AhR-MEDIATED ACTIVITIES AND CONTRIBUTION OF PAHs IN ROAD DUST FROM HANOI, VIETNAM

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Introduction

Industrialization, urbanization and high economic growth in recent years are accompanied by degradation of environmental quality in developing countries, where the pollution of the ambient air in large cities has become a major concern. It has been reported that the average concentrations of particulate matter ($PM_{2.5}$ and PM_{10}) in six Asian cities—Bandung (Indonesia), Bangkok (Thailand), Beijing (China), Chennai (India), Manila (Philippines), and Hanoi (Vietnam)—were in range of 18–168 and 33–262 µg m^{-3} and frequently exceeded the corresponding 24-h U.S. EPA standards (65 and 150 µg m^{-3}, respectively)[1]. The same study also found traffic as the most important contributor of PM emission. There is association between air particle pollution and polycyclic aromatic hydrocarbons (PAHs), a major source of which is vehicle exhaust, and high levels of PAHs have been found in road dust—a sink for traffic-related pollutants[2]—from Asian developing countries[3-5].

PAHs can cause various toxic impacts, the most well-known being carcinogenicity, mutagenicity and teratogenicity[6-10]. Their toxicities often involve a common mechanism: binding to the aryl hydrocarbon receptor (AhR), induction of AhR- related genes and subsequent transformation to toxic metabolites[6]. AhR has been associated with tumor-promotion and enhanced oxidative stress[7-10]. Many derivatives of PAHs occurring in ambient environment, such as methylated and oxygenated compounds, have also been reported to transactivate AhR[11-13]. Hence, an effect-based evaluation using AhR-specific bioassays is necessary to assess the total toxic activity of the mixture of PAHs and related compounds released from traffic-related processes, in addition to the conventional chemical analysis of PAHs.

This study analyzed road dust collected from Hanoi, a city with some of the highest levels of traffic-related air pollution in East Asia[1], to investigate the contamination status by AhR agonists including not only well-known PAHs but also other compounds. The AhR-mediated toxic activities were evaluated using two Chemical-Activated LUciferase gene eXpression (CALUX) assays: PAH-CALUX for rapidly metabolizable AhR agonists and DR-CALUX for more persistent compounds. PAHs also were analyzed to determine their contribution to the overall toxic activities.

Materials and methods

Sample collection
Road dust samples were collected from an urban area (Hanoi, $n = 15$) and a reference rural area (Duong Quang village, $n = 5$) in Vietnam during 2011 and 2012. The samples were kept in ice immediately after collection, shipped to CMES, Japan with gel ice and preserved at -25 °C until analysis.

Sample extraction and bioassay
Road dust samples were dried in oven at 35 °C, and then sieved to remove particles over 63 µm in size. One gram of sample was extracted with acetone/hexane (v/v; 50/50) in an ultrasonic device and then with toluene using a rapid solvent extractor (SE100, Mitsubishi Chemical Analytech, Japan). A 0.1-g portion of the crude extract was then concentrated, solvent-exchanged into 0.1 mL biochemical-grade dimethyl sulfoxide and stored at 4 °C for subsequent use in bioassays.

Pre-treatment and chemical analysis

The remaining extract was spiked with deuterated PAH surrogate standards and cleaned-up using 1.2% deactivated alumina chromatography, activated silica gel chromatography and gel permeation chromatography, and then finally spiked with chrysen-d_{12} as internal standard. PAHs (naphthalene (Nap), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fluh), pyrene (Pyr), benzo[c]phenanthrene (BcPh), cyclopenta[c,d]pyrene (CPP), benzo[a]anthracene (BaA), chrysene (Chy), benzo[b]-, benzo[k]-, and benzo[j]fluoranthene (BbF, bkF and BjF), 7,12-dimethylbenzo[a]anthracene (DMBA), benzo[e]- and benzo[a]pyrene (BeP and BaP), 3-methylchloranthrene (MCA), indeno[1,2,3-c,d]pyrene (IDP), dibenz[a,h]anthracene (DBA), benzo[g,h,i]perylene (BgP), dibenzo[a,h]-, dibenzo[a,i]-, dibenzo[a,l]pyrene (DBahP, DBaiP and DBalP) were identified and quantified by gas chromatography mass spectrometry (GC-MS). Every set of seven samples was accompanied with a procedural blank.

PAH-CALUX and DR-CALUX assays

The long-term and short-term AhR-inducing potencies of the crude extracts were determined by the DR-CALUX assay for 24 h and by the PAH-CALUX assay for 6 h, respectively; these method assays utilize the rate hematoma cell line H4IIE stably transfected with the firefly luciferase gene containing a multimerized DRE (dioxin response element) in front of a minimal promoter, using the protocols described elsewhere[14-15]. The results were expressed in amount of TCDD-equivalent (CALUX-TEQ) and BaP-equivalent (CALUX-BaPEQ) per gram dry weight, respectively.

Results and discussion

AhR-mediated activities of persistent and non-persistent compounds in road dust

AhR agonist activities were detected in DR-CALUX assay for all the road dust samples. Significant higher CALUX-TEQs were found in samples from the urban site Hanoi (median 36 ng/g dry wt) than in the rural site Duong Quang (median 17 ng/g dry wt) (Fig. 1). Compared with other TEQ results obtained using *in vitro* bioassay methods, AhR agonistic activity levels in crude extracts of road dust particles from Hanoi were found to be significantly higher than those of settled house dust collected in Vietnam (median: 12 ng CALUX-TEQ/g[16] and of flood-suspended particulate matter in Germany (7 ng EROD-TEQ/g[17]). The CALUX-TEQ in road dust from Hanoi also was comparable to those in crude extract of a reference urban dust particulate matter mixture SRM1649a (mean: 43 ng CALUX-TEQ/g)[18].
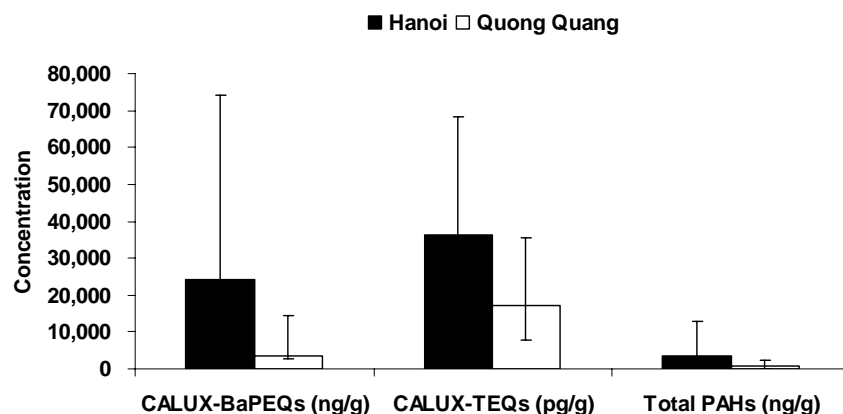

Figure 1. Concentration of CALUX-TEQ, CALUX-BaPEQ and Total PAHs in road dust

Marked AhR-mediated activities were also detected in PAH-CALUX assay in all the road dust samples. Significantly higher CALUX-BaPEQs were found in Hanoi road dust, with a median value of 25 µg/g dry wt, than those in Duong Quang road dust, with a median value of 3 µg/g dry wt (Fig. 1). Results from the two CALUX assays indicate that road dust from Hanoi area contains large amounts of AhR agonists, both labile and recalcitrant ligands.

PAHs concentrations and profiles

We also determined the concentrations of Σ_{24}PAHs found in urban and rural road dust samples and the values ranged from 1.0 to 13 µg/g dry wt. in urban and from 0.15 to 2.0 µg/g dry wt. in rural road dust samples (Fig. 1). Profile of PAHs in urban site in the order was Fluh > Phe > Pyr > Ant > CPP > DMBA > BbF+BkF > BgP > BeP > Nap > Chy > BaP > Flu whereas PAH profiles of rural road dust was Phe > BjF > BbF+BkF > CPP > DMBA > Flu > Fluh > Pyr > BeP > Acy > DBA > IDP > Ant (Fig. 2). The profile of PAHs confirmed that their major sources in the urban site are vehicle emissions.
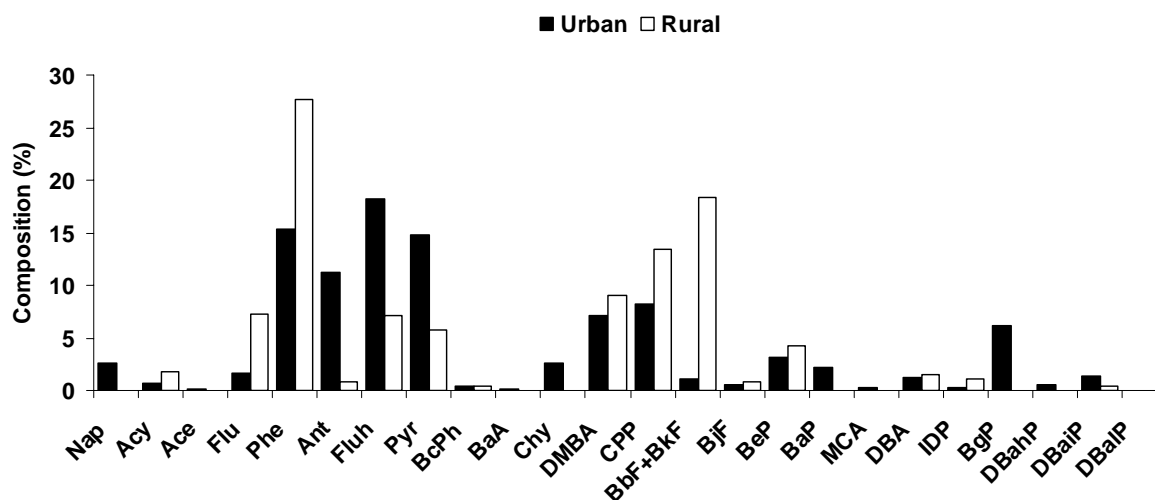

Figure 2. Profiles of PAHs in road dust

Contribution of PAHs to AhR-mediated activities

The toxic contributions of PAHs in samples were evaluated by comparison of the bioassay-derived CALUX-TEQ/CALUX-BaPEQ and chemically-derived theoretical equivalents (Theo-TEQs and Theo-BaPEQs). Theo-BaPEQs and Theo-TEQs were calculated by multiplying the PAH concentrations by appropriate relative potencies[18-20]. The contribution of theoretical AhR agonist activities of PAHs was only 8.4% (median) of the total CALUX-TEQs (Fig. 3) and 55% of the total CALUX-BaPEQ for Hanoi road dust (Fig. 4). Among the target PAHs, the major contributors to AhR agonism were BbF and BkF (total 87%), followed by Pyr (4%) and IDP (3%) for CALUX-TEQ. For CALUX-BaPEQ, the most important contributors were BbF and BkF (83%), followed by IDP (9%) and Chy (4%). Large differences between the observed AhR-mediated activities and the theoretical activities in all the samples indicate the occurrence of unknown AhR agonists in the road dust. Two other potential contributors to AhR agonist activities are oxygenated PAHs and other PAH derivatives[21].
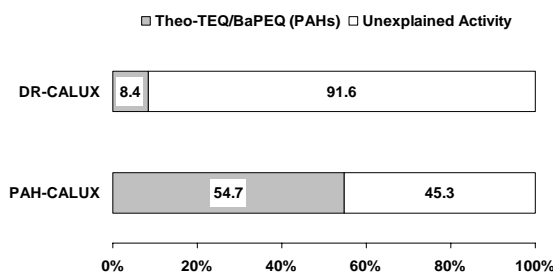

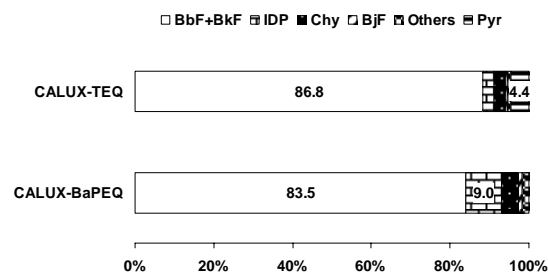*Figure 3. Contribution of PAHs to CALUX-TEQ/BaPEQ*

Figure 4. Contribution of PAHs to total Theo-TEQ/BaPEQ

To our knowledge, this is first report on the application of DR-CALUX and PAH-CALUX for evaluation of potential toxic activities by both labile and recalcitrant ligands in road dust from a developing country. Our

results indicate that AhR-mediated activities of other non-persistent and persistent compounds in road dust should be considered in further investigation.

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