDF	1.0 ± 0.3[a]	—	69	73	
23478-PeCDF	0.6 ± 0.3[a]	—	78	55	
123478-HxCDF	1.2 ± 0.7[a]	—	42	97	
123678-HxCDF	1.3 ± 0.7[a]	—	69	82	
234678-HxCDF	1.0 ± 0.8[a]	—	81	76	
1234678-HpCDF	1.5 ± 1.1[a]	—	75	90	

[a] Sampling rate obtained by Method 2. [b] Italicization means that results should be used with caution due to their high volatility.

techniques. Details are given in ESI.† The uncertainty of the risk assessment was estimated based on the results of the performance assessment of PUF–PAS as described above (*e.g.* detection, precision, and R_S).

Results and discussion

Indoor air concentrations and gas–particle partitioning

The reference AAS provided data on indoor air concentrations (gas and particle phase) and gas–particle distribution. The results showed consistent weekly air concentrations as well as gas–particle distributions throughout the 12-week sampling period for all SVOCs assessed in this study. This demonstrated that sampling did not result in progressively depleting concentrations of SVOC in the indoor air. Average air concentrations (gas + particle phase) are presented in Table S1 in ESI† together with the information on fraction associated with the gas phase and detection frequencies. The concentrations were generally low, ranging from a few fg m^{-3} for PCDD/Fs and some nBFRs to tens of pg m^{-3} for PCBs, OCPs, BDEs and nBFRs, and in the lower range of ng m^{-3} for PAHs. These concentrations are up to one order of magnitude lower than those previously reported for residential indoor environments.[3,5,29–31] Results from a simultaneous assessment of outdoor air at the same site (reported elsewhere[16]) showed PCBs, PBDEs and nBFRs to be a factor of 3–8 higher indoors, and OCPs, PAHs, and PCDD/Fs to be a factor of 2–3 lower indoors. This is in agreement with results from previous studies carried out in other locations.[4–6]

The gas–particle distribution data are in agreement with those from other indoor environments.[3,32,33] PCBs and DDTs were mainly found in the gas phase (80–100%) while PBDEs, nBFRs and PAHs were more widely distributed between the two phases (0–100% in gas phase, depending on the compound). Many of the PCDD/Fs compounds were below the MDL in one of the two phases and appropriate information could therefore not be obtained.

Performance of PUF–PAS

Detection. The obtained MDLs (pg per sample) and LDCs (pg m^{-3}) are shown in Table S2† together with the analytical limit of detection (LOD, pg per sample). It is important to emphasize that the presented MDLs and LDCs are a result of the instrumental sensitivity analysis and blank levels in this particular laboratory and not generally valid for other laboratories. However, the methodology adopted here followed strict QA/QC procedures consistent with those adopted by most of the reference users/developer of PUF–PAS.[34]

Results for MDLs and LDCs obviously varied across compounds and SVOC classes. Generally, the obtained MDLs were in the same range as LODs indicating that the field blank manipulation was not a source of contamination for the PUF–PAS. Very high MDLs were however found both in the PUF–PAS and the reference AAS for HCHs, BDE 209, *syn*- and *anti*-DP and BEHTBP. These compounds were therefore omitted from further evaluation. MDLs higher than LODs were also found for many of the volatile compounds but the levels in PUF–PAS samples (with the exception of those listed above) were more than one order of magnitude higher than the MDLs.

The detection frequencies were generally high in the reference AAS (Table S1†) except for a few compounds that were detected with low frequencies over the calibration period (*i.e.* 25–75%): BDE 154, 183, hexa CDDs, and tetra CDFs, or not at all (*i.e.* 0–17%): PCB 169, BDE 66, 85, 153, HCDBCO, and tetra–penta CDD. The latter group was excluded from further evaluation. The seven indicator PCBs were detected to the same high extent by PUF–PAS as the AAS. The rest of the SVOCs were detected to a significantly lower extent ($p < 0.01$) by the PUF–PAS. The differences in detection frequencies between the two sampler types were bigger for particle associated compounds than for gas phase compounds. Compounds with low detection frequencies in PUF–PAS (*i.e.* <30%) were omitted from further evaluation. This group included PCB 126, BDE 154, 183, DPMA, BTBPE, DBDPE, acenaphthylene, acenapthene, anthracene,

benzo(*a*)pyrene, indeno(123-*cd*)-pyrene, dibenz-(*ah*)anthracene, benzo(*ghi*)perylene, and hexa CDDs. This resulted in valid data for 60 out of a total number of 90 analyzed compounds.

The results show that even PUF–PAS in the double-dome chamber successfully provide detectable levels after only two weeks of exposure time for PCBs, PeCB, HCB, tri–tetra BDEs, nBFRs, and gas phase PAHs (*i.e.* 3–4 ring PAHs) in low level indoor scenarios. These results show that for this set of compounds PUF–PAS can be employed in medium to long term human exposure studies where an averaged exposure over one to two weeks often is used. In contrast, a longer exposure time (4–6 weeks) is required for DDTs and PCDFs, while penta–hepta BDEs, 5–6 ring PAHs, and PCDDs may not be detected at all with the double-dome chamber PUF–PAS under the conditions of this study. A minimum exposure time of 4 weeks is recommended for avoiding problem of detection and to obtain data for a broad range of compounds. The estimated minimum exposure times for individual compounds are presented in Table 1.

Precision. Starting from week 2, the precision for all SVOCs was independent of the exposure time ($p < 0.05$). An average precision, calculated using the data of eleven sets of triplicate (week two to 12), was therefore used as the representative for all exposure times (Table 1). The precision varied among the SVOC classes but generally good precision (<25% RSD) was found for PCBs, OCPs, PBDEs and PAHs (both in the gas and particle phase). Somewhat lower precision (20–50% RSD) was found for the detected nBFRs. Bad precision was found for the PCDD/Fs (>50%) as they were often found only in one of the three replicates, indicating inconsistent accumulation of these compounds by PUF–PAS. High precision has previously also been reported for PCBs (7% RSD) in PUF–PAS deployed in the same double bowl chamber indoors.[23] Overall, the factors limiting PUF–PAS precision appeared to be: (i) indoor air concentrations close to the MDL and (ii) particle partitioning.

Fingerprinting. Table S3† reports the results of linear regression analysis between the compound specific relative abundances (in relation to the total sequester mass of all compounds of the same SVOC class) determined by the PUF–PAS and the reference AAS (bulk phase and gas phase, respectively). Slopes of 0.8–1.0 were obtained for PCBs and OCPs suggesting high fingerprinting capacity for these classes of SVOC. This result suggests that PUF–PAS can provide sensible information on the congener or compound pattern even without the need for correcting the possible different uptake behaviour of more particle associated compounds. Poorer correlations were obtained for nBFRs, PAHs, and PCDDs showing the need for correcting their particle–gas partitioning behaviour in order to determine the fingerprint of these compounds. No significant correlation was found for SVOC classes with a higher content in the particle phase (*i.e.* PBDEs and PCDFs).

Indoor sampling rates (R_S)

General remarks on R_S. In theory, the two methods used to calculate R_S should provide consistent data. Indeed, the two methods have been used interchangeably in previous studies although the comparability of the results rarely have been analysed or questioned.

The obtained exposure-time-specific R_S from Method 2 was significantly higher (factor of 2–5) for short exposure times (1–3 weeks) than for longer exposure times. For periods longer than 3–4 weeks the R_S tended to reach relatively constant values, in the same range as those obtained from Method 1. High initial R_S values were also found in a concurrent outdoor calibration study[16] and can be observed also by analysing data reported in some previous studies.[23,27] This effect may be due to analytical issues as there is a greater uncertainty in derived sampling rates and potential bias for shorter exposure times when smaller amounts of compounds are collected by the PAS and data are near the MDL. However, the same effect was observed both for compounds well above MDL and for those close to MDL, which indicates that it may originate from a two-phase accumulation pattern with a rapid initial sorption onto the PUF–PAS surface in the first few weeks of deployment followed by a slower diffusion into the interior of the PUF disk to approach equilibrium. Such a model is largely adopted in describing the uptake of organic compounds in different hydrophobic environmental matrixes.[27,35,36] For PUF–PAS, it has not been described so far and would require further studies.

As a result of initial high R_S, the average R_S from Method 2 is significantly higher than the time-integrated R_S from Method 1 (factor of 2–5). This indicates that the two methods may provide inconsistent figures resulting in deviation of the estimated air concentration by a factor of two or more. This difference disappears when averaging R_S values from week 3 and up to the end of the linear uptake phase (obtained by Method 1). It shows that the initial fast uptake by PUF–PAS is not observed in Method 1 and should be taken into consideration if deploying PUF–PAS for short exposure times. In particular, Method 2 is recommended when short exposure times of 2 weeks are used.

Compound specific R_S for indoor monitoring. The suggested compound specific R_S for PUF–PAS deployed in the closed double-bowl chamber in non-industrial indoor environments are presented in Table 1. It has to be noted that differences in apparent R_S of the individual chemicals are driven by their particle–gas partitioning behaviour. While a difference in the R_S of various gas phase-associated chemicals is not significant, particle-associated compounds are sampled less efficiently due to limited ability of the double dome PUF–PAS to capture the atmospheric particles. Data available from some previous studies are not consistent which suggests that the particle sampling efficiency of PUF–PAS can be affected by many site- and time-specific factors. It is an area of on-going research.

Results from Method 1 were selected as a standard while results from Method 2, presented as the average of exposure-time-specific R_S, were chosen when Method 1 could not be applied. Method 1 provided valid and consistent R_S for the compounds of interest from indoor environments (Table 1), *i.e.* most PCBs, OCPs, and gas phase PBDEs, nBFRs and PAHs. The lack of R_S for the other compounds was due to: (i) low detection frequencies (*i.e.* <30%), or (ii) lack of a defined accumulation pattern with time (*i.e.* not appearing in the uptake curve). The

second point was the main reason for the lack of R_S for PCDD/Fs. This is explained by a random uptake caused by the low concentrations of PCDD/Fs and a high partition to particle phase.

Method 2 provided R_S for all compounds with a detection frequency above 20% (Table S4†). Exceptions were penta–hexa PCDDs and hepta–octa PCDFs for which the exposure time specific R_S were inconsistent from week to week. The presented results for PCDD/Fs should be treated with caution as results from Method 1 showed inconsistent accumulation patterns for these compounds in the PUF–PAS. In total, R_S was obtained only for 60% of the total number of target compounds.

The R_S for individual compounds varied within each SVOC class as follows (Table 1): 0.9–1.7 m^3 per day for individual PCB congeners (PCB-7 and dlPCBs), 1.1–3.4 m^3 per day for OCPs (1.1–1.4 m^3 per day excluding PeCB and HCB), 0.9–1.2 m^3 per day for PBDEs, 1.2–4.6 m^3 per day for nBFRs (1.2–2.1 m^3 per day excluding *p*-TBX), 0.03–5.5 m^3 per day for PAH-16 (0.03–1.7 m^3 per day excluding fluorene), and 0.4–1.5 m^3 per day for PCDD/Fs. The R_S for individual PCBs and PBDEs are in agreement with previously reported data for PUF–PAS deployed in a double-bowl chamber.[23] The results for the most volatile chemicals that may experience breakthrough in AAS (*i.e.* HCB, PeCB, fluorene), should be used with caution. When excluding these compounds the overall average R_S was 1.4 ± 0.7 m^3 per day. This is almost a factor of 2 lower than the R_S (2.5 m^3 per day) obtained for PUF–PAS deployed in a more open chamber design and commonly used in many indoor monitoring studies.[4,6,37] The variability between compounds within each SVOC class was of a factor of 2–4 with the exception of PAHs for which a larger inter-class variability was found as a result of broad variance in gas–particle partitioning. The inter-class variability for all classes was smaller than that obtained in a concurrent outdoor calibration study.[16] This was expected, since the meteorological conditions outdoors are more variable and the PUF–PAS is subjected to effects of wind speed and temperature variability.

Based on the obtained uncertainty ranges of the R_S for PCBs, DDTs, and some PAHs the expected relative error in concentration estimates does not exceed 20% while the error for nBFRs is up to 40%.

The length of the linear uptake phase (minimum to maximum) was estimated for all compounds using Method 1. The recommended ranges for exposure times are presented in Table 1. Generally, the uptake tended to be linear for most compounds during the full length of the sampling period (12 weeks). Exceptions were 3–4 ringed PAHs, nBFRs, and PCDFs for which the length of the linear uptake phase lasted for 4–9 weeks. This is in agreement with those reported in previous publications.[13,24]

Particle associated compounds

The evaluation of PUF–PAS performance for compounds with different gas–particle partitioning was based on two groups: (A) gas phase compounds and (B) particle associated compounds. The two groups encompass 55 and 30% of the total number of compounds respectively. The remaining 15% represent compounds below MDL in the AAS. The results confirm that PUF–PAS do collect, to some extent, particle associated compounds (group B), although with less consistent performance compared to group A (Fig. S2†). The detection frequencies for group B in PUF–PAS (average = 38%) were significantly lower than in the reference AAS (average = 84%) and significantly lower than group A in PUF–PAS (average = 84%). The precision for group B was significantly lower (average = 75% RSD) than for group A (average = 26% RSD). The results suggest inconsistent uptake behaviour for particle associated compounds in non-industrial indoor environments when using PUF–PAS in the closed double-bowl chamber design.

Time-integrated R_S (Method 1) could not be obtained for most of the particle associated compounds. The available R_S for group B were significantly lower (factor of 4) than for group A. The R_S for particle associated PAHs were up to a factor of 50 lower than for gas phase PAHs and the overall average R_S (1.4 m^3 per day). Additionally, a high uncertainty of R_S for group B (>50%) together with a low precision adds a significant overall error to estimated air concentrations. Lower R_S for particle associated compounds are in agreement with evaluations in urban and remote outdoor sites[16,19,24] but opposite to results from indoor and outdoor industrial sites.[18,26] Better PUF–PAS performance at these industrial sites is probably due to a much higher level of total suspended particles, different particle size modes and enhanced air turbulence or flow in these environments.

Better particle sampling efficiency may be achieved by deploying the PUF disks in a more open chamber design. This may either be open on all sides or covered only on the top.[4,6] In any case, we have to be aware that R_s values derived from the AAS–PAS co-employment studies are also affected by the AAS design. Active sampling of total suspended particles, PM10, PM5, PM2.5, or PM1 may result in slightly different R_s for particle-associated compounds.

Applicability for human health risk assessment

Human health risks were predicted for SVOCs with valid toxicity values (*i.e.* PCBs, OCPs, PAHs, and PCDDs/Fs) with a goal of assessing applicability of the double-dome PUF–PAS for human risk studies. The compound-specific human health risk level (*i.e.* estimated probability of developing cancer during lifetime) was calculated using the linear low-dose cancer risk equation and the total concentration of each compound obtained from the PUF–PAS.[28]

Fig. 1 shows the uncertainty of a PUF–PAS measurement (based on variability of replicates (%RSD)) and the quantified risk level (based on concentrations at this site) for individual SVOCs (boxes and whiskers). The influence of PUF–PAS uncertainty on the quantified risk is shown as the standard deviation of the risk probability level. The relative risk uncertainties were calculated from confidence intervals (±95% CI) of indoor R_S (in the section "Evaluation of sampling performance") and are presented as ±S.D. (upper-bound and lower-bound values; Fig. 1).

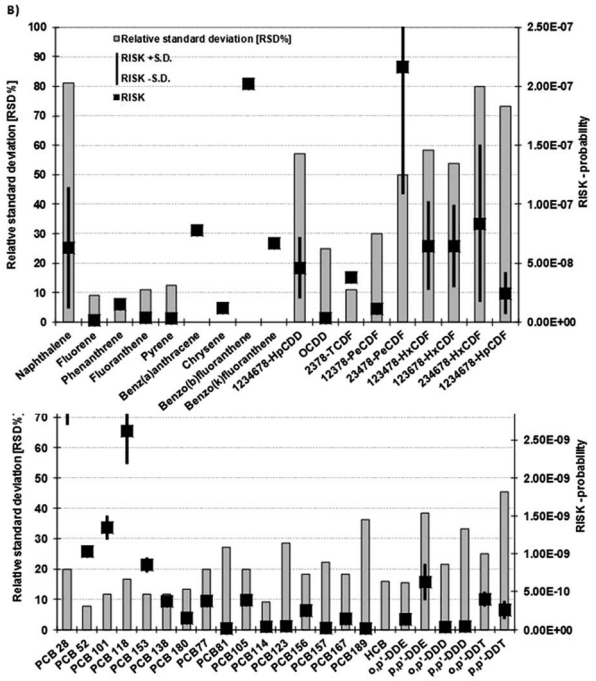

Fig. 1 Summary of potential human cancer risks related to the individual SVOCs, and their uncertainties. Black boxes quantify risks estimated on the bases of indoor passive sampling. Black lines represent the upper-bound and lower-bound values of such risk predictions (right *Y* axis). Grey bars show relative standard deviations (RSD%) of the risk prediction (left *Y* axis).

The highest risk was predicted for several PCDD/Fs and PAHs (10^{-8}–10^{-7}). Quantification of their risk values, however, was also associated with the highest uncertainties. Relative standard deviations of PCDDs/Fs were high because PCDDs/Fs were found at very low levels. Among PAHs, the highest health risks were predicted for those only partially associated with particles while the high molecular PAHs gave inconsistent results. Most consistent risk estimates were obtained for PCBs and OCPs (Fig. 1), for which the PUF–PAS performance is good. Among those, the highest (10^{-9}) risks were found for PCB 28 and PCB 118, while the risks associated with high molecular weight PCBs were an order of magnitude lower. The highest risk among the OCPs was assigned to *p*,*p*-DDE although still an order of magnitude lower than those of PCB 28, 52, and 118.

Conclusions and recommendations

A double-dome PUF–PAS design was tested in this study as a tool for the assessment of indoor concentrations and associated risks of various SVOC classes. It has been shown that even though this PAS design has been frequently applied indoors, results of such studies have to be interpreted with care. PUF–PAS can offer reasonable detection limits as well as precision for the gas phase associated SVOCs. It is also capable of providing representative compound fingerprints of their atmospheric mixtures. For the first time, it has been demonstrated that PUF–PAS performs well also for the gas phase nBFRs/NFRs indoors. Therefore, it can be used in future studies to enhance insufficient knowledge on the indoor occurrence and distribution of these emerging contaminants measured previously only in house dust.[38–40] In contrast, the double-dome PUF–PAS did not perform well for particle associated SVOCs indoors as the results found: (i) low detection frequencies, (ii) low precision, (iii) low ability to provide representative compound patterns, and (iv) few valid R_S. While deployment of the same samplers outdoors allowed for estimation of particle associated concentrations of some POPs, it was not an ideal solution indoors.

Several knowledge gaps related to the applicability of PUF–PAS for estimation of the atmospheric concentrations of high molecular weight chemicals have been identified previously. So far, we can only hypothesize on the particle size fraction that is efficiently sampled by the PUF–PAS. The particle sampling efficiency was reported to be between 10 and 100% (ref. 18 and 19) indicating that it is probably affected by many factors including the amount, material composition and size distribution of the atmospheric particles at specific sites. Size-specific distribution of various SVOCs among the particulate fractions can be another factor driving uncertainties when estimating the particle associated concentration of SVOCs as well as using various sampling heads in AAS–PAS calibration studies.

Uncertainties of these measurements are further enhanced indoors when the particle concentrations tend to be generally lower and stagnant air is responsible for decreased sampling rates of the PUF–PAS.

Higher particle sampling efficiency may be achieved indoors when more open designs of the PUF–PAS (tripod chamber or no protective chamber at all) are applied. These designs, however, still have to be carefully tested as there are no systematic data on the representativeness of the compound fingerprints detected in such samples for selected indoor environments. Not only various PUF–PAS but also AAS set ups have to be tested in an attempt to characterize the particle size fractions captured by the PUF–PAS.

All these uncertainties are complicating the use of the double-dome PUF–PAS for an assessment of human exposure and risk. While it works very well for estimation of the gas phase chemical exposure, an assessment of exposure to particle associated compounds is affected by the large deviations. In addition, PUF–PAS does not provide information on particle-size fractions crucial for assessment of inhalation risks. However, PUF–PAS can still be used for a semi-quantitative screening of chemicals suspected to present most significant risks. To increase the level of confidence in such studies, PUF–PAS should be preferably applied (i) for compounds >60% in gas phase; (ii) with exposure times between 4 and 9 weeks using time-integrated R_S from Method 1 (but R_S from Method 2 whenever exposure times are 3 weeks or shorter), and (iii) using a generic R_S for gas phase compounds and compound specific R_S for particle associated compounds. Specific recommendations for different SVOC classes are presented in Table 2.

Complementary sampling methods (for example, but not necessarily limited to dust or surface film sampling) should be

Table 2 Summary of PUF–PAS' performance and recommendations for its indoor applications for the SVOC classes targeted in this study

	Performance evaluation	Recommendations for application
PCBs	Good performance for all congeners except PCB 81, 126, and 169 due to low detection	Expose between 2 and 12 weeks Use generic R_S of 1.4 m^3 per day
OCPs	Good performance for CBs and DDTs No results obtained for HCHs due to high levels in blanks	Expose between 2 and 12 weeks Use generic R_S of 1.4 m^3 per day
PBDEs	Good performance for gas phase compounds (*i.e.* 28, 47 and 99)	Expose between 2 and 12 weeks
	Poor performance for particle associated compounds due to low detection	Use generic R_S of 1.4 m^3 per day
	No results obtained for BDE 209 due to high levels in blanks	Not recommended for particle-associated BDEs (*i.e.* Hexa–hepta BDEs)
nBFRs/NFRs	Good performance for gas phase compounds	Expose between 2 and 9 weeks
	Poor performance for DPMA, HCDBCO, BTBPE, BEHTBP and DBDPE due to low detection	Use compound specific for particle associated nBFRs R_S when detected at sufficient levels
	No results obtained for *anti*- and *syn*-DP due to high levels in blanks	Use generic R_S of 1.4 m^3 per day for gas phase nBFRs
PAHs	Good performance for gas phase compounds (*i.e.* 3–4 ring PAHs)	Expose between 2 and 9 weeks
	Poor performance for particle associated compounds (*i.e.* 5–6 ring PAHs) due to low detection	Use generic R_S of 1.4 m^3 per day for gas phase (3–4 ring) PAHs. Use compound specific R_S for particle associated (5–6 ring) PAHs when detected at sufficient levels Not generally recommended for particle-associated PAHs (*i.e.* 5–6 ring PAHs)
PCDD/Fs	Poor performance for most compounds R_S only obtained for tetra–penta CDFs	Expose between 5 and 10 weeks Use compound specific R_S when detected at sufficient levels
	Low precision	Not generally recommended for PCDDs

also considered as an option to obtain a more reliable and quantitative picture of exposure to particle associated contaminants in non-industrial indoor environments. These methods, however, also require further evaluation as there is a lack of consistent knowledge on the best sampling approach, comparability to airborne particle concentrations and compound profiles.

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Review

Environment and human exposure to persistent organic pollutants (POPs) in India: A systematic review of recent and historical data

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ABSTRACT

Persistent organic pollutants (POPs) have been used in a wide range of agricultural and industrial commodities, resulting in vigorous deterioration of environment and human health. A number of studies on the occurrence of POPs confirm their presence in various environmental compartments and human body. In order to deal with this global concern, India has recently prepared the National Implementation Plan (NIP) of the Stockholm Convention. Common beliefs point at India as a hot spot of POP contamination and human exposure; however no systematic analysis was ever performed so far considering all available past data on POP occurrence. This review aims to examine the distribution pattern of POPs in multicompartment environment and human samples, meta-analysis of time trends in exposure levels to environment and humans, and cross country comparison of POP contamination with China. Based on this review, it can be concluded that the Indian environment and human population are highly contaminated by DDTs and HCHs; however scarcity of data on other POPs makes it challenging to assess their nationwide human and environmental exposure. No evidence of a general decline in DDT and HCH residues in the environment and human body come out from the meta-analysis of time trend. While comparing contamination levels between India and China, tendency towards decline in POP contamination is visible in China, unlike India.

Contents

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1. Introduction

In the last half century, the global economy has developed vigorously in both the industrial and agricultural sectors resulting into exponential production and usage of industrial- and agro-chemicals which enter the environment often as potentially toxic contaminants. Persistent organic pollutants (POPs) are a group of pollutants posing a global concern. POPs are intentionally or unintentionally produced lipophilic chemicals (UNEP, 2003) which are capable of accumulating in different environmental compartments and organism tissues, resisting bio- and photochemical degradation, and undergoing long range atmospheric transport (Buccini, 2003; Wania and Mackay, 1993; Wong et al., 2005). In the past few decades, POPs have got global attention due to their bioaccumulation properties, high toxicity, and ubiquitous exposure of humans and wildlife (UNEP, 2007).

To acknowledge the global issue of POPs and to protect human and environmental health the UNEP Stockholm Convention on POPs entered into force in 2001 regulating or banning a preliminary list of twelve chemicals (including PCBs, dioxins and furans and a range of organochlorine pesticides OCPs) which fulfilled all the criteria of persistence, bioaccumulation, toxicity and potential for long range transport) setting the definition of POPs. In 2009 and 2012, ten new substances were added in the POP list (UNEP, 2003). The convention, ratified by India in 2006, established an obligation to take measures to eliminate and restrict the production and use of POPs (NIP, Govt. of India, 2011).

India is one of the most densely populated countries in the world, whose economy primarily depends on agriculture. In recent years, the country has gained a rapid boost in industrial development, which has adversely affected environment quality and human health (Galli et al., 2012; Parikh, 2012). Increased population and gross domestic product (GDP) of India has resulted in the rise of pesticide consumption and waste generation (Fig. S1). POPs, in particular organochlorine pesticides (OCPs) has unarguably played an important role for development of India, spanking as one of the historical most important producer of technical dichlorodiphenyltrichloroethane (DDT) and hexachlorocyclohexane (HCH), globally (Fig. S2).

Rapidly developing agricultural and industrial sectors have been accompanied by widespread application of organochlorine pesticides, particularly DDTs and HCHs. Although, the use of POPs in India has been banned or restricted during the last decade, therefore much later than most western countries, yet derived products such as DDT containing dicofol and anti-fouling paints and lindane still constitute active primary sources of contamination. In addition, waste of electrical and electronic equipment (WEEE) generated in India and in many other parts of the world has been related to high concentration of PCBs and PBDEs in various environmental compartments (Brigden et al., 2005; Eguchi et al., 2012).

POPs, and especially DDT, HCHs and HCB in India have been measured in all environmental compartments. Available information however is very fragmentary and often data are not directly comparable since they have been collected using different methods and without adopting a comprehensive and consistent monitoring design. Although the general belief is that India is a hotspot of POP contamination, it is currently not easy to inform such a statement since a critical assessment of all available data was not done so far. Hence, in order to develop a general view on the state of Indian environment and human exposure to POPs and inform future actions for Stockholm Convention implementation, it is fundamental to collect, compare and critically analyze historical data from the past monitoring. This review paper collects, organizes and critically discuss results from past studies on POP occurrence in the environment, biota and human samples in India. The paper is systematically organized presenting historically available data on POP occurrence in different environmental compartments (namely: surface water, ground water, soil, sediment and bioaccumulation in aquatic organisms and humans). It critically assesses comparability among different datasets searching for evidences of possible medium to long term time trends in the exposure levels. It discusses available data in various environmental matrices in relation to environmental quality guidelines set by some major international regulatory acts and compares Indian data with environmental contamination data from China, taken as an example of a geopolitical area that similar to India is interested by a recent rapid economic growth and chemical management development.

2. Environmental distribution of POPs

2.1. Surface water

In India, rivers play a key role in the economic growth by providing drinking water, water for irrigation and industrial purposes currently sustaining a growing human population of over 1.2 billion. India is endowed with thirteen major river basins covering an area of over 20,000 km^2 and witnesses high precipitation during months of June, July and August, associated to the southwest monsoon. Rivers and other resources of surface water provide a perfect integrative medium to monitor POPs from the perspective of transport and redistribution. In addition, POPs in surface water are keys in defining important exposure pathways for humans due to water use for drinking and food production purposes. Major sources of POPs for the surface water include direct discharges of waste and agricultural runoff, atmospheric depositions (Bidleman et al., 1998), and expectedly leaching from melting Himalayan glaciers.

A number of reports are available presenting a considerable crop of POP monitoring data in rivers and other internal waters, built during the last four decades (Tables 1, S1). However data lacks systematic consistency in the adopted sampling and analytical methodology, and basically no study ever reviewed these data to provide a comprehensive critical evaluation of India surface water exposure to POPs.

DDTs (sum of o,p′-DDT, p,p′-DDT, o,p′-DDE, p,p′-DDE, o,p′-DDD, and p,p′-DDD) and HCHs (sum of α-HCH, β-HCH, γ-HCH, and δ-HCH) were the most commonly monitored POPs in surface water. Other monitored POPs (although in a limited measure) include Σendosulfan (sum of α-Endo, β-Endo, and Endo-sulfate), heptachlor, aldrin, dieldrin, ΣPCBs (varied number of PCB congeners for different studies), chlordane, hexachlorobenzene, endrin, PFOS and mirex. In most of the research studies river segments crossing urban and semi-urban areas were considered for POP monitoring. This implies that available data may reflect to large extent active primary emissions especially from inappropriate urban, industrial or agricultural waste disposal rather than background exposure.

Concentration of DDTs and HCHs in the Ganges River and its tributary in Northern India varied from region to region, depending on the land use pattern, density of industrial area and consumption of pesticides for agricultural and controlling vector borne disease in the past (Mutiyar and Mittal, 2013). Most of the monitoring studies reported DDT and HCH levels in river water higher than the permissible limits (Anon., 1998; USEPA, 1986).

Monitoring the concentrations of DDTs, HCHs, aldrin, heptachlor and endosulfan at upstream Himalayan head water sites (Devprayag, Rishikesh and Haridwar in Uttrakhand) of the Ganges River, DDT levels were found approximately near the permissible limit (1.0 ng/L) and among the lowest measured levels in Indian surface waters (Mutiyar and Mittal, 2013). However, relatively high concentrations of HCHs were detected, possibly as a result of glacier runoff remobilizing long range transported contaminant previously deposited on Himalayan glaciers (Bizzotto et al., 2009; Blais et al., 2001; Kang et al., 2009). Higher concentrations of HCHs may be associated to historical use of lindane in farming in Indo-Gangatic plains. Another study on upstream tributaries (rainfed and snowfed) of the Ganges River in the Kumaon Himalayan region of Uttarakhand revealed that DDT and HCH levels in river water were relatively higher than the standards laid down by USEPA (Sarkar et al., 2003; USEPA, 1986). Lower levels of DDTs and HCHs in rainfed streams compared to those in snowfed streams clearly reflects

Table 1
Average concentrations (ng/L) of POPs in surface water resources in various regions of India.

Indian state	Source and sampling year	ΣDDT	ΣHCH/BHC	References
Devprayag, Uttrakhand[b]	Ganges River (2010)	ND	7.24	Mutiyar and Mittal (2013)
Rishikesh, Uttrakhand[b]		1.01	5.5	
Haridwar, Uttrakhand[b]		0.19	5.2	
Tributaries of Ganges river in Kumaon Himalayan Region, Uttrakhan	Sharda (Kali River) (1999)	20	10	Sarkar et al. (2003)
	Saryu (1999)	33	15	
	Gori (1999)	40	15	
	Ram Ganga (1999)	72	18	
	Dhuali (1999)	21	10	
	Kosi (1999)	9	4	
	Gomti River (1999)	25	26	
	Ladhiya (1999)	19	7	
	Lohawati (1999)	21	10	
Nainital, Uttrakhand[b]	Lake water (NA)	11,180	3655.5	Dua et al. (1998)
	Tap water (NA)	10,066	2446.8	
Haryana	Ghaggar River (1999–2000)	587.35	119.74	Kaushik et al. (2010)
Hisar, Haryana[a]	Rain water (2002)	3376.6	213.3	Kumari et al. (2007)
Haryana and Delhi[a,d]	Yamuna River (1999)	387.9	310.25	Kaushik et al. (2008)
Delhi[a,d]	Yamuna River (1995–2001)	0–1.44	–	CPCB (2000)
Delhi[a,d]	Yamuna River (NA)	0.12	–	Anbu (2002)
Jaipur, Rajasthan[a]	Ramgarh water reservoir (NA)	133,000	234,000	Bakore et al. (2004)
Kannauj, Uttar Pradesh[b]	Ganges River (2011)	0.05–0.12	0.1–1.0	Mutiyar and Mittal (2013)
Kanpur, Uttar Pradesh[a,d]		0.2	0.1–0.36	
Allahabad, Uttar Pradesh[a]		0.08–2.21	1.23–3.5	
Varanasi, Uttar Pradesh[a]		0.1–1.9	0.2–0.7	
Lucknow, Uttar Pradesh[a,d]	Gomti River (2011)	2.75	0.72	
Uttar Pradesh	Sharda River (NA)	–	220,000	Jit et al. (2011)
Uttar Pradesh	Gomti River (NA)	5.97	46.69	Malik et al. (2009)
Lucknow, Uttar Pradesh[a,d]	Rain water (NA)	0.53	23.48	Malik et al. (2007)
Unnao, Uttar Pradesh[b,d]	Streams, ponds and canal (2003)	8	60	Singh et al. (2007)
Kanpur, Uttar Pradesh[a,d]	Ganges River (NA)	ND	75	Sankararamakrishnan et al. (2005)
Varanasi, Uttar Pradesh[a]	Ganges River (NA)	0.135	–	Anbu (2002)
Farukkabad, Uttar Pradesh[b]	Ganges River (NA)	0.832	–	
Lucknow, Uttar Pradesh[a,e]	Stagnant rain water from HCH dumping sites and surrounding areas (NA)	–	816,666.7	Jit et al. (2011)
	From drainage near dumpsite (NA)	–	17,430,000	
	Reetha River (NA)	–	380,000	
Patna, Bihar[a]	Ganges River (2011)	ND	0.3–5.0	Mutiyar and Mittal (2013)
Bhagalpur, Bihar[b]		11.6–12.3	12.4–17.6	
Bhagalpur, Bihar[b]	Ganges River (2007–08)	227.85	61.17	Singh et al. (2012)
Greater Kolkata, West Bengal[a,d]	Tank water (NA)	193.33	1156.67	Ghose et al. (2009)
	River and canal water (NA)	210.91	1888	
Kolkata, West Bengal[a,d]	Ganges River (2005)	16,367,000	7,374,000	Purkait et al. (2009)
Kolkata, West Bengal[a,d]	Hooghly River (NA)	0.0015	–	Anbu (2002)
Nagaon, Assam[b]	River, streams, ponds, and wetlands (NA)	6121	4911	Mishra and Sharma (2011a)
Dibrugarh, Assam[b]		5402	4403	
Mumbai, Maharashtra[a]	Seawater (NA)	12.45	5.42	Pandit et al. (2006)
Goa[a]	Mandoei Sundarban River	0.0011	–	Anbu (2002)
Karnataka, Andhra Pradesh[a]	Streams of Cauvery River (NA)	1750	2430	Begum et al. (2009)
Andhra Pradesh	Kolleru lake, prawn ponds (NA)	25.2	86.9	Amaraneni (2006)
Bangalore, Andhra Pradesh[a,d]	Ulsoor Lake (NA)	0.0031	–	Anbu (2002)
Andhra Pradesh	Kolleru Lake (NA)	198	544	Amaraneni and Pillala (2000)
Tamil Nadu	Tamiraparani River (2008–09)	<0.01–0.72	<0.01–0.78	Kumarasamy et al. (2012)
Chennai, Tamil Nadu[a,d]	Cooum River (NA)	0.0016	–	Anbu (2002)
Chennai, Tamil Nadu[a,d]	Bay of Bengal (sea water) (1998)	12.549	–	Rajendran et al. (2005)
Cuddalore, Tamil Nadu[b]		5.63	–	
South India	Rivers (Kaveri and Coleroon) (NA)	0.75–4.17	3.2–182	Rajendran and Subramanian (1997)
Tamil Nadu	Vellar River water (1988)	0.057–4.8	26–3900	Ramesh et al. (1990a)
Permissible limits		1	100	Anon. (1998), USEPA (1986)

[a]Urban, [b]semi-urban, [c]rural, [d]industrial, and [e]direct discharge (for all tables).

higher persistency and absorption of pesticides in snowfed water sources (Sarkar et al., 2003). Overall, these data suggest that remobilization of old burden of POPs through snow and ice melt from Himalayan range can potentially represent a non-negligible input for the large downstream water systems.

In high altitude lakes and the tap water from the nearby area, levels of DDTs and HCHs were exceeding the permissible limits by many thousand folds. Study found that average total DDTs in high altitude lake and tap water from Nainital (Kumaon, Himalayan region) were 11,180.5 ng/L and 10,066.8 ng/L, respectively; while 3655.5 ng/L and 2446.8 ng/L for HCHs (Dua et al., 1998). These are very high numbers, exceeding several orders of magnitude the solubility of these compounds in the water (Mackay et al., 1997). These levels in the water are possible in bulk water samples characterized by the presence of elevated concentration of particulate and dissolved organic matter. These data, indeed refers to bulk water samples collected through the grab sampling methods. Unfortunately, no information on organic carbon content is reported by authors making impossible a better interpretation of these results. DDTs and HCHs were used extensively for the malaria control program in Terai and Bhabar area of Nainital (Bakre et al., 1990).

From two studies, conducted in 2007 and 2011 (Mutiyar and Mittal, 2013; Singh et al., 2012) in selected urban and semi-urban areas within

the Indo-Gangetic plains (Uttar Pradesh and Bihar), the levels of DDTs, HCHs and endosulfan ranged 12.3–227.85 ng/L, 17.6–61.17 ng/L and 17.9–241.7 ng/L, respectively, with the more recent data showing the lower levels.

Data are also reported for locally contaminated hotspot sites. Levels of HCHs in surface water from the premises of a lindane production facility were examined in a 2011 study (Jit et al., 2011). HCH isomers (380,000 ng/L) in the Reetha River, a tributary of the Ganges River, were exceeding permissible limits by many folds. This data shows how poor waste and old stockpile management can result in contamination hotspots, and serious hazard for the local environment and human population (Abhilash and Singh, 2008; Jit et al., 2011).

Incredibly high concentration of DDTs and HCHs in the small streams of Ganges River and ponds in West Bengal and Assam have also been reported being thousand fold higher than guideline values (USEPA, 1986). The highest concentration were observed in Ganges River at Kolkata, West Bengal with DDTs and HCHs in bulk water samples being in the incredible range of several g/L in the year 2005 (Purkait et al., 2009). Small water bodies near tea gardens in West Bengal were also highly contaminated by heptachlor (4300 ng/L) and endosulfans (2100 ng/L) (Bishnu et al., 2009). Similarly in Assam, small streams, ponds and wetlands near the tea gardens were adversely contaminated by DDTs (6121 ng/L) and HCHs (4911 ng/L) (Mishra and Sharma, 2011a). Assam and West Bengal are the top tea producing states of India. These are among the highest bulk water concentrations of these compounds ever reported in literature and indicate both high historical usage of DDTs and lindane in tea production and the possibility that stockpiles are still currently in use or inappropriately disposed despite the ban (Bishnu et al., 2009).

The hydrological characteristics of the river basins, such as seasonal variation of flow and activities related to construction of dams, can influence the spatial and temporal distribution of POPs in river water and sediments (Kumarasamy et al., 2012). Compared to the snowfed rivers of North India, low concentrations of pesticides were detected in the rainfed rivers of south Indian states (Karnataka, Tamil Nadu and Andhra Pradesh). Still, levels of contaminants were higher than the permissible limits (USEPA, 1986). Concentrations of DDTs and HCHs in bulk water samples of cauvery River in Karnataka and Andhra Pradesh were detected as 1750 ng/L and 2430 ng/L, respectively (Begum et al., 2009). These levels were several times higher than the permissible limits. High levels of contaminants may have resulted from extensive use of pesticides for crops such as paddy, banana, groundnut, cotton, sorghum, pulses, and ginger in the river basins. In contrast in Tamiraparani River, study reveals that ΣDDT (<0.001–0.72 ng/L), ΣHCH (<0.01–0.78 ng/L), chlordane (<0.01–0.49 ng/L), heptachlor (<0.06–2.1 ng/L) and mirex (<0.01–0.7 ng/L) were below environmental quality standards. However the concentration of aldrin (<0.02–1.5 ng/L), dieldrin (<0.03–7.5 ng/L) and endrin (<0.02–58 ng/L) in these sites were higher than the permissible limits (Kumarasamy et al., 2012).

2.1.1. Time trends in POP occurrence in surface water

Using the literature data on POP occurrence in surface water, a meta-analysis of time trends was performed. It is fully acknowledged here that data are often not directly comparable since they refer to different locations, different types of samples (e.g. bulk samples, vs. dissolved phase samples), they were never systematically collected to perform a time trend analysis, and they were generated by different research groups using different sampling and analytical methods lacking of inter-calibration. The scope of the time trend meta-analysis presented here is therefore to assess if literature data can highlight presence of any general increase or decline trend of contaminants in country-wise averaged surface water levels ranging across order of magnitude.

The availability of meta-data across 23 years for DDTs and HCHs allow performing the trend analysis only for these two pesticide classes (Fig. 1). Since the focus here is on diffuse contamination, the data relative to local hotspots sites (e.g. spills from pesticides production facilities and ponds in heavily treated agricultural catchments) were excluded from the analysis. These coincided with the samples that exceeded by several orders of magnitude compound solubility. Even after excluding those outliers, data for DDTs scattered across six orders of magnitude. This reflects the inclusion of samples from rural catchment where organochlorine pesticides were (or are still in case of DDTs) used and from areas in the sub-Himalayan mountain area which showed the lowest measured concentrations.

DDTs did not show any decline trend with time. Conversely, for HCHs a significant ($P < 0.05$) decline trend in the meta-data was observed, with average concentration values decreasing of about 3–4 orders of magnitude. Such an evident decline may directly reflect the ban for technical HCH production introduced in 1997 (Shetty and Sabitha, 2009). After that lindane was still produced and therefore officially in use basically until 2006, although the lindane production only represented about 1% of the yearly HCH production until the ban.

It appears that the different results for DDTs and HCHs capture the different regulatory history of these substances in India with overall levels of DDTs in the country stabilized and reflecting current usages. Despite these differences it is interesting to note that the levels of DDTs and HCHs were correlated. This may be due to the fact that they were co-analyzed in the same samples in different studies, however it also clearly reflects common sources and exposure pathways for the surface water ecosystem. It is remarkable to report that, as it will be showed later, such a correlation is preserved in other compartments including: ground water, sediments, aquatic organisms and human samples.

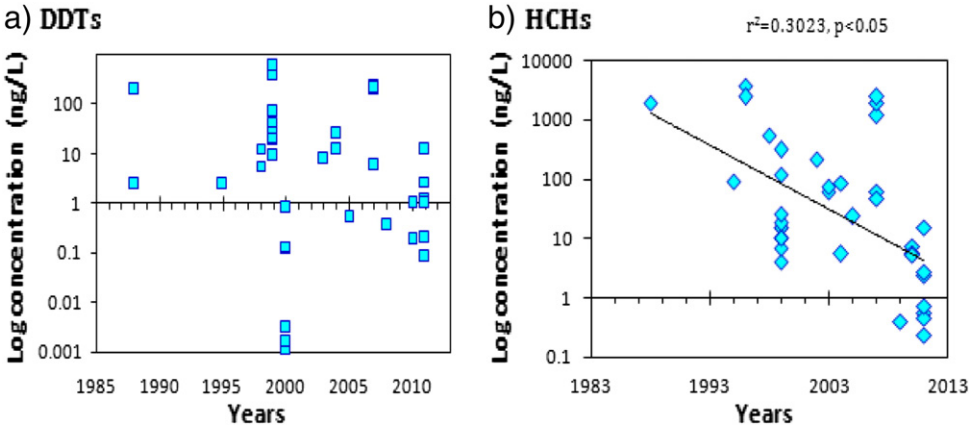

Fig. 1. Time trend meta-analysis of levels of (a) DDTs and (b) HCHs in surface water in India.

2.2. Ground water

The occurrence of POP residues in ground water mainly depends on compound specific solubility and binding of the chemicals to the soil organic matrix. POPs reach ground water through diffuse run-off, and leaching (Miliadis, 1994; Prakash et al., 2004) and many factors, including compound's physical chemical properties (solubility, degradability), soil properties (e.g. texture, permeability, organic carbon content, and depth of ground water level) concur in determining ground water exposure. POP contamination to ground water has been a matter of global concern (Foster et al., 1991; Papadopoulou-Mourkidou et al., 2004), especially in countries where contaminated ground water aquifers are the main sources of drinking water (Spliid and Koppen, 1998; Tuxan et al., 2000). This is the case of India, where water from the hand pump constitutes a major source of drinking water for rural and semi-urban areas. Ground water aquifers in India are tightly connected to surface water and they therefore were exposed to contaminant loads from agriculture (Agrawal, 1999).

Levels of POPs in ground water have been monitored in various regions of India (Tables 2, S2). DDTs and HCHs were most frequently monitored/detected POPs. Only few studies targeted other POPs, such as aldrin, dieldrin, heptachlor, and endosulfan. Concerning spatial distribution, the concentrations of DDTs and HCHs in ground water from Southern and Eastern-Indian states were many folds higher than those in northern and central Indian states. The highest concentration of DDTs (75,000 ng/L) were detected in bulk ground water samples from Jaipur, Rajasthan in 2003 (Bakore et al., 2004). The elevated organic matter content in the ground water (and therefore higher scavenging capacity for POPs) in this area favored these levels exceeding many folds theoretic solubility. The highest concentrations of POPs in Rajasthan were found in the summer season (13.83×10^6 ng/L) corresponding to the harvesting time, followed by rainy and winter seasons (Bakore et al., 2004). Heptachlor was used mostly on the standing crop just before harvesting so as to prevent it from the pest attack (Bakore et al., 2004). Highest concentration of HCHs (400,000 ng/L) was found in the ground water from HCH dumpsite areas of Lucknow City in Uttar Pradesh. These concentrations were many folds higher than the permissible limits (USEPA, 1986) and can be attributed to leaching of pesticides used in the past and currently stored in the soil. However, the observed seasonality in exposure levels lead to hypothesize that these forbidden substances were still in use at the time of the study (therefore after the official ban).

Analysis of water samples from open wells, bore-wells, dug wells and hand pumps in Kolkata, Assam, Andhra Pradesh and Tamil Nadu showed that DDTs and HCHs were present in all the ground water samples. The residual concentration of DDTs in ground water was found maximum in organic matter rich bulk water samples in Assam: 6904 ng/L and 6549 ng/L for Nagaon and Dibrugarh districts, respectively (Mishra and Sharma, 2011a). Total DDT and HCH residues were found to be higher in dug well water as compared to bore well water which may be attributed to the fact that dug wells are shallow with open tops, relatively more prone to direct contamination from the surrounding environment in comparison to bore well. Dug wells receive water from the top aquifer whereas the bore wells and hand pumps generally discharge water from relatively deep aquifer system (Mishra and Sharma, 2011a). Similar pattern for the concentration of DDTs were observed with the ground water samples from Andhra Pradesh and Tamil Nadu. In addition to DDTs, levels of HCHs were two to ten folds higher than the permissible limits prescribed by USEPA in all ground water samples except for Delhi (USEPA, 1986).

Presence of elevated concentrations of DDTs, HCHs, aldrin, dieldrin, heptachlor and endosulfan in ground water is likely associated to their past and possibly extensive use in agricultural and industrial activities round the year. Cumulative use of HCHs in India until 1985 was 575,000 tons and since then about 45,000 tons of HCHs have been used annually (Voldner and Li, 1995) (Fig. S2). In Assam, Andhra Pradesh, Tamil Nadu and West Bengal, paddy is the main crop. In Dibrugarh and Nagaon districts of Assam, around 166,309 ha (49.12%) and 234,633 ha (61.25%) area is under agriculture of which about 81,533 ha (24.09%) (NIC, 2004-05a) and 160,035 ha (38.94%) (NIC, 2004-05b) area is under paddy cultivation, respectively (summer, winter and autumn paddy). In Andhra Pradesh and Tamil Nadu, cotton, sugarcane, vegetables, jute, and tea are additional crops to paddy, which are subject to high pesticide applications. Although the use of DDTs, HCHs, and other POPs has been banned for agricultural purposes, still illegal use of these chemicals in agriculture and industries continues, may be due to lack of law enforcement as well as low cost and effectiveness of these pesticides (Agnihotri et al., 1996; Imphal Free Press, 2008; Mohapatra et al., 1995). India is not only country where the problem of illegal use of DDTs and other POPs in agriculture and industrial sector has been reported, the same has been found in several Asian and African nations (UNEP, 2008). Thus, illegal and permitted use of these pesticides in health, agricultural and domestic sectors may be the main reason for the presence of higher levels in ground water. No data for non-agricultural POPs (e.g. PCBs, Dioxines and Furans) are reported for Indian ground water.

2.2.1. Time trends in POP occurrence in ground water

Compared to the surface water compartment, limited data were available to perform a meaningful meta-analysis of time trend for ground water (Fig. 2). After excluding data associated to local source hotspot, only trend for DDTs and HCHs could be analyzed. DDT concentration in ground water did not display any relative evidence of decline, reflecting the behavior also observed in surface water. It has to be

Table 2
Average concentrations (ng/L) of POPs in ground water from various regions of India.

Indian state	Source and sampling year	ΣDDT	ΣHCH/BHC	References
Hisar, Haryana[b]	Tube well (2002–2003)	285.41	1090.33	Kumari et al. (2008)
Delhi[a,d]	ground water used for agricultural (NA)	62.25	88.43	Mukherjee and Gopal (2002)
Jaipur, Rajasthan[a]	Tube well (NA)	75,000	116,000	Bakore et al. (2004)
Jaipur, Rajasthan	Open wells/tube wells (NA)	ND	7240	Sharma and Khan (2009)
Lucknow, Uttar Pradesh[a]	HCH dumpsite areas(NA)		400,000	Jit et al. (2011)
Unnao, Uttar Pradesh[b,d]	Dug wells (NA)	16.5	208.9	Singh et al. (2005)
	Bore wells (NA)	14.1	144.6	
Kanpur, Uttar Pradesh[a,d]	Agricultural area (NA)	ND	418.5	Sankararamakrishnan et al. (2005)
	Industrial area (NA)	ND	233.4	
Agra, Uttar Pradesh[a,d]	Ground water (NA)	383.27	324.73	Singh (2001)
Farrukhabad, Uttar Pradesh[b]	Ground water (NA)	617	334	Mohapatra et al. (1995)
Greater Kolkata, West Bengal[a,d]	Ground water (NA)	10	100	Ghose et al. (2009)
Nagaon, Assam[b]	Bore wells, Dug wells and hand pumps (NA)	6904	5574	Mishra and Sharma (2011a)
Dibrugarh, Assam[b]		6549	5168	
Hyderabad, Andhra Pradesh[a]	Domestic well supply (NA)	162.05	906.62	Shukla et al. (2006)
Thiruvallur, Tamil Nadu[a]	Open wells (NA)	4743.1	10,013.8	Jayashree and Vasudevan (2007)
	Bore wells (NA)	2588.7	2939	

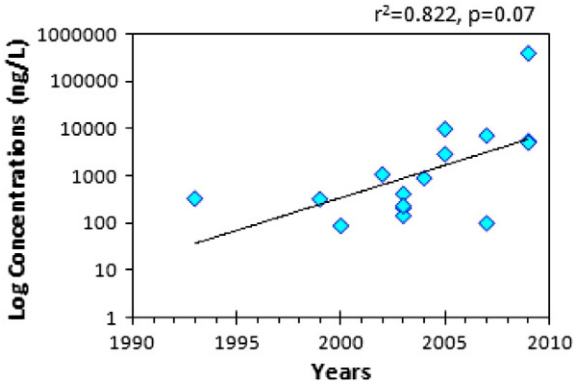

Fig. 2. Time trend meta-analysis of levels of HCHs in ground water in India.

highlighted however that only 8 data points were available for this meta-analysis, therefore its descriptive power is very limited and trends may be present (at least locally) even if not evidenced here. For HCHs the number of available data was higher ($N = 17$) and a non-significant ($P = 0.07$) increase trend in concentrations during the last decade (therefore after official banning of HCHs as commercial formulation) is appearing. Such a trend spans across 1.5–2 orders of magnitude and was opposite to the appearing decline observed in the meta-time trend analysis performed for surface water. In addition, concentrations from ground water were in average 1 to 2 orders of magnitude higher than those measured in surface water. Such a conflicting result may be associated to the different criteria adopted for selecting sampling locations for surface and ground water. Although surface water samples may reflect a broader spatial resolution (e.g. catchment scale), sampling locations for ground water may have systematically be selected to spot out local contamination peaks. Ground water levels higher than surface water levels (as observed here (1–2 orders of magnitude for both DDTs and HCHs) are indeed possible if the source for ground water is the leaching of the pesticides directly from agricultural soils. Ground water input to stream may therefore represent a significant source of contaminants for Indian surface water in area where surface water recharge is important. Interestingly, similar to what observed for surface water, the concentration of DDTs and HCHs was correlated ($P < 0.05$).

2.3. Soils

Pathways of POP accumulation in soils are either direct application in agricultural, domestic and disease control purposes or indirect deposition of airborne contaminants previously volatilized from areas of direct use. High persistence of POPs in soils and the affinity for soil organic matter provide the condition for soil to behave as important long-term storage compartments (Manz et al., 2001; Pereira et al., 2010). Soils can also serve as secondary sources whenever climate or anthropogenic disturbance produces the conditions for releasing POPs to the atmosphere or the water phase (Cheng, 1990; Nizzetto et al., 2010). The accumulation of POPs in soil results in increased exposure for soil organisms but also for higher organisms through the diet and respiratory pathways. This may result in adversely affected soil ecosystem, water quality (Kolpin et al., 1996; Kumar et al., 1995; Miglioranza et al., 1999), and human health (Kammenga et al., 2000).

Available information on soil contamination by POPs in India is noticeable for its paucity. DDTs and HCHs were the most frequently measured POPs. Only 3 studies focused on other OCPs. As discussed above, the distribution and fate of POPs in soil largely depends on the physicochemical characteristics of soil. Distribution of POPs in India has been surveyed in different types of soil (alluvial soil in Haryana, Delhi, Uttar Pradesh, and West Bengal; Mountain soil in Uttrakhand; and mixture of red and alluvial soil in Assam) and climatic conditions (Tables 3, S3). Majority of the monitoring have been done for the agricultural soil to correlate the use of organochlorine pesticides and their levels (Bishnu et al., 2009; Kumari et al., 2008; Nawab et al., 2003). Few studies investigated the concentrations of POPs in soil from the nearby POP production units to analyze the effectiveness of environmental protection and waste management measures and policies (Abhilash and Singh, 2008; Jit et al., 2011). DDTs and HCHs were once more the most monitored compounds. A very few studies monitored aldrin, dieldrin, endrin, chlordane, heptachlor, endosulfan, and HCB in a range of conditions/locations (Bishnu et al., 2009; Kumari et al., 2008; Singh, 2001; Singh et al., 2007). Spatially, levels of DDTs and HCHs were of lower magnitude in urban areas of Delhi and Haryana than in agricultural soil from Uttar Pradesh and Assam. This trend can be clearly attributed to their much higher use in agriculture and presence of pesticide production units in the former states. In all the studies DDTs was the POP with highest concentrations (Tables 3, S3).

DDTs, in Haryana was measured in different periods ranging between 13 ng/g and 45 ng/g, while HCHs ranged 27 ng/g to 162 ng/g; DDTs in Uttrakhand was found in concentrations between 117 ng/g and 270 ng/g. These levels were generally overtaken by 2–5 folds by those measured in Assam where major crop paddy (summer, winter and autumn paddy cultivation) and tea gardens are located; this indicates that intensive agricultural practices throughout the year clearly determined higher soil exposure.

Large scattering in data is obviously associated to soil property heterogeneity, as well as land use. Intensive and different agricultural practices have resulted into high organic carbon (OC) content and low pH of soil in these regions (Mishra et al., 2012; Wang et al., 2006; Yang et al., 2005). High concentrations of DDTs and HCHs in soil in Assam may be

Table 3
Average concentrations (ng/g) of POPs in soil from various regions of India.

Indian state	Source and sampling year	ΣDDT	ΣHCH/BHC	References
Hisar, Haryana[b]	From Fermers field (2002–03)	13.91	27.08	Kumari et al. (2008)
Haryana	Rice growing fields (NA)	45	162	Kumari et al. (1996)
Delhi[a]	Agricultural surface and sub-surface soil (NA)	–	382.97	Prakash et al. (2004)
Dehradun, Uttrakhand[a]	Rice growing fields (NA)	117	326	Babu et al. (2003)
Haridwar, Uttrakhand[b]	Malarious DDT-sprayed areas (NA)	270.51	61.12	Dua et al. (1996)
Lucknow, Uttar Pradesh[a]	Surrounding area of lindane production Unit (NA)	–	404,520.9	Jit et al. (2011)
Barabanki, Uttar Pradesh[b]	Agricultural area near HCH dumpsite (NA)	–	325,300	
	HCH dumpsite (NA)	–	3.36×10^5	
	Barren land with grazing cattle (NA)	–	29,775	
	Roadside close to dumpsite (NA)	–	165,762.5	
Lucknow, Uttar Pradesh[a]	Surroundings of Lindane production factory at Chinhat (NA)	–	61,100	Abhilash and Singh (2008)
Unnao, Uttar Pradesh[b]	Agricultural soils (NA)	13.81	1.65	Singh et al. (2007)
Aligarh, Uttar Pradesh[a,d]	Agricultural soils (NA)	34	88.9	Nawab et al. (2003)
Agra, Uttar Pradesh[a,d]	Agricultural soils (NA)	934	502	Singh (2001)
Farrukhabad, Uttar Pradesh[b]	Agricultural surface and sub-surface soil (NA)	337	158	Agnihotri et al. (1996)
Dibrugarh, Assam[b]	Agricultural fields (2009–10)	757	705	Mishra et al. (2012)
Nagaon, Assam[b]	Agricultural fields (2009–10)	903	835	

therefore attributed to high soil capacity and possible low biodegradation associated to acidic soils. Soil organic carbon was found to be positively correlated with high HCH and DDT levels in some studies (Mirian et al., 2008; Zhang et al., 2006) consistent with the findings from boreal soils (Meijer et al., 2003).

The paucity of available data doesn't allow any meaningful meta-analysis of time trends to be carried out for soil, even though data shows a potential decline tendency during last decade for both DDTs and HCHs (these trends were no significant). In addition concerning soil, no correlation was found between these two pesticides.

A few investigations have been conducted on occurrence of heptachlor and indosulfans in agricultural soil from different regions of India (Bishnu et al., 2009; Kumari et al., 2008; Singh, 2001; Singh et al., 2007). Much higher concentrations of heptachlors and endosulfans were detected in the soil from tea garden in West Bengal than the agricultural soil from wheat fields in Uttar Pradesh and Haryana. It may be because of high indiscriminate inputs of heptachlors and endosulfans in tea gardens to boost its production (Bishnu et al., 2009).

Two reports on a locally contaminated site are present in the literature. Studies on HCH distribution near their production units in Uttar Pradesh show that soil from industrial area is highly contaminated with all major isomers of HCH because of poor waste management. Concentrations of HCHs were found many thousand times higher than those in agricultural fields (Abhilash and Singh, 2008; Jit et al., 2011). The highest concentration (404,520 ng/g) of HCHs in soil was found in Lucknow City of Uttar Pradesh near Lindane production unit and from nearby barren land used for pasture (29,775 ng/g). Relative distribution of HCH isomers was found different for soil and the original waste from the production unit due to the spillage or outflow of lindane during manufacturing, packing, storage, and transportation. As the sampling sites were extending from the center of lindane production unit, the concentration of HCHs was also decreasing. For the same study the ratio of α-HCH/γ-HCH was found to be lower than one, indicating the fresh inputs of contaminants (Jit et al., 2011). In another study on the soil contamination in the nearby area of the lindane production unit, analysis of the soil samples from the adjoining rice fields to detect HCH levels, revealed that the average concentration of HCHs was 12,700 ng/g and higher residues (338,000–450,000 ng/g) in top layer of soil. This indicates that no measure has been taken to cover the contaminated site and nearby land to minimize direct wind erosion and rain water run-off. As a result, it is contaminating adjacent agricultural farms and water of two rivers (Reetha River and Sharda River) near the production unit.

2.4. Sediments

Sediments generally consist of detritus, inorganic and organic particles, and are generally spatially heterogeneous in terms of physic-biogeochemical characteristics (Hakanson, 1992). Similar to soil this heterogeneity can result in large spatial variability in POP levels. Sediments are integral part of aquatic systems representing it as major long-term capacitor for hosting contaminants. Reversibility of the contaminants exchange between sediment and the overlaying water column results in sediments playing as key secondary sources for the benthic and pelagic water ecosystem, effectively controlling biota exposure (Li et al., 2000; Zeng and Venkatesan, 1999). River and lake bed sediments are one of the major sink and reservoir for discharged POPs into the environment (Gomez-Gutierrez et al., 2006; Sodergren, 1997). Dynamics of accumulation of POPs in sediments is complex. POP residues in surface sediments of river, lake and ocean may reflect recent contamination, however residues in deeper layer of stable sediment cores provides historical record of water ecosystem exposure (Hong et al., 2003). These characteristics and the modality of the sampling clearly may affect the results of monitoring. Therefore, it is not easy to perform a comparative meta-analysis using sources from different studies due to methodological inconsistency. Nevertheless, the importance of research on polluted sediments to restrict impairment of aquatic system and biota has led to develop several methods for sediment quality assessment (USEPA, 2000). Available data on sediment contamination by POPs in India include data both from fresh water and the coastal marine systems. India has a coastal line of 8129 km where approximately 11% of global population lives (Amaraneni, 2006). 77 cities, including some of the largest and densely populated urban agglomerations like Mumbai, Kolkata, Chennai, Kochi, and Visakhapatnam are situated on the Indian coastal area. Occurrence of POPs in sediments from river, lakes, reservoirs, estuaries, bays, harbors and fish ponds were extensively investigated in different coastal and plan areas of India. All available concentration data of POPs in sediments have been reported in this section expressed in dry weight basis (Tables 4, S4).

As expected, huge variability in exposure levels is reflected, however, as a general trend, very high levels are reported for India compared to data from other regions of the planet. The range of DDTs (2–128,600 ng/g), HCHs (22,400–234,000 ng/g), dieldrin (1–19,600 ng/g), and endosulfan (89,600–238,000 ng/g) were found in the sediments of prawn ponds near Kolleru lake wetland in Andhra Pradesh. These concentrations were many thousand times higher than the Canadian sediment

Table 4
Average concentrations (ng/g) of POPs in sediments from various regions of India.

Indian state	Source and sampling year	ΣDDT	ΣHCH/BHC	References
Delhi	Yamuna River (NA)	–	38.047	Pandey et al. (2011)
Delhi	Yamuna River (2010)	<0.01–21.21	19.25–731.82	Kumar et al. (2011)
Keoladeo national park, Bharatpur, Rajasthan	Inside national park (NA)	1321.8	9039.9	Bhadouria et al. (2012)
	Outside national park (NA)	2028.1	10,335.4	
Uttar Pradesh	Gomti River (NA)	49.84	13.52	Malik et al. (2009)
Bhagalpur, bihar	Ganga River (2007–08)	589.15	36.21	Singh et al. (2012)
West bengal	Lower stretch of Hugli estuary (2003)	0.957	0.243	Guzzella et al. (2005)
Sunderban wetlands	Bay of Bengal (2005)	1.71	2.87	Binelli et al. (2007, 2009), Sarkar et al. (2008)
East coast of India	Mouth of Hugli River (1998, 1999, 2000)	58.25	152.7	Bhattacharya et al. (2003)
East coast of India		95.96	119.3	
East coast of India		75.75	190.6	
Mumbai, Maharashtra	Surface sediments of A. Marina (NA)	0.532	2.851	Shete et al. (2009)
Mumbai, Maharashtra	Marine surface sediments (NA)	5.86	9.9	Pandit et al. (2006)
Andhra Pradesh	Kolleru Lake, Prawn ponds (NA)	2–128,600	22,400–234,000	Amaraneni (2006)
Tamil Nadu, South India	Tamiraparani river basin (2008–09)	<0.01–857	<0.01–472	Kumarasamy et al. (2012)
Chennai, Tamil Nadu	Bay of Bengal (1998)	1.589	–	Rajendran et al. (2005)
Mahapalipuram, Tamil Nadu		0.141	–	
Pondicherry		1.731	–	
Cuddalore, Tamil Nadu		1.259	–	
Nagapattinum, Tamil Nadu		0.233	–	
Mandapam, Tamil Nadu		0.208	–	

quality guidelines for the protection of aquatic life (Amaraneni, 2006; CCME, 2002).

High DDT concentrations have been associated to the heavy use of DDT containing antifouling paints in fishing boat maintenance (Amaraneni and Pillala, 2000; Lin et al., 2009). High concentrations of POPs were also detected in Keoladeo National Park, Rajasthan which revealed the use of POPs in the past for controlling termites in the agricultural areas near to the national park in Rajasthan. Mean concentration of aldrin in sediment samples from outside the national park was detected to be 687 ng/g, while the same pesticide inside the national park was 478.4 ng/g. This reflects use of aldrin in agricultural area and Ajanbundh (the primary sources of water to national park) (Bhadouria et al., 2012; Muralidharan, 2000). Endosulfan exposure was particularly high due to the fact that in India after ban on pesticidal POPs; it was largely used as a substitute.

Concentrations of sediment POPs in river and estuaries from Delhi, West Bengal, were found comparatively lower than in wetlands from Rajasthan and Andhra Pradesh. However, these concentrations were still higher than the Canadian sediments quality guidelines for the protection of aquatic life (CCME, 2002). In Yamuna River, Delhi; Hugli estuary; sunderban wetlands and marine surface sediments from Mumbai, levels of HCHs were found relatively higher than DDTs. Concentrations of DDTs, HCHs and PCBs were very low in surface sediments of Bay of Bengal compared to other Indian regions. High chlorinated PCB congeners were predominant in sediments away from Bay (Rajendran et al., 2005). This may be associated to the higher affinity of highly chlorinated PCBs for suspended and settling particulate material leading to their enrichment in coastal sediments (Hong et al., 2003). Concentrations of DDTs in Bay of Bengal ranged from 0.04 to 4.79 ng/g. These concentrations were several orders of magnitude lower than the highest concentrations reported for same areas 15 years earlier possibly reflecting a significant decline in DDT usage and exposure in that area (Sarkar and Sen Gupta, 1988).

A study focused on the Bay of Bengal area reported data for PCBs in sediments, PCB levels (0.02–6.57 ng/g) were comparable to those found in the sediments of Caspian Sea (de Mora et al., 2001), Black Sea (Fillmann et al., 2002) and Xiamen Harbor, China (Hong et al., 1995). DDT levels (0.04–4.79 ng/g) were comparable to Black Sea–Turkey and Russian Coast (Fillmann et al., 2002), Caspian Sea (de Mora et al., 2001), but lower than Xiamen Harbor China (Hong et al., 1995). Similar trend was found with HCHs levels in Bay of Bengal. Mean concentrations of Most of the POPs in sediments generally follows the sequence: lake, wetlands > river sediments > estuary sediments > marine sediments; as lakes represent closed system, lake sediments are more efficient accumulator of POPs. Paucity and elevated scattering of available data did not allow drawing any conclusion on evident or significant time trends. The linear regression analysis between DDT and HCH data highlighted the occurrence of a significant ($P < 0.05$) direct correlation, reflecting the same results observed in the surface water and ground water dataset.

3. POPs in aquatic organisms

Fish and other aquatic species are one of the important sources of high-grade protein for Indian people (Huntington and Hasan, 2009). India is the third largest producer of fish and second for inland fish production (Feroz and Panikkar, 2006). Due to high bioaccumulation of POPs, aquatic biota is often used as time integrated matrix for biomonitoring. The link between aquatic organisms and human exposure is crucial and well documented (Colborn and Smolen, 1996; Jepson et al., 2005; Ylitalo, 2005). It has been observed that greater than 80% of the total intake of POP residues in human is via food chain (Martinez et al., 1997) with a significant fraction derived from contaminated fish consumption (Mwevura et al., 2002). Biomonitoring activity in India has been mainly focused on fish, prawns, and mussels. Most of the monitoring studies were concentrated on the exposure of DDTs, HCHs, dieldrin, chlordane, heptachlors, endosulfans, HCB, and PCBs (Tables 5, S5).

High levels of POPs in aquatic system have promoted the need for monitoring of bioaccumulation, biomagnifications, and vulnerable toxic effects from exposure to aquatic species. Levels of DDTs and HCHs were detected in all fish samples from rivers (snowfed and rainfed) in Kumaon Himalayas, Uttrakhand. However, no endosulfan residues were detected in the fish tissues probably also due to high method detection limits. Higher concentrations of DDTs (13–55 ng/g) were detected in fish tissues from snowfed rivers as compared to rainfed rivers (6–9 ng/g) (Sarkar et al., 2003). A study to compare the DDTs and HCHs bioaccumulation levels in fish (carp and cat fish) tissues from an unpolluted controlled pond and Ganges River was carried out in Uttar Pradesh (Singh et al., 2008) showing consistently higher levels of contaminants in fish from Ganges. Concentrations of DDTs and HCHs in fish tissues from Ganges River were thousand times higher than the permissible limits of USEPA. In general, HCH and DDT concentrations were found higher in cat fish than carp fish. Bioconcentration of γ-HCH was higher compared to other isomers, possibly due to isomerization of α- and β-HCHs (Singh et al., 2008).

As elevated concentrations of POPs extensively used in India, such as DDTs, PCBs and HCHs were detected in Ganges River dolphins (*Platanista gangetica*) (a top predator) from India in some studies performed during the decade of 90s (Kannan et al., 1993, 1997). In Irrawaddy dolphin adult blubber, an endangered cetaceans found in estuaries and sea coast of Orissa, DDT was the predominant POPs with concentrations as high as 10,000 ng/g lipid weight. These levels are 2–3 folds lower than those reported for bottlenose and spinner dolphins during 1990–1991 and 1997–1999 (Karuppiah et al., 2005; Tanabe et al., 1993). PCBs in Irrawaddy dolphins were also lower than the levels in dolphins from the Bay of Bengal (Karuppiah et al., 2005). PBDEs were also measured in dolphin blubber, levels ranged 0.98–18 ng/g lipid weight which was associated to the presence of an industrial e-waste recycling facilities in the nearby area of Chilka Lake, Orissa.

Green mussels have also been extensively analyzed as reference media for environmental monitoring of POPs. POPs in green mussels along the India coast (West Bengal, Orissa, Andhra Pradesh, Tamil Nadu, Pondicherry, Kerala, Karnataka, and Goa) were examined during 1994–1997 (Tanabe et al., 2000). Levels of POPs in green mussels were many folds lower than what was reported in biota from the streams and rivers of Northern India. In mussels as well, DDTs was the predominant POP. Comparing the PCB concentrations in green mussels with those from earlier studies (Kannan et al., 1995; Ramesh et al., 1990b; Tanabe et al., 1990), it was found that no significant decline was taking place (Tanabe et al., 2000). Interestingly, considering the mussel dataset, no large spatial variability in POP levels was evident, which contrast with a scenario in which many local contamination hotspot control environmental exposure. Despite this, higher levels of DDTs were detected in sampling sites in proximity of urbanized areas (Konarak, Orissa; Madras, Tamil Nadu; and Pondicherry).

Concerning the HCH isomers in mussels, α-HCH was the predominant isomer at many sampling sites, which reflects the recent usages of technical HCH in India. Relatively low levels of HCB in green mussels, compared to other OCPs also possibly reflect lower use this pesticide in India. Comparing these concentrations with other Asian countries, it was found that PCB levels in Indian mussels were higher than in green mussels collected from Philippines, while lower concentrations of PCBs were detected in India and Thailand (Tanabe et al., 2000). All Indian samples were however consistently characterized by relatively higher levels of DDTs and HCHs.

Levels of DDTs and HCHs in different marine fish species from Mumbai, Maharashtra have been studied (Pandit et al., 2006). Concentrations of total HCHs in different marine species ranged 0.87–33.73 ng/g while DDTs ranged 0.38–34.1 ng/g. These concentrations were lower than those in fish from temperate regions which partially may reflect different organism metabolism (Wania and Mackay, 1993). Concentrations of POPs (DDTs, HCHs, dieldrin, heptachlor, and endosulfans) were detected in nine species of wetland fish in Karnataka (Dhananjayan and

Table 5
Average concentrations (ng/g) of POPs in biota samples from various regions of India.

Sampling site, Indian state (sampling year)	Species name	ΣDDT	ΣHCH/BHC	References
Kumaon Himalayan Region[b,c] (1999)	*Tor Putitora, Schizothorax richardsonii*	35	3	Sarkar et al. (2003)
Gujartal, Jaunpur (Controlled site), Uttar Pradesh. Average in Liver, Brain and Ovary (NA)	Catfish — *Rita rita*	67.11	85	Singh and Singh (2008)
	Carp fish — *Cyprinus carpio communis*	25.33	35.5	
	Catfish — *Mystus tengara*	57.11	78.68	
	Carp fish — *Labeo rohita*	57.82	27.22	
Ganges river (Varanasi[a], Uttar Pradesh) (NA)	Catfish — *Mystus tengra*	5891.63	3536.72	
	Carp fish — *Labeo rohita*	2018.19	2428.28	
Ganges river Basin (India) (NA)	Cat fish	7.58	3.06	
	Carp fish	3.34	1.36	
Gomti River (Jaunpur[b]), Uttar Pradesh (NA)	Cat fish (*Rita rita*)	7582.68	3061.12	
	Carp fish (*Cyprinus carpio communis*)	3338.9	1360.24	
Gomti river, Uttar Pradesh (NA)	Cat fish	5.891	3.54	
	Carp fish	2.018	2.43	
Gomti River, Uttar Pradesh (2004–05)	Fish (*Channa punctatus*) muscles	1.53	1.496	Malik et al. (2007)
Ganges river Basin (India)	Fish tissues	0.42	1.108	Akhtar et al. (2009)
Chilika Lake[b], Orrisa (2000–01)	Irrawaddy dolphins	2721.81	270.8	Kannan et al. (2005)
Subarnarekkha and Konarak[b], Orissa (1995)	Green mussel (*Perna viridis*)	7.3	5	Tanabe et al. (2000)
Digha[b], West Bengal (1995)	Green mussel (*Perna viridis*)	3.5	2	
Mahim[b], Maharashtra (1995)	Green mussel (*Perna viridis*)	7.1	10	
Mumbai, Maharashtra[a,d] (NA)	Bangda (*Rastrelliger kanagurta*)	34.15	14.02	Pandit et al. (2006)
	Halwa (*Parastromateus niger*)	0.56	0.87	
	Dog fish (*Scoliodon laticaudus*)	32.56	33.73	
	Paplet (*Pampus argenteus*)	34.05	15.54	
	Rawas (*Eleutheronema tetradactylum*)	0.77	7.97	
	Prawn (*Panaeus monodon*)	0.38	2.64	
	Crab (*Portunus sanguinolentus*)	2.47	14.82	
	Clam (*Villorita cyprinoids*)	7.89	3.55	
Mangalore[a,d], Karnataka (1995)	Green mussel (*Perna viridis*)	18	10	Tanabe et al. (2000)
VisakhaPatnam[a,d], Kakinada[d], Machilipatnam[d], AndhraPradesh (1995)	Green mussel (*Perna viridis*)	6.13	3.36	
Goa, Goa[a] (1995)	Green mussel (*Perna viridis*)	5.8	9.5	Tanabe et al. (2000)
Inland Wetlands, Karnataka (2002–03)	*Anguilla bicolor bicolor*	12.3	51.7	Dhananjayan and Muralidharan (2010)
	Catla catla	5.9	6.2	
	Channa striatus	4.1	9.3	
	Cirrhinus mrigala	3.4	5.9	
	Clarias batrachus	<1.0	27.2	
	Cyprinus carpio	8.1	5.1	
	Heteropneustes	<1.0	2.1	
	Labeo rohita	6.6	24.8	
	Tilapia mossambica	6.9	16.1	
Kolleru Lake[b], Andhra Pradesh (NA)	Prawn	9800	32,200	Amaraneni (2006)
Coastal area (Tamil Nadu)(1995)	Green mussel (*Perna viridis*)	11.18	5.3	Tanabe et al. (2000)
Pondicherry[a] (1995)	Green mussel (*Perna viridis*)	26	7.1	
Cochin[a] and Calicut[b], Kerala (1995)	Green mussel (*Perna viridis*)	10.9	6.5	
Bay of Bengal, India (1997–99)	Bottlenose dolphin (*Tursiops truncatus*)	12,930	171	Karuppiah et al. (2005)
	Spinner dolphin (*Stenella longirostris*)	9025	311	
	Hump-backed dolphin (*Sousa chinensis*)	19,970	230	
Bay of Bengal, India (1997)	Cat fish (*Tachysurus thalassinus*)	534.16	88.12	Das et al. (2002)
Bay of Bengal, India (1990–91)	Bottlenose dolphin (*Tursiops truncatus*)	7250	131	Tanabe et al. (1993)
	Spinner dolphin (*Stenella longirostris*)	17,180	346	
	Hump-backed dolphin (*Sousa chinensis*)	12,330	507	

Muralidharan, 2010). Among all the monitored POPs, concentrations of HCHs were predominant. β- and γ-HCH were the most dominant among the isomers of HCHs due the stability of β- and γ-HCH isomers and isomerism of α-HCH (Kole et al., 2001). Endosulfan, heptachlor and dieldrin were the less abundant POPs in these samples; their levels were lower than the residue reported in fish samples from Kolkata, West Bengal (Kole et al., 2001).

Study on bioaccumulation of DDTs, HCHs, and PCBs in the dolphins from Bay of Bengal reveals very high levels when compared with data from other Asian regions (Das et al., 2002; Karuppiah et al., 2005; Tanabe et al., 1993). DDTs (ranging 3330–23,000 ng/g) and HCHs (ranging 141–1227 ng/g) suggest that these organisms are also exposed to different types of POP sources. Higher concentrations of PCBs were likely to be from ship breaking yards, as well as operational and defunct industrial equipments including transformers, capacitors and electrical appliances dismantled often in an inappropriate way in coastal areas of this region.

3.1. Time trends in aquatic organism data

Concerning aquatic organisms, sufficient data of DDTs and HCHs are available to attempt a meta-analysis of time trend (Fig. 3). The dataset includes concentrations of POPs in species at different trophic levels and both marine and fresh water organisms. The analysis was therefore performed by excluding top predators and benthic organisms. The analysis of the data (N = 30) including only marine coastal and fresh water fish did not provide any evidence of time dependent trends for DDTs and HCHs. An average value for each year in which a monitoring was performed is in the order of tenth of ng/g with value scattering within 1–3 orders of magnitude. Concentration data of blue mussels collected along Indian coastal areas unfortunately were only collected during a single year (1995) therefore no trend analysis could be performed. Their mean values were similar to the mean concentration value of fish. Data of fresh water and marine mammals were more frequent in the decade of the 90s. The levels were generally 1 to 2 orders of

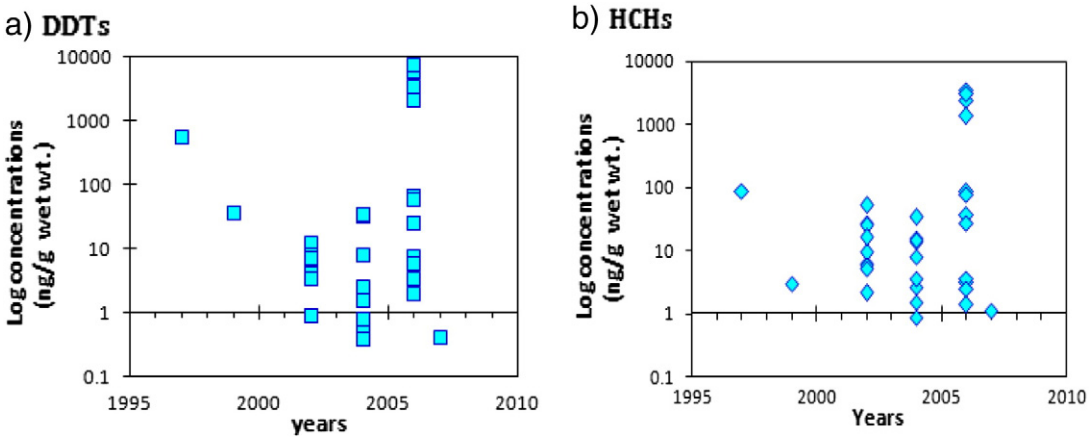

Fig. 3. Time trend meta-analysis of levels of (a) DDTs and (b) HCHs in aquatic organisms in India.

magnitude higher than those measured in fish, however, due to limited time span covered by the data and paucity of data no sound trend analysis could be performed for both DDTs and HCHs in aquatic organisms. Similar to what observed in the surface water sediment also in aquatic biota, DDT and HCH concentrations were highly correlated ($P < 0.05$).

4. POP exposure in humans

Humans are sensitive target of POP bioaccumulation and associated adverse effects (Falck et al., 1992; Krieger et al., 1994). Human can uptake POPs through different pathways bring the diet a major vector (John et al., 2001; Waliszewski et al., 1997). POPs, and in particular OCPs have been widely used by farmers in India due to their widespread availability and simplicity in application, therefore other pathways of exposure (e.g. inhalation, skin contact) are likely relevant in certain population (Dhananjayan et al., 2012).

The presence of POPs in human blood and milk is a matter of concern, globally (Massart et al., 2005). Human milk is, together with air, the compartment selected by UNEP for global monitoring of POPs. The available data on human exposure to POPs (namely through monitoring blood samples Section 4.1 and milk samples Section 4.2) are summarized in Tables 6 and 7; S6, and S7. The analysis categorizes the dataset into rural and urban contests of India.

4.1. Human blood

Few studies are available from different regions of India reporting data on POP residues in human blood. These have been summarized in Tables 6 and S6. Considering the size of the Indian population and the expected generally high exposure, these datasets appear to be relatively limited in number and basically reflecting mainly local population exposure rather than facing a general assessment of diffuse human exposure to POPs.

Once again the richest dataset refer to DDTs and HCHs with limited data for other POPs. Most of the available studies focused on rural population, or on the comparison of rural and urbanized populations.

A few datasets are also available on pesticides other than DDTs and HCHs. Concentrations of heptachlor have been found maximum in female blood samples from Rajasthan. Rajasthan and Haryana are important farming regions of India where likely the most intensive usages of these pesticides took place, as also indicated by contamination data from other environmental compartments. Concerning direct exposure on the site of pesticide applications, one study reported that inhalation represented an important uptake pathway (Kaushik et al., 2012). The study reported that even though the residues of DDTs and HCHs in farmer's blood have decreased during the decade of 1992–2002 in Haryana, still values are very high compared to other Indian regions and data from other part of the world (Kaushik et al., 2012; Mathur et al., 2002).

One study from this regions combined human exposure through blood monitoring with health outcomes in Rajasthan (Mathur et al., 2002). Study also reveals that female from rural areas have higher exposure to POPs as compared to their urban counterparts (Krieger et al., 1992; Mathur et al., 2002). Most of the samples in the study were vegetarian; however females with non-vegetarian diet had significantly higher levels of POPs (Mathur et al., 2002).

Another study reports POP data in blood samples of farmers engaged in agricultural and sheep wool work in the neighborhood of Bangalore City, Karnataka (Dhananjayan et al., 2012). POP residues in human blood were not significantly correlated with demography, dietary habits, sex, age and smoking status of blood donors. HCHs was the

Table 6
Average concentrations (μg/l) of POPs in human blood samples from various regions of India.

Indian state	Source and sampling year	ΣDDT	ΣHCH/BHC	References
Bhatinda, Roper district, Punjab[a,d]	Villages (NA)	65.2	57	Mathur et al. (2005)
Haryana	1992	644.5	536	Kaushik et al. (2012)
	2002	69	66	
Jaipur, Rajasthan[a]	Normal females (NA)	1489.3	366.7	Mathur et al. (2002)
	Female suffering from breast cancer (NA)	3856.7	2633.7	
Delhi[a,d]	(NA)	301	–	Saxena et al. (1987)
	(NA)	710	490	Ramachandra et al. (1984)
Lucknow, Uttar Pradesh[a]	(NA)	28	75	Kaphalia and Seth (1983)
Ahmedabad, Gujrat (Urban)[a,d]	(NA)	32.6	41.2	Bhatnagar et al. (2004)
Ahmedabad, Gujrat (Rural)	(NA)	47.7	148	Bhatnagar et al. (1992)
Nagaon, Assam[b]	Male and female of age between 9 and 76 years (2009–10)	743	627	Mishra et al. (2011)
Dibrugarh, Assam[b]		417	348	
Banglore, Karnataka (Rural)	Agriculture and sheep wool workers (NA)	10.6	26.7	Dhananjayan et al. (2012)
Madurai, Tamil Nadu[b]	Habitants from coast (NA)	8–26	6–61	Subramaniam and Solomon (2006)

Table 7
Average concentrations (ng/g lipid wt.) of POPs in human milk samples from various regions of India.

Indian state	Sampling year	ΣDDT	ΣHCH/BHC	References
Delhi[a,d]	(Age: 20–30 years) (NA)	–	380	Banerjee et al. (1997)
Delhi	(Age: 20–30 years)(NA)	344	–	Zaidi et al. (1989)
New Delhi	(2005–06)	1500	340	Devanathan et al. (2009)
Agra, Uttar Pradesh[a,d]	(NA)	175.5	127.25	Kumar et al. (2006)
Ahmedabad, Gujrat[a,d]	(Age: 18–30 years) (1981–82)	305.83	224.6	Jani et al. (1988)
Kolkata, West Bengal[a,d]	(2004–05)	1100	670	Devanathan et al. (2009)
Kolkata, West Bengal	Dumping site (2004–05)	665	265	Someya et al. (2010)
Kolkata, West Bengal	Reference site (2004–05)	1150	690	Devanathan et al. (2009)
Dibrugarh, Assam[b]	(2009–10)	2870	2330	Mishra and Sharma (2011b)
Nagaon, Assam[b]	(2009–10)	3210	2720	
Mumbai, Maharashtra[a,d]	(2005–06)	450	220	Devanathan et al. (2009)
Mumbai, Maharashtra[a,d]	(NA)	510.5	289.75	Sharma et al. (2001)
Chennai, Tamil Nadu[a,d]	(2002–03)	1200	4500	Subramanian et al. (2007)

dominant class in most of the blood samples accordingly also with other studies (Kaushik et al., 2012; Mathur et al., 2002, 2005; Mishra et al., 2011). In Assam, the concentrations of DDTs and HCHs in human blood were monitored in two districts (Nagoan and Dibrugarh) showing contrasting results. In this case POP levels were found to be related to age, gender, and habits of blood donors (Mishra et al., 2011). Relatively high levels of DDTs and HCHs were found in all the blood samples with p,p′-DDT and β-HCH being the most abundant POPs.

Residues of DDTs and HCHs were found to strongly directly correlate with age both in male and female donors (Bates et al., 2004; Zumbado et al., 2005). Another study reports higher levels of DDTs and HCHs in male population of Assam (Mishra et al., 2011). This may reflect higher participation of male population with agricultural practices. While relating the DDT and HCH residues in human blood with living areas, lower exposure of POPs was found in urbanized population than in the rural one. The situation may of course be different for other POPs, unfortunately, however paucity of data does not allow access these trends for PCBs or dioxins.

Concerning non-pesticidal POPs, direct and occupational exposure of PCBs and PBDEs to e-waste recycling workers in Bangalore, Karnataka was studied (Eguchi et al., 2012). Concentrations of PCBs in serum of residents of the coastal area were found lower (140 pg/g wet wt.) than those in non-vegetarian e-waste recycling workers (360 pg/g wet wt.), but higher than PCB concentrations in vegetarian e-waste recycling workers (60 pg/g wet wt.). This suggests that even under conditions of elevated direct exposure to airborne PCBs, dietary intake of contaminated fish and meat is major pathways controlling human exposure to PCBs (Eguchi et al., 2012; Fitzgerald et al., 2004; Sjodin et al., 2000). Similar results were reported for PCB residues in breast milk samples from e-waste recycling workers and common population in Vietnam (Tue et al., 2010). Accordingly, high PCB levels in human serum samples from e-waste locations were also detected in China (Xing et al., 2009). PBDE residues in serum of e-waste recycling workers in India averaged 340 pg/g wet wt. (Eguchi et al., 2012) and were somehow higher than that in the general population living on the coast. This indicates that e-waste recycling is an important economic activity in certain parts of India is a relevant source of PBDEs for humans. Similarly high PBDE residues from e-waste recycling workers were detected in China (Qiu et al., 2007; Sudaryanto et al., 2008).

4.2. Human milk

Available data on POP concentrations in human milk in India are summarized in Tables 7 and S7. The dataset include two studies performed in the late '80s, one study in 1997 and 14 studies performed after 2009.

Three studies were published for sampling site Delhi in 1989, 1997, and 2009 (Banerjee et al., 1997; Devanathan et al., 2009; Zaidi et al., 1989) which may serve to track possible long term trends of human exposure for that area. Concentrations of DDTs were ranged 344–1500 ng/g lipid wt., while HCH levels ranged 340–380 ng/g lipid wt. These values reported levels many times lower than those measured in women living in other areas of India (e.g. Punjab) (Kalra and Chawla, 1981). Study conducted on human milk contamination in Delhi found that the ratio of p,p′-DDT/p,p′-DDE was 1.44, indicating recent exposure to "fresh" DDTs inputs (Zaidi et al., 1989). In this study, the average daily intake of DDTs for breast-fed newly born was found to be approximately 0.062 mg/kg body wt. which is about 12 times higher than the guideline value (0.005 mg/kg/day) of FAO/WHO (FAO/WHO, 1987). Among HCHs isomers, β-HCH was the predominant one. Another study carried out in Delhi (Banerjee et al., 1997), calculated daily average intake of HCHs through mother's milk in the range of 0.065 mg/kg body wt., approximately 5 times higher than acceptance level (0.012 mg/day/kg body wt.).

Samples of human milk from rural areas in villages of Agra district, Uttar Pradesh (Kumar et al., 2006), were analyzed for DDTs, revealing DDT levels higher than HCH residues, reflecting the pattern of historical higher use in agricultural and for anti-malarial programs. Interestingly, α-HCH and β-HCH were detected in all the samples while γ-HCH was not detected in any sample. Higher levels of β-HCH were likely due to its more stability, lower mobility elimination rate (Jensen, 1983). Comparatively lower levels of chlordane, HCB and PCBs were detected reflecting lower exposure to these POPs in these rural areas. Conversely, high concentrations of PCBs, 15–1700 ng/g lipid wt. [reference value 1.2 mg/kg (Schulz et al., 2012)], were recently detected in human milk samples collected from industrial cities from states such as Delhi, West Bengal, Tamil Nadu and Andhra Pradesh (Devanathan et al., 2009, 2012; Someya et al., 2010; Subramanian et al., 2007). High residues of PCBs in human milk from Indian cities reveal the presence of significant active primary sources (Talyan et al., 2008). The influence of municipal dumping sites as drivers of PCB and PBDE contamination was analyzed in different urban locations of India (Devanathan et al., 2012).

The highest concentrations of PCBs and PBDEs were detected in human milk from population residing near municipal dumping site in Kolkata, followed by population from urban, urban slum, rural fishing village and suburban areas. This suggests that municipal dumping sites are relevant sources of PCBs exposure to humans in India. Elevated levels of POPs in ground water, soil, crow and fish collected near the dumping sites in India have also been reported confirming this result (Jit et al., 2011; Someya et al., 2010; Watanabe et al., 2005). Residues of PBDEs in human milk samples were significantly lower than those of PCBs and showed maxima in population residing in urban areas followed by those in fishing villages, suburban and slum areas. This trend was explained by relating exposure to PBDEs to higher use of electrical appliances, more sophisticated residential furnishings, rug, and drapery textiles in urban area than in rural areas (Kalantzi et al., 2004).

In Assam, where DDT is heavily applied for malaria vector control, DDT and HCH consumption has been monitored during the last 20 years (Dev et al., 2001). Assam is rich in forest and has vast tracts

of fertile land. More than 50% population of Assam is engaged in agricultural and allied activities. Thus in Assam, DDTs and HCHs have also been intensively used for agricultural purposes in Paddy, wheat and tea crops. Study conducted in Assam revealed that total mean DDT and HCH residues in human milk from Nagaon district, Assam were 3210 ng/g lipid wt. and 2720 ng/g lipid wt., respectively. While the concentration of the same were 2870 ng/g lipid wt. and 2330 ng/g lipid wt. in human milk collected from Dibrugarh district, Assam (Mishra and Sharma, 2011b). It was also found that β-HCH was the predominant isomer of HCH, detected in all the milk samples. The ratio of β-HCH/α-HCH was reported 0.74 and 0.63 in Nagaon and Dibrugarh, respectively (Mishra and Sharma, 2011b). These ratios were found higher than those in Iran (0.545) (Behrooz et al., 2009); Poland (0.037) (Szyrwinska and Lulek, 2007); China (0.0058) (Leng et al., 2009). p,p′-DDT (43–44%) was the predominant metabolite among DDT residues which was many times higher than those reported for other developing nations like Indonesia (18.3%) (Burke et al., 2003); China (8.09%) (Kunisue et al., 2004); and Vietnam (17%) (Minh et al., 2004), reflecting recent usages of DDTs for malaria eradication and agricultural purposes (Mishra and Sharma, 2011b).

The age of the mothers was found to be highly correlated with HCH and DDT residues in breast milk. Many studies in the past have reported that longer breast feeding period leads to lower residues of DDTs and HCHs in human breast milk due to their transfer to infant via breast milk feeding (Minh et al., 2004). According to this frame, many studies reported higher concentrations of HCHs in primipara mothers than those in multipara mothers. These results obtained in India are fully consistent with observations reported in many other countries (Bouwman et al., 1990; Chao et al., 2006; Czaja et al., 2001; Minh et al., 2004; Polder et al., 2009; Tan et al., 2008). Contrastingly, a study conducted on POP residues in mother's milk from Madras, Tamil Nadu reported that there is no variation in levels of POPs in milk samples of primipara and multipara mothers (Subramanian et al., 2007). This may depend on continuously ongoing exposure to high POP levels in this region.

4.3. Time trend of POP levels in human and cross-regional comparative analysis

Available data on human blood contamination from India (N = 18) span from 1981 to 2009, with most of the monitoring performed after year 2000. Data spread across 3.5 orders of magnitude and do not show any trend for either DDTs or HCHs (Fig. 4). For other POPs the lack of data prevents the possibility of performing the time trend meta-analysis.

Concerning human milk samples, the total available dataset include N = 23 data points for both DDTs and HCHs with the first observation performed during the decade of the '80s. Most of the dataset refers to the period following 1999. Obviously, different study considered different populations (in terms of age of the mothers and number of prior pregnancies, but also different living conditions, geographical locations and food habits) and different analytical methods (a brief summary of analytical methods have been provided in Table S8). This explains the broad variability in the concentrations. Overall, concentration trends in human milk (expressed on log scale) appear to follow a significant increase for DDTs and a non-significant increase for HCHs levels. This is also verified when the two early data points from the 1981 and 1987 studies (pulling downward the left hand side of the trend line) are excluded. The highest concentrations were recorded by the most recent study (Mishra and Sharma, 2011b) performed in Assam and including about 200 women with subpopulation categorized into rural/urban and primipara/multipara. This is the most articulated and solid dataset (due to the large number of monitored women). The data from this study influences the observed increasing time trends for both DDTs and HCHs. Since rural areas in Assam are hot spots of organochlorine pesticide exposure, the overall time trend may be a simple consequence of the fact that previous studies were performed in less exposed contests. However, in the case of DDTs the increasing time trend is still present (but not significant) even after excluding these results from the analysis.

Since organochlorine pesticides have been banned in India only in recent years (1989–2001) (while DDTs is still in use for malaria vector control), the lack of evidences in decline for human blood samples and the possible increasing trend in human milk concentrations emerged from the meta-analysis may very well reflect current exposure pattern for the general Indian population. These patterns are consistent with those observed in other environmental media. In addition, similarly to surface water, ground water, sediments and aquatic organisms, level of DDTs in blood and milk were highly correlated with the level of HCHs ($P < 0.01$).

5. Cross country comparison of POP contamination meta-data

India is generally regarded as a hot spot of POP contamination to human and environment, although no systematic comparative and critical analysis of environmental levels and human exposure was ever performed so far considering all available sources of information. The data presented in this review for DDTs and HCHs in particular, seem to support this view. In order to assess the state of Indian environment POP contamination, it is useful to compare available meta-data with other countries. To this regard China was chosen here as benchmark country with similar on-going rapid economic development and a recent past record of organochlorine pesticides usage to support agricultural production and malaria vector control. In addition, thanks to considerable investment in environmental monitoring, a number of dataset on environmental and human contamination by POPs are available for China (Tables S9, S10 and S11). Most of the POPs are banned to manufacture, use import in India (except DDT) and China (except DDT, HCB,

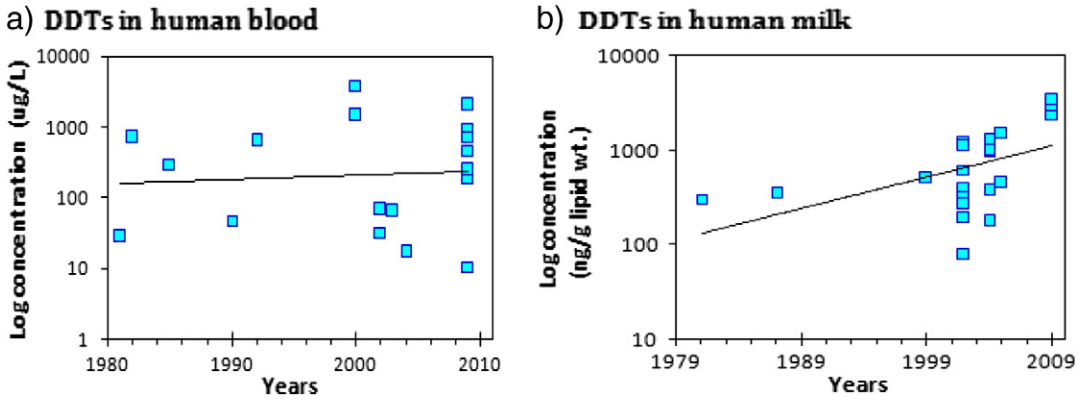

Fig. 4. Time trend of DDTs in (a) human blood and (b) human milk in India.

chlordane and mirex). India has been the largest producer/user of DDT in the world, followed by China (Li et al., 1998a; PANNA, 1990). Total production of technical HCH was 1.0 million tons (in India) and 4.5 million tons (in China) before it was banned in 1997 and 1983, respectively (Kannan et al., 1995; Li et al., 1998b). Present comparative analysis focuses on DDTs and HCHs in freshwater, aquatic organisms, and human blood and milk. Limited data availability for compartments such as air, ground water, soil and sediments; especially from India hampers the possibility of expanding the analysis of these compartments.

Data of surface water contamination showed similar ranges of exposure and median contamination levels in the two countries (Fig. 5a). The highest reported concentration data (exceeding solubility limits, and therefore likely referring to localized contamination hotspots) were excluded. Meta-data based time trend analysis showed that a decline of DDT and HCH levels in Chinese surface water is likely occurring. In India a similar trend is only visible for HCHs while DDT didn't show any decline trends in the surface water, likely as a result of ongoing primary emissions. Declining trends of HCHs in surface water are particularly evident in both countries starting from the year 2001 after the ban was introduced in both countries.

Data on levels of DDTs in human milk in India appeared to follow a significant increase trends during last three decades in India, while a similar meta-analysis in China did provide evidence of a non-significant decline trends (Fig. 5b). Median and maximum concentrations of DDTs in human milk tended to be higher in dataset from China until 2004, while more recent data fall within the same range in the two countries. Data of HCHs in human milk are highly scattered in both countries (no time trend visible) with similar ranges and median values. Similar figures also emerge for human blood samples where similar levels emerge from available datasets in the two countries and elevated data scattering prevented any assessment of time trends.

Levels of DDTs in aquatic organisms (fish and benthic organism, excluding top predators) were similar in India and China, while HCH data showed the tendency for higher exposure levels in India (Fig. 5c).

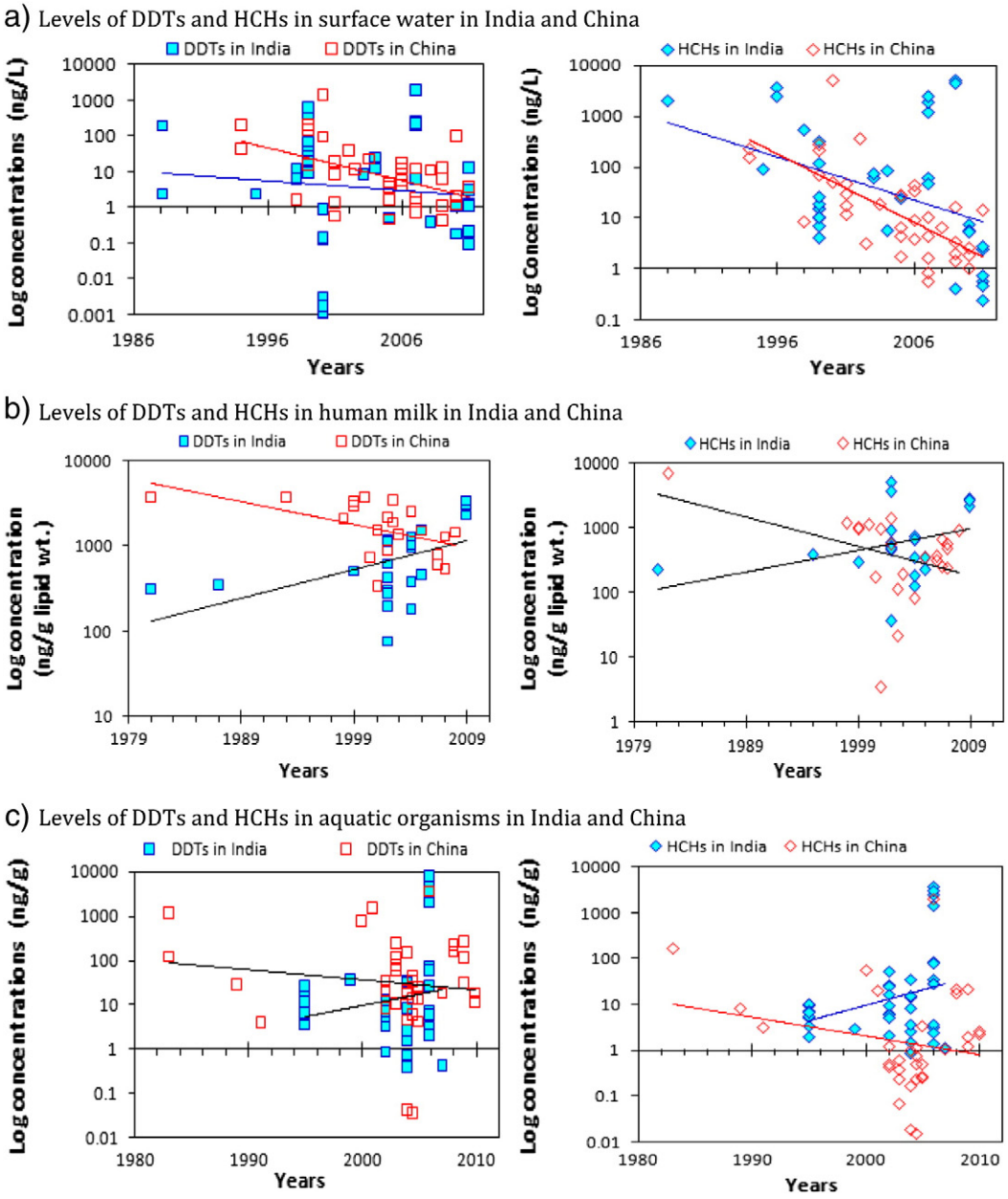

Fig. 5. a. Levels of DDTs and HCHs in surface water in India and China. b. Levels of DDTs and HCHs in human milk in India and China. c. Levels of DDTs and HCHs in aquatic organisms in India and China.

Altogether this comparative meta-analysis showed similar high DDT and HCH exposure levels for human and the environment in India and China, however from the Chinese dataset the tendency towards declining time trends for DDTs in human milk and surface water was evidenced, a behavior which is not reflected by Indian dataset.

6. Conclusions

From this comprehensive and systematic analysis of the full body of literature on POP environmental and human contamination in India following conclusions can be traced:

- Available data are highly fragmentary and typically refer to rural or urban areas. Little information is available on background environment contamination (e.g. remote and mountain areas) to be used for comparison term and for comparing background levels with other geographic contexts.
- Data are fairly abundant only for DDTs and HCHs and partially for HCB and PCBs. Concerning other legacy POPs, such as dioxins, furans and PBDEs, available data are still insufficient to trace a nationwide assessment of environmental and human exposure. This is of course also the case of those compounds which more recently included in the Stockholm Convention such as perfluorinated compounds.
- India is unarguably a hotspot of DDT and HCH contamination. Residues in all compartments considered in this study are often exceeding limits established by some international regulatory agencies. This is of course due to the elevated use of pesticidal POPs in agriculture carried out until recent years, and in the case of DDTs still ongoing applications for malaria control.
- No evidence of general decline in DDT and HCH levels in Indian environment (with exclusion of HCHs in surface water) emerged from the meta-analysis of time trends.
- Elevated environmental exposure is reflected by data from human biomonitoring. Basically all available data of DDTs and HCHs in human milk and blood were found to exceed safety limits, while no declining trends were observed after the introduction of regulation on POPs.
- Indian environmental and human contamination by DDTs and HCHs are comparable to those of china, although in China (unlike India) the tendency toward declining environmental contamination is visible, possibly due to earlier substitution and more restrictive measures adopted for pesticidal POPs.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.01.022.

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Příloha 24

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Size specific distribution of the atmospheric particulate PCDD/Fs, dl-PCBs and PAHs on a seasonal scale: Implications for cancer risks from inhalation

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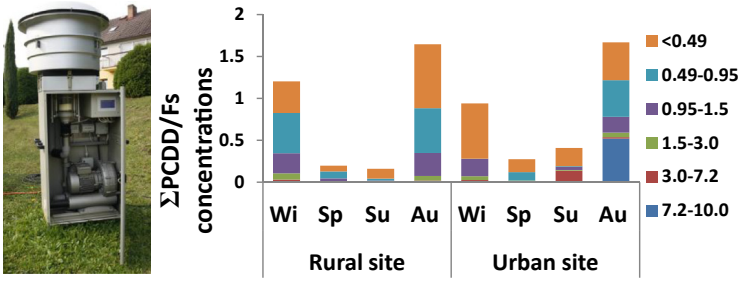
HIGHLIGHTS

- Seasonal size-specific concentrations were obtained for PAHs, PCDD/Fs and dl-PCBs.
- Combustion-related compounds showed highest concentrations in cold seasons.
- 60–73% of PAHs, PCDD/Fs and dl-PCBs were associated with particles <0.95 μm.
- Cancer risks from inhalation were 6.8–41 times higher in winter than in summer.

GRAPHICAL ABSTRACT



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ABSTRACT

This study presents the seasonal size distribution of particulate polycyclic aromatic hydrocarbons (PAHs), polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) and dioxin-like polychlorinated biphenyls (dl-PCBs) in the atmosphere. Particles were sampled from October 2009 to October 2010 on a seasonal basis using a cascade impactor collecting six size fractions at a rural and urban site in the Brno area, Czech Republic. Higher concentrations of PAHs, PCDD/Fs and dl-PCBs were observed in cold seasons at both sites, attributed to the seasonality of the gas-particle partitioning, the increase of emissions and the lower boundary mixing layer in winter. All of the compounds showed a strong accumulation in the fine fraction, with, on average, 71% of ΣPAHs, 73% of ΣPCDD/Fs and 60% of Σdl-PCBs associated with particles <0.95 μm. The human risk assessment via inhalation was addressed and followed the same pattern as for concentrations, with 41 and 7 times higher risk in winter compared to summer at the rural and urban sites, respectively. More than 70% of cancer risks of PAHs, PCDD/Fs and dl-PCBs was associated with particles <0.95 μm. Moreover, an overestimation of the cancer risk via inhalation of up to 50% occurred when the size distribution of related compounds was not considered.

1. Introduction

The atmosphere plays an important role in the transport and distribution of chemical compounds around the world. Air contains particulate matter (PM) created by both anthropogenic and natural sources, such as fossil fuel combustion, road dust, wind erosion,

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vegetation debris, sea spray and microbial degradation (Mastral and Callen, 2000; O'Dowd et al., 1997). Semi-volatile organic compounds (SVOCs) in the atmosphere partition between particle and gaseous phases depending on temperature, the characteristics of the atmospheric particles, and the vapor pressure of the compound. This partitioning influences the transport, deposition and degradation processes and thus the atmospheric residence time of SVOCs on local to global scales (Bidleman et al., 1986). PM is composed of both organic and inorganic compounds and is commonly grouped into ultrafine, accumulation and coarse size modes with aerodynamic diameters of <0.1 μm, 0.1–2.5 μm and >2.5 μm, respectively. While coarse particles are largely mobilized by mechanical processes such as construction activities, resuspension of road dust and sea spray (Allen et al., 1996), particles <2.5 μm ($PM_{2.5}$) are generally formed from combustion sources and gas-to-particle conversion (secondary aerosols) and have a longer residence time in the atmosphere (≈3–5 days) (Vecchi et al., 2004). The size of the particles and their composition is of great relevance for human health (Englert, 2004; Walgraeve et al., 2009). Finer particles penetrate deeper into the lungs and thus are linked with illness and deaths from heart or lung diseases (Englert, 2004; Walgraeve et al., 2009). Thus, a good knowledge of the particle size distribution of both particulate matter and associated pollutants is important for exposure and health risk assessment (Lohman and Seigneur, 2001).

Persistent organic pollutants (POPs) and polycyclic aromatic hydrocarbons (PAHs) have been widely recognized as being persistent, bioaccumulative and having health risks for humans and the environment (UNEP, 2009; WHO, 1997). Moreover, POPs such as polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) and polychlorinated biphenyls (PCBs) are listed in the Stockholm Convention, which restricts their emission to protect human health and the environment. Particle-bound PAHs, PCDD/Fs and PCBs are mainly associated with particles smaller than 1–2 μm (Chrysikou et al., 2009; Čupr et al., 2013; Kaupp and McLachlan, 2000). However, despite knowledge of significant seasonality in PM in temperate regions (Hrdličková et al., 2008; Van Dingenen et al., 2004), there is limited information on seasonal variation in the size distribution of these SVOCs (Chrysikou and Samara, 2009). Recent studies showed that the amount of particles, the particle composition, concentration of associated pollutants and the toxicological effects were size dependent (Čupr et al., 2013) and subject to large spatial variability (Landlová et al., 2014; Novák et al., 2014). Although the influence of local sources was hypothesized as the cause of this variability, seasonal variability may have been another contributing factor.

Thus, the aim of this study is to fill this gap by characterizing the seasonal size distribution of particle-bound PCDD/Fs, dl-PCBs and PAHs concentrations in both urban and rural air in Central Europe, and to assess human exposure via inhalation of particle-bound PAHs, PCDD/Fs and dl-PCBs.

2. Materials and methods

2.1. Air sampling

Samples were collected in the area of Brno (population ~400,000). Sampling was conducted simultaneously at two sites: one urban site (Kotlářská) and one rural site (Telnice). Kotlářská is an important traffic junction in the center of Brno (49°12′20″N, 16°35′50″E). The main sources of air pollution at this site are likely vehicle traffic and domestic heating. Telnice (49°6′21″N, 16°42′58″E) is close to a small airport in rural countryside, about 14 km south-east from the city centre of Brno. The airport has limited traffic (≈115 landings and take-offs per week), so the main source of pollution at this rural site is likely nearby domestic heating, pollution transported from the urban area, and long range transport.

Detailed information on the sampling and analysis can be found in the Supplementary Information (SI). From October 2009 to October 2010, a high volume air sampler (HV 100-P, Baghirra, CZ) equipped with a multi-stage cascade impactor (TE6001 PM_{10} Size Selective Inlet, Tisch Environmental, USA) was used to collect six particle size fractions at both sites. The sampler flow rates were calibrated before use and the sampler configuration was validated based on comparison with conventional high-volume active air samplers (Melymuk et al., 2014). Sampler flow rates were 68 m^3/h and samples were collected over one week, resulting in on average 8931 m^3 per sample. The fractions collected represented particles with aerodynamic diameters of <0.49 μm, 0.49–0.95 μm, 0.95–1.5 μm, 1.5–3.0 μm, 3.0–7.2 μm and 7.2–10 μm. They were collected on quartz fiber filters (QFFs). In total, forty-six weekly samples were collected at each site, each consisting of six QFFs with size-specific fractions of PM, and these samples were split for analysis of four different compound classes: (1) PAHs and (2) PCBs, PCDD/Fs, and (3) brominated flame retardants (Okonski et al., in prep) and (4) current use pesticides (Degrendele et al., in prep). For this study, 11 samples were analyzed for PAHs and 12 for PCDD/Fs and dioxin-like PCBs (dl-PCBs) from each site, with weekly samples grouped by season to ensure sufficient detection (Tables S1 and S2 in the SI). Four field blanks and nine laboratory blanks were analyzed as per the samples to evaluate any contamination during sampling or analysis.

2.2. Sample preparation and analysis

The target compounds were 17 PCDD/Fs, 12 dl-PCBs and 18 PAHs (listed in full in the SI). For the purposes of this paper, we refer to the sums of the corresponding compounds as ∑PAHs (excluding retene, listed separately as marker of wood combustion), ∑PCDD/Fs and ∑dl-PCBs.

QFFs were extracted with toluene using an automatic extractor (BUCHI Extraction System B-811, Switzerland). C-13 labeled compounds were spiked onto each QFF before extraction. The extracts were concentrated using a nitrogen stream. Samples were transferred to a silica column followed by a carbon column for PCDD/Fs and dl-PCBs. PCDD/Fs and dl-PCBs were analyzed using a 7890A gas chromatograph (GC) (Agilent, USA) equipped with a 60 m × 0.25 mm × 0.25 μm DB5-MS column (Agilent J&W, USA) coupled to an AutoSpec Premier high resolution mass selective detector (GC-HRMS) (Waters, Micromass, UK). The HRMS was operated in electron impact ionization (EI + mode) at a resolution of >10,000. PAHs were analyzed using a 6890 GC (Agilent, USA), equipped with the same column as mentioned above, coupled to a mass selective detector (MS 5975, Agilent USA) (Holoubek et al., 2007).

2.2.1. Quality assurance and quality control

Blank levels of individual analytes were in most cases below detection and very low otherwise (on average less than 1.13% of sample mass for all compounds). All quantities reported here have been corrected for blanks. Recovery efficiencies ranged from 66% to 100% for PCDD/Fs and dl-PCBs, respectively. PAHs recoveries were assessed using spike and recovery tests and ranged from 72 to 102% (Čupr et al., 2013).

2.3. Data analysis

A cumulative particle size distribution on log-probability axes was plotted for each collected data for all compounds. The mass

median diameter (MMD) and the geometric standard deviation (GSD) were determined (Hinds, 1999) and were calculated as the d50%, and as d50%/d16%, respectively, where d50% and d16% represents the diameters at the cumulative percentiles of 50% and 16%.

Particle size specific concentrations were also converted to inhalable, thoracic and respirable fractions. The inhalable fraction represents the particles of an aerodynamic diameter that can enter the nose and/or mouth during breathing, the thoracic fraction of the aerosol contains particles of aerodynamic sizes that can penetrate beyond the larynx and enter the lungs and the respirable fraction of the aerosol contains particles of aerodynamic sizes that can penetrate the gas exchange regions of the lungs (the alveolar region). The expressions used to derive those fractions (Ramachandran, 2005; Vincent, 1999) are presented in the SI. Inhalable, thoracic and respirable concentrations were estimated using the corresponding fractions.

2.4. Human health risk assessment

Human health risks resulting from outdoor workday inhalation exposure at two sites were evaluated with respect to the risk of developing cancer using a probabilistic approach. We applied the inhalation exposure model of the EPA baseline risk assessment approach (EPA, 2013). The chronic daily intakes (CDI) and cancer risk probabilities were calculated (detailed description in Čupr et al., 2013). Site specific exposure parameters were applied (air inhalation rate = 20 m^3 day^{-1}; exposure time = 8 h day^{-1} (working hours per day); exposure frequency = 251 days per year (only working days); exposure duration = 25 years (working years); body weight = 70 kg).

3. Results and discussion

3.1. Seasonality and size distribution of PM_{10}

Understanding PM behavior is crucial when studying the seasonal size distribution of particle-bound organic contaminants, as their respective concentrations are strongly correlated with the PM concentration (Pearson's r of 0.811, 0.602 and 0.606 for ∑PAHs, ∑PCDD/Fs and ∑dl-PCBs, respectively). The seasonal PM_{10} concentrations at both sites are given in Fig. 1 and Table S3.

Higher PM_{10} concentrations were found in winter at both sites, with average values of 49.8 and 49.2 μg m^{-3} at the rural and urban site, respectively. In comparison, PM_{10} concentrations measured in summer were on average 16.8 and 21.4 μg m^{-3} at the rural and urban sites, respectively. Higher winter PM_{10} concentrations have been reported for other European cities (Barmpadimos et al., 2012; Van Dingenen et al., 2004), and are attributed to higher emissions from domestic heating, a lower atmospheric boundary layer and higher gas-to-particle conversion due to lower temperatures.

In this study, the PM size distribution was dominated by the fine particles. The finest particle fraction (<0.49 μm) was highest at both sites, with an annual average of 8.42 and 11.1 μg m^{-3} at the rural and urban sites, respectively (accounting for 32.7 and 34.5% of the total PM, respectively), followed by the second finest fraction (0.49–0.95 μm) with an average of 7.99 and 7.14 μg m^{-3} at the rural and urban sites (accounting for 28.0% and 20.0%, respectively). On average, the contribution of coarse particles (particles >3.0 μm) was significantly higher ($p < 0.05$) at the urban site (≈26.1% of total PM) than at the rural site (≈15.5%). Moreover, the contribution of the coarse fraction to total PM in summer was 2.91 and 1.33 times higher than in winter at the rural and urban sites, respectively. The higher summer and urban concentrations of coarse particles are attributed to mechanical sources (Charron and Harrison, 2005), greater resuspension of coarse particles from the ground due to greater surface dryness and surface abrasion, and to more intense but shorter duration precipitation in summer compared to winter (Spindler et al., 2004). Moreover, the relative increase of coarse particles in total PM in summer could also be associated with more sources of 0.49–3.0 μm particles in winter compared to summer at both sites. Similar behavior was also found in Greece (Chrysikou and Samara, 2009) suggesting that seasonality on PM sources and/or the winter atmospheric conditions (e.g. lower temperatures, lower boundary mixing layer) could result in the higher fraction of 0.45–3.0 μm particles in winter.

We observed a clear bimodal distribution at the urban site in all seasons, peaking at the 0.49–1.5 μm and 7.2–10.0 μm size fractions. However, at the rural site, at colder temperatures the distribution was unimodal, peaking at 0.49–1.5 μm, while in warmer seasons the distribution was bimodal, similar to the urban site. Within the bimodal distribution, the fine peak shifted seasonally: the fine mode peak was in the 0.95–1.5 μm fraction in winter, and shifted to 0.49–0.95 μm fraction in summer at both sites. Particles <0.95 μm have a significant contribution from gas-to-particle conversion of young aerosols (Wu et al., 2006). Thus, the shift to larger sizes in the accumulation mode in cold months could be due to greater aerosol growth during longer winter accumulation times (Chrysikou and Samara, 2009), or the long-term aging of those particles between emission and deposition (Vanvaeck and Vancauwenberghe, 1985).

3.2. Seasonal variation of particle-bound PAHs, PCDD/Fs and dl-PCBs

3.2.1. PAHs concentrations

Both sites exhibited a large range of ΣPAHs concentrations, with maximum concentrations observed in winter (Fig. 2a, Tables S8 and S9). Higher winter PAHs levels are attributed to seasonal sources

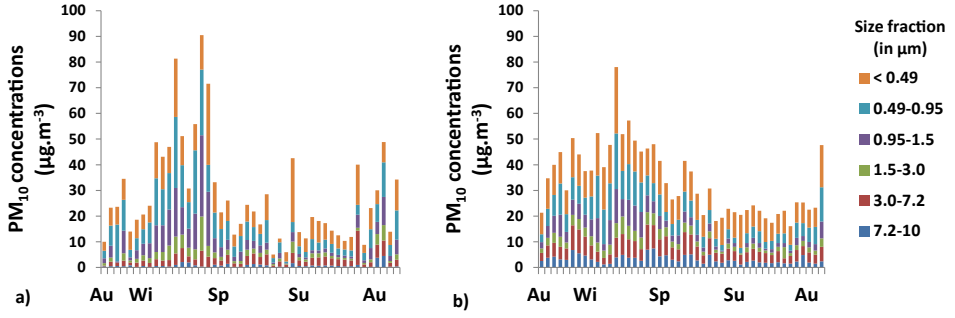

Fig. 1. Weekly PM_{10} concentrations (in μg m^{-3}) according to the size of particles (in μm) at the rural site (a) and the urban site (b). Au, Wi, Sp and Su represents autumn, winter, spring and summer, respectively.

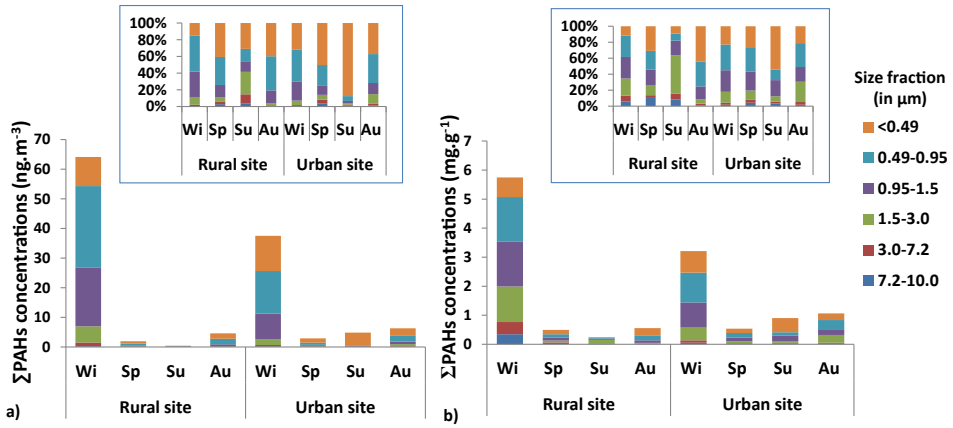

Fig. 2. PAHs concentration in ng m^{-3} (a) and in ng g^{-1} (b) at the rural and urban sites (Wi = winter; Sp = spring; Su = summer and Au = autumn).

which dominate in winter, such as domestic heating and vehicles cold starts, but also to the seasonal shifts in gas-particle partitioning, the lower atmospheric dispersion and the lower photolytic and thermal decomposition in winter compared with summer (Holoubek et al., 2007; Mantis et al., 2005). Domestic heating contributions are expected to be especially significant at the rural site, as demonstrated by the larger winter to summer concentration ratio (159 at the rural site vs. 7.75 at the urban site). The dominant PAHs were fluoranthene, benzo(b+j)fluoranthene, pyrene and chrysene, accounting on average for 19.2%, 16.7%, 14.9% and 10.3%.

The PAH concentrations observed in this study (($\sum_{17}$PAHs = 17.8 ± 31.0 and 12.9 ± 16.5 ng m^{-3} at the rural and urban sites, respectively) are within the range of concentrations observed in Prague, CZ ($\sum_{32}$PAHs = 55.1 ng m^{-3}) (Saarnio et al., 2008), Thessaloniki, Greece ($\sum_{18}$PAHs = 24.6 and 8.54 ng m^{-3} in winter and summer, respectively) (Chrysikou and Samara, 2009), and in Southern Germany ($\sum_{21}$PAHs = 23.0 ng m^{-3}) (Bari et al., 2010).

3.2.2. PAH size distribution

For all seasons except summer, 58.3–80.6% of particulate ΣPAHs were found on particles <0.95 μm at both sites (Fig. 2a). In contrast, the size distribution of ΣPAHs in summer differed between the two sites: at the urban site 87.6% of ΣPAHs were associated with the finest particles (<0.49 μm), while at the rural site, only 31.0% of ΣPAHs were associated with this particle fraction. The higher proportion of fine particles has been reported for other urban locations (Chrysikou and Samara, 2009; Schnelle-Kreis et al., 2001) and is attributed to the influence of PAHs from fresh emissions consisting mainly of fine particles (Yang et al., 1999) and/or the influence of non-exchangeable PAHs, which are ingrained within particles during the combustion processes (Harner and Bidleman, 1998).

As with PM, PAHs presented unimodal distributions in winter at both sites, with peaks in the 0.49–0.95 μm fraction. The majority of PAHs were associated with particles from 0.49 to 1.5 μm in winter, while this fraction was much less significant in summer (on average 67.6% of ΣPAHs in winter vs. 18.1% in summer) (Fig. 2a). In summer, medium and high molecular weight PAHs (MW > 200) had a unimodal distribution, with a peak at 1.5–3.0 μm at the rural site and at <0.49 μm at the urban site, while low molecular weight PAHs, such as fluorene, acenaphthylene and acenaphthene, had a bimodal distribution with a second peak at the 7.2–10.0 μm range. This bimodal distribution of low molecular weight species was expected, as they are more easily redistributed amongst coarse particles by rapid volatilization and condensation (Chrysikou and Samara, 2009), and therefore, although they are likely associated with the finest particles when emitted from vehicles, they can rapidly re-volatilize and then resorb to coarser particles. Several studies identified a unimodal distribution of PAHs in winter in the accumulation mode (<0.95 μm) (Kiss et al., 1998; Kleeman et al., 2008), while other studies performed on a seasonal scale have reported a bimodal distribution in fine and coarse particles (Duan et al., 2007; Hien et al., 2007). This suggests that no clear trend exists in the particle fraction distribution of PAHs, due to the high variability in source, seasonality and location. Moreover, this could be attributed to the variability of the material composition of particles of different sizes. Indeed, at locations near to those in the present study, coarse particles were mostly composed of mineral materials while the finest particles were dominated by carbonaceous matter (Čupr et al., 2013; Landlová et al., 2014).

In order to determine if the particle size distribution of PAHs was due solely to the distribution of PM, the PAH concentrations were normalized by the mass of PM for each size fraction, season and site (Fig. 2b, Table S9). Despite the general similarities between the trends of PM and PAH (e.g. unimodal distribution in winter), the fine fraction was still associated with a higher concentration of ΣPAHs (52.0% of ΣPAHs associated to particles >0.95 μm, regardless of site or season). Moreover, the contribution of particles >1.5 μm doubled compared with the concentrations in ng m^{-3}. These results suggest that the distribution of PM influences the distribution of PAHs to some extent (especially for the more volatile compounds) but that, even though there is some evidence of redistribution of PAHs amongst different particle fractions, the influence of either non-exchangeable PAHs, and/or the surface properties of the finer particles, are leading to preferential sorption to particles <1.5 μm.

The MMDs of individual PAHs ranged from 0.669 to 1.68 μm at the rural site and from 0.0167 to 1.09 μm at the urban site, with, in general, higher MMDs for low molecular weight PAHs (Table S10). At the rural site, individual PAHs exhibited larger MMDs than those calculated for PM, while at the urban site, the opposite was observed for all compounds and seasons, excepting the lowest molecular weight PAHs (acenaphthylene, acenaphthene and fluorene). The MMDs of high molecular weight PAHs were higher in summer than in winter at the rural site while the opposite trend was observed at the urban site (Table S10). The MMDs for the lowest molecular weight PAHs should be interpreted with caution, as only a very small fraction of these compounds is found in the particulate phase. However, we hypothesize that the urban MMDs reflect the influence of fresh emissions which have not redistributed amongst the other particle size fractions, while at the rural site, emitted PAHs have had more time for redistribution amongst

particles, and hence, MMDs are higher. It also may suggest a difference in primary sources between the sites, with traffic and natural gas combustion as the primary sources at the urban site, while the rural site has a greater influence from other biomass combustion (wood burning, domestic waste) that emits larger particles (Kocbach et al., 2005). Finally, the higher summer MMDs at the rural site suggest an influence of summer biomass combustion (e.g. barbeques, outdoor fires, burning of yard waste), activities which are more common in rural areas.

Retene, a marker of wood combustion, was detected in most of the size fractions, excepting in the 7.2–10.0 μm size fraction (Table S8). This compound followed the same trend observed for ΣPAHs, with higher concentration observed in winter at both sites and a strong accumulation in the fine fraction (on average, 57.9% and 73.4% associated to particles <0.95 μm at the rural and urban site, respectively). Moreover, in cold seasons, higher retene concentrations were found at the rural site, supporting the hypothesis of wood for domestic heating, widely used in rural areas.

3.2.3. PCDD/Fs and dl-PCBs

The annual average Σdl-PCBs concentrations suggest urban/rural differences in particle-bound PCBs, with concentrations higher at the urban site (0.776 pg m^{-3}) than at the rural site (0.419 pg m^{-3}). However, this trend is not consistent on a seasonal basis: all individual PCBs had higher concentrations at the urban site in spring, summer and autumn than at the rural site, however in winter, there were no spatial differences (Table S4). Similarly for PCDD/Fs, the annual average belies the seasonal trends. The annual average concentrations of ΣPCDD/Fs were similar at both sites (0.801 pg m^{-3} and 0.823 pg m^{-3} at the rural and urban sites, respectively). However, the majority of individual PCDD/Fs congeners had higher concentrations at the urban site in spring, summer and autumn and higher concentrations at the rural site in winter. These spatial differences in PCDD/Fs and dl-PCBs concentrations between the two sites may be due to differences in domestic heating (mainly gas and electricity in cities, and gas and wood in rural areas) and domestic waste burning. As with PAHs, higher PCDD/Fs and dl-PCBs concentrations during cold seasons (Fig. 3a, Table S4) are attributed to increased local combustion from domestic heating, seasonality of gas-particle partitioning and lower boundary layer (Ding et al., 2012; Lohmann and Jones, 1998).

The main contributors to ΣPCDD/Fs were OCDD, 1234678-HpCDD, 1234678-HpCDF and OCDF, accounting on average for 42.5%, 14.4%, 11.1% and 8.56%, respectively. Similarly, PCBs-118, -156 and -105 were the most abundant of the dl-PCB, accounting for about 39.8%, 15.8% and 15.4%, respectively, of the total ∑dl-PCBs concentrations.

It has been previously reported that 60% and 90% of ΣPCDD/Fs were associated with particles <0.41 μm and <2.1 μm, respectively (Kaupp and McLachlan, 1999, 2000; Kaupp et al., 1994; Oh et al., 2002) and that about 72% of PCBs are associated with particles <0.95 μm (Chrysikou et al., 2009). In the present study, we found a similar distribution, with a strong accumulation of PCDD/Fs and dl-PCBs in the fine fraction (Fig. 3a, Table S4). Indeed, at the rural site in autumn, winter and spring, 49.2% and 85.9% of the particulate ΣPCDD/Fs were associated with particles of diameter <0.49 μm and <1.5 μm, respectively, and 59.8% of the ∑dl-PCBs were associated with particles <0.95 μm. However, rural distributions differed in summer. For PCDD/Fs, the contribution of the finest particles (<0.49 μm) was larger (73.8% of particle-bound ΣPCDD/Fs, compared with 37.6% in other seasons) while 53.8% of dl-PCBs were in the coarse mode (compared with 8.86% in other seasons). At the urban site in spring, summer and winter, the highest concentration of particle-bound ΣPCDD/Fs was associated with particles <0.49 μm, accounting for about 53.5–70.2% and 49.1–59.5% of the total particle-bound ΣPCDD/Fs and dl-PCBs, respectively, while at the urban site in autumn, an unexpected high contribution of particles >7.2 μm was observed for both PCDD/Fs and dl-PCBs, attributed to some unknown short-term local source(s). In summer, 26.0% of dl-PCBs and 33.6% of PCDD/Fs were associated with coarse particles. Even though the average contribution of particles >1.5 μm is relatively small (14.1% for PCDD/Fs and 29.2% for dl-PCBs), when normalized by PM mass, the contribution of coarser particles is greater (29.3% > 1.5 μm for PCDD/Fs, and 52.6% for dl-PCBs; Fig. 3b, Tables S5 and S7).

While the accumulation of PCDD/Fs and dl-PCBs in the fine range is attributed to fresh emissions (in agreement with the higher proportion of finest particles at the urban site), the larger coarse fractions observed in summer are attributed to additional vehicle-related sources, in particular, particle resuspension from road surfaces, as has been noted for PAHs.

The average MMD of Σdl-PCBs at the rural site (0.990 μm) was slightly higher than for ΣPCDD/Fs (0.703 μm), while similar values were observed for both compounds at the urban site (0.818 and 0.822 μm, respectively). MMDs of individual dl-PCBs, excepting PCB-77 and PCB-81, were higher in summer than in winter at both sites (Table S12). The same trend was observed for individual PCDD/Fs, excepting 2378-TCDF, at the urban site, while higher MMDs in

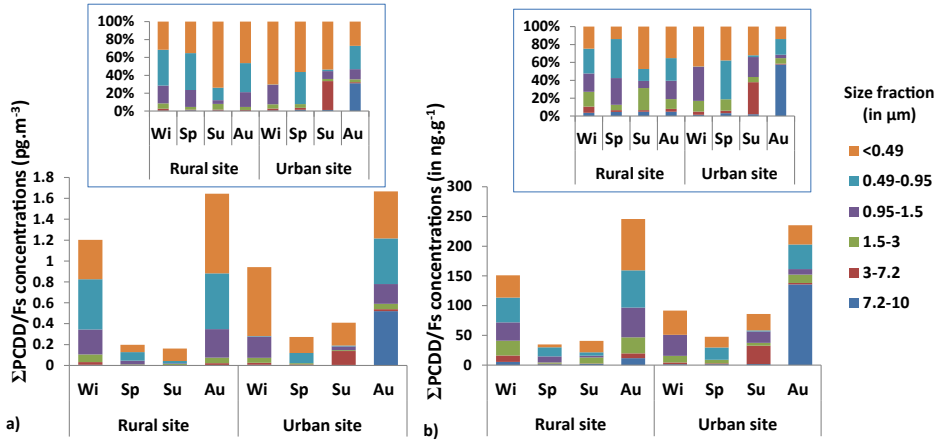

Fig. 3. PCDD/Fs concentration in pg m^{-3} (a) and in pg g^{-1} (b) at the rural and urban sites (Wi = winter; Sp = spring; Su = summer and Au = autumn).

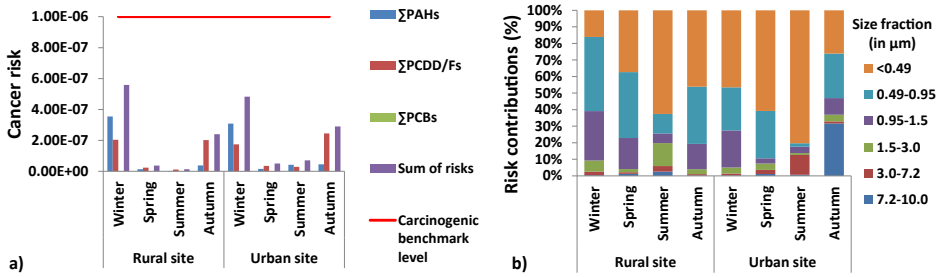

Fig. 4. Seasonal cancer risks of PAHs, PCDD/Fs and dl-PCBs at the rural and urban sites calculated using (a) size specific concentrations and (b) contribution of size fractions (in µm) to the total risk at the rural and urban sites.

winter vs. summer were found at the rural site (Table S11). The higher summer MMDs suggest volatilization and redistribution of the emitted PCDD/Fs and PCBs amongst all particle fractions, as was also observed for PAHs. The differing winter trend for rural PCDD/Fs may be indicative of the influence of biomass combustion, more common for domestic heating in rural areas, as shown for PAHs.

Most of the individual PCDD/Fs and dl-PCBs exhibited a unimodal distribution, with a peak in the 0.49–0.95 µm fraction. However, the peak shifted to the 0.95–1.5 µm size in winter at the urban site, reflecting the aerosol growth and/or the long-term aging from emission to deposition, as observed for PM.

3.3. Risk assessment

The inhalable, thoracic and respirable concentrations of PAHs and PCDD/Fs followed the same pattern as their particulate concentrations, with largest concentrations in winter for PAHs and in autumn for PCDD/Fs (Table S13). In cold seasons, the rural site showed slightly higher concentrations than the urban site, suggesting a higher exposure to PAHs and PCDD/Fs for the rural population. Calculations of the inhalable, thoracic and respirable concentrations are useful for exposure assessment, especially in cases where coarse particles dominates and non-particle size specific estimates could introduce bias. For example, while 93.7–94.0% of PCDD/Fs reach the respirable region of the lungs at the urban site in winter and spring, the corresponding values for the same site in summer and autumn, which were characterized by large amount of coarse fractions, are 65.9–67.4%.

The cancer risks from inhalation were estimated in two ways: (1) based on the atmospheric concentrations of PAHs, PCDD/Fs and dl-PCBs, measured in the six size fractions, and (2) based on the inhalable, thoracic and respirable concentrations estimated using size-fraction specific data and the inhalable, thoracic and respirable fractions. The cancer risk values for the selected exposure scenario (exposure to an outdoor worker) are presented in Fig. 4 and in Table S14. Cancer risks estimated in this study are significantly lower than the carcinogenic benchmark level (i.e. an exposure yielding an upper-bound lifetime excess cancer risk of 1.0E-6, or one cancer occurrence per one million people), and lower than what was found in an earlier study conducted in Brno (Čupr et al., 2013), as the previous PAH concentrations were higher and the exposure scenario was different (exposure time = 24 h per day, exposure frequency = 365 days per year, exposure duration = 70 years in Čupr et al., 2013, while in this study they were 8, 251 and 25, respectively).

When considering the size-specific concentrations of associated pollutants, PCDD/Fs and PAHs accounted for most of the total risk (on average 62.0% and 37.9%, respectively), and the contribution of PCBs was negligible (<0.05%). It is important to note that the values reported here are considering only the cancer risk from outdoor inhalation exposure, thus not including the cancer risk from ingestion or other inhalation exposure.

At both sites, the highest risk was in winter, due to the higher concentrations of combustion-related compounds. The sum of ∑PAHs, ∑PCDD/Fs and ∑dl-PCBs winter cancer risk was 41.4× higher at the rural site and 6.81× higher at the urban site than in summer. Examining the seasonality of individual compound classes, we see that the PAHs and PCDD/Fs risks were similar in warm seasons, while the PAHs risks dominated over the PCDD/Fs risks in winter and the opposite trend was observed in autumn, due to the higher autumn concentrations of PCDD/F (Fig. 4a).

At both sites, the majority of the total cancer risk was associated with particles <0.95 µm accounting for 53.1–89.4%, with higher contribution of the finest stage in warm seasons compared to cold seasons (Fig. 4b). Moreover, the contribution of the coarse fraction was small at both sites for most seasons (<12.6%), except in autumn at the urban site due to the high occurrence of PCDD/Fs and dl-PCBs associated with coarse particles (Section 3.2.3).

Cancer risks of PAHs, PCDD/Fs and dl-PCBs calculated using the inhalable, thoracic and respirable concentrations (Table S14) were very similar due to the strong accumulation of these compounds in the finest particle fractions. However, the inhalable, thoracic and respirable risks were on average 4.79%, 7.20% and 15.4% lower than the risks using PAHs, PCDD/Fs and dl-PCB concentrations, as use of the particulate concentrations assumes that all particles have potential to create cancer risk, even the ones that are too large to penetrate the human respiratory system. In some cases where the coarse fraction had a proportionally larger contribution to the overall concentrations, as in summer at the rural site for dl-PCBs, the risk estimated based on total concentrations overestimated the risk using the inhalable, thoracic and respirable concentrations by a factor of two. This supports the hypothesis that a good knowledge of the size distribution, thus using a cascade impactor or similar apparatus, is important for human risk assessment, especially in situations where large particles are common (e.g. industrial and agricultural areas).

Acknowledgments

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Appendix A. Supplementary data

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RESEARCH ARTICLE

An experimentally refined tool to assess the risks of the human dermal exposure to herbicide chlorotoluron

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Abstract Dermal absorption of the herbicide chlorotoluron was measured using ex vivo pig skin in Franz diffusion cells in an automated system. The steady-state flux was calculated, as well as the permeability coefficient, which is 0.0038 cm h^{-1}. The permeability coefficient (K_p) is a key factor when predicting human health risks resulting from dermal exposition to a substance. The experimental determination of this parameter filled data gaps regarding the dermal absorption of chlorotoluron. The experimental permeability coefficient was subsequently used to calculate the dermal absorbed dose during some exposure scenarios. Reference doses were revised, and screening risk assessment process was done to calculate the risks resulting from exposure to chlorotoluron. This refined new approach proved to be a useful tool for human health risk assessment in the areas with these herbicide applications.

Keywords Franz cell · Dermal exposure · Chlorotoluron · Pesticides · Ex vivo permeability coefficient · Dermal absorbed dose · Recommended reference dose · Human health risk assessment

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Highlights
Kp of chlorotoluron was defined experimentally for the first time
Nonoccupational exposure (via dermal route) scenarios to chlorotoluron in Czech population were identified
Reference doses were revised, and a reference dose for chlorotoluron was recommended
Useful tool for human health risk assessment of chlorotluron was presented

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Introduction

The prevention of environmentally induced diseases requires the implementation of experimental measures based on a comprehensive analysis of human exposure. An experimental approach in this field is crucial (Kefeni and Okonkwo 2014).

Despite the growing numbers of papers reporting on exposure to elevated levels of some chemicals, there are many data gaps with respect to experimental kinetics and transfer coefficients for different dermal exposure scenarios, for example, in the case of polar pesticides.

Today, many pesticides are used in the environment. The pollution of soil and plants and the drift that occurs when applying pesticides can present a risk of exposure to bystanders and local residents. Also, the exposure of operators and workers can be considerable when these come into contact with contaminated facilities or they are not using appropriate personal protective equipment (PPE). The dermal exposure route can be an important route of exposure to pesticides not only for operators and workers. Further action on assessing the contribution of this route to uptake of pesticides, including dermal exposure, is needed (Beamer et al. 2009).

According to Regulation (EC) No. 1107/2009 (EC 2009), the impact of an active substance on human health has to be studied prior to the inclusion of the active substance in Annex 1, which allows it to be used in plant protection products in EU countries. Annex I to Regulation (EC) No. 1107/2009 (EC 2009) is a list of active substances that are allowed to be used in plant protection products in countries of the European Union. The producer or distributor of a given active substance must apply for this substance to be included in Annex I. Documentation about its toxicity and other characteristics is prepared, and a decision is made about whether the substance will or will not be included in Annex I. When included, the substance can be used for 10 years, after which it is re-evaluated.

Statements about the necessity urgency of dermal absorption studies on active substances in Commission Regulations (EU) No. 544/2011 (OECD 2011) and (EU) No. 546/2011 (EU, 2011) are not very clear. Dermal absorption studies should be done "where appropriate" (EU 2011). This gives considerable freedom to assessors regarding the dermal absorption studies.

For this reason, experimental information about the fraction of a dose absorbed dermally is not available for all currently used pesticides. For some pesticides, the default value (theoretical/estimated/computed) for the dermal absorption of an active substance is used, which can lead to underestimation or overestimation of the absorbed dose. Also, many of the data are generated using rat skin. Pig skin is a better surrogate for human skin in human dermal absorption experiments (Dick and Scott 1992), and it was used in this study to study the dermal absorption of the pesticide chlorotoluron.

Chlorotoluron is a phenylurea herbicide used to control annual grasses and broad-leaved weeds in winter cereals (WHO 1996a). It is supplied as a soluble concentrate or as wettable granules that are mixed with water and applied as a spray (PPDB 2009–2013). At the national level, chlorotoluron is authorized in 16 countries of the European Union (Austria, Belgium, Bulgaria, Czech Republic, Germany, Spain, France, Hungary, Italy, Latvia, Poland, Portugal, Romania, Slovenia, Slovakia, UK) (DG SANCO 2008–2013). For example, eight plant protection products containing chlorotoluron as an active substance are currently (17/09/2014) registered in the UK according to the Health and Safety Executive (https://secure.pesticides.gov.uk/pestreg/ProdSearch.asp), and seven plant protection products containing chlorotoluron as an active substance are registered in Germany. Six products containing chlorotoluron as an active substance are currently registered in the Czech Republic. Two of the six currently registered products contain more than one active substance. In the Czech Republic, 90.403342 tonnes of chlorotoluron was used in 2011 (SRS 2009–2011).

The toxic effects of chlorotoluron include the induction of chromosomal aberrations (Federico et al. 2011) and pathological changes of mouse testis (Hong et al. 2007). Endocrine disrupting effects are expected because of its structural similarity to other phenylurea herbicides with known endocrine disrupting effects (Orton et al., 2009).

In this study, dermal absorption of chlorotoluron using excised pig skin and Franz diffusion cells was measured in an automatic system (Babu et al. 2003; Csóka et al. 2005; Franz 1975; Mircioiu et al. 2013; Ouypornkochagorn and Feldmann 2010).

The permeability coefficient and other absorption kinetics parameters were measured and calculated. The dermal absorbed dose for some exposure scenarios in Czech Republic was calculated and then used to calculate the hazard quotient using different available reference doses. The available reference doses for chlorotoluron have been revised, and the most appropriate reference dose has been recommended. We offer a new refined approach for human health risk assessment of chlortolurone herbicide application.

Materials and methods

The assay was performed following the OECD guidelines (OECD 2004a, 2004b) in accordance with Wellner et al. (2008) and Lademann et al. (2008).

Chemicals

All chemicals, chlorotoluron (CAS number 15545-48-9) analytical standard with 99.5 % purity, 0.01 M phosphate-buffered saline (containing 0.138 M NaCl; 0.0027 M KCl, pH=7.4 at 25 °C), and bovine serum albumin (BSA, CAS number 9048-46-8) of ≥98 % purity were purchased from Sigma-Aldrich.

Skin source and skin membrane preparation

The skin of the dorsolateral part of a domestic pig (*Sus scrofa f. domestica*), male, 15.75 kg, was removed after the animal had been euthanized and washed. The skin was packed in aluminum foil, stored in a transportable fridge at 4 °C, and transported to the laboratory, where the subcutaneous fat was removed. The skin was packed in the aluminum foil and a zip-lock bag and stored in a freezer in −20 °C until the beginning of the experiment (for 1 week). Before the experiment, the skin was defrosted at ambient laboratory temperature. Subsequently, split-thickness skin membranes (450 μm) were prepared with an electric dermatome (Humeca D42). The skin was cut into small pieces of uniform size, and the integrity of membranes was checked visually before the experiment, and post-study data analysis integrity evaluation was done (OECD 2004a; Wellner et al. 2008; Stahl et al. 2012).

Dermal absorption experiment

An automatic MicroettePlus system (Hanson Research) containing six amber glass Franz cells (with volume of the receptor chamber approximately 4 ml, opening for skin surface approximately 0.64 cm^2) was used for the dermal absorption experiment. Receptor chambers of Franz diffusion cells were filled with the receptor fluid containing the phosphate-buffered saline (pH=7.4) and 2.5 % of bovine serum albumin. The skin membrane was placed between the receptor and donor chamber (with the stratum corneum in the donor chamber), and the receptor chamber was checked for bubbles. At the start of the experiment, 200 μl of chlorotoluron dissolved in water (10 μg ml^{-1}) was applied to the skin. The whole

system was heated to a temperature of 32 °C, reflecting the temperature of the body surface. Over the experiment duration of 24 h, 1 ml of the receptor fluid (in six replicates) was collected every 2 h into HPLC vials and analyzed at the end of the experiment.

Analysis of the receptor fluid

Analyses were performed with an Agilent 1290 HPLC (Agilent Technologies, Waldbronn, Germany) consisting of a vacuum degasser, a binary pump, an thermostated autosampler (10 °C), and a thermostated column compartment kept at 30 °C. The column was a Phenomenex Synergi Fusion C-18 endcapped (4 μm) 100×2 mm i.d., equipped with a Phenomenex SecureGuard C18 guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 5 mM ammonium acetate in water (A) and 5 mM ammonium acetate in methanol (B). The binary pump gradient was nonlinear (increasing from 20 % B at 0 min to 80 % B at 1 min, then increasing to 90 % B at 5 min, then 90 % B for 8 min, and 5-min column equilibration back to initial conditions (20 % B)); the flow rate was 0.25 ml min^{-1}. Ten microliters of individual sample was injected for the analyses.

The mass spectrometer was an AB Sciex Qtrap 5500 (AB Sciex, Concord, ON, Canada) with electrospray ionization (ESI) in positive ionization mode. The ionization parameters were as follows: capillary voltage, 5.5 kV; desolvation temperature, 400 °C; curtain gas, 15 psi; Gas 1, 40 psi; Gas 2, 30 psi. In scheduled MRM mode, m/z transitions (213.1) were monitored (with values of declustering potential (DP=81 V), entrance potential (EP=10 V), collision cell exit potential (CXP=4 V), and collision energy (CE=31 V)). Quantification of analytes was based on isotope-labeled internal standards. The method quantification limit (MQL) was 0.50 ng ml^{-1}, and retention time was 3.4 min.

Results

Figure 1 shows the plot of the median of the cumulative permeated amount at the 25, 75, 10, and 90 % quantiles with the trend line and regression equation. Analysis of the regression lines of the fluxes showed that fluxes could be considered constant over the period from 4 to 24 h. The trend line was fit through the cumulative permeated amount of all cells (replicates), and the regression equation was calculated from it. The plot is a typical plot of infinite dose dermal absorption experimental results, when the cumulative permeated amount is linear over time after the lag time elapsed, indicating that steady-state was achieved.

The permeability coefficient (K_p) was calculated by dividing the steady-state flux (J_{ss}) by the concentration of chlorotoluron in the donor chamber (10 μg ml^{-1}). The lag time was calculated extrapolating the trend line to y=0. The steady-state flux values, permeability coefficient, and lag time are in Table 1. The standard deviation of the steady-state flux was calculated in program STATISTICA as the standard deviation of the regression coefficient, and the standard deviation of the lag time was calculated from the standard deviation of the regression coefficient and the intercept.

Discussion

In this study, the permeability coefficient was calculated from the steady-state flux measured ex vivo using pig skin. The permeability coefficient is concentration independent. Once measured, it can be used for exposure calculations for any donor concentration (Wester and Maibach 2005). However, the permeability coefficient is formulation and membrane specific (Roberts and Anissimov 2005), and it is a key parameter when assessing the risks that result from the dermal exposure.

The experimental K_p of chlorotoluron in water is 3.8 * 10^{-3} cm h^{-1}.

Chlorotoluron is a nonvolatile substance, and its $LogK_{ow}$ is 2.41. The water solubility of chlorotoluron is 70 mg l^{-1} in 20 °C, meaning that it is poorly water-soluble (NLM 2006). Concentrations of chlorotoluron in surface water ranging from 0.4 to 0.6 μg l^{-1} were found in the UK (Lees and McVeigh 1988). In a German study, chlorotoluron was detected in surface and groundwater in concentrations of 0.2 and 0.3 μg l^{-1} (Reupert and Ploger 1989). Concentrations ranging from tens to hundreds of nanograms per liter have been measured in surface waters in the Czech Republic in 2008 (ČHMÚ).

Chlorotoluron has the potential to induce chromosomal aberrations (Federico et al. 2011) and concentration-dependent pathological changes of mouse testis (Hong et al. 2007). Endocrine disrupting effects of some phenyl-urea pesticides (for example diuron) have been reported (Orton et al. 2009). Chlorotoluron belongs to the group of phenyl-urea herbicides, and its structure is similar to that of diuron (one molecule of clorine in diuron is substituted by methyl group in chlorotoluron). Thus, chlorotoluron's similar endocrine effects are expected. It is a very effective inducer of benzo(a) pyrene monooxygenase and enhancer of uridine-diphosphate-glucuronyltransferase and glutathione-S-transferase activity (Schoket and Vincze 1990).

According to the information from the PPDB database (PPDB 2009–2013), the LD50 for rat is 10 000 mg kg^{-1}, 2000 mg kg^{-1}, and 5.3 mg l^{-1} for acute oral, dermal, and inhalation toxicity, respectively. According to Zak and Sachsee (1971), the oral LD50 is greater than 5 g kg^{-1} in dogs and 10 g kg^{-1} in rats.

The results of an experiment testing the metabolism of chlorotoluron by humans using cells of *Saccharomyces cerevisiae* expressing human cytochrome P450 showed that

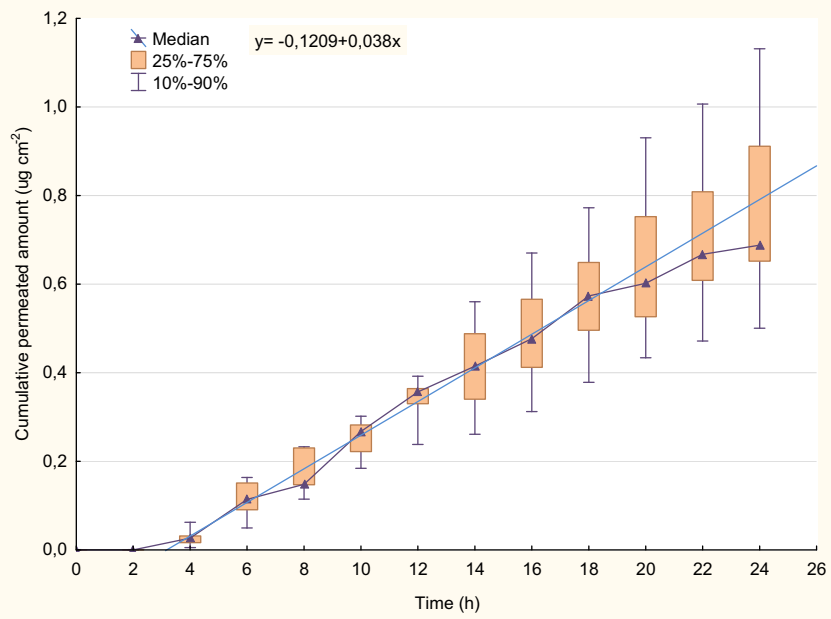

Fig. 1 Median and 25 and 75 % quantiles (*boxes*), 10 and 90 % quantiles (whiskers) of the cumulative permeated amount of chlorotoluron through the pig skin ex vivo. The plot is typical of an infinite dose flux curve. Values of cumulative permeated amount from 4 to 24 h were used to calculate the trend line and regression equation. The slope of the linear part of the cumulative permeated amount (the regression coefficient) is considered to be the steady-state flux (in units of μg $cm^{-2}h^{-1}$)

almost 45 % of chlorotoluron was metabolized in 30 min by the microsomal fraction and around 75 % of chlorotoluron in approximately 50 h was transformed by the whole cells (Mehmood et al. 1995).

These data are evidence that although chlorotoluron is quickly metabolized, it can cause negative effects on the health of the nontarget organisms, including humans.

There is a strong rafting and canoeing tradition in natural waters in the Czech Republic, as well as the use of natural waters for swimming and other recreation. Pollution with chlorotoluron has been measured in rivers frequently used for recreation. One of the highest concentration found in these rivers was 0.202 μg l^{-1} in the Cidlina River (locality Sány) in March 2008 (ČHMÚ). The limit of quantification for the method used was 0.03 μl l^{-1},; thus, this value was considered the lower limit of concentrations found in the river. However, concentrations of chlorotoluron from 0.02 to 0.202 μg l^{-1} were found in the river Cidlina in this locality during a whole year 2008, when the mnonitoring of the water quality was carried out. Even higher concentrations have been found in the Rakovnický potok (Rakovnický Creek; locality Křivoklát, 0.86 μg l^{-1} in April of 2008). The Figures S1 and S2 of the support information show the concentrations of chlorotoluron detected in two aforementioned localities (Sány—river Cidlina and Křivoklát—Rakovnický creek, respectively) during monitoring in 2008. Risk due to dermal absorption of chlorotoluron during rafting, canoeing, or swimming was calculated from these concentrations of chlorotoluron: 0.202 and 0.03 μl l^{-1}, 0.86 μg l^{-1}. The risk was calculated for 1 week (7 days) of active holiday being exposed to the contaminated water 5 h a day. The exposure duration was 10 years. Also, we calculated the dermally absorbed dose during the worst scenario. A concentration of chlorotoluron of approximately 7 μg l^{-1} was found in October 1999 in the surface waters of Almar river basin during the monitoring of the agricultural pollution of waters in an area of Salamanca and Zamora (Spain) (Carabias-Martínez et al. 2003). We considered that some people can be exposed to this concentration due to using this river for recreational activities, e.g., 5 h a day during summer months (90 days—the worst case scenario). The average adult body weight is 71.8 kg, and the mean of the 50th percentile for total adult body surface is 18,150 cm^2 (USEPA 1997, 2004). The mean adult body weight between the ages of 18 and 75 is 71.8 kg (USEPA 1997).

The dermal absorbed dose (DAD) was calculated using Eq. 1, the USEPA equation for calculating the DAD (USEPA 2004).

$$DAD = \frac{DA_{event} \times EV \times ED \times EF \times SA}{BW \times AT} \quad (1)$$

where:

DAD	is the dermal absorbed dose (mg kg^{-1} day^{-1})
DA_{event}	is the absorbed dose per event (mg cm^{-2} $event^{-1}$)
SA	is the skin surface area available for contact (cm^2)
EV	is the event frequency (events day^{-1})
EF	is the exposure frequency (days $year^{-1}$)
ED	is the exposure duration (years)
BW	is body weight (kg)

Table 1 Steady-state flux (J_{ss}), permeability coefficient (K_p), and lag time with the standard deviations (SD)

Parameter (unit)	Value	±1xSD
J_{ss} (μg cm^{-2} h^{-1})	0.038	0.0027
K_p (cm h^{-1})	0.0038	0.00027
Lag time (h)	3.19	0.96

AT is the averaging time (days), which is 70 years (70×365 days) for compounds with carcinogenic effects and AT is equal to ED (ED×365 days) when the substance shows no carcinogenic effects.

The DA_{event} is calculated according to the US EPA equations (USEPA 2004). The calculation is based on the concentration in the donor formulation, duration of the exposure event, and the measured K_p, and it can be obtained from Eq. 2 if $t_{event} \leq t^*$ and from Eq. 3 if $t_{event} > t^*$, where the t* is the time to reach steady-state and $t^* = 2.4\,\tau$, where τ is the lag time.

$$DA_{event} = 2FA \times K_p \times C_w \sqrt{\frac{6\tau \times t_{event}}{\pi}} \quad (2)$$

$$DA_{event} = FA \times K_p \times C_w \left[\frac{t_{event}}{1+B} + 2\tau \left(\frac{1+3B+3B^2}{(1+B)^2} \right) \right] \quad (3)$$

where:

DA_{event}	is the absorbed dose per event (mg cm^{-2} event)
FA	is the fraction absorbed water (dimensionless)
K_p	is the permeability coefficient (in these calculations, the experimental K_p was used) in cm h^{-1}
C_w	is the concentration of chemical in water
T	is the lag time
T_{event}	is the event duration
T*	is the time to reach steady state and $t^* = 2.4\,\tau$

B is the dimensionless ratio of the permeability coefficient of a compound through the stratum corneum relative to its permeability coefficient across the viable epidermis ($_{ve}$). B is calculated according to Eq. 4 (USEPA 2004):

$$B = \frac{K_p}{K_{p,ve}} \cong K_p \frac{\sqrt{MW}}{2.6} \; (as\ an\ approximation) \quad (4)$$

Table 2 gives the overview of exposure scenarios with calculated DAD.

According to the PPDB database (PPDB 2009–2013), chlorotoluron resistance to the hydrolysis is pH and temperature sensitive. While chlorotoluron is stable and very persistent at 20–30 °C and pH 5–9, its DT50 is 22 days at pH 5 and 69 days at pH 9 both at 50 °C. DT50 is 2–3 days at 70 °C.

However, chlorotoluron is degradated by direct and indirect photolysis. It has been proven that the rate of indirect photolysis is strongly inversely correlated with the nitrate concentration in waters. This may be an important way of degradation of chlorotoluron in natural waters, especially in agricultural areas, where the concentration of nitrate ions is high due to the application of nitrate fertilizers to the field (Wallace et al. 2010; Oliver et al. 2013). On the other hand, the concentration of pesticides found in waters is likely to be higher in agricultural areas, which both of the aforementioned areas are.

According to the study by Oliver et al. (2013), the photodegradation DT_{50} mean values of chlorotoluron in ntural waters in the UK were 26 days in a lake and a pond, 6.8 days in the river and 7.3 days in a stream. Shorter DT50 values in the river and stream were proven to be caused by the higher concentration of nitrates in these waters.

The PPDB database (PPDB 2009–2013) declares the aqueous photolysis D50 of chlorotoluron at pH 7 to be 0.12 days.

The rate of photolysis degradation depends on many factors, like the intensity of sunlight, presence, and concentration of other compounds (nitrates, nitrites, humic substances, etc.) and others (Oliver et al. 2013; Shankar et al. 2008).

Different reference doses were found or calculated based on the data from the scientific literature.

The World Health Organization (WHO) established a tolerable daily intake (TDI) for chlorotoluron of 11.3 μg kg^{-1} bw day^{-1} (RfD_1=11.3 μg kg^{-1} bw day^{-1}) based on the 11.3 mg kg^{-1} bw day^{-1} NOAEL for systemic effects in a 2-year feeding study in mice (Ciba-Geigy, unpublished data, 1989, according to the WHO (WHO 1996a)), using an uncertainty factor of 1000 (100 for interspecies and intraspecies variation and 10 for evidence of carcinogenicity) (WHO 1996b). According to the same authors (Ciba-Geiby, unpublished, 1989, according to the WHO (WHO 1996a)), the NOAEL in a 2-year rat study was 100 mg kg^{-1}, which is equivalent to a daily intake of 5 mg kg^{-1} of body weight. This NOAEL is lower than that of the study with mice.

During the re-evaluation of chlorotoluron as an active substance to decide whether it will be included in Annex I to the Regulation (EC) No. 1107/2009 (EC 2009), the NOAEL for chlorotoluron was determined in a 2-year study on rats, and it was 3.7 mg kg^{-1} bw day^{-1}. The acceptable daily intake (ADI) was set to be 0.04 mg kg^{-1} day^{-1} in the Review Report for the active substance chlorotoluron by DG SANCO (furthermore just Review Report), using a safety factor of 100, which is 40 μg kg^{-1} bw day^{-1} (DG SANCO 2005) (RfD_2=40 μg kg^{-1} bw day^{-1}).

The safety factor of 10 was used by the WHO to calculate the TDI from the NOAEL (RfD_1) of chlorotoluron because of evidence of carcinogenicity. According to Ciba-Geiby (unpublished data, 1989, according to the WHO (WHO 1996b), no carcinogenic effects were observed in the 2-year rat study with chlorotoluron, and carcinogenic effects were observed only at the highest doses (2500 and 500 mg kg^{-1}) in a 2-year mouse study. No other evidence of carcinogenic effects of chlorotoluron has been found. According to the PPDB database (PPDB 2009–2013), chlorotoluron is a class 3 carcinogen (a substance which causes concern for humans due to possible carcinogenic effects, according

Table 2 Overview of exposure scenarios used for DAD calculation

No.	Description of scenario	Concentration	Event duration (h event^{-1})	Event frequency (events day^{-1})	Exposure frequency (days year^{-1})	Exposure duration (years)	Skin surface area available for contact (cm^2)	Body weight (kg)	Averaging time (days)	Calculated DAD (mg kg^{-1} bw day^{-1})
1.	Recreationist exposed to the maximum river concentration	0.86 μg l^{-1}	5	1	7	10	18150	71.8	3650	4.8×10^{-7}
2.	Recreationist exposed to to the maximum Cidlina River concentration	0.202 μg l^{-1}	5	1	7	10	18150	71.8	3650	1.13×10^{-7}
3.	Recreationist exposed to the minimum river concentration	0.03 μg l^{-1}	5	1	7	10	18150	71.8	3650	1.7×10^{-8}
4.	Exposure during the worst case scenario	7 μg l^{-1}	5	1	90	10	18150	71.8	3650	$5{,}1\times10^{-5}$

to the EU classification of chemicals). The safety factor of 10 for evidence of carcinogenicity was not used to calculate the ADI value in the Review Report (DG SANCO 2005). There is no strong evidence of the carcinogenic effects on humans; therefore, we modified the TDI value from the WHO by dividing it by 10; so, the safety factor of 10 was removed. We thereby obtained another reference dose (RfD_4=113 μg kg^{-1} bw day^{-1}).

If the value of NOAEL, 5 mg kg^{-1}, from the 2-year rat study from Ciba-Geiby (unpublished, 1989, according to the WHO (WHO, 1996b)), was used to calculate the reference dose, using the safety factor of 100 for extrapolating from an animal to human, but not using the safety factor of 10 (as there is no evidence enough of carcinogenicity), we would obtain the value of TDI 0.05 mg kg^{-1} bw day^{-1}, which is 50 μg kg^{-1} bw day^{-1} (RfD_3=50 μg kg^{-1} bw day^{-1}). It is obvious that this value is closer to the value of ADI (RfD_2) published in the Review Report (DG SANCO 2005). The reason that the TDI from the WHO based on the mouse study differs from the ADI in the Review Report for chlorotoluron (DG SANCO 2005) may be that each value was derived from different animals and also different safety factors were used. Considering all available toxicological data, use of the reference dose of 40 μg kg^{-1} bw day^{-1} from the Review Report for chlorotoluron (DG SANCO 2005) is recommended for the risk assessment process.

The hazard quotient (HQ) for each exposure scenario was calculated. The hazard quotient is the ratio of the average daily dose (which in this case is the calculated DAD) and the reference dose (which in this case is the ADI or TDI) (Eq. 5).

$$HQ = \frac{D}{RfD} \tag{5}$$

where D is the average daily dose and RfD is the reference dose. If the value of the HQ is higher than 1, this indicates that the average daily dose is higher than the reference dose and that this can lead to adverse effects from the given exposure.

The calculated HQ values when considering aforementioned exposure scenarios were 1.21×10^{-5}, 2.83×10^{-6}, and 4.21×10^{-7} for the concentrations 0.86 μg l^{-1}, 0.202 μg l^{-1}, and 1.68×10^{-5}, respectively. For the worst case scenario (concentration 7 μg l^{-1}, 5-h exposition a day, 90 days a year, 10 years), the HQ is 0.0013.

For the worst case scenario, the concentration of chlorotoluron in water that would yield the HQ=1 would be 5.54 mg l^{-1}. The hazard index (HI) for these scenarios is unknown, as only the HQ for the dermal exposure pathway was calculated in this study. The HI is the sum of all the HQs for all the exposure pathways and/or multiple contaminants with deterministic effects. Thus, if all the exposure pathways were included, the value of the HQ and therefore HI would be higher, resulting in a higher possibility of adverse health effects from this exposure (Fjeld et al. 2007).

Regarding the exposure scenarios for swimming, rafting, and canoeing in contaminated rivers, none of the concentrations in given exposure scenarios seem to be preconditions for adverse health effects from chlorotoluron, as the HQs of all the DADs were lower than 1. However, as was mentioned before, the total HI may be higher, especially due to ingestion of contaminated water. Also, other exposure pathways and the presence of other chemicals may lead to a higher probability of adverse effects from chlorotoluron on humans. Therefore, assessment of aggregate and cumulative risks resulting from the human exposure to pesticides, with an appropriate uncertainty analysis (Moschandreas and Karuchit 2002; Ragas et al. 2011) would yield more complex information about exposure rates and health risks of chlorotoluron and compounds with similar mechanisms of action.

In this study, scenarios of 1-week exposition a year during 10 years were considered. However, the exposition

to cholorotoluron can greatly increase for those individuals that are living close to the contaminated water body and use this for everyday recreation (e.g., children during summer holiday). Although chlorotoluron can be degraded fast by photolysis, this depends on many factors and the DT_{50} of chlorotoluron in water can vary depending on them. Although this degradation half-time can decrease with the presence of nitrates and other compounds present in water bodies near agricultural areas, it must be considered, that the concentration of pesticide is also higher in these areas. It is necessary to emphasize that the risks resulting from exposure to chlorotoluron can be higher especially in vulnerable population groups, such as pregnant women, children, and the elderly.

Conclusions

The permeability coefficient of chlorotoluron in water was defined experimentally ex vivo, using pig skin. The value of this permeability coefficient was 0.0038 cm h^{-1}. This coefficient can be used in risk assessment, as it was defined following the strict standardized methodology.

Standard and precise methods for setting the ADI or TDI values should be set, and one value should be recommended by scientific authorities to be used for one compound. The process of generating the reference doses should be uniform for all the compounds; so, the reference doses of different compounds are comparable, which could be useful, for example, in deciding which alternative compounds may be less toxic for the human population.

Human exposure risks were assessed for certain exposure scenarios. None of the dermally absorbed dose for none of the aforementioned scenarios should result in toxic effects. However, we did not consider the cumulative exposure and doses absorbed by other exposure pathways, nor most risky exposure scenarios. This study shows that chlorotoluron can be absorbed through skin, offeres the experimental K_p, and gives examples how this can be used in risk assessment process.

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RESEARCH ARTICLE

Bioavailability and mobility of organic contaminants in soil: new three-step ecotoxicological evaluation

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Abstract A novel approach was developed for rapid assessment of bioavailability and potential mobility of contaminants in soil. The response of the same test organism to the organic extract, water extract and solid phase of soil was recorded and compared. This approach was designed to give an initial estimate of the total organic toxicity (response to organic extractable fraction), as well as the mobile (response to water extract) and bioavailable fraction (response to solid phase) of soil samples. Eighteen soil samples with different levels of pollution and content of organic carbon were selected to validate the novel three-step ecotoxicological evaluation approach. All samples were chemically analysed for priority contaminants, including aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH) and dichlordiphenyltrichloroethane (DDT). The ecotoxicological evaluation involved determination of toxicity of the organic, mobile and bioavailable fractions of soil to the test organism, bacterium *Bacillus cereus*. We found a good correlation between the chemical analysis and the toxicity of organic extract. The low toxicity of water extracts indicated low water solubility, and thus, low potential mobility of toxic contaminants present in the soil samples. The toxicity of the bioavailable fraction was significantly greater than the toxicity of water-soluble (mobile) fraction of the contaminants as deduced from comparing untreated samples and water extracts. The bioavailability of the contaminants decreased with increasing concentrations of organic carbon in evaluated soil samples. In conclusion, the three-step ecotoxicological evaluation utilised in this study can give a quick insight into soil contamination in context with bioavailability and mobility of the contaminants present. This information can be useful for hazard identification and risk assessment of soil-associated contaminants.

Keywords Bioavailability · Mobility · Ecotoxicological evaluation · Toxicity · Soil contamination · Bioremediation · Risk identification/assessment

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Introduction

One of the major current challenges in context with ecological risk assessment of contaminants in soil is the assessment of their bioavailability. There are two approaches of environmental risk assessment, the biological and chemical, which complement each other (Kwan and Dutka 1995). The overview of existing studies comparing ecotoxicological assays with chemical analysis is presented in Cachada et al. (2014). Chemical analysis are focused on selected 'priority' pollutants such as polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), hexachlorocyclohexane (HCH) and dichlordiphenyltrichloroethane (DDT) and ignore other potential pollutants. The concentration determined from the chemical analysis is often significantly different from the amount of a chemical actually available to organisms in the environment.

Bioavailability is a function of the interaction between an organism and the environment as well as the physico-chemical interactions between the chemical and the matrix. The application of ecotoxicity tests, based on the exposure of test organisms

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to all bioavailable forms of a chemical, is being widely used for evaluating the toxicity of soils (Kwan and Dutka 1992). For the majority of toxicity tests involving bioassays, either aqueous or organic extracts of soil samples are used (Chen et al. 2005; Heinlaan et al. 2007; Pohren et al. 2012; Roig et al. 2011). These procedures estimate the amount of water-soluble or extractable organic toxicants. Solid-phase tests (also indicated as solid-contact test, contact bioassay, contact test, solid-phase test, solid-phase toxicity test, solid-phase microbial assay, solid-phase contact toxicity test) allow the test organisms to come in direct contact with the entire untreated/undisturbed soil sample. The response of the test organism to the solid-phase test can reflect the actual amount of bioavailable chemicals presented in the sample (Ahlf 2006; Brouwer et al. 1990; Day et al. 1995; Feiler et al. 2013; Kwan 1993; Kwan and Dutka 1995, 1992; Rönnpagel et al. 1995; Weber et al. 2006).

Nowadays, batteries of solid-phase tests are used for the ecotoxicological evaluation of the behaviour or interactions of the substances contained in the soils and sediments (Feiler et al. 2013; Höss et al. 2010; Shaw et al. 2000). Solid-phase tests have been performed using bacteria (*Arthrobacter globiformis* (Neumann-Hensel and Melbye 2006), *Bacillus cereus* (Rönnpagel et al. 1995) and *Vibrio proteolyticus* (Heise and Ahlf 2005)), yeasts (*Saccharomyces cerevisiae* (Weber et al. 2006)), nematodes (*Caenorhabditis elegans* (Traunspurger et al. 1997)), oligochaetes (*Lumbriculus variegatus* (Phipps et al. 1993)), zebrafish (*Danio rerio*) embryos (Hollert et al. 2003; Zielke et al. 2011) and plants (*Myriophyllum aquaticum* or *Lemna Minor* (Feiler et al. 2004)). It is recommended to conduct bioassays in combination with chemical analysis for a comprehensive evaluation of hazards, as toxicity results will include considerations of chemical exposure and mixture toxicity (chemical interactions) (Ma et al. 2014). Many of the tests that compare effects of water or organic extract tests with solid-phase tests use different organisms to evaluate the toxicity of contaminants in soil (Ivask et al. 2004; Kołtowski and Oleszczuk 2015; Mouchet et al. 2006; Olajire et al. 2005).

The novel approach presented in this study involves complementary testing of tree different fractions of solid sample contamination which provides a complex toxicological assessment using the same test organism (*B. cereus* CCM 2010). Because of using the same organism, there are not any interspecies uncertainties and also matrix effects and influences of the organism are eliminated. *B. cereus* was exposed to three different phases (organic extract, water extract and solid phase) that represent the total toxicity and mobile and bioavailable fractions, respectively. Our three-step ecotoxicological evaluation is a useful tool to provide a fast evaluation of mobility and bioavailability of toxic compounds in complex matrices such as soils. The results of our three-step ecotoxicological evaluation and chemical analysis of selected soil samples were compared and interpreted in the context of bioavailability and mobility of present contaminants.

Materials and methods

Sample collection and handling

Eighteen soil samples were collected from different sampling sites in Southern Moravia, Czech Republic. The sample collection and handling was performed according to ISO 10 381-6 (1993). Detailed sampling and analytical procedures have been published previously (Holoubek et al. 2009; Cupr et al. 2010). Sampling sites, in the locality near a cement factory, South Moravia, CZ, were selected according to different level of POPs contamination and physico-chemical characteristic, from various geographical regions, soil qualities and land uses. Sampling sites included three arable soils, seven grasslands and eight forest soils (Table 1). Total nitrogen (N_{tot}), total organic (C_{org}) and pH was measured in each of the soil samples according to ISO 11 261 (1995); ISO 14 235 (1998) and ISO 10 390 (1994), respectively. Information on the soil profile and basic soil parameters were recorded for all sites at the beginning of each sampling event. Each site was represented by ten sub-samples collected from an area of 25×25 m (approximately 1.5 kg). The mixed plough layer (0–25 cm) was sampled for arable soil, while the top 10 cm of soil was collected on grassland and forest soils. In the forest, the litter (O_L horizon) was carefully removed before sampling, so the sample was a mixture of the overlaying organic horizons O_F+O_H. At the sampling sites with the organic horizon layers thinner than 10 cm, this mixture also contained a fraction of mineral horizon A_H. This sampling strategy results in high variability of the physicochemical properties of the soil samples.

All soil samples were transported to the laboratory in polyethylene bags, air-dried at laboratory temperature sieved through 2-mm mesh and stored at 4 °C until use (Seiler et al. 2008).

Chemical analysis

The soil samples were analysed for PAHs (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benz(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(123cd)pyrene, dibenz(ah)anthracene, benzo(ghi)perylene), PCBs (PCB 28, 52, 101, 118, 153, 138, 180) and organochlorine pesticides ((OCPs) α-, β-, γ-, δ-HCH, p,p′-DDE, p,p′-DDD, p,p′-DDT) according to the method (Holoubek et al. 2009). Five grams of dry soil sample was extracted using 120 ml dichloromethane (DCM) in an automatic extraction system (40 min warm extraction, 20 min washing; Büchi B-811, Flawil, Switzerland). Concentrated extracts for PAHs analysis were fractionated on activated silica (activation 250 °C, 3 h). The unused aliphatic fraction was eluted with hexane and then dichloromethane to obtain PAHs. Quantitative analysis was carried out on gas

Table 1 Description of the soil samples, characterisation of the soil and other properties

Sample	Soil texture	Soil type	pH (H_2O)	N_{tot} (%)	C_{org} (%)	Clay (%)	Sand (%)
Soil 1	Sandy	Forest soil	5.90	0.32	4.48	22.50	27.20
Soil 2	Sandy loam	Grasslands	4.50	0.14	1.10	37.20	16.10
Soil 3	Loamy sand	Forest soil	4.50	0.26	4.10	19.30	33.60
Soil 4	Sandy	Forest soil	6.40	0.32	4.38	26.70	31.60
Soil 5	Sandy	Grasslands	5.40	0.34	2.76	22.70	35.20
Soil 6	Sandy loam	Arable soil	6.48	0.18	1.21	32.00	16.30
Soil 7	Loamy sand	Forest soils	4.90	0.21	4.00	22.60	30.40
Soil 8	Sandy	Grasslands	6.50	0.23	1.79	20.60	41.70
Soil 9	Sandy	Grasslands	6.70	0.34	3.59	14.80	45.30
Soil 10	Sandy	Forest soil	5.50	0.43	5.00	8.70	57.00
Soil 11	Sandy loam	Grasslands	4.90	0.11	1.17	29.40	22.00
Soil 12	Sandy	Grasslands	7.12	0.28	2.60	6.60	69.50
Soil 13	Loamy sand	Arable soils	6.95	0.17	1.11	10.80	52.30
Soil 14	Loamy sand	Forest soil	5.80	0.48	7.06	10.70	48.10
Soil 15	Sandy	Forest soil	6.47	0.34	3.91	6.10	68.40
Soil 16	Sandy loam	Arable soils	6.70	0.15	1.05	21.30	37.40
Soil 17	Loamy sand	Forest soil	5.30	0.55	5.85	11.60	47.60
Soil 18	Sandy	Grasslands	6.56	0.48	3.39	8.30	75.60

chromatograph mass spectrometer (GC-MS). The GC was a HP 6890 equipped with mass selective detector HP 5972 (Hewlett Packard, Les Ulis, France) supplied with fused silica column DB-5MS (J&W Scientific, Folsom, CA, USA) 60 m × 0.25 mm with 0.25 μm film of stationary phase with 5 % phenyl in 95 % methyl polysiloxane. The second extract of 5 g dry soil was cleaned-up for determination of PCBs and OCPs on column content acid-modified silica (44 wt.% of concentrated sulphuric acid). PCBs and OCPs were eluted with 5 % dichloromethane in hexane. Samples were analysed on GC-ECD instrument HP 5890 (Hewlett Packard, Palo Alto, CA) equipped with capillary column Quadrex 007-5, 40 m × 0.18 mm × 0.25 μm film thickness.

Quality assurance and quality control were ensured by spiking surrogate standards (recovery and internal standards). The appropriate standards were quantified for all samples. Analyses of four blanks (two solvent and two using empty extraction cartridges) were performed for every week of extraction, so that the total number of blanks was 20. Blank levels were below the detection limit for all compounds and in all cases. Standard recoveries varied between 75 and 105 % for deuterated PAHs (D10-phenanthrene, D12-perylene) and 85 and 105 % for PCB 30 and PCB 185 congeners.

Ecotoxicological tests

Toxicity of (i) water extract, (*ii*) organic extract and (*iii*) untreated soil sample (solid-phase test) was evaluated using *B. cereus* toxicity assays. Every sample was tested in three-step dilution, and two true replicates per treatment group were applied. In the water extract test (*i*), the presence of toxicity in the mobile phase was assessed. Toxicants can leach from the soil during wet conditions (mobile contamination); hence, bacterial inhibition by the water extract exposure would indicate mobility of toxic compounds. The organic extract test (*ii*) was used to demonstrate the potential maximum organic toxicity potential of soil samples. The contact toxicity test (*iii*) was used to provide information on bioavailable portion of contaminants in the soil.

The water extracts were extracted from soils by Milli-Q water at ratio 1:1 (d*w*/*v*) following standardised procedures (Dutka et al. 1993, 1994; Prokop et al. 2003). The suspension was shaken (200 rpm) for 1 h at 20 °C, and then filtered and applied for toxicity testing. In case of the organic extract test, the collected soil samples were extracted with DCM using a Soxtec extraction system (Büchi B-811, Flawil, Switzerland; aliquot of DCM extraction for chemical analysis was applied). The solvent was evaporated under a nitrogen stream, and then reconditioned with 1 ml dimethyl sulfoxide (DMSO) for toxicity testing.

Dehydrogenase activity

Dehydrogenases are directly involved in many of the vital anabolic and catabolic processes of living organisms, and their activity is subjected to inhibition by chemical toxicants. Microbial dehydrogenase activity was determined using the oxidation-reduction dye resazurin. Resazurin (λ_{max} = 601.2 nm) is reduced by the microbial electron transport systems to resorufin (λ_{max} = 571.4 nm), and the reaction is

irreversible in biological systems, thus increasing the accuracy of the assay. Microbial dehydrogenase activity is proportional to the amount of resazurin reduced, which can be quantitatively measured at 601 nm using a spectrophotometer or colorimeter.

All three tests were based on determination of microbial dehydrogenase activity inhibition. Test bacteria (*B. cereus*) dehydrogenase activity is proportional to the amount of reduced resazurin (oxido-reduction indicator) (Rönnpagel et al. 1995; Ahlf 2006; Liu 1989). The test procedure was performed according to Prokop et al. (2003).

In the solid-phase test, 2 g of each sample was transferred into a centrifuge screw-cap tube and resuspended in 2 ml of buffer medium. Incubation was performed for 4 h, at 30 °C and 70 rpm. The inoculum was prepared from actively growing biomass by adjusting the cell concentration to $OD_{610}=1.0$ (optical density at 601 nm). After the incubation, 2 ml of a resazurin solution (with a concentration of 0.2 mg ml^{-1} of phosphate buffer) was added to indicate the activity of bacterial dehydrogenases. The mixture was shaken for 1 h and centrifuged (3500×*g*, 5 min). The reaction was stopped by membrane filtration (pore size, 0.2 pm). The concentration of unreduced resazurin was measured in the filtrate by spectrophotometry at 601 nm.

Data analysis

The toxic effect was expressed as the effective sample concentration causing a 50 % inhibition of the dehydrogenase activities (EC_{50}) compared with a negative control in these three tests (Ahlf 2006). Linear models were fitted to the linear parts of dose–response relationships. EC_{50} values were calculated by inverse prediction from these linear models (Meddings et al. 1989). All statistical calculations were performed in Statistica for Windows 11.0 (StatSoft Inc., Tulsa, USA). Toxic units (TU) were calculated according to Newman (1995) as the ratio $100/EC_{50}$ (Anderson et al. 2003; Blaise and Férard 2005; Hunt et al. 2003; Junghans et al. 2006; Thomas et al. 2001). The bioavailable fraction (F_{Bio}) and mobile (F_{Mob}) of the soil contaminants was expressed as follows:

$$F_{Bio} = \frac{TU_{Solid}}{TU_{Organic}} \times 100 \quad (1)$$

$$F_{Mob} = \frac{TU_{Water}}{TU_{Organic}} \times 100 \quad (2)$$

where, TU_{Solid} is the bioavailable toxicity of the soil (toxicity of the solid phase), TU_{Water} is the toxicity of mobile fraction of the soil (toxicity of the water extract) and $TU_{Organic}$ represents the total organic toxicity of the soil (toxicity of organic extract) in toxic units TU.

Canonical analysis was carried out to assess the correlation between chemical analysis ecotoxicological tests.

Spearman's correlation test was carried out to assess correlation between individual toxicological analyses (correlation F_{Bio} and C organic, correlation F_{Mob} and C organic, correlation F_{Bio} and pH of water extract).

Results and discussion

It is important to highlight that the main aim of our study was to present the new approach verification of a three-step ecotoxicological evaluation and not the pollution sources

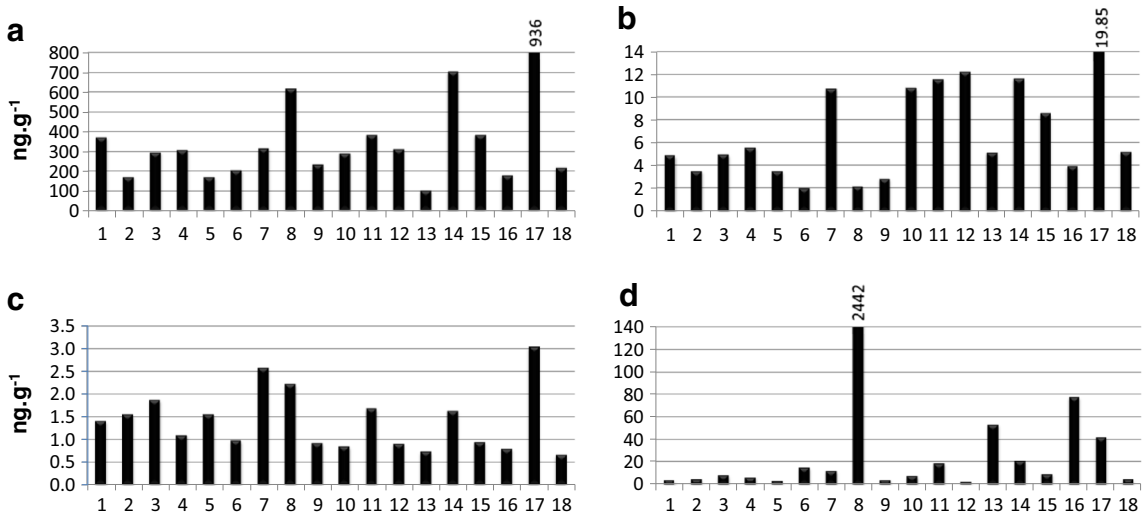

Fig. 1 The results of chemical analysis of PAHs (**a**), PCBs (**b**), HCHs (**c**) and DDTs (**d**) present in the soil samples; *y*-axis: concentration of the chemicals (ng g^{-1}), *x*-axis: the soil sample number

Table 2 Toxicity of the soil sample contaminants

Sample	*Bacillus cereus* Toxicity Test (TU)									
	Water Extract Toxicity		Solid Phase Toxicity		Organic Extrakt Toxicity		Bioavailable Fraction [%]		Mobile Fraction [%]	
	TU_{Water}	S.D.	TU_{Solid}	S.D.	$TU_{Organic}$	S.D.	F_{Bio}	S.D.	F_{Mob}	S.D.
Soil 1	0,66	0,08	5,47	1,09	29,24	7,46	18,71	0,85	2,26	0,24
Soil 2	1,30	0,35	5,71	0,76	26,51	1,37	21,52	1,67	4,89	1,03
Soil 3	0,69	0,25	5,32	0,45	28,23	3,13	18,86	0,44	2,44	0,56
Soil 4	-1.12[a]	0,16	3,90	0,01	14,70	1,75	26,52	2,75	0,00	0,00
Soil 5	2,90	0,16	5,16	0,10	28,14	0,53	18,35	0,02	10,30	0,38
Soil 6	0,81	0,20	12,50	1,27	31,28	2,75	39,96	0,49	2,58	0,37
Soil 7	0,77	0,45	6,38	0,53	67,15	4,38	9,51	0,15	1,14	0,56
Soil 8	2,18	0,29	20,42	1,68	25,31	2,49	80,67	1,17	8,62	0,26
Soil 9	0,32	0,09	3,90	1,19	32,22	3,76	12,12	2,04	1,00	0,16
Soil 10	-0.42[a]	0,07	5,51	0,45	21,35	5,77	25,80	3,82	0,00	0,00
Soil 11	-1.43[a]	0,23	6,31	0,13	26,72	4,83	23,62	3,19	0,00	0,00
Soil 12	0,19	0,07	5,31	1,43	9,80	0,55	54,20	10,89	1,92	0,57
Soil 13	-1.28[a]	0,09	3,70	0,07	5,02	0,08	73,66	0,33	0,00	0,00
Soil 14	-0.04[a]	0,07	5,90	0,84	61,26	4,64	9,64	0,60	0,00	0,00
Soil 15	-0.17[a]	0,11	1,54	0,79	21,39	1,05	7,20	3,17	0,00	0,00
Soil 16	-2.17[a]	0,32	3,91	0,05	3,31	0,72	100,00	1,85	0,00	0,00
Soil 17	0,49	0,08	6,47	0,39	33,97	9,97	19,04	3,42	1,43	0,15
Soil 18	0,35	0,16	8,38	0,96	14,27	3,29	58,74	5,53	2,43	0,47

The biological responses of the bacteria *Bacillus cereus* to the presence of toxic compounds in untreated solid samples (solid-phase) and in their water and organic extracts (in toxic units (TU))

[a] Growth stimulation effect

identification of each samples. Our intention was to choose the most diverse samples with different contamination and physico-chemical properties; these differences were confirmed by the chemical analysis (Fig. 1). One of the highest PAHs and HCHs contamination was found by soil sample 8, where the extremely high levels of DDTs were also determined. The highest contamination was found by soil sample 17, which showed the highest levels of PAHs, PCBs and HCHs contamination. High contamination was found also by samples 7 and 14 due to the relative high levels of PAHs, PCBs and HCHs. In comparison, the lowest contamination was found by samples 13 and 16. These samples showed relative low levels of PAHs, PCBs and HCHs, and only DDTs levels were slightly elevated. This diverse set of samples was used for the demonstration of suitability of the proposed methodology providing essential information about mobility and availability of contaminants presented in solid-phase matrices.

The organic extract is intended to monitor the maximum amount of organic contaminants in the sample. The efficiency of organic extraction procedure used in this study was >95 % (Skarek et al. 2007). The toxicity of the organic extracts was observed to be high relative to the mobile and bioavailable factions for almost all of the soil samples.

The water extracts showed low toxicity for all soil samples (Table 2; Fig. 2), which indicates that only low fraction of overall contamination is readily water soluble and mobile (F_{Mob}) *(Table 2; Fig. 3). This observation suggests that the organic contaminants present in the samples can be strongly*

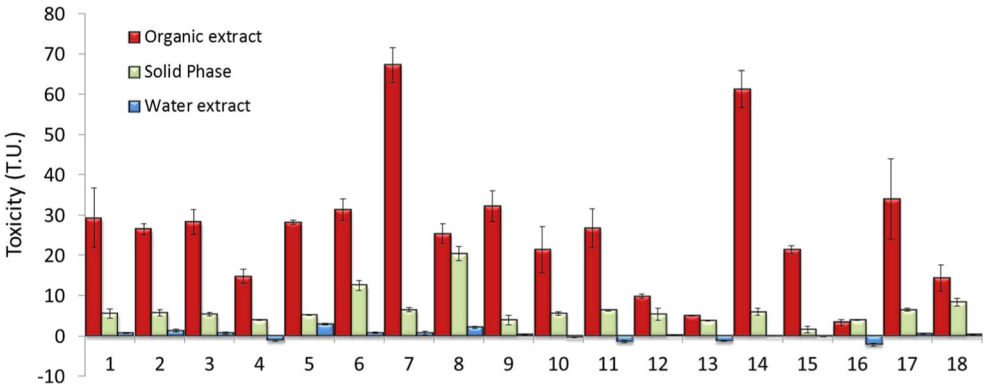

Fig. 2 Toxicity of the soil sample contaminants assessed using three-step ecotoxicological evaluation. Organic extract (*red*), solid-phase (*green*) and water extract (*blue*). *Bars*, average of extracts toxicity in toxic units (*TU*). *Whiskers*, standard deviation (SD)

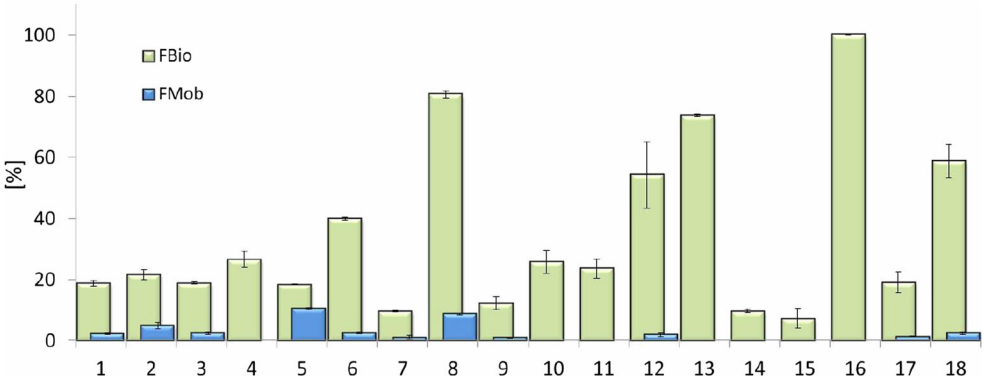

Fig. 3 Bioavailability (F_{Bio}; *green*) is the ratio of toxicity of solid fraction (TU_{Solid}) and toxicity of organic extract ($TU_{Organic}$) in percentage (%); mobility (F_{Mob}; blue) is the ratio of water extract toxicity (TU_{Water}) and organic extract toxicity ($TU_{Organic}$) in percentage (%). *Whiskers*, standard deviation (SD)

bound to solids and were not available for water extraction. This effect can be clearly seen for sample 14, which showed a weak response on the water extract; however, the organic extract showed one of the highest toxicity and also levels of PAHs and PCBs, measured by chemical analysis, were high. This indicates the strong binding of organic toxicants to the solid particles, i.e., these compounds are unlikely to be very mobile.

he solid-phase test indicates the fraction of the contamination which is available for biota (F_{Bio}). The overall level of toxicity of solid phase was higher in comparison with water extract toxicity but still significantly lower than overall contamination evaluated by organic extract toxicity (Table 2; Fig. 2). This indicates that the contaminants available for organisms living in solid environment do not need to be well soluble in water and mobile. This effect can be explained by the effect of 'driven' desorption. The microorganisms bind on solid-phase surfaces and interact with both the aqueous and the particle-bound factions of potential toxicants (Feiler et al. 2013; Shaw et al. 2000). Still, large fraction of contamination is bound to solid particles strong enough not to be biologically available. This scenario could be seen in sample 7, which had high organic extract toxicity and high level of contamination, while the bioavailable fraction of presented toxicants (F_{Bio}) was very low. Interestingly, samples 13 and 16 with very low level of contamination showed stimulation in the water extract and the organic extract showed the lowest toxicity of all the organic extracts. Overall, the canonical analysis showed good correlation between the chemical and toxicological analyses (R^2=0.84, p=0.0005).

The elevated level of bioavailability and mobility was observed for sample 8. This sample belongs to the highest contaminated samples, where high levels of PAHs and PCBs and extremely high levels of DDTs were determined. The high level of bioavailable (F_{Bio}) and mobile (F_{Mob}) fraction is likely due to limited adsorption capacity of sample 8. The correlation analysis indicates that the bioavailability of contaminants in tested samples was affected by the amount of organic carbon content of the soil (Fig. 4). The negative relationship between bioavailability and amount of organic carbon in the soil samples (r=−0.60, p<0.05) suggests that bioavailability of organic contaminants in the tested samples decreased with increasing amount of organic matter.

Organic matter has a high affinity to bind organic compounds, reducing their bioavailability. The greater the hydrophobicity or lipophilicity of an organic contaminant, the greater the potential for its sorption to organic matter (Christman and Pfaender 2006). Another indirect effect of soil organic matter is its role on limiting contaminant mass transfer. The rate of mass transfer of an organic contaminant from soil particles to the surrounding pore water is inversely proportional to the contaminant's soil-water distribution coefficient (Pignatello 1999). Therefore, with increasing organic matter content, retention of an organic contaminant increases and rates of release decrease, thereby, decreasing overall contaminant bioavailability (Stokes et al. 2006; Hayat et al. 2010). Another correlation analysis indicates dependence of bioavailability on pH (Fig. 5). In this case, the bioavailability increases with increasing value of pH (r=0.52, p<0.05); pH is one of the most important parameters, which can strongly influence the bioavailability of organic contaminants (Loibner et al. 2006). It was found that bioavailability of soil contaminants should be increased at low pH, due to the reduced competing influence of protons at high pH, which facilitates uptake of free ions of contaminants in the test organism (Ardestani and van Gestel 2013).

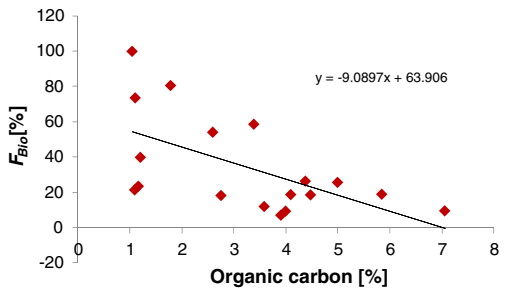

Fig. 4 Relationship between bioavailability F_{Bio} of contaminants and amount of organic carbon in tested soils (r=−0.60, p<0.05)

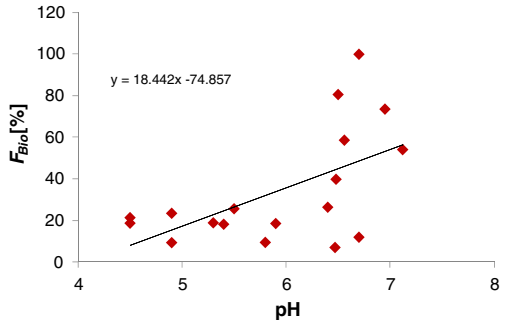

Fig. 5 Relationship between bioavailability F_{Bio} of contaminants and pH (r=0.52, p<0.05)

Conclusions

A novel approach was used for the fast assessment of bioavailability and potential mobility of contaminants in soil. The procedure used the same test organism and was designed to give an early estimate of the total organic toxicity (organic extractable fraction), mobile (water extract) and bioavailable (solid phase) fraction of contaminants in soil samples. The selected soil samples with different levels of contamination (PAHs, PCBs, HCH and DDT) and different amounts of organic carbon were used for demonstration of efficiency of proposed method for analysis of fractionation of contaminants in solid samples. The results showed a low potential mobility of organic toxicants in tested soils. The bioavailable fraction was significantly greater than the mobile fraction. Still, the large fraction of overall contamination was strongly bound to the solid samples showing significant dependence on the amount of organic carbon. The fraction bioavailable to the test microorganism was likely elevated by the effect of driven desorption.

The presented new tool of 'multi-component three-phase exposure' utilise the exposure of the same test organism and is useful for evaluation of total toxicity, water solubility and biologically available fraction of the solid sample contamination. The three-step ecotoxicological evaluation provides early information about behaviour of contaminants in soils in the context of mobility and bioavailability. The knowledge of mobility and bioavailability of chemical compound in a soil is useful for the hazard identification (provides useful toxicity and exposure information) and environmental risk assessment for organic contaminants in soils.

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New experimental data on the human dermal absorption of Simazine and Carbendazim help to refine the assessment of human exposure

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HIGHLIGHTS

- Absorption kinetics of Simazine and Carbendazim were measured for the first time.
- Human skin was used to experimentally determine the permeability coefficient.
- Risks due to dermal exposure to polluted water were assessed probabilistically.
- Two exposure scenarios were considered and no increased risks were found out.
- New refined tool to assess the risks of dermal exposure to pollutants is presented.

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ABSTRACT

Due to their widespread usage, people are exposed to pesticides on a daily basis. Although these compounds may have adverse effects on their health, there is a gap in the data and the methodology needed to reliably quantify the risks of non-occupational human dermal exposure to pesticides.

We used Franz cells and human skin in order to measure the dermal absorption kinetics (steady-state flux, lag time and permeability coefficient) of Carbendazim and Simazine. These parameters were then used to refine the dermal exposure model and a probabilistic simulation was used to quantify risks resulting from exposure to pesticide-polluted waters.

The experimentally derived permeability coefficient was 0.0034 cm h^{-1} for Carbendazim and 0.0047 cm h^{-1} for Simazine. Two scenarios (varying exposure duration and concentration, i.e. environmentally relevant and maximum solubility) were used to quantify the human health risks (hazard quotients) for Carbendazim and Simazine. While no risks were determined in the case of either scenario, the permeability coefficient, which is concentration independent and donor, formulation, compound and membrane specific, may be used in other scenarios and exposure models to quantify more precisely the dermally absorbed dose during exposure to polluted water.

To the best of our knowledge, the dermal absorption kinetics parameters defined here are being published for the first time. The usage of experimental permeability parameters in combination with probabilistic risk assessment thus provides a new tool for quantifying the risks of human dermal exposure to pesticides.

1. Introduction

Today, many pesticides are used in the environment to protect crops against pests and to help increase agricultural production. Their use poses risks to both surface and ground water (Rojas et al., 2015). Pesticides are emitted into the environment by diffusive pathways and direct losses (De Wilde et al., 2009). While these compounds have toxic effects on target organisms, they also influence non-target organisms – including humans. Consequently, the use of personal protective equipment (PPE) is recommended for workers and operators who are occupationally exposed to them, in order to avoid or minimize risks resulting from such exposure. However, since other population groups, such as residents and bystanders, may also be exposed to these compounds due to environmental pollution, a greater degree of attention should be

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dedicated to human risk assessment associated with non-occupational exposure. Moreover, many people are vulnerable to non-agricultural sources of pesticide exposure and more data and research are thus needed to quantify such exposure (McKinlay et al., 2008). Nevertheless, there is a lack of experimental data on pharmacokinetics, including the transdermal penetration parameters of pesticides, and dermal non-occupational exposure thus cannot be quantified. Dermal exposure to pesticides may be an important source of exposure. Further actions focusing on clarifying the contribution of the dermal route to the total uptake of pesticides is needed (Beamer et al., 2009) and an experimental approach in assessing human exposure is crucial (Kefeni and Okonkwo, 2014).

This study focuses on two compounds: Simazine and Carbendazim. These two compounds were selected from among other pesticides according to their parameters (e.g. molecular weight, octanol–water partitioning coefficient and acceptable daily intake values), which influence their dermal absorption rate and thus risk of human exposure. In addition, no experimental data on their dermal absorption parameters has been published to date. As a result, it has been impossible to quantify their dermal exposure and the risks these compounds pose to humans.

Carbendazim is a fungicide from the benzimidazole group used to control plant diseases by inhibiting mitosis and cell division. Carbendazim has been found to result in maternal and developmental toxicity in mammals (Sitarek, 2001; Minta et al., 2004). It exhibits toxicity by affecting the liver in rats (Muthuviveganandavel et al., 2008). Carbendazim has also been found to increase estrogen production by increasing aromatase activity (Morinaga et al., 2004), increase the androgen receptor mRNA in male rats (Hsu et al., 2011) and induce other endocrine disrupting effects (Maranghi et al., 2003; Rajeswary et al., 2007; Yu et al., 2009; Prashantkumar et al., 2012; Rama et al., 2014). It has also been found to cause hepatic and slight kidney dysfunctions in male goats (Waghe et al., 2013). Moreover, Carbendazim is a metabolite of Benomyl, another benzimidazole pesticide. Carbendazim was previously used in the European Union; however, its inclusion on the list of active compounds approved in the EU (EC, 2009) expired in November 2014 (EC, 1995–2015). Nevertheless, plant protection products containing Carbendazim as an active compound are still available on the market and their use is approved until 2021, e.g. in the UK (HSE, 2015). Carbendazim can persist in the environment due to its benzimidazolic ring (Hernandez et al., 1996; Pourreza et al., 2015).

Simazine is a herbicide from the triazine group used to control broad-leaved weeds and annual grasses on deep-rooted crops, as well as on non-crop areas such as farm ponds and fish hatcheries (University of Hertfordshire, 2013). Its mode of action consists of photosynthesis inhibition. In mammals, Simazine is suspected of endocrine disruptive effects and there is evidence that it may be capable of inducing aromatase activity and thus increase estrogen production (Sanderson et al., 2000). It also exhibited developmental toxicity in female offspring mice by disturbing cellular apoptosis and proliferation during exposure *in utero* (Park et al., 2014). Simazine is on the list of 33 priority substances of the Directive 2008/105/EC (EC, 2008) amended by Directive 2013/39/EU (EU, 2013). This list was established in order to provide measures designed to reduce water pollution by such compounds by the Water Framework Directive (EC, 2000), which aims to reduce the negative impact of water pollution on the environment and on humans. Although the use of Simazine in the European Union was banned in 2004 (EC, 2004) due to environmental concerns (EC, 2003), Simazine and other triazine herbicides are still detected in the environment due to their long retention time in the soil and aquifers. Thus, due to leaching processes, levels of Simazine may be detected in the environment even years after their prohibition (Lorente et al., 2015). Also, Simazine is still used in a number of countries such as Australia (https://portal.apvma.gov.au).

In view of the above, measuring human exposure to pesticides such as Simazine and Carbendazim is necessary. Human skin *ex vivo* is currently the best available model for measuring the human transdermal penetration of chemical compounds (Barbero and Frasch, 2009). In a system of diffusion Franz cells, it presents an optimal tool for assessing human dermal exposure to Simazine and Carbendazim. The dermal absorption kinetics (steady state flux, permeability coefficient, lag time) of these pesticides measured by this system can serve as inputs for pharmacokinetic models; e.g. the US EPA exposure model allows for the calculation of dermally absorbed doses during defined common exposure scenarios. Refining this model by using experimentally derived pharmacokinetic parameters and probabilistic modeling can yield more realistic results when quantifying human non-occupational exposure to Carbendazim and Simazine in polluted water.

This study thus uses the above mentioned approach to quantify non-occupational dermal exposure to polluted water and thus assess the risks posed to humans by Simazine and Carbendazim and suggest measures for alleviating potential human health risks.

2. Materials and methods

2.1. Chemicals

All chemicals, Carbendazim (CAS number: 10605-21-7) analytical standard with 99.2% purity, Simazine (CAS number: 122-34-9) analytical standard with 99.8% purity, 0.01 M phosphate-buffered saline (PBS, containing 0.138 M NaCl; 0.0027 M KCl, pH = 7.4 at 25 °C), and bovine serum albumin (BSA, CAS number 9048-46-8) of ≥98% purity were purchased from Sigma-Aldrich. Milli-Q water was used to prepare all aqueous solutions.

2.2. Skin membranes source and preparation

The assay was performed following OECD guidelines (OECD, 2004a; OECD, 2004b) in accordance with Wellner et al. (2008) and Lademann et al. (2008).

For each compound, *ex vivo* human abdominal skin from 3 patients (males and female) was used. The patients were aged 27, 28 and 42 in the case of Carbendazim and 24, 27 and 46 in case of Simazine. The skin was obtained from plastic surgery with the informed consent of all patients.

After surgery, the skin was packed in a plastic bag, stored in the fridge and transported the laboratory at 4 °C. In the laboratory, the subcutaneous fat was removed with a scalpel. The hairs were thin and it was therefore not necessary to remove them. The skin was washed in tap and then distilled water, gently dried with a cotton tissue, cut into slices and packed in aluminum foil and a zip-lock bag. Finally, the skin was stored in a freezer at −20 °C for no longer than 6 months.

Immediately before the experiment, the skin was defrosted at ambient laboratory temperature and split-thickness membranes (350 μm) were prepared with an electric dermatome (Braun Acculan® 3Ti). The skin was cut into small pieces of uniform size and the integrity of the membrane was checked visually before the experiment. After the experiment, a post-study data analysis integrity evaluation was carried out (OECD, 2004a; Wellner et al., 2008; Stahl et al., 2012).

2.3. Dermal absorption experiment

An automatic MicroettePlus system (Hanson Research) with six

amber glass Franz cells was used. The volume of the receptor chamber was approximately 4 mL, the opening for the skin surface was approximately 0.64 cm^2. Saturated water solutions of Carbendazim and Simazine were used as the donor formulations. These solutions were obtained by preparing oversaturated aqueous solutions. After ultrasonification (30 min) and shaking at room temperature (30 min), the solutions were filtered using regenerated cellulose syringe filters. Concentrations of Carbendazim and Simazine in the donor fluid were then analyzed. A phosphate buffered saline (pH = 7.4) with 2.5% bovine serum albumin dissolved in Milli-Q water was used as a receptor fluid for both compounds.

Before the experiment, the receptor chamber was filled with the receptor fluid. To avoid the formation of air bubbles in the receptor fluid, the storage beaker with the receptor fluid was degassed with helium. The skin membranes were placed between the receptor and the donor chamber with the uppermost layer to the upper side and the whole system was left to stabilize for 30 min before the experiment. The receptor chamber was visually checked for bubbles. At the start of the experiment, 200 μL of the donor formulation were applied to the skin in the donor chamber (two independent experiments for each compound). The whole system of Franz cells was heated to 32 °C, i.e. body surface temperature. The experiment duration was 24 h for Simazine and 22 h for Carbendazim with the aim of reaching steady-state flux. Every 2 hours 1 mL of receptor fluid from each cell was collected into HPLC vials and subsequently analyzed.

2.4. Donor formulations and receptor fluid analysis

Analyses were performed using the Agilent 1290 series HPLC apparatus consisting of a vacuum degasser, a binary pump, a thermostated autosampler (10 °C), and a thermostated column compartment heated to 30 °C. The column was a Phenomenex Synergi Fusion C-18 endcapped (4 μm) 100 × 2 mm i.d., equipped with a Phenomenex SecureGuard C18 guard column (Phenomenex, Torrance, CA, USA). The mobile phase consisted of 5 mM ammonium acetate in water (A) and 5 mM ammonium acetate in methanol (B). The binary pump gradient was non-linear (increased from 20% B at 0 min to 80% B at 1 min, then increased to 90% B at 5 min, then 90% B for 8 min and 5 min column equilibration to initial conditions (20% B)); the flow rate was 0.25 mL min^{-1}. 10 μL of individual samples was injected for the analyses.

The mass spectrometer used in this study was an AB Sciex Qtrap 5500 (AB Sciex, Concord, ON, Canada) with electrospray ionization (ESI). Ions were detected in the positive mode. The ionization parameters were as follows: capillary voltage, 5.5 kV; desolvation temperature, 400 °C; Curtain gas 15 psi, Gas1 40 psi, Gas2 30 psi. The *m/z* transitions were monitored in scheduled MRM (multiple reaction monitoring) mode; they are included in Table 1 along with additional parameter settings.

2.5. Data evaluation

The cumulative permeated amount of pesticides was calculated from the concentrations of pesticides detected in receptor fluid samples, taking into consideration the dilution factor. Data were analyzed using STATISTICA software. Post-study data analysis integrity evaluation was carried out and extreme and outlying values were excluded. The median, 10th, 25th, 75th and 90th percentiles were calculated and a regression analysis was performed. The trend line was fitted for the linear part of the cumulative permeated amount and a regression equation was generated. The regression coefficient of the regression equation is the value of steady-state flux. By the extrapolation of the regression line to the y = 0, the lag time was calculated. The permeability coefficient was calculated by dividing the steady-state flux values by the concentration of the pesticide in the donor formulation.

2.6. Probabilistic risk assessment

The experimentally derived dermal absorption parameters were used in US EPA equations (USEPA, 2004; USEPA, 2011) to calculate the dermally absorbed dose during two water exposure scenarios. The probabilistic approach using Monte Carlo simulation software Oracle Crystal Ball was used. The distribution function was fitted for the set of values or using the descriptive statistics of the input parameters, where possible. Where no such data were available, a single value of the input parameter was used. The hazard quotients were calculated by simulating the two exposure scenarios for each compound. The number of trials in the simulation was 50,000 (Manová et al., 2015). A detailed descriptions of the parameters used in the simulation and the process of simulation is available in Table S2 (support information).

3. Results and discussion

Figs. 1 and 2 show the results of the percutaneous penetration of Carbendazim and Simazine, respectively. The cumulative

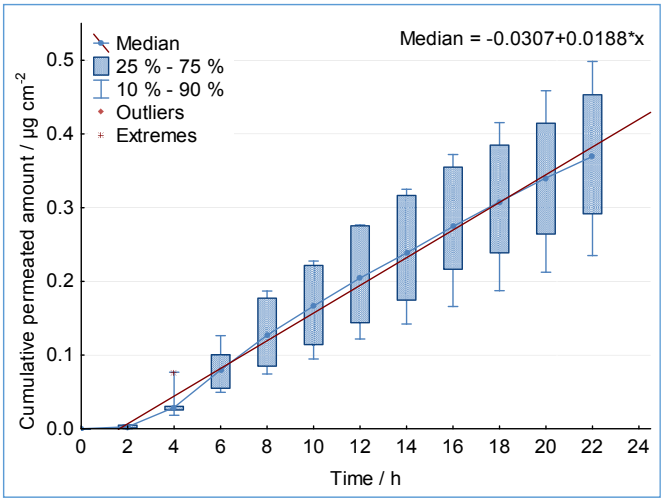


Fig. 1. Percutaneous penetration of Carbendazim expressed as a median with the 25th, 75th (boxes), 10th and 90th (whiskers) percentiles of cumulative permeated amounts of the compound through human skin in 22 h. Median values of the cumulative permeated amount were used to calculate the regression equation. Steady-state flux, permeability coefficient and lag time were calculated from the regression coefficient and the intercept of the regression equation. N = 6.

Table 1
Parameter settings of mass spectrometer used during the analysis. SRM – Selected reaction monitoring, R_t – retention time, DP – declustering potential, EP – entrance potential, CE – collision energy, CXP – collision cell exit potential, MQL – method quantitation limits, *m/z* – mass-to-charge ratio.

Analyte	*m/z*	SRM 1	SRM 2	R_t (min)	DP	EP	CE	CXP	MQL (ng mL^{-1})
Carbendazim	192.0	160.0	131.9	2.9	51	10	25	14	0.05
Simazine	202.0	132.0	124.1	3.2	61	10	27	12	0.10

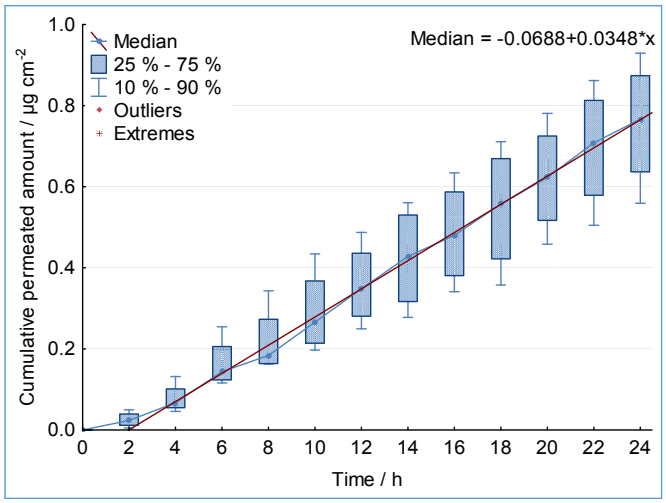


Fig. 2. Percutaneous penetration of Simazine expressed as a median with the 25th, 75th (boxes), 10th and 90th (whiskers) percentiles of cumulative permeated amount of the compound through human skin in 24 h. Median values of the cumulative permeated amount were used to calculate the regression equation. Steady-state flux, permeability coefficient and lag time were calculated from the regression coefficient and the intercept of the regression equation. N = 4.

permeated amounts of Carbendazim and Simazine were calculated from the concentrations of these compounds measured in receptor fluid samples at given time intervals (every 2 h). Detailed information on the individual cumulative permeated amounts of Simazine and Carbendazim for each cell is included in Figs. S1 and S2 of the Supplementary information. Median values with the 10th, 25th, 75th and 90th percentiles of the cumulative permeated amounts were calculated. These values were plotted against time, as shown in Figs. 1 and 2. The plots of both compounds show a typical trend for infinite dose dermal absorption experimental results. It means that the cumulative permeated amount is linear over time after the lag time elapsed, indicating that steady-state flux of the compound through human skin was achieved. The trend line was fitted through the median values in the linear part of the cumulative permeated amount (from 2 h for both compounds) and regression equations were generated. The regression equations can be seen in Fig. 1 for Carbendazim and Fig. 2 for Simazine. The regression coefficient is the value of steady-state flux. By extrapolating the regression line to the value of y-axis = 0, lag time was calculated from the regression equation. The permeability coefficient was calculated by dividing the steady-state flux values by the concentration of pesticide in the donor formulation. During all calculations, where possible, the parameters were calculated probabilistically, using Monte Carlo simulation and distribution functions. The dermal absorption kinetic parameters of Carbendazim and Simazine are listed in the Table 2.

During experiments with Carbendazim and Simazine, steady-flux was achieved for both compounds approximately after 2 h of experiment duration. Steady-state flux is concentration dependent, i.e. its value varies depending on the concentration of the compound in the donor compartment. In addition, the steady-state flux values of the two compounds cannot be compared when the donor concentrations used in the experiment are of different thermodynamic activity (Pugh and Chilcott, 2008). While steady-state flux is concentration dependent, the permeability coefficient is not (Wester and Maibach, 2005). Therefore, it may be applied to calculate the dermally absorbed dose from any donor concentration of the compound. The measured permeability coefficient (K_p) is 0.0034 cm h^{-1} for Carbendazim, and 0.0048 cm h^{-1} for Simazine. The lag time (the time it takes to reach the steady-state flux) of Carbendazim is 1.6 h, the lag time of Simazine is 2 h.

The permeability coefficient is donor formulation and membrane specific (Roberts and Anissimov, 2005). Therefore, the permeability coefficient measured for a compound dissolved in water and using human skin, can only be used to calculate the dermally absorbed dose of the compound in an aqueous solution in humans. However, the presence of other compounds, such as permeation enhancers in the donor formulation, can indicate a higher absorption rate. In this case, a risk assessor can assume that the dermally absorbed dose may be higher, though it is impossible to quantify precisely. The advantage of the use of a permeability coefficient and lag time in risk assessment is that once they are measured, they may be used in exposure models to calculate the dermally absorbed dose in any scenario in case donor formulation and membrane specificity remains the same. On the other hand, a certain degree of uncertainty is associated with the usage of physical diffusion laws (Fick's first law from which the calculation of K_p is derived) for the assessment of the diffusion of chemical compounds through a complex membrane such as skin (Korinth et al., 2007).

The dermal absorption kinetics measured in this study constitute the first available experimental data on the dermal absorption of Carbendazim and Simazine using human skin. They can be used in exposure models to quantify human dermal exposure to pesticides in water and the risks resulting from such exposure. We used these parameters in two exposure scenarios we designed in order to refine exposure quantification towards more realistic results. These scenarios model human exposure to water polluted by Carbendazim or Simazine. To quantify human dermal exposure risks posed by Carbendazim and Simazine in polluted water, the US EPA exposure model was used (USEPA, 2004; USEPA, 2011), though other models may be used as well. In addition, we refined this model using a probabilistic approach and the Monte Carlo Simulation to obtain more realistic data on the exposure and risks posed by Carbendazim and Simazine, including all population groups and using probabilistic distribution functions of parameters. The complete detailed equations of this refined exposure model used to quantify human exposure and risks posed by Carbendazim and Simazine may be found in the Supplementary information (Eqs. S1–S3). The exposure scenarios were defined as follows:

The first scenario models human exposure when swimming in water polluted by pesticides for 1 h, 12 days a year for 10 years. The second scenario models exposure when swimming in water polluted by pesticides for 3 h, 182 days a year for 30 years, which is a worse-case scenario in comparison with scenario number one.

Table 2
New dermal absorption kinetic parameters of Carbendazim and Simazine through human skin. J_{ss} – Steady-State Flux, K_p – Permeability Coefficient, LagT – Lag Time.

	Carbendazim			Simazine		
	J_{ss} (µg cm^{-2} h^{-1})	K_p (cm h^{-1})	LagT (h)	J_{ss} (µg cm^{-2} h^{-1})	K_p (cm h^{-1})	LagT (h)
Mean	0.0188	0.0034	1.6	0.0348	0.0048	2.0
Standard deviation	0.0004		0.3	0.0005		0.2
Coefficient of variation		0.06	0.2		0.11	0.1

Table 3
Exposure scenarios, for which the dermally absorbed dose and hazard quotient were calculated using probabilistic modeling. T_{event} – Event Duration, EV – Event Frequency, ED – Exposure Duration, EF – Exposure Frequency.

Scenario no.	Concentration of pesticide in water	T_{event} (h)	EV (event/day)	ED (years)	EF (days/year)
1	Distribution of concentrations found in literature and IS Arrow	1	1	10	12
2	Distribution of concentrations found in literature and IS Arrow	3	1	30	182
3	Water solubility of Carbendazim	1	1	10	12
4	Water solubility of Carbendazim	3	1	30	182

Human body surface area and body weight are crucial parameters with respect to calculating the dermally absorbed dose. The mean and standard deviation values of the ratio of body surface and body weight of all ages and sexes (USEPA, 2011) were used to model the distribution of these parameters in the population and thus to include all population groups in the risk assessment (50,000 trials according to Manová et al. (2015)).

To model human dermal exposure to Carbendazim and Simazine, the concentrations of these compounds measured in surface fresh waters in the years 2000–2015, found and reviewed in the Czech IS Arrow system of the Czech Hydrometeorological Institute and in available literature, were used. The list of these concentrations with references to their sources may be found in Tables S1 (for Simazine) and S2 (for Carbendazim) of the Supplementary information. Where no absolute concentrations were available, only their descriptive statistics, mean, median, maximum and minimum concentration values were used. Based on these concentrations, the concentration distributions of Carbendazim and Simazine were modeled using the Monte Carlo Simulation and subsequently used as an input for the calculation of the dermally absorbed dose during defined exposure scenarios. These distributions can be seen in the Figs. S3 and S4 of the Supplementary information.

An overview of exposure scenarios is presented in Table 3.

Following the calculation of the dermally absorbed dose, the hazard quotients for both scenarios and for both compounds were calculated using Eq. (1).

$$HQ = \frac{DAD}{RfD} \quad (1)$$

where HQ is the hazard quotient, DAD is the dermally absorbed dose and RfD is the reference dose. As a reference dose, acceptable daily intake values were used (EC, 1995–2015). The HQ was expressed as a probabilistic distribution function. If the dermally absorbed dose is higher than the reference dose, the hazard quotient is higher than one and the risks resulting from this exposure are expected. If the hazard quotient is less than one, no adverse effects due to such exposure are expected. However, the hazard quotient is calculated only for one exposure route and one scenario, other exposure routes, such as inhalation and ingestion, have not been considered. The advantage of using the hazard index (hazard quotient) to quantify the risks is its transparency, understandability and the fact that it relates directly to a frequently used and well-understood acceptable risk approach.

Physico-chemical properties and Acceptable Daily Intake (ADI) values of Carbendazim and Simazine are listed in Table 4.

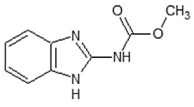
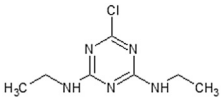
The probability plots of hazard quotients for Carbendazim and Simazine calculated for both scenarios are included in Fig. 3. The results of the sensitivity analysis for the aforementioned exposure scenarios are provided in Figs. S5–S20 of the Supplementary information. The overview of other input parameters used in the risk assessment is in the Table S3 of the Supplementary information.

For each defined scenario and compound, the hazard quotients were below zero. Hence, none of the aforementioned dermal exposure scenarios present any risk according to our results. To see if these compounds could be dangerous during dermal exposure defined by these two scenarios at higher concentrations, water solubility concentrations of Carbendazim and Simazine were used to calculate the dermally absorbed dose and the hazard quotient, though these concentrations are barely environmentally relevant. Nevertheless, even with these concentrations, the hazard quotients were lower than one. However, we must emphasize that this study considers only two scenarios and only one exposure route.

In a deterministic risk assessment approach, variability in population and uncertainty are treated by using conservative estimates of parameters, which might lead to less precise results (Bosgra et al., 2009). To a certain extent, this problem may be dealt with by probabilistic risk assessment, as it was done in this study, where the model takes into consideration variability in body surface and weight across the population. However, it does not include variability in the varying susceptibility of population subsets to the toxicity of individual chemicals (a single ADI value is used).

Bias in the risk assessment results may be caused by the concentrations used, as only the concentrations above LODs were included in the model, i.e. not all concentrations found during the monitoring phase mentioned in literature. On one hand, risk

Table 4
Physico-chemical properties and Acceptable Daily Intake (ADI) values of Carbendazim and Simazine.

	Carbendazim	Simazine
Chemical class	Carbamate	Triazine
Group	Fungicide	Herbicide
CAS number	10605-21-7	122-34-9
Molecular formula	C9–H9–N3–O2	C7–H12–Cl–N5
Structural formula		
Molecular weight	191.1891	201.66
Log K_{ow}	1.52	2.18
Water solubility (mg l^{-1})	29	6.2
ADI (mg kg^{-1} day^{-1})	0.02	0.005

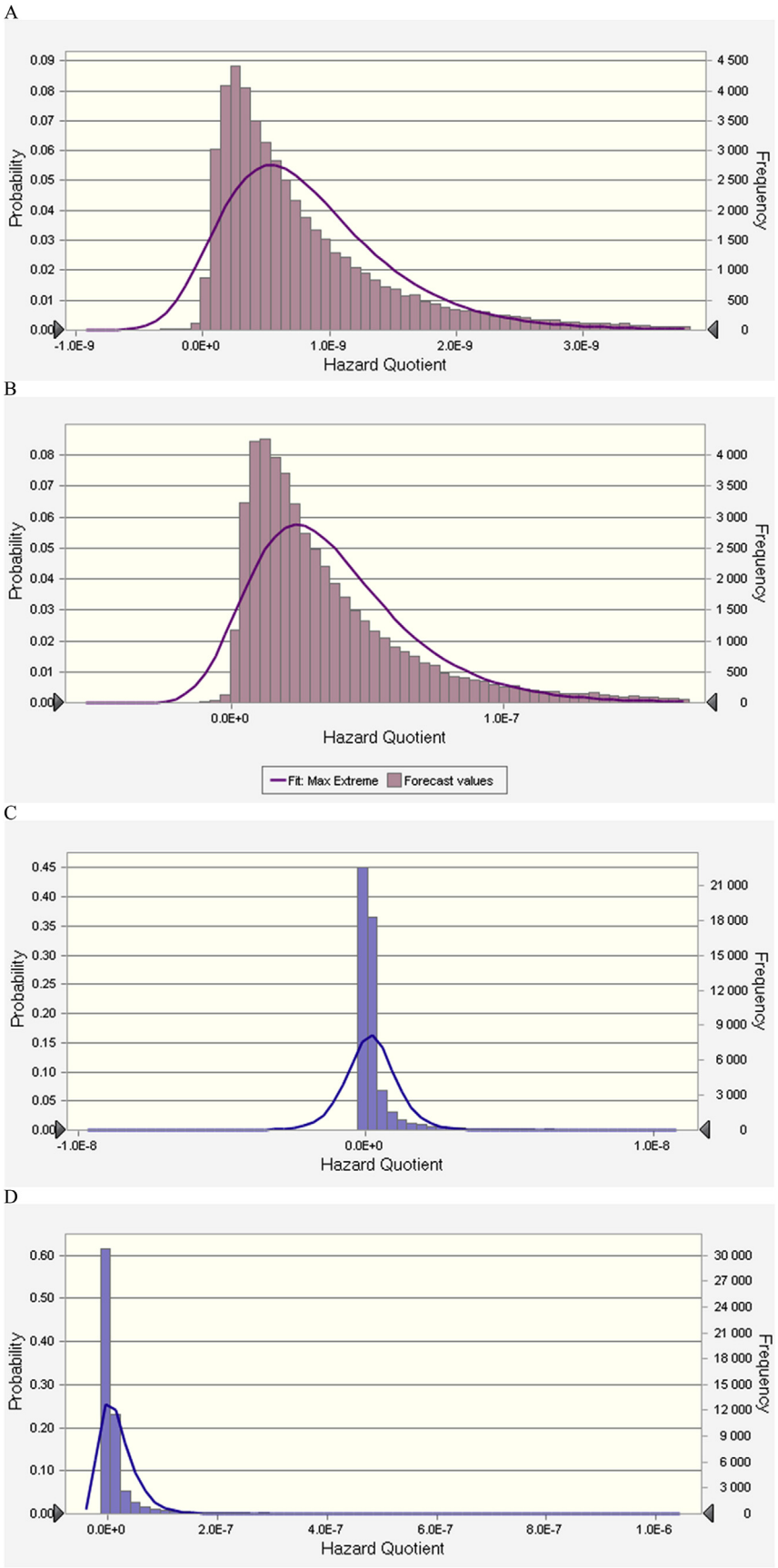


Fig. 3. Probability plots of forcast values of hazard quotients calculated for both scenarios (presented in Table 3) and both compounds. Fit: Max Extreme. A – Simazine, scenario 1 (48,950 out of 50,000 trials displayed, Fit: Max Extreme); B – Simazine, scenario 2 (48,912 out of 50,000 trials displayed, Fit: Max Extreme); C – Carbendazim, scenario 1 (49,642 out of 50,000 trials displayed, Fit: Logistic); D – Carbendazim, scenario 2 (49,849 out of 50,000 trials displayed, fit: Lognormal).

assessment focuses on that part of a population which is exposed to polluted waters (where increased concentrations were found). On the other hand, a concentration below the limits of detection (LODs) does not necessarily constitute zero and thus no effect. There is evidence of pesticides with endocrine disrupting activity being active *in vivo* at extremely low doses (Mankame et al., 2004; Weltje et al., 2005; McKinlay et al., 2008; Muthuviveganandavel et al., 2008). However, analytical methods used in the monitoring are not necessarily able to detect concentrations which correspond to these low but active doses.

The results of the probabilistic risk assessment of our two scenarios are influenced by the variability of input parameters as well as by uncertainties resulting from different knowledge of each parameter, the extent of a particular data set or information on the distribution of each parameter. For instance, the concentration of a pesticide in water will have a greater impact on the hazard quotient calculation, as the variability of the concentration will be wide-ranging in comparison with e.g. the acceptable daily intake value where only a single value is used.

Acceptable daily intake values are the amounts of a substance that can be taken every day for an entire lifetime without any harm. They are derived from the compound's NOAEL (No Observed Adverse Effect Level) or NOEL (No Observed Effect Level), established on the basis of animal studies and taking into account uncertainty factors (usually 100 for animals–humans extrapolation). In spite of using the uncertainty factor, it should not be omitted that these values are derived from animal studies and there may be a certain uncertainty in the estimation of relevant human exposure patterns (Damalas and Eleftherohorinos, 2011). There is a concern that the acceptable intake values for these compounds have not been set in view of more susceptible population subsets, such as fetuses, infants and children (Birnbaum and Fenton, 2003; Goldman et al., 2004; Sharpe, 2006; McKinlay et al., 2008).

Furthermore, the use of the permeability coefficient measured on the skin of adult individuals can be a source of uncertainty in population risk assessment, as children's skin differs from adult's in physiology (Fluhr et al., 2000). Also, skin of different body sites differs in its thickness and other parameters that can influence the permeation rate and there might be uncertainty caused by only using the abdominal skin of people aged 24–46 years (Sandby-Moller et al., 2003; Dick et al., 1997a; Farahmand and Maibach, 2009). However, the use of three different skin samples to measure the absorption kinetics of each compound helps include the inter- and intra-individual skin permeability variability to a certain extent. Other factors influencing skin condition and barrier function (stress, skin diseases, age) are not taken into consideration. Using the entire body surface area may also lead to a slight overestimation of exposure, as during swimming, the head is not fully or always submerged.

There are some differences between *in vitro* and *in vivo* conditions that might have influence on the flux, e.g. air temperature and humidity, skin reservoir formation (Wilkinson and Williams, 2005), the presence of the dermis in the *in vitro* testing (Wilkinson and Williams, 2005; Reifenrath et al., 1991; OECD, 2004), the perfusate flow rate and compound solubility (Chang and Riviere, 1991; Dick et al., 1997a; Dick et al., 1997b; Roberts et al., 2004; Mai and Howard, 2012; Reifenrath et al., 1991) and predicted RfD levels (USEPA, 2004).

In this study, only a single compound was considered when calculating the dermally absorbed dose, i.e. either Carbendazim or Simazine. However, in the environment, none of the compounds is present by itself, there is always a mixture of compounds which interact with each other even in terms of human exposure and chemical uptake. The presence of other compounds might lead to a different dermal absorption rate of each compound. Moreover, the compounds present in the mixture may also interact with each other, resulting in a different effect than that which could be expected from each individual compound. Although detailed information on mixture composition, mechanism of action, toxicity and exposure data is not available, it is crucial in order to assess the impact of the mixture on human health (Reffstrup et al., 2010). The introduction of the physiologically based toxicokinetic (PBTK) modeling as a tool for the risk assessment of chemical mixtures is recommended (Reffstrup et al., 2010), because not only the absorption rate, but also the rate of excretion determines the steady-state concentration of a compound in body (Wester and Maibach, 1983; Mai and Howard, 2012). We think that this dermal exposure model, refined using a probabilistic approach and experimental data on absorption kinetics may be of use.

In the EU, a standardized method designed to assess the exposure of operators, workers, residents and bystanders is missing. EFSA Guidance document on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products (EFSA, 2014) partially covers this gap by providing guidance on this assessment. However, while the EFSA Guideline (EFSA, 2014) admits that there are many data gaps regarding non-dietary exposure pathways, it does not consider some exposure pathways (**such as dermal contact with a contaminated water**) and it recommends the use of default values (single values or percentiles) to calculate exposure, though it is known, that a probabilistic approach to risk assessment would yield more reliable data (EFSA, 2008). Further actions are needed to increase the representativeness of the assessments proposed and that the Guidance document should hereafter be reviewed periodically. There is a lack of data needed to establish the impact of single exposure routes on overall exposure assessment.

4. Conclusions

In this study, the dermal absorption kinetics of Carbendazim and Simazine from water were measured using human skin. Simazine and Carbendazim were absorbed by the human skin, although two refined scenarios we defined in this paper did not show any potential risks connected with dermal exposure to these compounds. However, the risks posed by these pesticides during other exposure scenarios are not out of question. The dermal absorption kinetics of Carbendazim and Simazine presented in this paper are the first to be published. They can be used in exposure models to quantify human dermal exposure to Carbendazim and Simazine in water and to assess the risks resulting from such exposure. While they cannot be used for other donor formulations, or other compounds, as they are compound, membrane and donor formulation specific, the methods and approach used in this study to experimentally measure them and to quantify exposure and risks may be used for other compounds, including pesticides. This methodology could be included among approaches used to evaluate pesticide exposure and risk, especially in the case of non-occupational population groups, and to contribute to the overall body of knowledge on human exposure to pesticides, not only in the sense of EFSA guidelines.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2015.11.018.

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Current implications of past DDT indoor spraying in Oman

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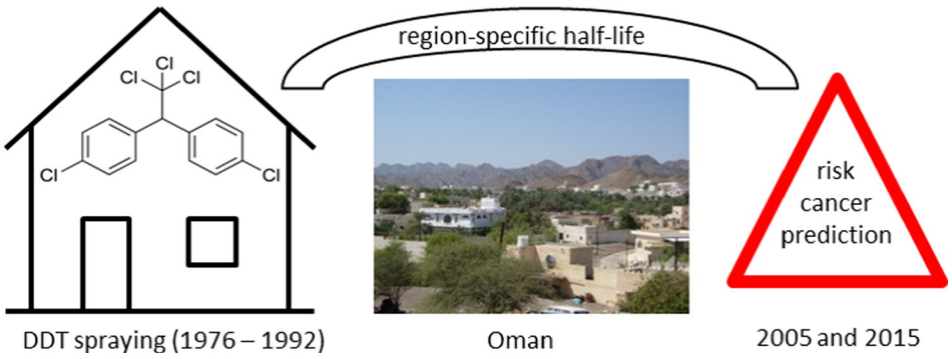
HIGHLIGHTS

- Indoor air and dust are important exposure routes for DDT.
- Significant DDT, DDE and DDD levels were found years after indoor spraying.
- A novel risk assessment model identified site-specific potential cancer risks in Oman.
- Region-specific half-life is important for proper cancer risk assessment.
- More data linking indoor exposure to relevant health outcomes are needed.

GRAPHICAL ABSTRACT



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ABSTRACT

In Oman, DDT was sprayed indoors during an intensive malaria eradication program between 1976 and 1992. DDT can remain for years after spraying and is associated with potential health risk. This raises the concern for human exposure in areas where DDT was used for indoor spraying. Twelve houses in three regions with a different history of DDT indoor spraying were chosen for a sampling campaign in 2005 to determine *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT), *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD) levels in indoor air, dust, and outdoor soil. Although DDT was only sprayed indoor, *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD were also found in outdoor soil. The results indicate that release and exposure continue for years after cessation of spraying. The predicted cancer risk based on concentrations determined in 2005, indicate that there was still a significant cancer risk up to 13 to 16 years after indoor DDT spraying. A novel approach, based on region-specific half-lives, was used to predict concentrations in 2015 and showed that more than 21 years after spraying, cancer risk for exposure to indoor air, dust, and outdoor soil are acceptable in Oman for adults and young children. The model can be used for other locations and countries to predict prospective exposure of contaminants based on indoor experimental measurements and knowledge about the spraying time-schedule to extrapolate region-specific half-lives and predict effects on the human population years after spraying.

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1. Introduction

The Sultanate of Oman, situated in the southeastern part of the Arabian Peninsula, is characterized by a generally hot and dry climate with its southern part affected by the summer monsoon. The spectrum of infectious and tropical diseases in Oman is wide, however, incidence and prevalence rates are usually low (Scrimgeour et al., 1999). A National Malaria Eradication Program was established from 1976 to 1992 in Oman and all malaria areas in the country were exposed to an intensive eradication treatment. As a result, the number of malaria cases decreased substantially from 14,827 in 1992 to 882 in 1998 (Scrimgeour et al., 1999). However, even malaria-free regions still have the potential for malaria introduction, as was demonstrated in 1998 by a malaria outbreak (Baomar and Mohamed, 2000).

The Global Malaria Eradication Campaign, started in 1955 by the World Health Organization, was based on the widespread use of antimalarial drugs and dichlorodiphenyltrichloroethane (DDT) wettable powder (WP) for indoor and outdoor spraying against mosquitoes (Trigg and Kondrachine, 1998). In Oman, 137,034 kg of DDT (75% WP) were sprayed indoors in selected villages in 28 districts between 1976 and 1992 according to WHO rules. Shinas, Dank and Rustaq were the districts were DDT was sprayed most and over a longer period. DDT was not used for outdoor mosquito control in Oman. In 1992 the use of DDT was stopped (except for some isolated uses for the control of sand flies from 1994 to 1998) until it was completely banned in Oman in 2001. After 1992, DDT has been replaced by organophosphates and synthetic pyrethroids in Oman (Al Zadjali et al., 2014; UNEP, 2008).

Human exposure to DDT and its breakdown product dichlorodiphenyldichloroethylene (DDE) may be associated with breast cancer, diabetes, decreased semen quality, spontaneous abortion, preterm birth, early weaning and impaired neurodevelopment in children (Rogan and Chen, 2005; Eskenazi et al., 2009). Toxic effects on humans together with long-lasting effects on wildlife (Dauwe et al., 2009) made DDT subject of various regulations. Inhabitants in areas where DDT was sprayed indoor can show high DDT levels in blood and breast milk and therefore it is of high importance not only to monitor DDT levels, but also to focus on determining actual health effects from DDT (Gyalpo et al., 2012).

The Stockholm Convention on Persistent Organic Pollutants allows the use of DDT for vector control only, provided that no safe, effective and affordable alternatives are locally available. The Sultanate of Oman joined the Stockholm Convention in 2004 and therefore, a national inventory on the amounts and emissions of persistent organic pollutants (POPs) including DDT had to be elaborated for Oman (UNEP, 2008).

Based on an agreement between the Omani Ministry of Regional Municipalities, Environment and Water Resources and TOCOEN, Ltd. (a company in Czech Republic in close cooperation with RECETOX, MU, Brno), a project with the aim to study the current DDT burden in areas historically exposed to DDT indoor spraying was conducted with the aim to provide supporting information for the elaboration of the National Implementation Plan (NIP) under the Stockholm Convention for Oman.

Inhalation exposure is a relevant exposure pathway for people living in homes treated by indoor residual spraying (IRS) of DDT (Ritter et al., 2011). Dust sampling identifies specific active ingredients to which a person may have been exposed (Colt et al., 2004). DDT is reasonably anticipated to be a human carcinogen based on sufficient evidence of carcinogenicity from studies in experimental animals (United States Department of Health and Human Services, 2014). Evidence was provided to suggest that DDT and DDE may pose a risk to human health, however little is known about human exposure and health effects in communities where DDT was sprayed for malaria control (Eskenazi et al., 2009). Concentrations of DDT, DDD and DDE in human serum, placenta, maternal and cord blood indicate that humans are exposed in spraying areas and even suggest an adverse effect on newborns (Al-Saleh et al., 2012).

In this study the concentrations of DDT and degradation products DDE and DDD were determined in selected indoor air, indoor dust and outdoor soil samples at the three most sprayed districts in Oman in order to correlate indoor and outdoor exposure and to investigate the potential human health effects, several years after IRS. It was carried out as a contribution to the Global Monitoring Plan of the Stockholm Convention on POPs. According to the authors knowledge, the here presented study results constitute the only information on the indoor environmental POP burden in Oman currently available.

2. Methodology

2.1. Sampling

The sampling campaign was conducted in October 2005, in Shinas, Dank, and Rustaq district in northern Oman (Fig. 1). In each area three houses subject to historical DDT indoor spraying and one house built after the last spraying, to obtain a reference sample, were chosen for the sampling campaign. The buildings subject to sampling did not undergo major reconstruction after the last DDT indoor application. Indoor air samples were completed with at least one indoor dust sample in each house and an outdoor soil sample wherever possible. Altogether, 12 indoor air, 17 indoor dust and 10 outdoor soil samples were taken (Table 1). Sample codes, coordinates of the sampling locations, years in which DDT was sprayed, the total number of sprayings and a description of where the samples were taken in house are shown in Table 1. Compared to the use of DDT for malaria control between 1976 and 1992, relatively small amounts of DDT were used between 1994 and 1998 to control sandflies (Table 2).

Air samples were taken by the MWS-6 (Leckel, Germany) low-volume air sampler equipped with polyurethane foam and quartz filters, the sampling time was 20 h. Household dust was sampled usually under carpets and sometimes from sprayed ceilings. Soil samples were pooled out of 10 point samples taken in ca 10 m distance (Čupr et al., 2010) from each house (10 cm depth). The samples were analyzed on the content of *p,p′*-dichlorodiphenyltrichloroethane (*p,p′*-DDT) and its metabolites *p,p′*-dichlorodiphenyldichloroethylene (*p,p′*-DDE) and *p,p′*-dichlorodiphenyldichloroethane (*p,p′*-DDD).

2.2. Extraction and chemical analysis

p,p′-DDT, *p,p′*-DDE and *p,p′*-DDD in indoor air filters (equivalent to 23.87–55.73 m^3 air), indoor dust (equivalent to 1.28–5 g) and outdoor soil (5 g) samples were extracted using automated Soxhlet extraction for 1 h with dichloromethane (DCM) in a B-811 extraction unit (Büchi, Switzerland). The extract was cleaned on a H_2SO_4 modified (44% *w/w*) silica column, and analytes were eluted with 40 mL DCM/*n*-hexane mixture (1:1, *v/v*). The volume was reduced under a gentle nitrogen stream at ambient temperature and transferred into a vial, finally PCB 121 was added as an internal standard. For details on chemical analysis and quality control see Holoubek et al. (2007). In short, chemical analysis of *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD and PCBs was performed using a HP 5890 gas chromatograph (GC) coupled to an electron capture detector (GC-ECD, Hewlett Packard), with a Quadrex fused silica column (5% Ph, length 40 m, internal diameter 180 μm, film thickness of 0.25 μm). Helium was used as a carrier gas at a flow rate of 1.5 mL min^{-1}. Sample volumes of 1 μL were injected in splitless mode at 280 °C. The oven program was 80 °C (1 min), 20 °C min^{-1} to 160 °C, 3 °C min^{-1} to 300 °C (10 min). ECD was kept at 310 °C with N_2 as auxiliary gas. ΣDDT was considered as the total concentration of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD. Quality assurance and quality control were ensured by spiking surrogate standards (recovery and internal standards). One laboratory blank and one reference material were analyzed with each set of ten samples. Surrogate recovery standards (PCB

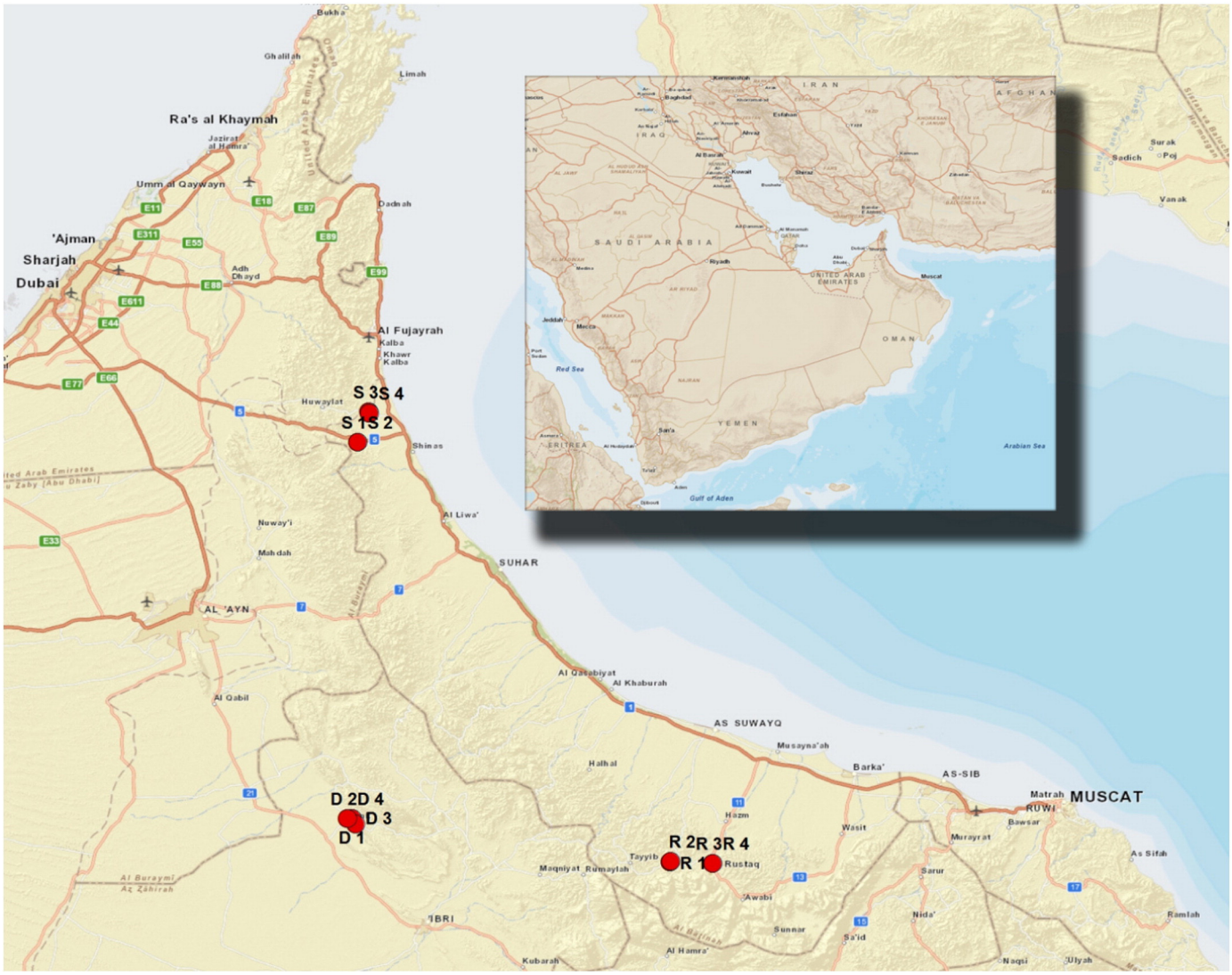


Fig. 1. Location of sampling sites. S, D and R are Shinas, Dank and Rustaq, respectively. Numbers refer to the samples taken at the specific location (Table 1).

Table 1
Sampling locations and description of samples.

Code	Area	Coordinates			Years in which DDT was sprayed indoor	Description of samples
		x	y	Z		
S1	Shinas—Agiib	24.77474	56.28862	147	1976, 1977, 1978, 1979, 1980, 1981, 1984 (in total 7 sprayings)	Dust under carpets (same room with air samplers), soil around the house
S2	Shinas—Agiib	24.77415	56.28877	151	1976, 1977, 1978, 1979, 1980, 1981, 1984 (in total 7 sprayings)	(a) Dust under carpets (same room with air samplers) (b) Dust from hoover
S3	Shinas—Aswad	24.87142	56.32361	75	No indoor spraying	Dust under carpets (same room with air samplers), soil around the house
S4	Shinas—Aswad	24.86767	56.32579	88	1976, 1977, 1978, 1979, 1980, 1981, 1984 (in total 7 sprayings)	Dust under carpets (same room with air samplers), soil around the house
D1	Dank—Al Moatha	23.56427	56.26989	350	1978, 1979, 1980, 1981, 1982, 1988, 1989 (in total 7 sprayings)	Dust under carpets (same room with air samplers), soil around the house
D2	Dank—Al Moatha	23.56315	56.26948	347	1978, 1979, 1980, 1981, 1982, 1988, 1989 (in total 7 sprayings)	(a) Dust from the surface of carpet (same room with air samplers), soil around the house (b) Dust under carpets (same room with air samplers)
D3	Dank—Al Alaya	23.54593	56.28194	369	1978, 1979, 1980, 1981, 1982, 1988, 1989 (in total 7 sprayings)	Dust under carpets (same room with air samplers), soil around the house
D4	Dank—Dank	23.56311	56.25439	349	no indoor spraying	Dust under carpets (same room with air samplers), soil around the house
R1	Rustaq—Khafdi	23.42504	57.29386	459	1984, 1985, 1986, 1990, 1992 (in total 5 sprayings)	(a) Dust under carpets (same room with air samplers), soil around the house (b) Dust under carpets and contaminated wooden roof
R2	Rustaq—Khafdi	23.42522	57.29718	447	1984, 1985, 1986, 1990, 1992 (in total 5 sprayings)	(a) Dust under carpets (same room with air samplers) (b) Dust under carpets, wooden roof, and walls (same room with air samplers) (c) Dust under carpets (same room with air samplers)
R3	Rustaq—Ghaushb	23.41916	57.43156	318	1984, 1985, 1986, 1990, 1992 (in total 5 sprayings)	Dust under carpets (same room with air samplers), soil around the house
R4	Rustaq—Ghaushb	23.41908	57.4316	338	No indoor spraying	Dust under carpets (same room with air samplers), soil around the house

Table 2
Amount of DDT used for indoor spraying.

Year	Location	Number of houses	Total kg DDT (75% WP)	Average kg DDT (75% WP)/house
1976	Shinas	748	353	0.4713
1977	Shinas	1496	701	0.4686
1978	Shinas, Dank	5461	3227	0.5909
1979	Shinas, Dank	9965	4839	0.4856
1980	Shinas, Dank, +1 location	11,957	5054	0.4227
1981	Shinas, Dank, +2 locations	8758	3575	0.4082
1982	Dank, +6 locations	20,304	9847	0.4850
1983	5 locations	29,216	20,088	0.6876
1984	Shinas, Rustaq, +13 locations	43,770	32,755	0.7483
1985	Rustaq, +7 locations	30,890	20,626	0.6677
1986	Rustaq, +5 locations	10,053	8612	0.8567
1987	3 locations	8043	4725	0.5875
1988	Dank, +3 locations	8788	5238	0.5960
1989	Dank, +4 locations	12,371	7093	0.5734
1990	Rustaq, +2 locations	5799	3620	0.6242
1991	3 locations	7851	5337	0.6798
1992	Rustaq, +3 location	5595	1344	0.2402
1993	Shinas, Rustaq, +6 locations	6596	Other insecticide	No DDT
1994	Dank, +8 locations	13,595	348 (sf)	0.0256
1995	Shinas, Rustaq, +21 locations	37,176	19 (sf)	0.0005
1996	Dank, +17 locations	6811	14 (sf)	0.0021
1997	Shinas, Dank, Rustaq, +22 locations	12,451	Other insecticide	No DDT
1998	Shinas, Dank, Rustaq, +29 locations	16,031	8 (sf)	0.0005
1999	Shinas, Dank, Rustaq, +14 locations	6542	Other insecticide	No DDT
2000	Dank, +8 locations	1244	Other insecticide	No DDT

DDT 75% WP = DDT 75% wettable powder.
(sf) DDT used in small amounts to control sand flies.
Other insecticide (organophosphates and synthetic pyrethroids) as a replacement of DDT was used.

30 and PCB 185) were spiked on each filter prior to Soxhlet extraction. The blank samples didn't contain significant levels of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD and recoveries of PCB 30 and 185 were acceptable (between 75% and 102%).

2.3. Degradation of DDT in indoor air, indoor dust and outdoor soil

The half-life ($t_{1/2}$) of DDT (the time it takes for half of the substance to degrade) was calculated by using Eq. (1) in which C_t represents the concentration at time t (the years after the last spraying) and C_0 is the concentration at time t_0.

$$t_{1/2} = t^{*}\ln(2)/\ln\frac{C_0}{C_t} \quad (1)$$

The half-life was used to calculate the *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD concentrations in air, dust and soil and to predict the human health risk in 2015. DDT was sprayed for several years at different locations, however the average amount of DDT/house is in the same order of magnitude (Tables 1 and 2). We assumed that *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD levels increased during the years of spraying and concentrations in air, dust and soil were highest after the last spraying at the specific location. The last spraying of DDT in Shinas was in 1984, Dank in 1989, and Rustaq in 1992. The years between the last spraying and sampling in 2005 were 21, 16, and 13 in Shinas, Dank and Rustaq, respectively. These years after the last spraying were plotted versus the concentration of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD, determined in houses in 2005 where DDT was sprayed, to calculated half life time in air, dust, and soil (Eq. (1)).

2.4. Human health risk assessment

The risk for a human developing cancer after inhalation exposure of air and dermal exposure to indoor dust and outdoor soil on the investigated locations was evaluated. To estimate the risk to develop cancer after inhalation of DDT, the approach of Čupr et al. (2013), based on the EPA baseline risk assessment was used. The chronic daily intake (CDI) was calculated using Eq. (2), where C_{air} is the concentration of the compound determined in air (mg m^{-3}) in 2005 and IF is the intake factor (m^{-3} kg^{-1} day^{-1}).

$$CDI_{air} = C_{air} * IF \quad (2)$$

The IF was calculated with Eq. (3), where IR-A is the inhalation breathing rate (m^3 day^{-1}), EF is the exposure frequency or number of exposes per year, ED is the exposure duration or duration of the exposures in years over a life time, ET is the exposure time or number of hours per exposure, BW is the body weight and AT is an average exposure time over a lifetime.

$$IF = \frac{IR\text{-}A * EF * ED * ET}{BW * AT} \quad (3)$$

Exposure parameters were obtained from the Exposure Factors Handbook (US EPA, 2011); IR-A = 20 m^3 day^{-1}, EF = 365 days, ED = 70 years, ET = 8 h day^{-1}, BW = 70 kg, and AT = 25,550 days. The chemical specific cancer risks were calculated by multiplying the CDI, for carcinogenic substances also called "Life Average Daily Dose" (LADD), by the slope factor (SF) [(mg kg^{-1} $day^{-1})^{-1}$] (Eq. (4)).

$$\text{Cancer risk} = 1\text{-}\exp^{(-CDI*SF)} \quad (4)$$

The SF values used were 0.34 (mg kg^{-1} $day^{-1})^{-1}$ for DDT and DDE and 0.24 (mg kg^{-1} $day^{-1})^{-1}$ for DDD (US EPA, 2015). The cancer slope factor is an upper bound estimate of the increase in cancer risk per unit of dose. For inhalation, carcinogens are also expressed in terms of increased cancer risk unit (IUR — inhalation unit risk; US EPA, 2015) for estimating risks from exposure to substances found in air, which were 9.70E−5, 6.90E−05 and 9.70E−05 ($\mu g m^{-3})^{-1}$ for DDT, DDD and DDE, respectively (US EPA, 2015).

To calculate the cancer risk level in air, two different scenarios were used. Scenario 1 (Eq. (4)), describes a worst case exposure scenario, assuming 24 h exposure for 365 days a year and a life expectancy of 70 years for an adult. Scenario 2, describes a more realistic exposure, assuming 8 h per day exposure for 350 days per year after spraying of DDT.

For dermal exposure from soil and dust the cancer risk assessment needs to be corrected for the fraction that is absorbed (Bányiová et al., 2015), the absorption factor (ABS). To calculate the chronic daily intake for soil and dust, Eq. (5) was used.

$$CDI_{dust/soil} = \frac{ABS * C_{dust/soil} * AF * CF * SA * EF * ED}{BW * AT} \quad (5)$$

The adherence factor (AF) describes the amount of solid material that adheres to the skin per unit of surface area. The skin surface area (SA) describes the area that is available for contact with the contaminants. The average time (AT) in days specifies the length of time over which the average dose is calculated. Exposure parameters were obtained from (US EPA, 2011); ABS = 0.1 for DDD and DDE, 0.03 for DDT, AF = 0.2 mg cm^{-2}, CF = 1E−06 kg mg^{-1}, SA = 5000 cm^{-2} day^{-1}, EF = 350 days $year^{-1}$, BW = 70 kg, AT = 25,550 days. For exposure duration (ED) in Eq. (5), the years after spraying at each location was used: 21 years for Shinas, the average of 16 years for Dank, and 13 years for Rustaq.

The potential cancer risk through ingestion of soil and house dust for children of age 1 to 6 years was calculated with Eq. (6) (US EPA, 2014)

Table 3
Levels of DDTs in and around households with and without historical indoor spraying.

Code	DDT spraying	*p,p′*-DDE	*p,p′*-DDD	*p,p′*-DDT	ΣDDTs	*p,p′*-DDT/ *p,p′*-DDE
Shinas district						
Indoor air — gaseous phase [ng m^{-3}]						
S1	Yes	1.68	1.09	5.28	8.05	3.1
S2	Yes	2.99	1.20	3.35	7.53	1.1
S3	No	0.70	0.45	3.01	4.16	4.3
S4	Yes	0.48	0.11	1.15	1.740	2.4
Indoor air — particulate phase [ng m^{-3}]						
S1	Yes	0.06	0.02	0.18	0.25	3.0
S2	Yes	1.04	0.29	0.58	1.91	0.6
S3	No	0.01	0.01	0.07	0.10	7.0
S4	Yes	0.02	0.02	0.06	0.10	3.0
Indoor air — total concentration [ng m^{-3}]						
S1	Yes	1.74	1.11	5.46	8.30	3.1
S2	Yes	4.03	1.49	3.92	9.44	1.0
S3	No	0.71	0.46	3.08	4.25	4.3
S4	Yes	0.50	0.13	1.22	1.84	2.4
Indoor dust [μg g^{-1}]						
S1	Yes	0.01	0.01	0.04	0.06	3.1
S2a	Yes	0.02	0.04	0.17	0.23	7.3
S2b		0.01	0.003	0.01	0.02	1.8
S3	No	0.08	0.07	0.48	0.63	6.1
S4	Yes	0.05	0.01	0.17	0.23	3.3
Outdoor soil [ng g^{-1}]						
S1	Yes	11.69	4.15	11.16	27.01	1.0
S3	No	5.67	2.95	8.63	17.24	1.5
S4	Yes	22.27	2.50	4.80	29.57	0.2
Dank district						
Indoor air — gaseous phase [ng m^{-3}]						
D1	Yes	13.27	1.96	7.00	22.23	0.5
D2	Yes	9.12	1.66	4.23	15.02	0.5
D3	Yes	94.95	11.49	80.19	186.63	0.8
D4	No	2.23	0.98	2.87	6.08	1.3
Indoor air — particulate phase [ng m^{-3}]						
D1	Yes	0.07	0.01	0.13	0.22	1.9
D2	Yes	0.21	0.05	0.11	0.36	0.5
D3	Yes	2.17	0.91	7.39	10.48	3.4
D4	No	0.07	0.03	0.11	0.21	1.6
Indoor air — total concentration [ng m^{-3}]						
D1	Yes	13.34	1.97	7.13	22.44	0.5
D2	Yes	9.33	1.71	4.34	15.38	0.5
D3	Yes	97.13	12.39	87.58	197.10	0.9
D4	No	2.29	1.01	2.98	6.28	1.3
Indoor dust [μg g^{-1}]						
D1	Yes	3.73	0.96	50.24	54.93	13.5
D2a	Yes	0.27	0.04	0.89	1.20	3.3
D2b		0.54	0.03	0.61	1.18	1.1
D3	Yes	2.87	0.63	10.63	14.13	3.7
D4	No	0.05	0.01	0.09	0.14	1.8
Outdoor soil [ng g^{-1}]						
D1	Yes	26.73	11.78	40.82	79.34	1.5
D2	Yes	36.11	12.18	78.57	126.86	2.2
D3	Yes	4.22	4.82	12.52	21.56	3.0
D4	No	58.46	12.94	103.10	174.50	1.8
Rustaq district						
Indoor air — gaseous phase [ng m^{-3}]						
R1	Yes	358.69	16.11	67.22	442.02	0.2
R2	Yes	88.75	104.96	253.16	446.87	2.9
R3	Yes	139.21	18.80	149.93	307.94	1.1
R4	No	7.19	1.95	7.75	16.89	1.1
Rustaq district						
Indoor air — particulate phase [ng m^{-3}]						
R1	Yes	275.83	20.01	596.27	892.11	2.2
R2	Yes	29.40	9.19	71.68	110.27	2.4
R3	Yes	0.73	0.45	3.39	4.56	4.6
R4	No	0.12	0.06	0.59	0.77	4.9
Indoor air — total concentration [ng m^{-3}]						
R1	Yes	634.5	36.12	663.49	1334.13	1.0
R2	Yes	118.15	114.15	324.84	557.14	2.7
R3	Yes	139.93	19.25	153.32	312.51	1.1
R4	No	7.31	2.02	8.33	17.66	1.1
Indoor dust [μg g^{-1}]						
R1a	Yes	147.29	16.70	619.26	783.25	4.2
R1b		34.04	8.74	263.09	305.87	7.7
R2a	Yes	12.74	17.74	263.56	294.03	20.7
R2b		66.05	47.69	1704.40	1818.14	25.8
R2c		29.16	13.48	480.09	522.74	16.5
R3	Yes	0.45	0.16	3.32	3.92	7.5
R4	No	0.06	0.09	0.49	0.64	8.0
Outdoor soil [ng g^{-1}]						
R1	Yes	36.10	18.48	177.96	232.53	4.9
R3	Yes	409.36	78.96	2151.94	2640.26	5.3
R4	No	66.07	7.21	53.38	126.65	0.8

followed by Eq. (4) (US EPA, 1998). To calculate the life time average daily dose (LADD), Eq. (6) was used.

$$\mathrm{LADD} = \frac{C_{soil/dust} * CF * IR_{soil/dust} * EF * ED}{BW * LT} \quad (6)$$

$C_{soil/dust}$ is the concentration determined in soil or dust in mg/g. CF is a conversion factor, 0.001 g/mg. The recommended central tendency intake rate of soil and dust ($IR_{soil/dust}$) (for young children (1 to <6 years old) is 100 mg/d. The exposure frequency (EF) is 350 days per year, assuming that young children are away from home (e.g., on vacation), the source of contamination, for two weeks per year. The home and surrounding yard are assumed to be the only sources of contamination. The exposure duration (ED) is 5 years (from age 1 to <6 years), based on the assumption that after 5 years of age, children no longer play in outdoor soil or crawl on the floor, and their soil and dust ingestion is limited compared to that of younger children. The average body weight (BW) for children between the ages of 1 and 6 is 16.2 kg. Because the contaminant used in this example is assumed to be a carcinogen, the dose is averaged over the lifetime (LT). A lifetime (LT) of 70 years for a member of the general population is used as a reference value. For use in the calculations, this value is converted to 25,550 days (i.e., 70 years × 365 days year^{-1}).

The calculated cancer risk levels were compared to US EPA (2015) risk levels. U.S. EPA considers excess cancers risk that are above 1 chance to 1,000,000 (1E−06) to be acceptable, a risk between 1E−06 and 1E−04 are generally considered to be significant. A risk above 1E−04 is considered sufficiently large that some remediation is desirable (US EPA, 2015).

3. Results

3.1. p,p′-DDT, p,p′-DDE, and p,p′-DDD levels in indoor air, dust and outdoor soil

Concentrations of *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD determined in the gaseous and particulate phases in indoor air, indoor dust and outdoor soil are shown in Table 3. Due to the high standard deviation observed for *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD concentrations in indoor

air, indoor dust and outdoor soil at the three different locations, a significant difference between the locations was not observed. Overall the Rustaq district contained the highest concentrations of the ΣDDT, ranging from 442.02 to 446.87 ng m^{-3} for indoor air gaseous phase, 4.56 to 892.11 ng m^{-3} for indoor particulate phase, 312.51 to 1334.13 ng m^{-3} for total indoor air, 294.03 to 1818.14 μg g^{-1} for indoor dust and 232.53 to 2640.26 ng g^{-1} for outdoor soil. In general, indoor gaseous phase concentrations were higher compared to indoor particulate phase concentrations, except of Rustaq area location 1. Although DDT was only sprayed indoor, *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT were also determined in outdoor soil. Even in houses in which DDT was not sprayed, significant concentrations of *p,p′*-DDE, *p,p′*-DDD and *p,p′*-DDT were detected indoor. In Shinas area location 3, DDT was not sprayed indoor and concentrations of the ΣDDT in indoor air and indoor dust were 2.3 and 1.8 times higher, respectively, compared to Shinas location 4 where DDT was sprayed seven times between 1976 and 1984. This is an indication that DDT contamination is not limited to the houses directly affected by IRS. DDT and its metabolites are quite mobile, especially in the warm climates. Additional factors as house furniture and equipment, cleaning, ventilation as well as behavior of residents should be assessed to explain differences in the indoor levels. Therefore, the entire area where DDT was applied has to be considered in the risk assessment.

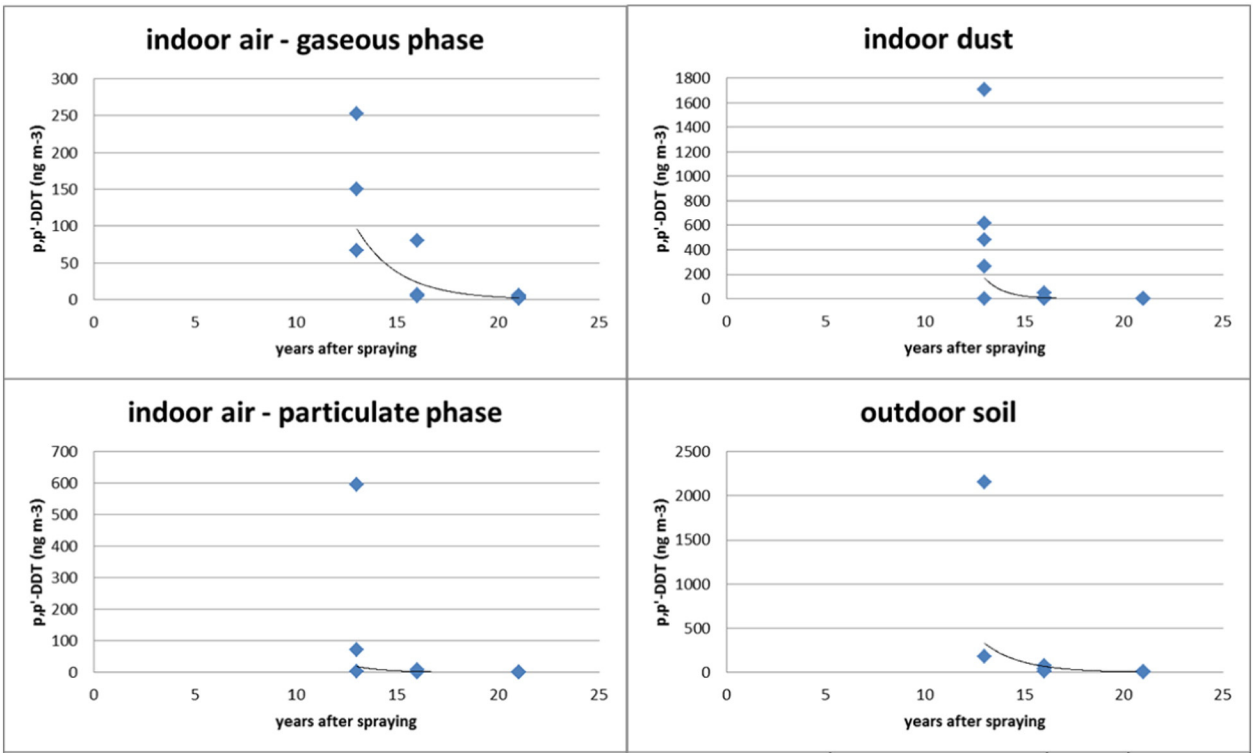
3.2. Degradation of DDT

The DDT/DDE ratio can indicate the degradation rate of DDT, however, in this study there was no exponential relation between the DDT/DDE ratio and the years after spraying. Instead *p,p′*-DDT concentrations were plotted versus the years after spraying and indicated an exponential relation, with coefficients of determination (R^2) above 0.508 (Fig. 2). DDT was sprayed 5 to 7 times at the three locations. During this period degradation of DDT already started, however we assumed that during the period of spraying the concentration of DDT increased and was highest after the last spraying.

The calculated region-specific half-life for *p,p′*-DDT in indoor air, dust and soil was between 0.7 years for indoor dust and 1.5 years for indoor air — gaseous phase (Fig. 2). Half-lives for *p,p′*-DDE in indoor air dust and soil varied between 0.8 and 3.2 (Supporting information Fig. S1) and for *p,p′*-DDD between 0.8 and 4.7 (Supporting information Fig. S2).

3.3. Human health risk assessment

Determination of the health risk was based on cumulative cancer risk values for *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in 2005 and shown in Fig. 3 for air and Fig. 4 for dust.



	Exponential relation	R^2	Half-life (years)
Indoor air – gaseous phase	$y = 44254e^{-0.472x}$	0.719	1.5
Indoor air – particulate phase	$y = 44254e^{-0.472x}$	0.508	1.1
Indoor air – total concentration	$y = 255965e^{-0.557x}$	0.731	1.2
Indoor dust	$y = 106214587e^{-1.027x}$	0.785	0.7
Outdoor soil	$y = 290513e^{-0.523x}$	0.699	1.3

Fig. 2. Correlation between *p,p′*-DDT concentrations determined in various years after spraying and calculated half-life of *p,p′*-DDT in indoor air, indoor dust and outdoor soil.

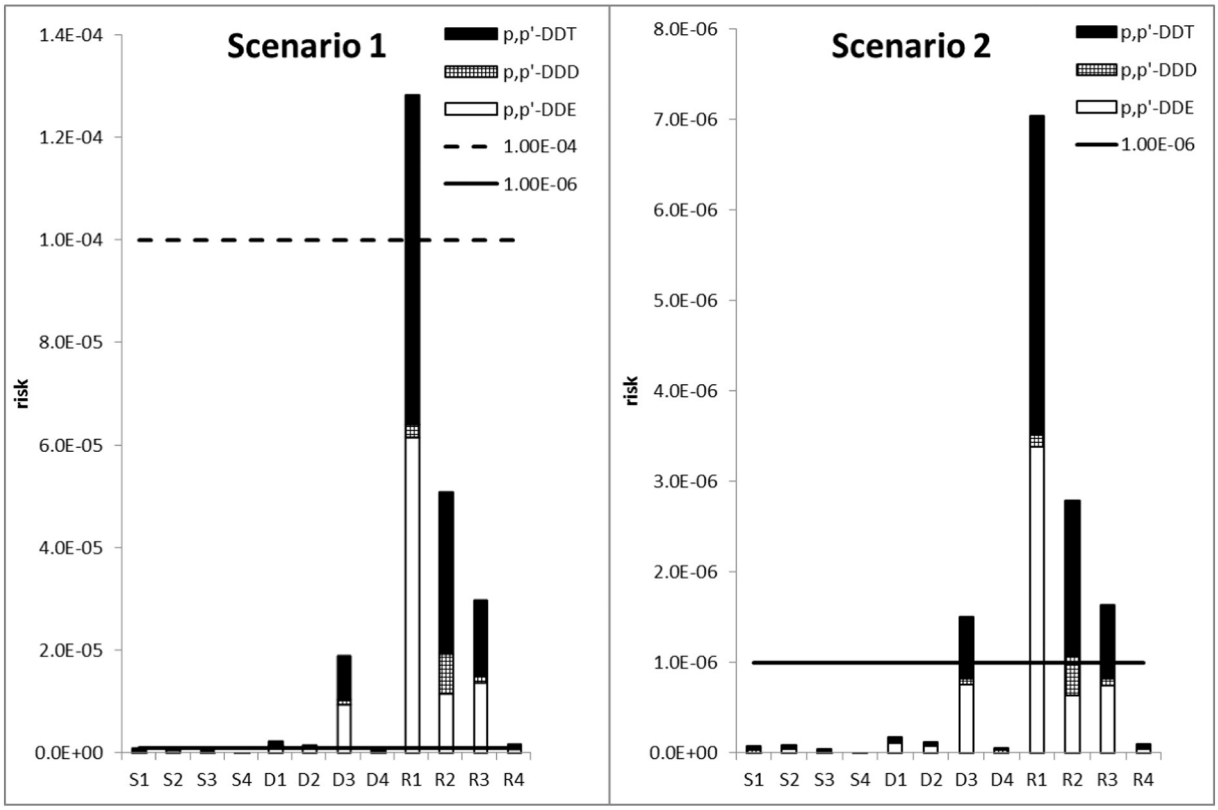


Fig. 3. Calculated cancer risks for human inhalation exposure to *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in Oman in 2005: scenario 1 (worst case) and scenario 2 (realistic). X-axis: S, D and R are Shinas, Dank and Rustaq, respectively. For detailed description of sampling locations see Table 1. The 1.00E−6 line is the border between an acceptable and a significant risk, 1.00E−04 is the border between a significant and sufficiently large risk.

In 2005, three locations in Rustaq (R1, 2 and 3) and one in Dank (D3) showed significant cancer risk from air in a realistic scenario (cancer risk between 1E−06 and 1E−04, right side Fig. 3), at the other locations cancer risk from air exposure was acceptable. In scenario 1, a worst case scenario, one location in Rustaq (R1) indicated a sufficiently large cancer risk from air exposure (cancer risk >1E−04, left side Fig. 3). Three locations in Rustaq (R2, 3, and 4) and three locations in Dank (D1, 2, and 3) showed a significant cancer risk (cancer risk between 1E−06 and 1E−04) for the worst case scenario in air. At locations in Shinas the risk was acceptable in both scenarios.

Two locations in Rustaq (samples R1a, R1b, R2a, R2b, and R2c) and one location in Dank (D1) showed a significant cancer risk from dust exposure (Fig. 4). At the other locations the cancer risk from dust exposure was acceptable. From soil, a potential dermal cancer risk was not expected based on the concentrations determined in soil in 2005. Both in dust and indoor air, *p,p′*-DDT and *p,p′*-DDE are the main contributors to the cancer risk.

Determination of the cancer risk through ingestion of soil and dust for children between the age of 1 and 6 years was based on cumulative cancer risk values for *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in 2005 and the results for dust are shown in Fig. 5.

Five locations in Rustaq (R1a, R1b, R2a, R2b and R2c) showed a sufficiently large cancer risk for ingestion for children exposed to indoor dust and one location in Rustaq (R3) and four locations in Dank (D1, D2a, D2b and D3) showed a significant cancer risk in 2005. For outdoor soil the ingestion cancer risk for children was low, only one location in Rustaq (R3) showed a significant risk (see supporting information Fig. S3) in 2005.

4. Discussion

4.1. p,p′-DDT, p,p′-DDE and p,p′-DDD levels in indoor air, dust and outdoor soil

The content of *p,p′*-DDT in the 75% WP used for indoor spraying in Oman is assumed to be 72–75% with the balance made up of *o,p′*-DDT (Bouwman et al., 2006). Table 2 shows the amount of DDT that was used at each location and the average of kg DDT 75% WP that was sprayed in each house. The average total amount of DDT sprayed indoor between 1976 and 1992 in Shinas, Dank and Rustaq was 3.6, 3.6 and 3.1 kg DDT 75% WP/house, respectively. The amount of DDT used during 1994 and 1998 for control of sand flies was relatively low compared to the amount used for malaria control and therefore not considered in the calculations. The mixture of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD was considered in this study to predict the cancer risk. Since *o,p′*-DDT, *o,p′*-DDE, and *o,p′*-DDD were not analyzed in this study, the calculated cancer risk might be underestimated up to 28%, assuming that the technical mixture contained minimum 72% of *p,p′*-DDT.

Several studies reporting on DDT and metabolite concentrations following indoor spraying were already published, however, to the author's knowledge, human risk assessment to predict the IRS effects on the human population has not been performed so far.

In a study performed in India, indoor air concentration of DDT reached 14,600 ng m^{-3} 1 h after the spraying, and dropped to 5900 ng m^{-3} after 10 days (Singh et al., 1992). In the study performed by Van Dyk et al. (2010) in South Africa, *p,p′*-DDT concentrations reached 2200 ng m^{-3} in indoor air and 1 ng g^{-1} in outdoor soil two months after spraying. Indoor air concentration were higher compared

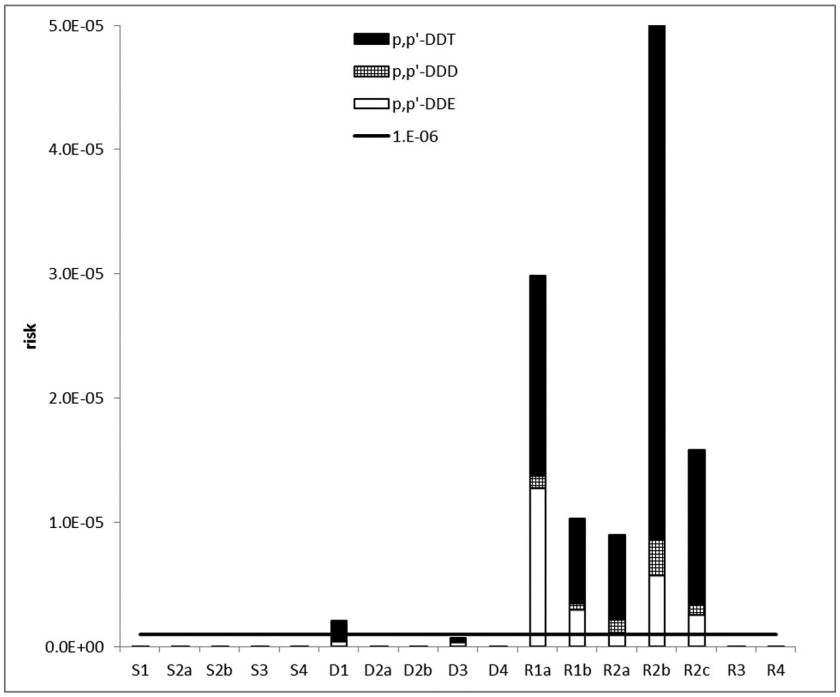


Fig. 4. Calculated cancer risks for human dermal exposure to dust in Oman in 2005. X-axis: S, D and R are Shinas, Dank and Rustaq, respectively. For detailed description of sampling locations see Table 1. The 1.00E−6 line is the border between an acceptable risk and a significant risk.

to our study while outdoor soil concentrations were lower, which corresponds to extended time period after the spraying in our study. Indoor air concentrations of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in houses in Mexico were 0.61, 0.07 and 0.05 μg g^{-1}, respectively, 23 years after indoor spraying of DDT (Herrera-Portugal et al., 2005) while outdoor soil concentrations in the same study were 160, 20 and 20 ng g^{-1}, respectively. Both indoor dust and outdoor soil concentration were in the same order of magnitude as houses in Shinas area of our study, 21 years after spraying. Indoor dust DDT concentrations in houses measured in Mexico only 9 years after spraying ranged between 0.001 and 95.87 μg g^{-1} while outdoor soil concentrations varied between 1 and 788 ng g^{-1} (Martínez et al., 2012). The results indicate that DDT, DDE and DDD are persistent in indoor air, indoor dust and outdoor soil for many years after spraying at levels relevant for human exposure assessment. This has been also confirmed in the study from South Africa where significantly higher concentrations of DDT and metabolites were found in maternal plasma of woman residing in areas with indoor DDT spraying when compared to other locations. This raised a concern about

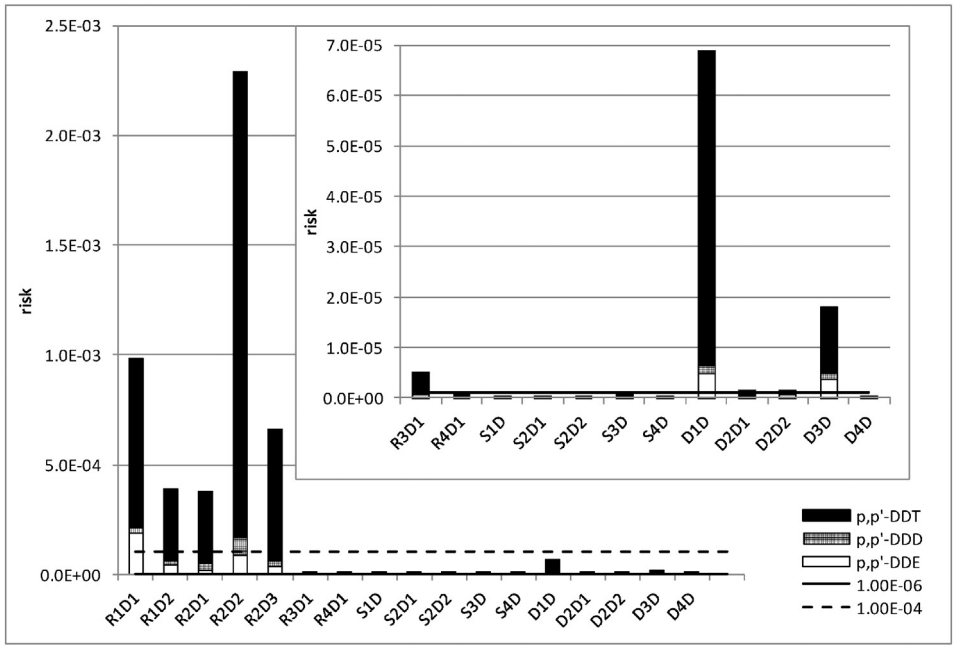


Fig. 5. Calculated cancer risks for children exposure by ingestion of indoor dust to *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in Oman in 2005: X-axis: S, D and R are Shinas, Dank and Rustaq, respectively. For detailed description of sampling locations see Table 1. The 1.00E−6 line is the border between an acceptable and a significant risk, 1.00E−04 is the border between a significant and sufficiently large risk.

the fetus exposure to DDT and potential health impacts (Channa et al., 2012).

A high standard deviation in DDT levels in environmental matrices has been reported in several studies (Martínez et al., 2012). This variability in DDT level within communities might be dependent on how often the houses were cleaned and ventilation within the house. Nevertheless, different location showed a potential cancer risk and therefore care should be taken.

In the future DDT, DDE and DDD on ceilings and walls where DDT was sprayed should be determined as well to calculate the partitioning between the source and air or dust. This information is needed to explain the sorption, degradation and transport of DDT to air, dust and soil. The temperature and ventilation indoor can affect the volatilization of DDT, DDE and DDD.

4.2. Degradation of DDT in indoor air, indoor dust and outdoor soil

In the gaseous phase, DDT reacts with photochemically produced hydroxyl radicals. The half-life of DDT, DDE, and DDD in the gaseous phase is approximately 17–37 h (ATSDR, 2002). The estimated half-lives for gaseous-phase DDT, DDE, and DDD do not necessarily reflect the lifetimes of these compounds in real air conditions. DDT, DDE, and DDD can be adsorbed on particulate matter. Once bound to particulate matter, these compounds do not degrade rapidly by photo oxidation, and therefore transport to indoor dust and outdoor soil is possible. The predicted half-lives for DDT in air in our study were much higher, between 1.1 and 1.5 years. The DDT concentrations in air in the house in Oman most likely reached equilibrium with DDT sprayed on walls and ceilings in the past, which is constantly slowly leaking to the air and can therefore not be compared to half-life in air.

The persistence of DDT in soil is highly variable. Photo oxidation of DDT and DDE is known to occur on soil surfaces or when adsorbed to sediment. During biodegradation of DDT, both DDE and DDD are formed in soils. Both metabolites may undergo further transformation but the extent and rate are dependent on soil conditions and, possibly, microbial populations present in soil (ATSDR, 2002). DDT, DDE, and DDD strongly bind to soil, dust and sediments. The half-life of DDT in soil can vary between 2 and 15 years, depending on the soil acidity and temperature (ATSDR, 2002). Degradations rates for DDT in dust and soil in Oman, predicted in our study, were in the lower range of 0.7 and 1.3 years, respectively. The high temperatures and large amounts of sunlight in Oman probably increased the degradation.

Soil-air partition coefficient (K_{SA}) values are significantly dependent on soil temperature and soil organic matter quantity, and to a minor extent on organic matter type (Cabrerizo et al., 2011). However, a relation between outdoor soil or dust and indoor air concentrations for DDT in this study was not observed, probably due to the indoor air concentrations which were influenced by the DDT still leaking from walls and ceilings within the houses years after spraying.

4.3. Human health risk assessment

The fact that DDT, DDE and DDD were still found several years after spraying indicates that they are still relevant to human risk assessment. Our study on potential human exposure to DDT pollution sources identified location-specific potential cancer risks. However, it must also be noted that uncertainties are inherent in quantitative risk assessment because of assumptions required to extrapolate from one species to another (Slope Factor SF definition), from high to low doses and because of the statistical modeling techniques required to fit data points.

Indoor air and indoor dust seem to be important sources for exposure in 2005; at some locations in Rustaq and Dank a significant cancer risk was predicted in 2005 for adults, and especially for young children. In Shinas samples were taken 21 years after spraying, in Dank 16 years and in Rustaq 13 years after indoor DDT spraying. The results indicate that 13 to 16 years after spraying there was still a significant cancer risk, only 21 years after spraying cancer risk reached acceptable level.

Children are a very vulnerable group, especially since most of their activities take place in and around the house. Small children have more hand-to-mouth contact than adults and are therefore more exposed to indoor dust and soil particles.

Comparing the risk assessment of indoor air, indoor dust and outdoor soil associated with DDT levels for exposure to adults and children indicates that public health managers need to pay more attention to inhalation of contaminated indoor air, dermal exposure from indoor dust, and ingestion of indoor dust for children as important exposure routes for DDT in Oman.

The potential health effects in residents living in the immediate vicinity of DDT spraying areas was calculated based on measurements obtained in 2005. Concentration of *p*,*p*′-DDT, *p*,*p*′-DDE and *p*,*p*′-DDD in air, soil and dust in 2015 were predicted based on half-lives for *p*,*p*′-DDT (Fig. 2), *p*,*p*′-DDE and *p*,*p*′-DDD (Supporting information Figs. S1 and S2). Based on the calculated half-lives and concentration predicted in 2015, risk levels for indoor air and indoor dust are below 1E−06, which indicated that the cancer risk in 2015 for indoor air and indoor dust is acceptable. However, if half-lives for soil of 2 to 15 years (ATSDR, 2002) are used to predict risk levels for dust in 2015, a significant cancer risk for dermal exposure was predicted based on half-lives of 15 years at 5 locations in Rustaq and 1 location in Dank (Supporting information Table S1). Based on a half-life of 2 years risk levels for dermal exposure were acceptable for indoor dust, except for one location in Rustaq where there was a significant cancer risk. Based on half-life of two years for indoor dust a sufficiently large effect is predicted at 5 locations in Rustaq and a significant risk for young children at one location in Dank (Supporting information Table S2) If half-life of 15 years is used, a sufficiently large risk is predicted for 5 locations in Rustaq and 2 locations in Dank, for 1 location in Rustaq the predicted risk was significant. Half-lives are region specific and dependent on temperature, sunlight and biodegradation. Therefore, a proper prediction of the half-life is important to predict risk assessment. The approach used in this study can be used for other locations and countries to predict prospective exposure of contaminants based on indoor experimental measurements and knowledge about the spraying time-schedule to extrapolate region-specific half-lives and predict effects on the human population years after spraying. Based on the region-specific half-lives calculated in our study, the cancer risk via inhalation or dermal exposure for adults and ingestion exposure for young children in 2015 is acceptable for indoor air, dust and soil.

The fact that people spend most of their time indoor and that DDT was sprayed indoor probably means that inhalation of indoor air and dermal/ingestion exposure to dust are the main exposure pathways. However, DDT and metabolites have also been found in food and drinking water after indoor residual spraying to control malaria vectors in South Africa (Van Dyk et al., 2010). Since food was not included in our study, the human risk assessment might be underestimated. Further research is necessary to determine the potential cancer risk for ingestion from food and drinking water and the contribution of the cancer risk compared to inhalation and dermal exposure pathways. The amount of malaria cases decreased significantly after spraying of DDT, however at the time of spraying the long term effects of DDT were not considered. Actual data on the number of people who developed cancer as a consequence of DDT spraying are not available. Therefore further research is necessary to provide information about the ethical aspects of DDT spraying in relation to the cancer risk assessment and the decrease of malaria cases. After the use of DDT for malaria control was stopped in 1992, organophosphates and pyrethroids were used as alternatives for indoor spraying against mosquitos. However these substances are toxic as well, and may cause health and environmental hazards as much as caused by DDT (Rahman, 2013).

5. Conclusions

Significant levels of *p,p′*-DDT, *p,p′*-DDE and *p,p′*-DDD in indoor air, dust and outdoor soil were found up to 21 years after indoor spraying in Oman. To the authors knowledge this is the first study in which the cancer risk on human population was predicted years after DDT spraying indoor in Oman. Results have shown that up to 16 years after indoor spraying of DDT there is still a significant cancer risk for adults and for young children. Region-specific half-lives showed to be important for the risk assessment prediction. The novel approach can be used for other locations and countries to predict prospective exposure of contaminants based on indoor experimental measurements and knowledge about the spraying time-schedule to extrapolate region-specific half-lives and predict effects on the human population years after spraying. More attention should be paid to the long term consequences of indoor spraying of DDT and alternative insecticides.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.12.044.

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