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Bioavailability and potential carcinogenicity of polycyclic aromatic hydrocarbons from wood combustion particulate matter *in vitro*

Susanne Gauggel-Lewandowski [a,*], Alexandra H. Heussner [a], Pablo Steinberg [b], Bart Pieterse [c], Bart van der Burg [c], Daniel R. Dietrich [a]

[a] *Human and Environmental Toxicology, University of Konstanz, D-78457 Konstanz, Germany*
[b] *Institute for Food Toxicology and Analytical Chemistry, University of Veterinary Medicine Hannover, 30173 Hannover, Germany*
[c] *BioDetection Systems b.v., Science Park 406, 1098 XH Amsterdam, The Netherlands*

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ABSTRACT

Due to increasing energy demand and limited fossil fuels, renewable energy sources have gained in importance. Particulate matter (PM) in general, but also PM from the combustion of wood is known to exert adverse health effects in human. These are often related to specific toxic compounds adsorbed to the PM surface, such as polycyclic aromatic hydrocarbons (PAH), of which some are known human carcinogens. This study focused on the bioavailability of PAHs and on the tumor initiation potential of wood combustion PM, using the PAH CALUX® reporter gene assay and the BALB/c 3T3 cell transformation assay, respectively. For this, both cell assays were exposed to PM and their respective organic extracts from varying degrees of combustion. The PAH CALUX® experiments demonstrated a concentration–response relationship matching the PAHs detected in the samples. Contrary to expectations, PM samples from complete (CC) and incomplete combustion (IC) provided for a stronger and weaker response, respectively, suggesting that PAH were more readily bioavailable in PM from CC. These findings were corroborated via PAH spiking experiments indicating that IC PM contains organic components that strongly adsorb PAH thereby reducing their bioavailability. The results obtained with organic extracts in the cell transformation assay presented the highest potential for carcinogenicity in samples with high PAH contents, albeit PM from CC also demonstrated a carcinogenic potential. In conclusion, the *in vitro* assays employed emphasize that CC produces PM with low PAH content however with a general higher bioavailability and thus with a nearly similar carcinogenic potential than IC PM.

1. Introduction

Due to concerns regarding climate change and finite fossil fuel resources, the demand for renewable, CO_2-neutral energy sources, increased [1]. In order to achieve economic but carbon–neutral energy use some countries, e.g. Germany, support the combustion of wood, even in small-scale furnaces with the effect that in 2010 92% of renewable energy consumed consisted of wood [2]. Yet, increasing domestic wood combustion also enhances particulate matter (PM) emissions. PM from various combustion processes, e.g. diesel exhaust particles (DEP), biomass smoke particles (BSP) and gasoline exhaust emissions have gained importance regarding possible adverse health effects in humans [3–5]. DEP has been classified as a human carcinogen [6]. BSP emissions were associated with approximately 1.5 million premature deaths per year due to secondary acute lower respiratory tract and chronic obstructive pulmonary disorders (COPD) [7]. Both evaluations heavily rely on epidemiologic data. Consequently, the IARC evaluation, differing in interpretation with another recent expert team evaluation [8], nearly entirely relies on epidemiology studies derived from populations exposed to DEP (i.e. DEP generated in 1950–1980s) and not from cohorts exposed to new technology diesel exhaust particles (NTDEP). However, NTDEP, due to novel combustion and filter technologies, differ dramatically from DEP. This demonstrates the discrepancy between ongoing technological development and epidemiological evaluation of latent long-term effects.

The alternative to epidemiological studies, i.e. using animal experimentation, has historically proven to be difficult as e.g. lung tumors in rats resulting from exposure to DEP are a reflection of

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Abbreviations: PM, particulate matter; PAH, polycyclic aromatic hydrocarbon; ANF, alpha-naphthoflavone; IC, incomplete combustion; CC, complete combustion.

[*] Corresponding author. Address: Human and Environmental Toxicology, Department of Biology, University of Konstanz, P.O. Box X-918, 78457 Konstanz, Germany. Tel.: +49 (0)7531/88 3518; fax: +49 (0)7531/88 3170.

E-mail addresses: susanne.gauggel@uni-konstanz.de (S. Gauggel-Lewandowski), alexandra.heussner@uni-konstanz.de (A.H. Heussner), Pablo.Steinberg@tiho-hannover.de (P. Steinberg), Bart.Pieterse@bds.nl (B. Pieterse), Bart.van.der.Burg@bds.nl (B. van der Burg), Daniel.Dietrich@uni-konstanz.de (D.R. Dietrich).

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PM lung overload rather than the true carcinogenic potential of the DEP, which has not been observed in other rodent species (reviewed by [8]). Moreover, evaluating adverse health characteristics of PM following each step of a technical development using animal studies is ethically, economically, and technically impossible. Thus a general reduction strategy, as achieved with diesel exhaust (>99% removal efficiency for a number of carcinogenic polycyclic aromatic hydrocarbons (PAH) and nitro-PAH in NTDEP compared to traditional DEP), could be envisioned for all combustion PM. However, biomass combustion technology is extremely variable, ranging from small private furnaces to large industrial biomass burners. With the increasing trend to burn biomass, total PM will most likely increase. Indeed, residential wood combustion has been calculated to significantly contribute to the amount of total PAH in emissions in Sweden and the US [9], while source appointment studies in Denmark demonstrated that residential wood burning was responsible for 47% of national $PM_{2.5}$ emissions in 2002 [4]. Consequently, technological development of biomass burners require fast, reliable and economically feasible testing of PM generated to allow prospective assessment of potential risks. In view of the fact that, at least for the moment, the carcinogenic potential of PM from combustion processes appears of primary importance [6], one emphasis can be laid on the assessment of PM contaminants e.g. carcinogenic PAHs [10,11]. The current working hypothesis for technological advancement of wood burners is that the more efficient and complete the combustion, the lower the hazard potential of PM and by-products, e.g. PAHs. However, the prediction of PAH toxicity is difficult to assess on the basis of mere chemical analysis with a limited number of prioritized PAHs, because in most cases the effects observed result from exposure to PAH mixtures, with different PAHs having different toxic potentials. As the actual risk to humans also depends on the bioavailability of PAH in inhalable PM, test systems are required allowing to understand the bioavailability as well as a toxicological summary endpoints (e.g. receptor interaction, foci formation).

The PAH CALUX® enables the quantification of PAH-induced, AhR-mediated activity and is based on the CALUX® technology, which has been used for screening of AhR-active compounds (e.g. dioxins) in food, feed, sediments, pore water, waste, consumer products as well as marine biological matrices [12–16]. The BALB/c 3T3 is an established cell transformation assay, simulating a two-stage animal carcinogenicity test, to identify potential tumor initiating or tumor promoting substances [17,18].

Consequently, to test whether complete combustions provide for toxicologically less potent PM than PM generated from incomplete combustion processes, organic extracts of wood combustion PM samples as well as the original PM samples were tested in the PAH CALUX® and the BALB/c 3T3 cell transformation assay.

2. Materials and methods

2.1. Materials

Unless stated otherwise, materials were purchased as follows: Steady Glo® (Promega, #E2520), AhR antagonist alpha-naphthoflavone (ANF) (#N5757-1G), benzo[a]anthracene (#1600917825), chrysene (#101128480), benzo[a]pyrene (B[a]P) (#087K0733), benzo[k]fluoranthene (#101077504) and benzo[b]fluoranthene (#1001015267), Sigma–Aldrich; Indeno[c,d]pyrene (#LB82005V), dibenzo[a,h]anthracene (#4-8574), Supelco. Carbon black (CB), (Printex® 90) was obtained from Degussa (Evonik industries, Germany), encompasses 0.4% ash content (following ASTM D 1506), a BET surface area of 350 m^2/g (following ASTM D 6556) and an average primary particle size of 14 nm (following internal method TGZ3). Activated charcoal (AC) was purchased from Riedel de Haën (#18002). All other materials were of the highest grade commercially available.

2.2. PM sampling

Sampling of PM#01–PM#10 and their respective combustion characteristics are detailed in Table 1. Further information on sampling of PM#01, PM#02 and PM#05 was recently described by Gauggel et al., 2012 [19]. Sampling of PM#03 and PM#04 was carried out in a test station of the German Biomass Research Centre in Leipzig, over the duration of 2–3 days and represent samples from an incomplete combustion. Common wood stoves (PM#03–PM#04: nominal output 10 kW), were fired with commercially available non-pretreated standard beech split logs (1.3 kg, specific humidity: 8.9%) via hand loading every ½ h. PM was collected in the ash pan of a standard electrostatic precipitator, 3–4 m away from the furnace, connected to the smoke tube. PM#06 and PM#07, representing samples from incomplete combustions, were collected after 270 and 245 min duration of combustion, respectively. High quality wood stoves (9.0 kW nominal output) in a test station of the German Biomass Research Centre were fired with commercially available non-pretreated standard beech split logs (1.3 kg, specific humidity: 8.9%) via hand loading every ½ h. PM collection was carried out as described for PM#01, PM#03 and PM#04. PM#08–PM#10 were obtained from separate combustions in an automatic pellet boiler at the German Biomass Research Centre, using "DIN plus" wood pellets. Whereas PM#08 and PM#10 represent complete combustion samples, PM#09 resulted from the combustion under suboptimal conditions (15 kW nominal output). All samples were obtained via an electrostatic precipitator.

2.3. Particle characterization

PM samples were characterized using several different methods, as previously described [19]. Scanning electron microscopy (SEM) for PM#03, PM#04 and PM#06–PM#10 was performed with a LEO 1530 at a voltage of 5 kV.

2.4. Elementary (EC), organic carbon (OC), salt (Ion) and metal analysis

EC was analyzed on the basis of the German VDI 2465-1 protocol [20]. EC/OC and ion analysis were carried out as previously described [19]. Because of limited sample amount, determination of OC and EC could not be performed for PM#03 and PM#04. For metal analysis the material was digested with conc. HNO_3 supra-pure in a microwave oven and analyzed for a series of metals with a ContrAA 700 atomic absorption spectrophotometer.

2.5. PAH analysis

PM#01–PM#10 were extracted according to DIN EN 15549 [21] and resulted in Extract#01–Extract#10, respectively (Table 2). Extracts were generated as previously described [19]. A subset (LPM#06–LPM#10) of the extracted (leached) PM, now described as leached particulate matter (LPM) were employed for direct experimentation in the PAH CALUX® system as well as for PAH spiking experiments (see below). The PAH content was also determined in activated charcoal and carbon black. While the PAH content of carbon black could not be measured with the available chromatograph, activated charcoal had a very low degree of PAH contamination (Table 2). The limit of detection (LOD) using GC–MS was 40 ng of each PAH ml^{-1}.