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Investigating unmetabolized polycyclic aromatic hydrocarbons in adolescents' urine as biomarkers of environmental exposure

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HIGHLIGHTS

- 12 of 16 parent PAHs were quantified in urine of Flemish adolescents.
- Benzo(*a*)pyrene showed a positive association with DNA damage.
- Parent PAH congeners do not correlate with 1-hydroxypyrene.
- Parent PAHs are useful as exposure biomarkers for biomonitoring studies.

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) are of interest to human biomonitoring studies due to their carcinogenic potential. Traditionally metabolites of these compounds, like 1-hydroxypyrene, are monitored in urine, but recent methods allow the determination of the parent compounds in urine, which give additional information regarding sources and toxicity of PAHs. In order to assess the feasibility of incorporating these methods in a human biomonitoring study, the 16 USEPA parent PAHs were determined in 20 urine samples. These samples were obtained from 10 boys and 10 girls aged 14–16 years, participating in the third Flemish Environment and Health Study (Flanders, Belgium).

Of these 16 parent PAHs, nine could be determined in more than 95% of the samples and three (including benzo(*a*)pyrene) in more than 50%. Several correlations were found between different PAHs, but not between pyrene and its metabolite 1-hydroxypyrene. Diagnostic PAH ratios in urine and air samples pointed towards combustion sources and are in line with the ratios in environmental samples. Benzo(*a*)pyrene, naphthalene and fluorene have the highest carcinogenic potential in our cohort, when using toxic equivalency factors. Some associations between PAH congeners and determinants of exposure were found, while fluorene and acenaphthylene were positively associated with thyroid hormone levels and benzo(*a*)pyrene showed a positive correlation with DNA damage by comet assay. These results confirm that parent PAHs in urine are useful as biomarkers of exposure in biomonitoring studies.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a family of toxic

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compounds that are mainly of anthropogenic origin. As their name implies, PAHs are a family of compounds consisting of at least 2 aromatic carbon rings, without any incorporated heteroatoms. They are predominantly produced as a side product during incomplete combustion and are present in, amongst others, exhaust from fossil fuel engines, in smoke and soot from furnaces and cigarettes, and in charcoal grilled food. They are also present in asphalt, crude oil, coal and tar, and there is some deliberate production for applications such as medicine and research (ATSDR, 1995).

Although PAHs have common sources and similar properties, their environmental and toxicokinetic behaviour largely depends on their volatility and water solubility, which decreases with increasing molecular weight (Kamal et al., 2015; Li et al., 2010; Nagpal, 1993). After production, atmospheric PAHs will be distributed between the gaseous and particulate phases, where NAP is found in the gaseous phase, three-ring PAHs partitioned between gas and particulate phases, and heavier PAHs fully absorbed on the particulate phase (Kamal et al., 2015). Deposition, dry or wet, can then lead to uptake in the human food chain, both directly or via soil and surface water. Food can also be contaminated during preparation phases, either at home or by industrial preparation (EFSA, 2008). In a review Phillips (1999) concluded that ingestion after deposition on consumable plants is likely the most important exposure pathway of humans to PAHs, except for smokers and individuals that are occupationally exposed. However, inhalation and dermal exposure to PAHs are not necessarily negligible, especially when occupationally exposed (Kim et al., 2013). PAH have lipophilic properties, the logarithm of their octanol-water partition coefficient being between 3 and 8 (Nagpal, 1993). For this reason, PAH can easily cross cell membranes through passive diffusion both on the skin and inside the body. Parent PAH congeners do not directly induce DNA damage, but exert their effects mainly through metabolisation and aryl hydrocarbon receptor (AhR) activation. PAHs will be metabolized by the human body into metabolites like diol-epoxides, radical cations and reactive redox active *o*-quinones, which can all react with DNA to form DNA adducts, and can also alter proteins and lipids. On the other hand, several parent PAHs can associate with the AhR transcription factor that is present in all tissues, inducing AhR activity, which alters expression of many genes, indirectly causing changes in hormonal pathways, tumorigenesis, inflammation, cell proliferation and loss of cell adhesion (Moorthy et al., 2015; Murphy et al., 2007). The carcinogenic effects of PAHs are expected to be stronger when they have four or more rings, of which several are classified as proven, possible or probable carcinogens, but naphthalene is a notable exception that is classified by the IARC as a possible carcinogen, even though it is the lightest PAH (IARC, 2012a).

Over the last decades atmospheric emissions of PAHs have globally decreased, and will probably continue to do so (Shen et al., 2013). However, this trend is not followed in Belgium, where the levels are still high (Gao et al., 2013). Moreover, the concentrations of PAHs tend to be the highest in the most populated areas. Monitoring of atmospheric concentrations and of internal exposure of the general populations, as well as identification of important sources and potential health effects are still highly relevant in areas such as Flanders (Northern part of Belgium). Human biomonitoring is relevant since it integrates exposure by various routes.

Human biomonitoring of PAH exposure is usually done by measuring PAH metabolites in urine. Typically, 1-hydroxypyrene (1-OHPYR) is measured, but naphthalene (NAP), phenanthrene (PHE) and fluorene (FLU) metabolite measurements have also been reported in the literature (Li et al., 2008). On the other hand, parent PAHs in urine are rarely measured, possibly because measurement of the metabolites was already a somewhat established method before viable techniques for parent PAHs became available (Waidyanatha et al., 2003). Investigating them alongside metabolized PAHs could have certain benefits. Often the concentration of one or a small amount of metabolites is extrapolated to the total PAH exposure. This ignores the fact that not all sources produce the same mixture of PAHs and the differences in environmental behaviours, so by only measuring one metabolite important information might be missed (Rossella et al., 2009). Additionally, parent PAHs are expected to be less sensitive to variability of urine concentration (creatinine content) than their metabolized counterparts, because they are eliminated from the kidney by passive rather than active diffusion. As such, no corrections for urinary dilution using either urine creatinine or specific gravity (SG) would be necessary (Boeniger et al., 1993; Campo et al., 2007; Waidyanatha et al., 2001). However, some correlation between creatinine and PHE or NAP levels was observed in the past, although it was much weaker than that between creatinine and PHE or NAP metabolites (Sobus, 2008). Initially, analysis of urinary parent PAHs was conducted in occupationally exposed individuals (Campo et al., 2007, 2006; Rossella et al., 2009), but recently an Italian study focused on cohorts with differing environmental exposures in which 10 PAH congeners were determined (Ranzi et al., 2013).

In Flanders, the Flemish Environment and Health Study (FLEHS) comprises human biomonitoring studies since 1999 and 1-OHPYR has been traditionally used to monitor for PAH exposure. In the present study, urine samples of a small subpopulation of the reference adolescent population of the 2012–2015 cycle of FLEHS (FLEHS III) were analysed for parent PAHs (the 16 US-EPA priority PAHs) to assess if they could be detected and monitored within this population. These PAHs are, by total number of rings:

2 rings: naphthalene(NAP), acenaphthylene(ACY), acenaphthene(ACE)
3 rings: anthracene(ANT), phenanthrene(PHE), fluorene(FLU)
4 rings: chrysene(CHR), fluoranthene(FLT), pyrene(PYR), Benz(*a*)anthracene(BAA)
5 rings: benzo(*a*)pyrene(BAP), benzo(*p*)fluoranthene(BPF), benzo(*k*)fluoranthene(BKF)
6 rings: benzo(*ghi*)perylene(BghiP), indeno (1,2,3-cd)pyrene(IP)

We calculated diagnostic PAH ratios in urine to distinguish between possible sources and compared them with those observed in the atmosphere in Flanders. We tried to determine the relative toxicological contribution of PAHs, and tested if levels of PAH congeners are correlated with each other, with 1-OHPYR and with t,t'-Muconic acid (TTMA). TTMA in urine is a benzene metabolite and proxy for benzene exposure. Since benzene shares some important sources with PAHs, like motor exhaust and cigarette smoking, its correlations with PAHs in urine were tested.

We also studied associations of parental PAH levels in urine with possible determinants of exposure and with biomarkers of health effects. Although the sample size in this study is quite small for such analyses, this was an explorative test to see if results in accordance with the literature could be generated, indicating the use of parental PAH in this kind of analysis. Due to the carcinogenicity of PAH and their effect on the thyroid hormonal pathways through AhR activation(Murphy et al., 2007), associations of PAH exposure with markers of DNA damage and thyroid hormones were examined.