Environmental Pollution xxx (2017) 1–11

Contents lists available at ScienceDirect

Environmental Pollution

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

Biomass burning particles in the Brazilian Amazon region: Mutagenic effects of nitro and oxy-PAHs and assessment of health risks☆

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ARTICLE INFO

Article history:
Received 17 March 2017
Received in revised form
31 July 2017
Accepted 20 September 2017
Available online xxx

Keywords:
Brazilian Amazon
Biomass burning
Nitro-PAHs and oxy-PAHs
Salmonella/microsome assay
Cytokinesis-block micronucleus (CBMN)

ABSTRACT

Emissions from burning of biomass in the Amazon region have adverse effects on the environment and human health. Herein, particulate matter (PM) emitted from biomass burning in the Amazon region during two different periods, namely intense and moderate, was investigated. This study focused on: i) organic characterization of nitro- and oxy-polycyclic aromatic hydrocarbons (PAHs); ii) assessment of the excess lifetime cancer risk (LCR); and iii) assessment of the *in vitro* mutagenic effects of extractable organic matter (EOM). Further, we compared the sensitivity of two mutagenicity tests: *Salmonella*/microsome test and cytokinesis-block micronucleus (CBMN) with human lung cells. Among the nitro-PAHs, 2-nitrofluoranthene, 7-nitrobenz[*a*]anthracene, 1-nitropyrene, and 3-nitrofluoranthene showed the highest concentrations, while among oxy-PAHs, 2-metylanthraquinone, benz[*a*]anthracene-7,12-dione, and 9,10-anthraquinone were the most abundant. The LCR calculated for nitro-PAH exposure during intense biomass burning period showed a major contribution of 6-nitrochrysene to human carcinogenic risk. The EOM from intense period was more mutagenic than that from moderate period for both TA98 and YG1041 *Salmonella* strains. The number of revertants for YG1041 was 5–50% higher than that for TA98, and the most intense responses were obtained in the absence of metabolic activation, suggesting that nitroaromatic compounds with direct-acting frameshift mutagenic activity are contributing to the DNA damage. Treatment of cells with non-cytotoxic doses of EOM resulted in an increase in micronuclei frequencies. The minimal effective dose showed that *Salmonella*/microsome test was considerably more sensitive in comparison with CBMN mainly for the intense burning period samples. This was the first study to assess the mutagenicity of EOM associated with PM collected in the Amazon region using *Salmonella*/microsome test. The presence of compounds with mutagenic effects, particularly nitro- and oxy-PAHs, and LCR values in the range of 10^{-5} indicate that the population is potentially exposed to an increased risk of DNA damage, mutation, and cancer.

Abbreviations: B[*a*]P_{eq}, benzo[*a*]pyrene equivalency concentration; CBMN, cytokinesis-block micronucleus assay; EOM, extractable organic matter; LCR, excess lifetime cancer risk; MED1.5, minimal effective dose to increase the effect 50%; MN, micronuclei; NDI, nuclear division index; PAHs, polycyclic aromatic hydrocarbons; PM, Particulate matter; TEF, toxicity equivalency factor.

☆ This paper has been recommended for acceptance by Dr. Chen Da.

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https://doi.org/10.1016/j.envpol.2017.09.068
0269-7491/

1. Introduction

The Amazon represents over half of the remaining tropical rain forests on the planet and contains one of the largest biodiversity in the world. Further, it corresponds to 61% of the area of Brazil (Malhi et al., 2008). Deforestation and land use have changed about 18% of the original tropical rain forest, mostly in the southern and western Amazonia. The land is used mainly for establishing pastures and preparing for crops, consequently causing many alterations in atmospheric dynamics (Artaxo et al., 2013). Interestingly, most of the forest fire hotspots in the Brazilian Amazon are located in an area of approximately 500,000 km^2, with a population of over 10 million, known as the deforestation arc (de Oliveira Alves et al., 2015).

In October 2013, the International Agency for Research on Cancer (IARC) classified outdoor air pollution as carcinogenic to humans (Group 1) (IARC, 2016). In addition, IARC considers indoor emissions from household combustion of biomass fuel (primarily wood) as probably carcinogenic to humans (Group 2A) (IARC, 2010). Particulate matter (PM) is a heterogeneous mixture of chemicals that changes in space and time according to the emission source, and it is considered one of the main air pollutants. PM, with adsorbed chemical species such as polycyclic aromatic hydrocarbons (PAHs) and other mutagenic compounds, is suspected to increase the risk of human lung cancer and cardiovascular diseases (Brown et al., 2013; Raaschou-Nielsen et al., 2013; IARC, 2013). The PAHs are formed by incomplete combustion or pyrolysis of organic material, together with their nitrated (nitro-PAHs) and oxygenated (oxy-PAHs) derivatives, represent one of the largest contributors to the biological effects of PM (Boström et al., 2002; Kelly and Fussell, 2012; Nemmar et al., 2013).

Many of the biological effects of PAHs, including mutagenesis and carcinogenesis, are believed to be mediated by activation of a series of enzymatically-catalyzed reactions to form their active metabolites. The mutagenic potential of PAHs is probably associated with the structural differences between DNA adducts and the consequent effects of their removal by DNA repair mechanisms (Boström et al., 2002; Jarvis et al., 2014). It is speculated that nitro- and oxy-PAHs are more mutagenic than unsubstituted PAHs because of their ability to act as direct mutagens; however, such quantification of risk is limited by sparse availability of toxicological data (Fu, 1990; Jung et al., 1991; Jariyasopit et al., 2014a, 2014b; Tomaz et al., 2016; Bandowe and Meusel, 2017).

The combination of chemical-analytical methods, along with specific extraction strategies, and short-term bioassays has been successfully used to evaluate effects of a variety of mutagenic compounds in complex environmental samples such as air (Kessler et al., 2012; Jarvis et al., 2013; Palacio et al., 2016). Cell-based bioassays that target relevant biological endpoints complement chemical analysis for environmental quality assessment (Escher et al., 2014). Many mutagenicity assays, such as *Salmonella*/microsome test (Umbuzeiro et al., 2008a; Alves et al., 2016), micronuclei analysis using plants as *Tradescantia pallida* (de Oliveira Alves et al., 2011; de Oliveira Galvão et al., 2014), human lung cell lines (de Oliveira Alves et al., 2014), and exfoliated buccal cells from exposed human populations (Bruschweiler et al., 2014; Wultsch et al., 2015; de Oliveira Galvão et al., 2017), have been used to evaluate the effects of different pollutants emitted by biomass burning. The exposure to fine particulate matter ($PM_{2.5}$) generated in the Amazon region was reported to contribute to an increased micronuclei frequency in exfoliated buccal mucosa cells from schoolchildren (Sisenando et al., 2012).

Recently, de Oliveira Alves et al. (2015) determined the human health risk associated with exposure to PAHs emitted due to burning of biomass in western Amazon. The estimated cancer risk calculated during the dry seasons (17×10^{-5}) exceeded the WHO health-based guideline (8.7×10^{-5}). These facts point out the need to understand mutagenic mechanisms associated with the exposure to emissions from Amazon biomass burning.

The *Salmonella*/microsome test has long been used to detect potentially mutagenic environmental compounds. It is a short-term bacterial test that is able to detect mutagens that may directly alter the DNA, using selected mutant strains of *Salmonella enterica* serovar Typhimurium[2] (Mortelmans and Zeiger, 2000). In environmental studies, TA98 and TA100 strains are usually employed for the detection of frameshift and basepair mutagenic activity, respectively. More recently developed strains with different metabolic capacities such as YG1041 and YG1042, with enhanced nitroreductase and acetyl transferase activities have been adopted in air pollution monitoring of specific classes of PAHs present in environmental samples (Claxton et al., 2004; DeMarini et al., 2004; Umbuzeiro et al., 2014). However, the *Salmonella*/microsome assay is able to detect solely point mutations. The inclusion of a test that detects chromosome damage is, therefore, a good strategy to supplement the information on the mutagenic hazards of a substance or complex mixture (Thybaud et al., 2007; Kirkland et al., 2011).

The cytokinesis-block micronucleus assay (CBMN) is a well-established *in vitro* genetic toxicology assay and has become an accepted standard method to assess the genotoxic hazard of chemicals (OECD, 2016). This protocol has been used in environmental studies investigating air quality with several different cell lines, as reported by Bocchi et al. (2016) and de Oliveira Alves et al. (2014) for A549 cells; León-Mejía et al. (2016) for V79 cells; Xin et al. (2014) for HepG2 cells; and Zhai et al. (2012) for HBE cells. Micronuclei (MN) are biomarkers of mutagenicity that may originate from whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division and/or acentric chromosome fragments, both induced by aneugenic or clastogenic substances, respectively (Kirsch-Volders et al., 2011, 2014). The cytome approach of this assay provides information about chromosome breakage and/or loss (e.g., MN), gene amplification (e.g., nuclear buds) and chromosome rearrangement, as dicentric chromosomes (e.g., nucleoplasmic bridges) (Kirsch-Volders et al., 2011, 2014).

The aims of this study were: i) to identify and quantify nitro-PAHs and oxy-PAHs present in the PM emitted from burning of biomass in the Amazon region; ii) to estimate the excess lifetime cancer risk for the exposed population; and iii) to assess the *in vitro* mutagenic effects of extractable organic matter collected during the periods of moderate and intense biomass burning, comparing the sensitivity of a prokaryote (*Salmonella*/microsome test) and a eukaryote (CBMN with human lung cells) mutagenicity assays.

2. Materials and methods

2.1. Air sampling and solvent extraction

PM_{10} fraction was collected from the rural area of Porto Velho, state of Rondônia, in the western part of the Amazon region. The sampling site (8.69° S, 63.87° W) is located in a region with large land use and associated regional biomass burning (de Oliveira Alves

[2] We adopted the taxonomy revision that occurred for the genus Salmonella, where *Salmonella typhimurium* was revised as *Salmonella enterica* serovar Typhimurium (Brenner et al., 2000).

Please cite this article in press as: de Oliveira Galvão, M.F., et al., Biomass burning particles in the Brazilian Amazon region: Mutagenic effects of nitro and oxy-PAHs and assessment of health risks, Environmental Pollution (2017), https://doi.org/10.1016/j.envpol.2017.09.068