Science of the Total Environment 491–492 (2014) 246–254

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Aryl hydrocarbon receptor mediated activities in road dust from a metropolitan area, Hanoi—Vietnam: Contribution of polycyclic aromatic hydrocarbons (PAHs) and human risk assessment

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HIGHLIGHTS

- First assessment of total AhR-mediated toxic activities in road dust using DR-CALUX.
- PAHs known as carcinogens were found at high levels in Hanoi.
- PAHs contributed only 0.8–76% to AhR agonist activities in road dust.
- Exposure to PAHs in road dust may pose high cancer risk for Hanoi residents.

ARTICLE INFO

Article history:
Received 15 November 2013
Received in revised form 23 January 2014
Accepted 23 January 2014
Available online 9 February 2014

Keywords:
Ah-receptor
Dioxins
PAHs
CALUX
Road Dust
Vietnam

ABSTRACT

Dioxin-Responsive Chemical-Activated LUciferase gene eXpression assay (DR-CALUX) was applied to assess the total toxic activity of the mixture of PAHs and related compounds as well as dioxin-related compounds in road dust from urban areas of Hanoi, Vietnam. Road dust from Hanoi contained significantly higher DR-CALUX activities (3 to 39, mean 20 ng CALUX-TEQ/g dw) than those from a rural site (2 to 13, mean 5 ng CALUX-TEQ/g dw). The total concentrations of 24 major PAHs (Σ_{24}PAHs) in urban road dust (0.1 to 5.5, mean 2.5 μg/g dw) were also 6 times higher than those in rural road dust (0.08 to 1.5, mean 0.4 μg/g dw). Diagnostic ratios of PAHs indicated vehicular engine combustion as the major PAH emission source in both sites. PAHs accounted for 0.8 to 60% (mean 10%) and 2 to 76% (mean 20%) of the measured CALUX-TEQs in road dust for Hanoi the rural site, respectively. Benzo[b]-/benzo[k]fluoranthenes were the major TEQ contributors among PAHs, whereas DRCs contributed <0.1% to CALUX-TEQs for both rural and urban sites. These results suggest TEQ contribution of other aryl hydrocarbon receptor agonists in road dust. Significant PAH concentrations in urban dust indicated high mutagenic and carcinogenic potencies. Estimated results of incremental life time cancer risk (ILCR) indicated that Vietnamese populations, especially those in urban areas such as Hanoi, are potentially exposed to high cancer risk via both dust ingestion and dermal contact. This is the first study on the exposure risk of AhR agonists, including PAHs and DRCs, in urban road dust from a developing country using a combined bio-chemical analytical approach.

1. 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. Located in the Red River delta, Hanoi, Vietnam's capital is one of the biggest cities in Asia with over 3.4 million inhabitants in the city proper and its urban districts. Hanoi urban area consists of 7 urban districts that cover 918.5 km^2 in a total of 3344 km^2 of the Hanoi metropolitan area. Among the six thickly populated Asian cities including Vietnam (Hanoi), Bandung (Indonesia), Bangkok (Thailand), Beijing (China), Chennai (India), and Manila (Philippines), Hanoi has been reported as the most polluted city by dust with the mean of atmospheric particulate matter in the range of 18–168 ($PM_{2.5}$) and 33–262 μg m^{-3} ($PM_{10-2.5}$) and frequently exceeded the

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http://dx.doi.org/10.1016/j.scitotenv.2014.01.086

corresponding 24-h U.S. EPA standards (65 and 150 μg m^{-3}) (Oanh et al., 2006). The hourly average of suspended particulate matter in Hanoi has been reported by yet another study as 0.4–1.5 mg/m^3 exceeding the Vietnamese standard of 0.2 mg/m^3 (Saksena et al., 2006). According to the Ministry of Natural Resources and Environment, the dust density on Hanoi streets and roads is in the range of 20–40 g/m^2/year, especially with higher values on the ring roads with figures up to 100–400 g/m^2/year whereas 10 g/m^2/year is the normal average in developed countries. The traffic in Hanoi has been reported as the most important contributor of particulate matter emission (Oanh et al., 2006). A previous study on the traffic volume has been performed using video camera recording in the year 2004 and the results showed that there were around 10,000 motorbikes passed a selected point per hour. This traffic volume has reached over 18,000 motorcycles in 2005. Additionally, the number of cars and light trucks has also increased (Yen et al., 2007). In highway, the motorcycles' density is 9100 vehicles per hour. Meanwhile the corresponding values are 13,600 and 3500 vehicles per hour for the arterial and residential areas, respectively (Oanh et al., 2012). Moreover, diesel vehicles were reported as the highest contributors of particulate matter in Hanoi (Oanh, 2009). Air particle pollution by vehicles has been associated with polycyclic aromatic hydrocarbons (PAHs), a major source of which is vehicle exhaust, and high levels of PAHs in road dust from Asian developing countries were reported (Wang et al., 2009; Boonyatumanond et al., 2007; Lee et al., 2001). Road dust is also a sink for complex mixtures of traffic-related pollutants (Keller and Lamprecht, 1995) and may contain aliphatic hydrocarbons, PAHs and their derivatives, as well as other organic substances (Bodzek et al., 1993; Yassaa et al., 2001; Lee et al., 2001). However, contaminations by traffic-related pollutants including PAHs in Hanoi road dust and related human health risk have not been investigated comprehensively.

PAHs are well-known for their carcinogenic, mutagenic and teratogenic characteristics and they can cause various toxic impacts to human and wildlife (Behnisch et al., 2001; Sjiigren et al., 1996; Poland et al., 1982; Senft et al., 2002; Nebert et al., 2004). Their toxicities usually involve a common mechanism such as binding to the aryl hydrocarbon receptor (AhR), induction of AhR-related genes and subsequent transformation to toxic metabolites (Behnisch et al., 2001). AhR has been associated with tumor-promotion and enhanced oxidative stress (Sjiigren et al., 1996; Poland et al., 1982; Senft et al., 2002; Nebert et al., 2004). Not only PAHs but also many of their derivatives occurring in ambient environment, such as methylated and oxygenated compounds, have been reported to transactivate AhR (Trilecová et al., 2011; Sonneveld et al., 2007; Sovadinová et al., 2006). Moreover, some other chemicals often occur in the environment at low concentrations compared with PAHs, but with a high AhR-mediated activity (e.g. dioxin-related compounds). It is important to know not only concentration levels of organic compounds in the environment but also their toxicities to evaluate the integrated risk for human health effects and environmental risk assessment. Regarding this matter, reporter gene assays such as DR-CALUX have been known as a useful method to evaluate the AhR-mediated toxicities of contaminants in the environment (Behnisch et al., 2003; Machala et al., 2001). Therefore, an approach that aims to establish a causal link between chemical substances and biological effects in environmental samples 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 the highest levels of traffic-related air pollution in East Asia (Oanh et al., 2006) and Duong Quang as a rural reference site, to investigate the contamination status by AhR agonists including not only the well-known PAHs and dioxin-related compounds (DRCs) but also other potential compounds. The AhR-mediated toxic activities were evaluated using Chemical-Activated LUciferase gene eXpression (CALUX) assays. PAHs and DRCs were also analyzed to determine their contribution to the overall toxic activities. Finally, US-EPA incremental lifetime cancer risk model were applied to identify the human health risk for PAH exposure to road dust via ingestion and dermal contact.

2. Material and methods

2.1. Sampling site description

In recent years, as a result of its economic growth, infrastructure in Hanoi, capital of Vietnam has seen considerable change. Though urban and suburban road extension has been marginal, traffic is generally increasing, leading to higher traffic density and congestion. According to the Transport Police Department of Hanoi, registered motorcycles in Hanoi are increasing at ~13.5% per year and car ownership at ~10% a year. Duong Quang, a mostly agricultural commune located at approximately 50 km to the east of Hanoi in My Hao district, Hung Yen province, was chosen as the reference site. The population of this commune was approximately 6500, and motorbikes were the main transport vehicles.

2.2. Sample collection

We collected 24 road dust samples from Hanoi urban area and 6 samples from Duong Quang in January 2011 (Fig. S1). Each dust sample of approximately 300 g was collected from an area of 20–50 m in length and 0.5 m in width at the side of the road using straw broom. After collection, the samples were preserved at −25 °C until analysis.

2.3. Sample pre-treatment and extraction

Road dust samples were air-dried, and then sieved through a 500 μm stainless sieve to remove coarse particles. Two grams of the sample was extracted with an acetone/hexane mixture and then toluene using a rapid solvent extractor (SE100, Mitsubishi Chemical Analytech, Japan) according to a previously reported method (Tue et al., 2010). A 0.2 g-equivalent portion of the crude extract was then concentrated, solvent-exchanged into 0.1 ml biochemical-grade dimethyl sulfoxide (DMSO) and stored at 4 °C for in vitro determination of AhR-mediated activities using DR-CALUX assay. The remaining extract was used for chemical analysis of PAHs. Every set of seven samples was accompanied with a procedural blank.

2.4. DR-CALUX

The AhR-inducing potencies of the crude extracts were determined by the DR-CALUX assay. This method assays utilize the rat hematoma cell line H4IIE (BioDetection Systems, The Netherlands) stably transfected with the firefly luciferase gene containing a multimerized dioxin response element in front of a minimal promoter. All assays were performed following the BioDetection Systems' protocol described elsewhere (Suzuki et al., 2004, 2006). Briefly, 80,000 cells/well were seeded on 96-well plates. After 24 h of incubation at 37 °C and 5% CO_2, the cells were treated with exposure medium contained DMSO solutions (0.8% DMSO in final wells) of either the reference standard 2,3,7,8-tetrachlorobenzo-*p*-dioxin (TCDD, 0 to 37.5 nM) or the samples (diluted by a factor of 1 to 1000). After another 24 h of incubation, the cells were subjected to luminescence measurement. The AhR agonist activities were derived from the diluted samples with similar response to 1–3 pM TCDD (usually 300 to 1000 time dilution), and expressed in amounts of TCDD equivalent (CALUX-TEQ) per gram dry weight (dw). Each experiment was done in triplicate. No significant effect on cell viability was observed in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (Suzuki et al., 2013) for these diluted samples.

2.5. Chemical analysis

The extract for PAH analysis was spiked with deuterated PAH surrogate standards and cleaned-up using 1.2% deactivated alumina