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

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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.

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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).

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et al., 2015). The sampling conditions and instrumentation details were previously described by Brito et al. (2014). Sample collection was conducted during two distinct periods: intense biomass burning – dry season (August–October/2011), and moderate biomass burning – wet season (November/2011–March/2012).

The aerosol samples were collected using a high-volume air sampler with a flow rate of 1.13 m^3/min. Quartz fiber filters were pre-cleaned by heating in an oven at 800 °C for 8 h. Thereafter, the filters were weighed after equilibrating them in a room with constant temperature and relative humidity (50%) for 24 h. The extractable organic matter (EOM) was obtained with dichloromethane using the Soxhlet apparatus, as previously described by de Oliveira Alves et al. (2015). In addition, the EOM mass concentration contained in the PM_{10} was determined according to Valle-Hernández et al. (2010). Previously, each vial was pre-cleaned by heating at 400 °C for 24 h, until constant weight. After, 200 μL of the EOM was added and was completely concentrated with a high purity N_2 gas. Finally, the vials were weighed again using an electronic micro-analytic 1 μg precision Mettler scale. The amount of EOM was assessed taking into consideration the difference of mass between the vial with and without organic extract as well as the air volume sampled.

The filters obtained for each period – moderate and intense biomass burning – were extracted individually for chemical analyses and pooled together to perform biological assays.

2.2. Organic chemical analysis

Non-substituted PAHs' results were published in de Oliveira Alves et al. (2015). The detection of their nitro- and oxy-derivatives was performed later, and is described here. Eleven nitro-PAHs (9-nitrophenanthrene, 3-nitrophenanthrene, 2-nitrophenanthrene, 2-nitroanthracene, 3-nitrofluoranthene, 2-nitrofluoranthene, 1-nitropyrene, 4-nitropyrene, 7-nitrobenz[*a*]anthracene, 6-nitrochrysene, 6-nitrobenzo[*a*]pyrene) and four oxy-PAHs (9-fluorenone, 9,10-anthraquinone, 2-methylanthraquinone, benz[*a*]anthracene-7,12-dione) were detected. The quantitative and qualitative analyses of nitro-PAHs (Crimmins and Baker, 2006) and oxy-PAHs (Wei et al., 2012) were performed using gas chromatography with mass spectrometer (GC-MS; Agilent 7820A + 5975 MSD, USA). An inert 5% phenyl - methyl column, VF - 5ms (30 m × 0.250 mm, 0.25 μm film thickness) was used for separating the compounds and 1 μL of the sample was injected in splitless mode. For plotting the calibration curve, the nitro-PAHs were procured from NIST (SRM 2265). The oven temperature program was: 40 °C (held 1.7 min) ramped to 150 °C at 20 °C. min^{-1}, held for 10 min, then to 220 °C at 10 °C min^{-1}, held for 10 min and finally ramped to 310 °C and held for 15 min. The oxy-PAHs standard was procured from Sigma-Aldrich and the chromatographic conditions were as follows: injector temperature 290 °C and detector temperature 250 °C. The temperature ramp was: an initial oven temperature of 80 °C maintained for 4 min, and increased to 150 °C at 40 °C min^{-1}, maintained for 5 min, then increased at 5 °C min^{-1} to a maximum of 300 °C for 10 min. Helium was used as carrier gas (at a flow rate of 1.2 mL min^{-1}). The limits of detection (LOD) and quantification (LOQ) for the nitro and oxy-PAH compounds are presented in Table S1.

2.3. Cancer risk assessment

To estimate the excess lifetime cancer risk (LCR) for inhalation exposure to nitro-PAHs, the benzo[*a*]pyrene equivalency concentrations (B[*a*]P_{eq}) were determined using the current relative potency factors (RPF) scales with known toxicity equivalency factor (TEF) values, Table 2. The cancer risk was determined by multiplying the concentrations of each nitro-PAHs compound with its corresponding TEF and then by the unit risk, as:

$$\text{Cancer risk} = \sum ([\text{nitro-PAHs}] \text{ x } \text{TEF}_{\text{nitro-PAHs}}) \text{ x unit risk}$$

The unit risk used was defined by the World Health Organization (WHO) for B[*a*]P as 8.7×10^{-5} ng/m^3 (theoretical upper limit of the possibility of contracting cancer when exposed to B[*a*]P at a concentration of 1 ng/m^3 of air for a 70-year lifetime, based on an epidemiological study of coke oven workers in Pennsylvania) (OEHHA, 1994; 2005, 2011; WHO, 2000; Callén et al., 2013).

2.4. Salmonella/microsome microsuspension assay

The *Salmonella*/microsome assay was performed using the microsuspension version of the method, as described by Kado et al. (1983, 1986), where overnight bacteria cultures are centrifuged to obtain a higher cell density compared to the traditional protocol. The exposure of a higher number of bacteria enhances the detection of mutagenic compounds in complex mixtures (Mortelmans and Zeiger, 2000), and it is a good strategy when sample quantity is limited. Due to limited amount of the extracts, only *Salmonella enterica* serovar Typhimurium strains TA98 (*his*D3052, *rfa*, Δ*bio*, Δ*uvr*B, Δ*chl*D, plasmid pKM101) that preferably detects frameshift mutations and its derivative YG1041 (=TA98 with plasmid pYG233), with high levels of both nitroreductase and acetyltransferase activity, were tested (Maron and Ames, 1983; Hagiwara et al., 1993).

All assays were performed in the absence and presence of *in vitro* metabolic activation, S9 mix (4%, Moltox Inc., NC, USA). Three independent tests were conducted for each extract, with doses varying between 1 and 500 μg of EOM equivalent per plate. Positive controls used were 0.125 μg/plate of 4-nitroquinoline-1-oxide (4NQO - Alfa Aesar), 2.5 μg/plate of 4-nitro-*o*-phenylenediamine (4NOP – Sigma-Aldrich), 0.625 μg/plate and 31.25 ng/plate of 2-aminoanthracene (2AA – Sigma-Aldrich) for TA98-S9, YG1041-S9, TA98 + S9 and YG1041 + S9, respectively.

2.5. Cell culture and exposure

Human lung adenocarcinoma epithelial cells, A549 (American Type Culture collection) were cultured in Dulbecco's modified Eagle's medium (DMEM) – high glucose, supplemented with fetal bovine serum (FBS) 10% (v/v), antibiotics (100 U/mL of penicillin and 100 μg/mL of streptomycin), L-glutamine (2 mM) and maintained at 37 °C in a humidified 5% CO_2 atmosphere. The EOM from intense and moderate biomass burning periods were dissolved in dimethyl sulfoxide (DMSO) at the time of use, never exceeding 0.1% of DMSO in the culture. The final concentrations used were 50 μg/mL, 100 μg/mL, 200 μg/mL, and 400 μg/mL for 24 h of treatment period. The untreated cells, vehicle (DMSO 0.1%), and a mix of PAHs (10 ng/mL, Supelco/Sigma SS EPA 610 PAH MIX) were used as control groups, similar to Roubicek et al. (2007) and de Oliveira Alves et al. (2014). The experiments were conducted in triplicate.

2.6. Viability assay

Cell viability was determined by the mitochondria-dependent reduction of MTT (3-(4,5-dimethyl-thiazol-2y)2,5-diphenyl-tetrazolium bromide, Sigma) to formazan as described by Mosmann (1983). Briefly, the cells were plated at a density of 4×10^3 cells per well in 96-well plates until cell adhesion (24 h). After treatment, the cells were washed with PBS followed by incubation in serum- and phenol-red free medium containing 0.5 mg/mL of MTT solution for 4 h. Thereafter, the medium was removed and 100 μL of

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DMSO was added to each well to dissolve the formazan crystals. The plates were then protected from light and kept under agitation for 5 min. After shaking, the absorbance was read at a wavelength of 570 nm in a μQuant-microplate spectrophotometer (Biotek, USA). Data were expressed as percent of control.

2.7. Cytokinesis-block micronucleus assay (CBMN)

The CBMN assay was conducted using the standard technique proposed by Fenech (2000, 2007), with some modifications. Briefly, A549 cells were plated in 6-well plates at a density of 1.5×10^5 cells per well until cell adhesion (24 h). Subsequently, the cells were exposed to the different concentrations of EOM. After the exposure time (24 h), EOM was removed, and cytochalasin B (final concentration 5 μg/mL, Sigma-Aldrich) was added. The cells remained in the incubator during 24 h, followed by trypsinization and centrifugation at 300*g* for 5 min. Finally, the cells were fixed in three centrifugation cycles (300 g, 5 min) with freshly made cold methanol: acetic acid (9:1) fixative solution, and then homogenized. A drop with 20–30 μL of the material was evenly dropped onto a previously washed slide, placed above a hot water bath (40–60 °C) for 10–20 s, and stained using 10% Giemsa in potassium phosphate buffer solution for 8 min (Cornélio et al., 2014). Three thousand binucleated cells were scored per treatment for evaluation of nuclear alterations such as micronuclei, nucleoplasmic bridges, and nuclear buds, as classified by Fenech et al. (2003).

The nuclear division index (NDI) provides important information about cytostatic properties of the test chemical or mixture. NDI was calculated as follows:

$$NDI = [M1 + 2(M2) + 3(M3) + 4(M4)]/N$$

where M1–M4 indicate the number of cells with 1–4 main nuclei and N is the total number of viable cells scored as recommended by Fenech (2007).

2.8. Minimal effective dose – MED 1.5

To be able to compare such different endpoints, as the *Salmonella* test and the micronucleus assay results, the MED1.5 was calculated. The MED1.5 corresponds to the concentration/dose that can cause a 50% increase of the effect observed in comparison to the control values. The MED1.5 can be derived using the linear regression curve for each of the assays. Although a ratio of 2 is considered as a positive criterion for the *Salmonella* test, which would be a 100% increase in the number of revertants above the control values, the MED1.5 was chosen because, as pointed out by Escher et al. (2014), it is employed in several guideline documents, is close to the detection limit of many biological assays, and is an interpolation not an extrapolation of the dose-response curve.

2.9. Statistical analysis

The *Salmonella*/microsome microsuspension test results were statistically analyzed by applying the Bernstein model (Bernstein et al., 1982) with the *Salanal* computer program (Salmonella Assay Analysis, version 1.0, Integrated Laboratory Systems, RTP, NC, USA). Samples were considered mutagenic when there was a significant difference between doses (ANOVA, $p < 0.05$), positive dose-response curve ($p < 0.05$), and mutagenicity ratio (MR) ≥ 2. The mutagenic potency of a sample is the slope of the regression curve obtained, and is expressed as revertants per unit of mass or volume of the sample tested. In this study, to allow comparisons with other studies alike, the *Salmonella*/microsome results were expressed as the number of revertants per mg of EOM (rev/mg EOM), per ng of PAHs (rev/ng PAH), per μg of PM_{10} (rev/μg PM_{10}), and per m^3 (rev/m^3), as shown in Table 3.

For cytotoxicity and mutagenicity tests with A549 cells, one-way ANOVA followed by Dunnett's test was used to determine the significance level between the treated and control group. Statistical differences were considered significant at $p < 0.05$.

3. Results

3.1. Airborne PM and EOM concentrations

Time series of PM_{10}, EOM, and fire spots are shown in Fig. 1. In accordance with the results obtained with the MODIS sensor, larger number of fire spots occurred between July and October/2011 (INPE, 2014). Higher peaks of PM_{10} concentrations were observed during intense biomass burning, with concentrations reaching close to 60 μg/m^3.

Table 1 shows the number of filters, total collected air volume, concentration of PM_{10}, EOM and PAHs for each of the two samples tested for mutagenicity. The concentration in Table 1 corresponds to the sum of the mass of the all filters by the total air volume and was used to calculate the concentrations in Table 3.

3.2. Analysis of organic compound content in air PM extracts

The unsubstituted PAHs profile for the moderate (retene > phenanthrene > anthracene > chrysene > dibenzo[*a,h*] anthracene) and intense biomass burning period (retene > anthracene > dibenzo[*a,h*] anthracene > phenanthrene > benzo[*e*]pyrene) were previously published by our group (de Oliveira Alves et al., 2015). Nitro- and oxy-PAHs are a part of the complex mixture in the organic extract of PM_{10} fraction collected. The concentrations of these compounds are shown in Fig. 2. Among the nitro-PAHs identified, 2-nitrofluoranthene > 7-nitrobenz[*a*]anthracene > 1-nitropyrene > 3-nitrofluoranthene > 6-nitrochrysene were most abundant for the intense biomass burning period. On the other hand, 2-methylanthraquinone > benz[*a*]anthracene-7,12-dione > 9,10-anthraquinone > 9-fluorenone showed highest concentrations among the oxy-PAHs. The average concentrations of 1-nitropyrene and 6-nitrochrysene during intense biomass burning

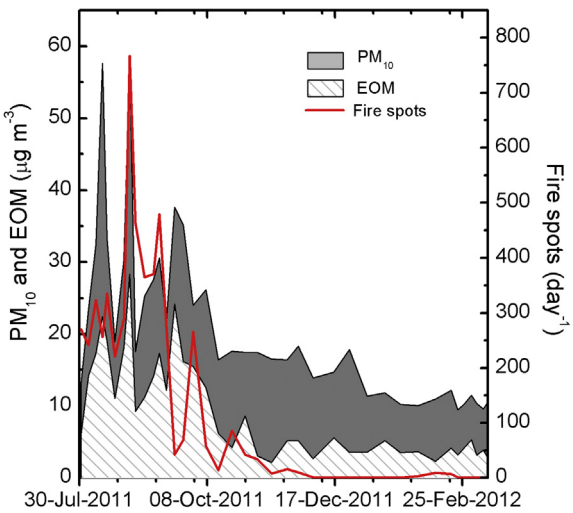

Fig. 1. Concentration time series of PM_{10}, extracted organic matter (EOM) and fire spots obtained by MODIS sensor for intense biomass burning (August–October/2011) and moderate biomass burning (November/2011–March/2012) periods in the Brazilian Amazon region.

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Table 1
Number of filters, total collected air volume, concentration of PM_{10}, EOM, and PAHs for each of the two samples tested.

Sample	# filters	Volume (m^3)	Mass (mg)	PM_{10} ($\mu g/m^3$)	EOM ($\mu g/m^3$)	∑PAHs* (ng/m^3)
Moderate	23	189,874.1	2889.1	13.7	4.4	1.3
Intense	17	103,813.6	2597.3	27.8	14.9	2.9

EOM: Extractable Organic Matter. *PAHs - fluorene, phenanthrene, anthracene, fluoranthene, pyrene, retene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indene[1,2,3-*c*,*d*]pyrene, dibenz[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]pyrene.

period were 125 ± 28 pg/m^3 (range 83–206) and 83 ± 27 pg/m^3 (range 53–131), respectively. Whereas, the average concentration of 4-nitropyrene for the same period was 39 ± 13 pg/m^3 (range 30–62), which was approximately twice the concentration found during the moderate biomass burning period. Furthermore, high levels of oxy-PAH, 9,10-anthraquinone, were observed during fire incidences. The average concentration values of all nitro- and oxy-PAHs are shown in Fig. 2.

3.3. Cancer risk assessment

The mean, minimum, and maximum concentrations of the nitro-PAHs with known TEF, B[*a*]P_{eq} values and LCR for nitro-PAH exposure are shown in Table 2. Although 11 nitro-PAHs were quantified, only 3 of them have defined TEF values. The excess lifetime cancer cases per 100,000 people exposed were 4.2 and 7.3 for moderate and intense biomass burning seasons, respectively (Table 2).

3.4. Salmonella mutagenicity assay

The *Salmonella*/microsome results for TA98 and YG1041 are presented in Table 3. The significant difference between doses, positive dose-response curve, and mutagenicity ratio for both periods and stains are presented in Tables S2 and S3. The EOM from the intense burning period was more mutagenic than that from the moderate burning period, for both the *S.* Typhimurium strains. Interestingly, potencies were higher in the absence of metabolic activation, regardless of the strain used, showing a large contribution of direct acting mutagens. The much higher potencies observed with YG1041 demonstrate the significant presence of nitro-compounds (Tables 3 and S3).

3.5. Cell viability

The cytotoxic effects of EOM are shown in Fig. 3. Significant differences in cytotoxicity were found neither at any of the EOM concentrations tested, nor between the different organic extracts. The mix of PAHs (10 ng/mL) did not significantly influence the cell viability and, thus, cells could be exposed to all concentrations in the mutagenicity assays.

Table 3
Mutagenicity potencies in *Salmonella* strain TA98 and YG1041 per unit of mass of extracted organic matter (rev/mg EOM), per ng of PAHs (rev/ng PAH), per μg of PM_{10} (rev/μg PM_{10}), and per m^3 (rev/m^3), with and without *in vitro* metabolic activation (S9) for moderate and intense Amazon biomass burning periods.

strain	Moderate biomass burning				Intense biomass burning			
potency	TA98		YG1041		TA98		YG1041	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
rev/mg EOM	64	27	730	99	680	240	3800	1500
rev/ng PAHs[a]	0.4	0.2	4	0.6	30	10	166	65
rev/μg PM_{10}	4	2	44	6	280	99	1570	620
rev/m^3	0.3	0.1	3	0.4	10	4	57	22

[a] PAHs - fluorene, phenanthrene, anthracene, fluoranthene, pyrene, retene, benz[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*e*]pyrene, benzo[*a*]pyrene, indene[1,2,3-*c*,*d*]pyrene, dibenz[*a*,*h*]anthracene and benzo[*g*,*h*,*i*]pyrene.

3.6. Micronuclei and nuclear abnormalities tests

Micronuclei frequencies and NDI are presented in Fig. 4. A dose-dependent increase in the MN frequency was observed for both moderate period and intense burning period extracts, the differences were not statistically significant. Moreover, no significant increase in the nucleoplasmic bridges and nuclear buds formation compared with negative controls were observed (data not shown), regardless of the extract tested.

The NDI ranged from 1.60 to 1.78, and 1.65 to 1.70 for moderate and intense burning period, respectively, indicating no significant difference between the samples and the negative control. Thus, it was concluded that the samples did not have any cytotoxic effect in the concentrations used (Fig. 4). These results corroborate those of cytotoxicity data obtained by MTT assay (Fig. 3).

3.7. MED1.5

The minimal effective doses that were able to induce a 50% increase in the number of revertants and in the frequency of micronuclei induced by the extracts are shown in Fig. 5. They varied from 11 to 383 μg of EOM depending on the test and condition used (with/without metabolic activation). The *Salmonella*/microsome test was extremely sensitive to the mutagens contained in the intense burning extract, as only 11 μg of the EOM was enough to

Table 2
Estimation of the excess lifetime cancer risk (LCR) for inhalation exposure to nitro-PAHs during intense and moderate Amazon biomass burning periods.

Nitro-PAHs (ng. m^{-3})	TEF[a]	Moderate biomass burning	B[*a*]P_{eq}[b]	Intense biomass burning	B[*a*]P_{eq}[b]	Excess lifetime cancer cases/100,000 people[c]	
		Mean (Min - Max)	Mean (Min - Max)	Mean (Min - Max)	Mean (Min - Max)	Moderate	Intense
1-nitropyrene	0.1[d]	0.07 (0.044–0.102)	0.007 (0.004–0.01)	0.125 (0.082–0.205)	0.012 (0.008–0.020)	–	–
4-nitropyrene	0.1[d]	0.02 (0.014–0.032)	0.002 (0.001–0.003)	0.04 (0.030–0.062)	0.004 (0.003–0.006)	–	–
6-nitrochrysene	10[d]	0.048 (0.028–0.085)	0.48 (0.28–0.85)	0.083 (0.053–0.131)	0.83 (0.53–1.31)	–	–
∑3Nitro-PAHs	–	0.138	–	0.248	–	–	–
∑B[*a*]P_{eq}	–	–	0.489	–	0.846	–	–
Cancer risk	–	–	–	–	–	4.2	7.3

[a] TEF is toxicity equivalency factor of individual nitro-PAHs relative to benzo[*a*]pyrene.
[b] B[*a*]P_{eq} is the B[*a*]P equivalency concentrations.
[c] The unit risk used was defined by the WHO for B[*a*]P set to 8.7×10^{-5}ng/m^3.
[d] Obtained from OEHHA (1994, 2005, 2011).

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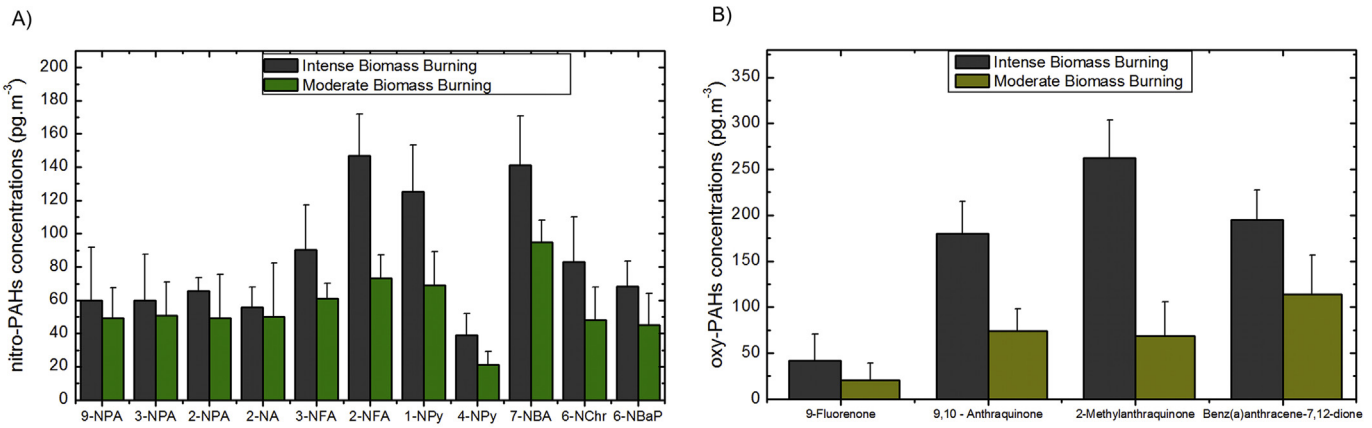

Fig. 2. Average concentrations of (**A**) nitro-PAHs and (**B**) oxy-PAHs for intense and moderate biomass burning periods. 9-NPA (9-nitrophenanthrene); 3-NPA (3-nitrophenanthrene); 2-NPA (2-nitrophenanthrene); 2-NA (2-nitroanthracene); 3-NFA (3-nitrofluoranthene); 2-NFA (2-nitrofluoranthene); 1-NPy (1-nitropyrene); 4-NPy (4-nitropyrene); 7-NBA (7-nitrobenz[*a*]anthracene); 6-NChr (6-nitrochrysene); 6-NBaP (6-nitrobenzo[*a*]pyrene).

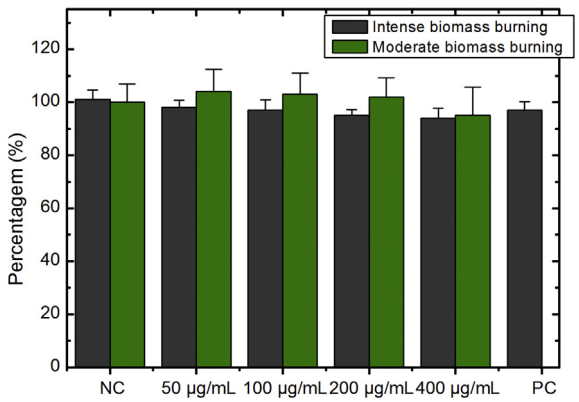

Fig. 3. A549 cells viability after 24 h of exposure to different concentrations of extractable organic matter (EOM) for moderate and intense biomass burning samples. NC = negative control (DMSO 0.1%); PC = positive control (10 ng/mL of a PAHs mixture).

induce 50% increase in mutations. In comparison, the micronucleus assay required 176 µg of the EOM to induce a 1.5-fold increase in the micronucleus rate. For the moderate burning period extract, the two tests showed only small differences in sensitivity.

4. Discussion

In this study, we quantified the nitro-PAHs and oxy-PAHs present in the emitted PM from the Amazon biomass burning and estimate excess lifetime cancer risk as well as the *in vitro* mutagenic effects of the extractable organic matter. We also compared the sensitivity of a prokaryote (*Salmonella*/microsome test) and a eukaryote (CBMN with human lung cells) mutagenicity assays.

PM from burning of biomass can substantially degrade air quality, leading to adverse effects on human health (Jacobson et al., 2014; Reddington et al., 2015). Johnston et al. (2012) demonstrated that landscape fire emissions are an important contributor to global mortality. Around 15% of the Brazilian Amazon was deforested between 1976 and 2010 (Aragão et al., 2014) and the emissions resulting from burning of biomass from the Amazon region significantly enhanced the organic content of the fine aerosol above to 90% (Artaxo et al., 2013). The PM toxicity seems to be strongly influenced by the organic fraction, and is probably associated with organic components in addition to the commonly measured unsubstituted PAHs (Bølling et al., 2012).

During the intense period of biomass burning in Amazon, 7-nitrobenz[*a*]anthracene, 1-nitropyrene and 3-nitrophenanthrene outdoor concentrations were 141 ± 30 pg/m^3 (range 63–174), 125 ± 28 pg/m^3 (range 83–206), and 60 ± 28 pg/m^3 (range 34–97),

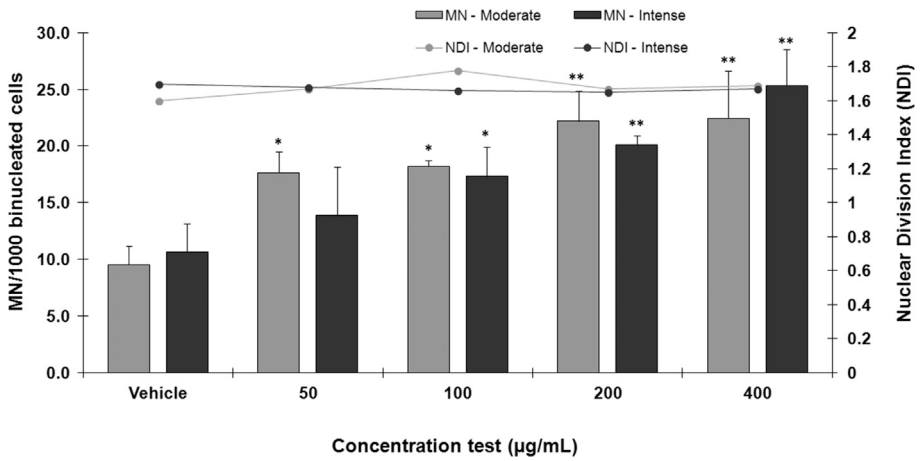

Fig. 4. Micronuclei frequencies and nuclear division index (NDI) in A549 cells for moderate and intense biomass burning samples, at all testes concentrations of Amazon extractable organic matter. Vehicle: DMSO 0.1%. $^*p < 0.05$ e $^{**}p < 0.01$, statistically significant compared to negative control according to Dunnett's test.

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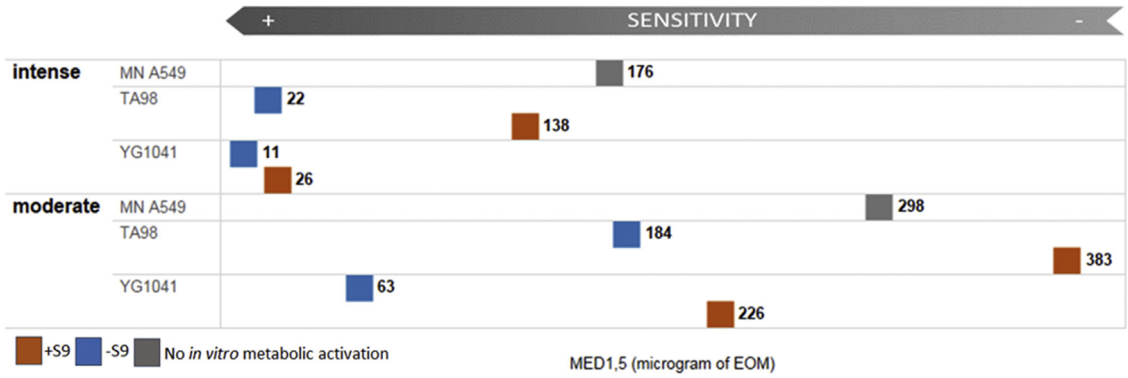

Fig. 5. Minimal effective doses (MED1.5) in μg of extractable organic matter (EOM), able to induce a 50% increase of the observed effect (revertants or micronuclei) in comparison with control values, for the intense and moderate biomass burning extracts.

respectively, in the ambient air (Fig. 2A). Shen et al. (2016) assessed the personal inhalation exposure to nitro-PAHs from residential solid fuel combustion in rural China and found values for 7-nitrobenz[*a*]anthracene (<30 pg/m^3), 1-nitropyrene (<30 pg/m^3), and 3-nitrophenanthrene (<15 pg/m^3) below those found in the current study.

The LCR for nitro-PAH exposure was of 4.2×10^{-5}, and 7.3×10^{-5}, for moderate and intense burning periods, respectively (Table 2). Although these values did not exceed the WHO health-based guideline value (8.7×10^{-5}), they are relevant because LCR might be underestimated as only the three nitro-PAHs (1-nitropyrene, 4-nitropyrene, 6-nitrochrysene) with established TEFs were taken into account (OEHHA, 1994; 2005, 2011). To the best of our knowledge, no other study has demonstrated the inhalation cancer risk estimated for nitro-PAHs from biomass burning. The LCR calculated for the ∑8PAHs identified in the same samples were 7×10^{-5}, and 17×10^{-5} for moderate and intense seasons, respectively (de Oliveira Alves et al., 2015). While LCR values between 10^{-4}–10^{-6} suggest potential risks, values smaller than 10^{-6} are considered as a negligible risk, and above 10^{-4} represent a high risk (Chen and Liao, 2006).

Interestingly, studies that estimated LCR for exposure to PAHs in urban PM samples in New York, USA (4.2×10^{-5}) (Jung et al., 2010); Nanjing, China (1.44×10^{-5} for male adults) (Wang et al., 2016), and Madrid, Spain (Mirante et al., 2013) estimated a lower carcinogenic risk than the established standards by environmental and health agencies. Higher LCR values were obtained for Kandy, Sri Lanka (3.31×10^{-3}) (Wickramasinghe et al., 2011); Zaragoza, Spain ($1.9–8.2 \times 10^{-5}$) (Callén et al., 2014) and Cordoba, Argentina ($5.13–12.3 \times 10^{-5}$) (Amarillo et al., 2014).

Despite the more pronounced toxic effects of several nitro-PAHs than those of PAHs, among the derivatives PAHs identified in this study, only few of them have IARC classification (Bandowe and Meusel, 2017). 1-nitropyrene and 6-nitrochrysene are classified as human probable carcinogens (Group 2A), and 4-nitropyrene and anthraquinone as possibly carcinogen (Group 2B) (IARC, 2014). Further, despite the knowledge about genotoxicity and/or carcinogenicity of PAHs, there are limited/few studies concerning isolated nitro- and oxy-PAH compounds (Table S4).

Amongst the nitro-PAHs found in Amazon biomass burning in the current study, 1-nitropyrene and 6-nitrochrysene were reported as mutagenic for TA98 and TA100 *Salmonella* strains, mostly with a higher response without S9 than with S9 (Tokiwa et al., 1981; Salmeen et al., 1982; Fu et al., 1988). 1-nitropyrene is the most studied compound which has also showed a positive response in mutagenesis assays (Durant et al., 1996). Bonnefoy et al. (2010) showed increase of mutagenicity for 1-nitropyrene in human lymphocytes and the majority of micronuclei (63%) were generated without centromeres, suggesting a clastogenic effect of this compound. Recently, Cervena et al. (2016) found higher micronuclei frequencies in human bronchial epithelial cells (BEAS-2B) exposed to 1-nitropyrene.

Although oxy-PAHs are classified as possibly carcinogens by IARC, they are less studied than the nitro-derivatives. In addition, anthraquinone has been shown to lack any mutagenic effect in multiple models such as the *Salmonella* test, mouse lymphoma forward mutation assay, chromosomal alterations, and *in vivo* mouse bone marrow MN assay (Butterworth et al., 2001). Similar negative results were obtained by Durant et al. (1996) in the forward mutagenesis assay using h1A1v2 cells. Further, no significant DNA damage, as measured by the Comet Assay, was found in A549 cells exposed to 9,10-anthraquinone (Shang et al., 2013). 2-methylanthraquinone was identified at high concentrations in our samples, especially during intense biomass burning period (Fig. 2B). Shang et al. (2014) exposed A549 cells to this compound, and did not find statistically significant changes in the level of DNA tail moment (Comet assay) (Shang et al., 2014). This suggests that nitro- and oxy-PAH fractions present in the complex mixtures such as EOM, evaluated in this study, contribute differently to the mutagenic effects observed. Further, the contribution of nitro-PAHs seems to play a major role in the effects of EOM.

There are very few genetic toxicology studies that have assessed nitrated and oxygenated PAHs emitted from biomass burning. The use of different strains in the *Salmonella*/microsome assay is a strategy to detect compounds from different chemical groups that induce mutagenic activity (Mortelmans and Zeiger, 2000; Marvin and Hewitt, 2007; Mutlu et al., 2013). The YG *Salmonella* strains are more sensitive indicators of nitroaromatic mutagens than the classic TA strains (Claxton et al., 2004). In our study, the mutagenic responses were higher with *Salmonella* strain YG1041 compared to TA98 for both moderate and intense burning extracts, indicating the possible contribution of nitroaromatic compounds contained in the EOM from the air sampled in the Amazon region (Hagiwara et al., 1993; Umbuzeiro et al., 2008b). This can be explained by the fact that the elevated levels of nitroreductase and *O*-acetyltransferase in YG1041 could activate low concentration compounds present in the samples that could not be activated by the normal levels of nitroreductase present in TA98 (Einistö et al., 1991; DeMarini et al., 2004).

In addition, nitro-PAHs are known to be direct-acting mutagens (Fu, 1990; Jung et al., 1991; Claxton et al., 2004; Jariyasopit et al., 2014a, 2014b), and the higher mutagenic potencies were obtained for both strains (TA98 and YG1041) and both samples (moderate and intense) in the absence of metabolic activation (Table 3,

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TablesS2 and S3).

Umbuzeiro et al. (2008a) used TA98 and YG1041 *Salmonella* strains to investigate the mutagenicity of organic fractions of PM from sugar cane harvesting season. The most polar fractions analyzed (nitro and oxy-PAHs) possessed highest mutagenicity. Consistent with the results obtained in the current study, a strong increase in the mutagenic activity using YG1041 stain and a decrease of response in the presence of *in vitro* metabolic activation were also observed. Similar results were obtained for urban atmospheric PM samples from other Brazilian cities, as São Paulo (Umbuzeiro et al., 2008b), Limeira (Alves et al., 2016), Montenegro and Santo Antônio da Patrulha (Pereira et al., 2013), and Esteio, which is under the influence of atmospheric emissions from an oil refinery (Coronas et al., 2009).

In this study, although the results point out to a strong involvement of nitro-PAHs in the mutagenicity observed, the experiments were done using whole extracts. From the *Salmonella*/microsome data it is not possible to directly correlate the increase of the mutagenic activity with only a specific class of compounds such as nitro-PAHs derived from the Amazon biomass burning. For this purpose, fractionation of the extracts and evaluation of the specific PAH derivatives should be performed.

The CBMN has become one of the standard cytogenetic assays for genetic toxicology testing in cultured human cells because scoring is restricted to binucleated cells, i.e. those that have divided once (Fenech, 2000, 2007). Moreover, the increase in MN formation is associated with early events in carcinogenesis, is predictive of cancer (Bonassi et al., 2011), and is a sensitive and specific predictor especially of lung cancer risk (El-Zein et al., 2006, 2008; Lloyd et al., 2013).

There are few reports assessing CBMN biomarkers in cultured cell lines exposed to PM from environmental air samples. The EOM from both moderate and intense Amazon biomass burning were able to induce an increase in the frequency of MN in A549 cells (Fig. 4). A pioneer study carried out by our group demonstrated genotoxic effects of organic PM collected in Alta Floresta city, also belonging to Brazilian Amazon deforestation arc (de Oliveira Alves et al., 2014). Despite the differences in the PAH concentrations between both regions, the data obtained for Porto Velho in this study are in consonance with those from Alta Floresta. Similar to treatment with samples from Porto Velho, a significant increase in the MN frequencies without an increment in the nucleoplasmic bridges and nuclear buds induction was observed on treatment of A549 cells with samples from Alta Floresta.

According Gutiérrez-Castillo et al. (2006) and Roubicek et al. (2007) the water-soluble transition metals and the organic-soluble fraction of PM are both important in inducing DNA damage. However, organic extracts appeared to be more effective for the biological effects induced by the different wood combustion particles than the corresponding washed particles (Bølling et al., 2012). León-Mejía et al. (2016) assessed the genotoxic effects in Chinese hamster lung fibroblast cell line (V79) exposed to coal fly ash particles at concentrations similar to the ones used in this study (37.5–800 μg/mL). They found a dose-dependent increase in micronuclei formation, with statistical significance only at the concentration of 600 μg/mL. Our results show significantly increased MN formation due to exposure to organic extracts at concentrations ranging from 50 to 400 μg/mL (Fig. 4). Similar increase in MN frequencies was observed for the organic fraction from coke oven emissions in HepG2 cells (Xin et al., 2014) and for an urban sample from Italy in A549 cells (Bocchi et al., 2016).

The MED1.5 values showed that the *Salmonella*/microsome test was extremely sensitive to the mutagens contained in the intense burning period extract compared with CBMN in A549 cells (11 μg vs. 176 μg) (Fig. 5). Even bioassays that are indicative of the same mode of action have slightly different numeric trigger values due to differences in their inherent sensitivity (Escher et al., 2015). As metabolism is the most crucial modifier of the biological effects of organic pollutants, detoxifying many chemicals and activating others, the metabolic capacity of a bioassay needs to be considered when selecting or designing a bioassay (Escher et al., 2014). The A549 cell line was chosen mostly because it maintains many features of normal type II cells from human pulmonary epithelium, expresses wild-type p53 and mRNAs for CYPs 1A1, 1B1, 2B6, 2C8-19, 2E1, 3A5, and 3A7 (Jia et al., 1997; Foster et al., 1998; Hukkanen et al., 2000; Roubicek et al., 2007). MED1.5 data (Fig. 5) reinforce the idea that majority of organic compounds contained in the samples act as direct mutagens, and suggest possible detoxification reactions in A549 cells.

It is important to point out that most of the PM_{10} measurements did not exceed the 50 $\mu g/m^3$ limit established by the World Health Organization (WHO, 2005). Nevertheless, we observed mutagenic effects that can be associated with PAHs and their derivatives found in the particles from emissions due to biomass burning in the Brazilian Amazon region.

5. Conclusions

This study addressed the effects of extractable organic matter of PM emitted from Amazon biomass burning on DNA damage and showed its mutagenic activity using different bioassays. The *Salmonella*/microsome test was considerably more sensitive to the organic matter in comparison with the CBMN using human lung cells mainly for the intense burning period samples. This was the first study to assess the mutagenicity of the samples collected in Amazon region using *Salmonella*/microsome test. The data suggest that nitroaromatic compounds with direct-acting frameshift mutagenic activity contribute significantly to DNA damage. This study revealed that even though majority of PM_{10} concentrations were lower than the air quality standards set by the World Health Organization, the strong presence of compounds with mutagenic effects, particularly nitro-PAHs 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.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.envpol.2017.09.068.

References

Alves, D.K., Kummrow, F., Cardoso, A.A., Morales, D.A., Umbuzeiro, G.A., 2016. Mutagenicity profile of atmospheric particulate matter in a small urban center subjected to airborne emission from vehicle traffic and sugar cane burning. Environ. Mol. Mutagen 57, 41–50. https://doi.org/10.1002/em.21970.

Amarillo, A.C., Busso, I.T., Carreras, H., 2014. Exposure to polycyclic aromatic hydrocarbons in urban environments: health risk assessment by age groups. Environ. Pollut. 195, 157–162. https://doi.org/10.1016/j.envpol.2014.08.027.

Aragão, L.E.O.C., Poulter, B., Barlow, J.B., Anderson, L.O., Malhi, Y., Saatchi, S., Phillips, O.L., Gloor, E., 2014. Environmental change and the carbon balance of Amazonian forests. Biol. Rev. 89, 913–931. https://doi.org/10.1111/brv.12088.

Artaxo, P., Rizzo, L.V., Brito, J.F., Barbosa, H.M.J., Arana, A., Sena, E.T., Cirino, G.G., Bastos, W., Martin, S.T., Andreae, M.O., 2013. Atmospheric aerosols in Amazonia and land use change: from natural biogenic to biomass burning conditions. Faraday Discuss. 165, 203–235. https://doi.org/10.1039/C3FD00052D.

Bandowe, B.A.M., Meusel, H., 2017. Nitrated polycyclic aromatic hydrocarbons (nitro-PAHs) in the environment – a review. Sci. Total Environ. https://doi.org/10.1016/j.scitotenv.2016.12.115.

Bernstein, L., Kaldor, J., McCann, J., Pike, M.C., 1982. An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test. Mutat. Res. 97, 267–281. https://doi.org/10.1016/0165-1161(82)90026-7.

Bocchi, C., Bazzini, C., Fontana, F., Pinto, G., Martino, A., Cassoni, F., 2016. Characterization of urban aerosol: seasonal variation of mutagenicity and genotoxicity of PM2.5, PM1 and semi-volatile organic compounds. Mutat. Res. Toxicol. Environ. Mutagen 809, 16–23. https://doi.org/10.1016/j.mrgentox.2016.07.007.

Bølling, A.K., Totlandsdal, A.I., Sallsten, G., Braun, A., Westerholm, R., Bergvall, C., et al., 2012. Wood smoke particles from different combustion phases induce similar pro-inflammatory effects in a co-culture of monocyte and pneumocyte cell lines. Part. Fibre Toxicol. 9, 45. https://doi.org/10.1186/1743-8977-9-45.

Bonassi, S., El-Zein, R., Bolognesi, C., Fenech, M., 2011. Micronuclei frequency in peripheral blood lymphocytes and cancer risk: evidence from human studies. Mutagenesis 26, 93–100. https://doi.org/10.1093/mutage/geq075.

Bonnefoy, A., Chiron, S., Botta, A., 2010. Environmental nitration processes enhance the mutagenic potency of aromatic compounds. Environ. Toxicol. 27, 321–331. https://doi.org/10.1002/tox.20644.

Boström, C.-E., Gerde, P., Hanberg, A., Jernström, B., Johansson, C., Kyrklund, T., Rannug, A., Törnqvist, M., Victorin, K., Westerholm, R., 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. Environ. Health Perspect. 110 (Suppl), 451–488. https://doi.org/10.1289/ehp.02110s3451.

Brenner, F.W., Villar, R.G., Angulo, F.J., Tauxe, R., Swaminathan, B., 2000. Salmonella nomenclature. J. Clin. Microbiol. 38, 2465–2467.

Brito, J., Rizzo, L.V., Morgan, W.T., Coe, H., Johnson, B., Haywood, J., Longo, K., Freitas, S., Andreae, M.O., Artaxo, P., 2014. Ground-based aerosol characterization during the South American biomass burning analysis (SAMBBA) field experiment. Atmos. Chem. Phys. 14, 12069–12083. https://doi.org/10.5194/acp-14-12069-2014.

Brown, J.S., Gordon, T., Price, O., Asgharian, B., 2013. Thoracic and respirable particle definitions for human health risk assessment. Part. Fibre Toxicol. 10, 12. https://doi.org/10.1186/1743-8977-10-12.

Bruschweiler, E.D., Hopf, N.B., Wild, P., Huynh, C.K., Fenech, M., Thomas, P., Hor, M., Charriere, N., Savova-Bianchi, D., Danuser, B., 2014. Workers exposed to wood dust have an increased micronucleus frequency in nasal and buccal cells: results from a pilot study. Mutagenesis 29, 201–207. https://doi.org/10.1093/mutage/geu003.

Butterworth, B.E., Mathre, O.B., Ballinger, K., 2001. The preparation of anthraquinone used in the National Toxicology Program cancer bioassay was contaminated with the mutagen 9-nitroanthracene. Mutagenesis 16, 169–177. https://doi.org/10.1093/mutage/16.2.169.

Callén, M.S., López, J.M., Iturmendi, A., Mastral, A.M., 2013. Nature and sources of particle associated polycyclic aromatic hydrocarbons (PAH) in the atmospheric environment of an urban area. Environ. Pollut. 183, 166–174. https://doi.org/10.1016/j.envpol.2012.11.009.

Callén, M.S., Iturmendi, A., López, J.M., 2014. Source apportionment of atmospheric PM2.5-bound polycyclic aromatic hydrocarbons by a PMF receptor model. Assessment of potential risk for human health. Environ. Pollut. 195C, 167–177. https://doi.org/10.1016/j.envpol.2014.08.025.

Cervena, T., Rossnerova, A., Sikorova, J., Beranek, V., Vojtisek-Lom, M., Ciganek, M., Topinka, J., Rossner, P., 2016. DNA damage potential of engine emissions measured in vitro by micronucleus test in human bronchial epithelial cells. Basic Clin. Pharmacol. Toxicol. https://doi.org/10.1111/bcpt.12693.

Chen, S.-C., Liao, C.-M., 2006. Health risk assessment on human exposed to environmental polycyclic aromatic hydrocarbons pollution sources. Sci. Total Environ. 366, 112–123. https://doi.org/10.1016/j.scitotenv.2005.08.047.

Claxton, L.D., Matthews, P.P., Warren, S.H., 2004. The genotoxicity of ambient outdoor air, a review: Salmonella mutagenicity. Mutat. Res. - Rev. Mutat. Res. 567, 347–399. https://doi.org/10.1016/j.mrrev.2004.08.002.

Cornélio, D.A., Tavares, J.C.M., Pimentel, T.V.C.D.A., Cavalcanti, G.B., Batistuzzo de Medeiros, S.R., 2014. Cytokinesis-block micronucleus assay adapted for analyzing genomic instability of human mesenchymal stem cells. Stem Cells Dev. 23, 823–838. https://doi.org/10.1089/scd.2013.0383.

Coronas, M.V., Pereira, T.S., Rocha, J.A.V., Lemos, A.T., Fachel, J.M.G., Salvadori, D.M.F., Vargas, V.M.F., 2009. Genetic biomonitoring of an urban population exposed to mutagenic airborne pollutants. Environ. Int. 35, 1023–1029. https://doi.org/10.1016/j.envint.2009.05.001.

Crimmins, B.S., Baker, J.E., 2006. Improved GC/MS methods for measuring hourly PAH and nitro-PAH concentrations in urban particulate matter. Atmos. Environ. 40, 6764–6779. https://doi.org/10.1016/j.atmosenv.2006.05.078.

de Oliveira Alves, N., Matos Loureiro, A.L., dos Santos, F.C., Nascimento, K.H., Dallacort, R., de Castro Vasconcellos, P., de Souza Hacon, S., Artaxo, P., de Medeiros, S.R.B., 2011. Genotoxicity and composition of particulate matter from biomass burning in the eastern Brazilian Amazon region. Ecotoxicol. Environ. Saf. 74, 1427–1433. https://doi.org/10.1016/j.ecoenv.2011.04.007.

de Oliveira Alves, N., de Souza Hacon, S., de Oliveira Galvão, M.F., Simões Peixoto, M., Artaxo, P., de Castro Vasconcellos, P., de Medeiros, S.R.B., 2014. Genetic damage of organic matter in the Brazilian Amazon: a comparative study between intense and moderate biomass burning. Environ. Res. 130, 51–58.

de Oliveira Alves, N., Brito, J., Caumo, S., Arana, A., de Souza Hacon, S., Artaxo, P., Hillamo, R., Teinilä, K., Batistuzzo de Medeiros, S.R., de Castro Vasconcellos, P., 2015. Biomass burning in the Amazon region: aerosol source apportionment and associated health risk assessment. Atmos. Environ. 120, 277–285. https://doi.org/10.1016/j.atmosenv.2015.08.059.

de Oliveira Galvão, M.F., Cabral, T. de M., de André, P.A., Andrade, M. de F., de Miranda, R.M., Saldiva, P.H.N., Vasconcellos, P. de C., de Medeiros, S.R.B., 2014. Cashew nut roasting: chemical characterization of particulate matter and genotocixity analysis. Environ. Res. 131, 145–152. https://doi.org/10.1016/j.envres.2014.03.013.

de Oliveira Galvão, M.F., de Queiroz, J.D.F., Duarte, E. de S.F., Hoelzemann, J.J., de André, P.A., Saldiva, P.H.N., Menezes Filho, J.A., Batistuzzo de Medeiros, S.R., 2017. Characterization of the particulate matter and relationship between buccal micronucleus and urinary 1-hydroxypyrene levels among cashew nut roasting workers. Environ. Pollut. 220, 659–671. https://doi.org/10.1016/j.envpol.2016.10.024.

DeMarini, D.M., Brooks, L.R., Warren, S.H., Kobayashi, T., Gilmour, M.I., Singh, P., 2004. Bioassay-directed fractionation and Salmonella mutagenicity of automobile and forklift diesel exhaust particles. Environ. Health Perspect. 112, 814–819. https://doi.org/10.1289/ehp.6578.

Durant, J.L., Busby, W.F., Lafleur, A.L., Penman, B.W., Crespi, C.L., 1996. Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. Mutat. Res. 371, 123–157.

Einistö, P., Watanabe, M., Motoi Ishidate, J., Nohmi, T., 1991. Mutagenicity of 30 chemicals in Salmonella typhimurium strains possessing different nitroreductase or O-acetyltransferase activities. Mutat. Res. Toxicol. 259, 95–102. https://doi.org/10.1016/0165-1218(91)90113-Z.

El-Zein, R.A., Schabath, M.B., Etzel, C.J., Lopez, M.S., Franklin, J.D., Spitz, M.R., 2006. Cytokinesis-blocked micronucleus assay as a novel biomarker for lung cancer risk. Cancer Res. 66, 6449–6456. https://doi.org/10.1158/0008-5472.CAN-06-0326.

El-Zein, R.A., Fenech, M., Lopez, M.S., Spitz, M.R., Etzel, C.J., 2008. Cytokinesis-blocked micronucleus cytome assay biomarkers identify lung cancer cases amongst smokers. Cancer Epidemiol. Biomarkers Prev. 17, 1111–1119. https://doi.org/10.1158/1055-9965.EPI-07-2827.

Escher, B.I., Allinson, M., Altenburger, R., Bain, P.A., Balaguer, P., Busch, W., et al., 2014. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. Environ. Sci. Technol. 48, 1940–1956. https://doi.org/10.1021/es403899t.

Escher, B.I., Neale, P.A., Leusch, F.D.L., 2015. Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. Water Res. 81, 137–148. https://doi.org/10.1016/j.watres.2015.05.049.

Fenech, M., 2000. The in vitro micronucleus technique. Mutat. Res. Mol. Mech. Mutagen 455, 81–95. https://doi.org/10.1016/S0027-5107(00)00065-8.

Fenech, M., Chang, W.P., Kirsch-Volders, M., Holland, N., Bonassi, S., Zeiger, E., 2003. HUMN project: detailed description of the scoring criteria for the cytokinesis-block micronucleus assay using isolated human lymphocyte cultures. Mutat. Res. - Genet. Toxicol. Environ. Mutagen 534, 65–75. https://doi.org/10.1016/S1383-5718(02)00249-8.

Fenech, M., 2007. Cytokinesis-block micronucleus cytome assay. Nat. Protoc. 2, 1084–1104. https://doi.org/10.1038/nprot.2007.77.

Foster, K., Oster, C., Mayer, M., Avery, M., Audus, K., 1998. Characterization of the A549 cell line as a type II pulmonary epithelial cell model for drug metabolism. Exp. Cell Res. 366, 359–366.

Fu, P.P., Heflich, R.H., Unruh, L.E., Shaikh, A.U., Wu, Y.-S., Lai, C.-C., Lai, J.-S., 1988. Relationships among direct-acting mutagenicity, nitro group orientation and polarographic reduction potential of 6-nitrobenzo[a]pyrene, 7-nitrobenz[a] anthracene and their derivatives. Mutat. Res. Lett. 209, 115–122. https://doi.org/10.1016/0165-7992(88)90027-9.

Fu, P.P., 1990. Metabolism of nitro-polycyclic aromatic hydrocarbons. Drug Metab. Rev. 22, 209–268.

Gutiérrez-Castillo, M.E., Roubicek, D.A., Cebrián-García, M.E., De Vizcaya-Ruíz, A., Sordo-Cedeño, M., Ostrosky-Wegman, P., 2006. Effect of chemical composition on the induction of DNA damage by urban airborne particulate matter. Environ. Mol. Mutagen 47, 199–211. https://doi.org/10.1002/em.20186.

Hagiwara, Y., Watanabe, M., Oda, Y., Sofuni, T., Nohmi, T., 1993. Specificity and sensitivity of Salmonella typhimurium YG1041 and YG1042 strains possessing elevated levels of both nitroreductase and acetyltransferase activity. Mutat. Res. 291, 171–180. https://doi.org/10.1016/0165-1161(93)90157-U.

Hukkanen, J., Lassila, A., Päivärinta, K., Valanne, S., Sarpo, S., Hakkola, J., Pelkonen, O., Raunio, H., 2000. Induction and regulation of xenobiotic-metabolizing cytochrome P450s in the human A549 lung adenocarcinoma cell line. Am. J. Respir. Cell Mol. Biol. 22, 360–366. https://doi.org/10.1165/ajrcmb.22.3.3845.

INPE, 2014. Portal do Monitoramento de Queimadas e Incêndios. [WWW Document]. Inst. Nac. Pesqui. Espac. http://www.inpe.br/queimadas (accessed 9.22.16).

International Agency for Research on Cancer, 2016. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: vol. 109, Outdoor Air Pollution. IARC, Lyon, France, 2016.

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

International Agency for Research on Cancer, IARC, 2014. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: vol. 105, Diesel and Gasoline Engine Exhausts and Some Nitroarenes, vol. 2014. IARC, Lyon, France.

International Agency for Research on Cancer, 2013. In: Cancer, Straif, K., Cohen, A., Samet, J. (Eds.), IARC Scientific Publication No. 161: Air Pollution, vol. 2013. IARC, Lyon, France.

International Agency for Research on Cancer, 2010. In: IARC Monographs on the Evaluation of Carcinogenic Risks to Humans: vol. 95, Household Use of Solid Fuels and High-temperature Frying, vol. 2010. IARC, Lyon, France.

Jacobson, L.D.S.V., Hacon, S.D.S., Castro, H.A., De, Ignotti, E., Artaxo, P., Saldiva, P.H.N., De Leon, A.C.M.P., 2014. Acute effects of particulate matter and black carbon from seasonal fires on peak expiratory flow of schoolchildren in the Brazilian Amazon. PLoS One 9. https://doi.org/10.1371/journal.pone.0104177.

Jariyasopit, N., McIntosh, M., Zimmermann, K., Arey, J., Atkinson, R., Cheong, P.H.-Y., Carter, R.G., Yu, T.-W., Dashwood, R.H., Massey Simonich, S.L., 2014a. Novel nitro- PAH formation from heterogeneous reactions of PAHs with NO2, NO3/N2O5, and OH radicals: prediction, laboratory studies, and mutagenicity. Environ. Sci. Technol. 48, 412–419. https://doi.org/10.1021/es4043808.

Jariyasopit, N., Zimmermann, K., Schrlau, J., Arey, J., Atkinson, R., Yu, T.-W., Dashwood, R.H., Tao, S., Massey Simonich, S.L., 2014b. Heterogeneous reactions of particulate matter-bound PAHs and nitro-PAHs with NO_3/N_2O_5, OH radicals, and O_3 under simulated long-range atmospheric transport conditions: reactivity and mutagenicity. Environ. Sci. Technol. 48, 10155–10164. https://doi.org/10.1021/es5015407.

Jarvis, I.W.H., Bergvall, C., Bottai, M., Westerholm, R., Stenius, U., Dreij, K., 2013. Persistent activation of DNA damage signaling in response to complex mixtures of PAHs in air particulate matter. Toxicol. Appl. Pharmacol. 266, 408–418. https://doi.org/10.1016/j.taap.2012.11.026.

Jarvis, W.H., Dreij, K., Mattsson, A., Jernström, B., Stenius, U., 2014. Interactions between polycyclic aromatic hydrocarbons in complex mixtures and implications for cancer risk assessment. Toxicology 321, 27–39.

Jia, L.Q., Osada, M., Ishioka, C., Gamo, M., Ikawa, S., Suzuki, T., Shimodaira, H., Niitani, T., Kudo, T., Akiyama, M., Kimura, N., Matsuo, M., Mizusawa, H., Tanaka, N., Koyama, H., Namba, M., Kanamaru, R., Kuroki, T., 1997. Screening the p53 status of human cell lines using a yeast functional assay. Mol. Carcinog. 19, 243–253. https://doi.org/10.1002/(SICI)1098-2744(199708)19:4<243::AID-MC5>3.0.CO;2-D.

Johnston, F.H., Henderson, S.B., Chen, Y., Randerson, J.T., Marlier, M., DeFries, R.S., Kinney, P., Bowman, D.M.J.S., Brauer, M., 2012. Estimated global mortality attributable to smoke from landscape fires. Environ. Health Perspect. 120, 695–701. https://doi.org/10.1289/ehp.1104422.

Jung, H., Heflich, R.H., Fu, P.P., Shaikh, A.U., Hartman, P., 1991. Nitro group orientation, reduction potential, and direct-acting mutagenicity of nitro-polycyclic aromatic hydrocarbons. Environ. Mol. Mutagen 17, 169–180.

Jung, K.H., Yan, B., Chillrud, S.N., Perera, F.P., Whyatt, R., Camann, D., Kinney, P.L., Miller, R.L., 2010. Assessment of benzo(a)pyrene-equivalent carcinogenicity and mutagenicity of residential indoor versus outdoor polycyclic aromatic hydrocarbons exposing young children in New York city. Int. J. Environ. Res. Public Health 7, 1889–1900. https://doi.org/10.3390/ijerph7051889.

Kado, N.Y., Langley, D., Eisenstadt, E., 1983. A simple modification of the Salmonella liquid-incubation assay. Increased sensitivity for detecting mutagens in human urine. Mutat. Res. 121, 25–32. https://doi.org/10.1016/0165-7992(83)90082-9.

Kado, N.Y., Guirguis, G.N., Flessel, C.P., Chan, R.C., Chang, K.I., Wesolowski, J.J., 1986. Mutagenicity of fine (less than 2.5 microns) airborne particles: diurnal variation in community air determined by a *Salmonella* microPreincubation (microsuspension) procedure. Environ. Mutagen 8, 53–66.

Kelly, F.J., Fussell, J.C., 2012. Size, source and chemical composition as determinants of toxicity attributable to ambient particulate matter. Atmos. Environ. 60, 504–526. https://doi.org/10.1016/j.atmosenv.2012.06.039.

Kessler, N., Schauer, J.J., Yagur-Kroll, S., Melamed, S., Tirosh, O., Belkin, S., Erel, Y., 2012. A bacterial bioreporter panel to assay the cytotoxicity of atmospheric particulate matter. Atmos. Environ. 63, 94–101. https://doi.org/10.1016/j.atmosenv.2012.09.048.

Kirkland, D., Reeve, L., Gatehouse, D., Vanparys, P., 2011. A core in vitro genotoxicity battery comprising the Ames test plus the in vitro micronucleus test is sufficient to detect rodent carcinogens and in vivo genotoxins. Mutat. Res. Toxicol. Environ. Mutagen 721, 27–73. https://doi.org/10.1016/j.mrgentox.2010.12.015.

Kirsch-Volders, M., Plas, G., Elhajouji, A., Lukamowicz, M., Gonzalez, L., Vande Loock, K., Decordier, I., 2011. The in vitro MN assay in 2011: origin and fate, biological significance, protocols, high throughput methodologies and toxicological relevance. Arch. Toxicol. 85, 873–899. https://doi.org/10.1007/s00204-011-0691-4.

Kirsch-Volders, M., Bonassi, S., Knasmueller, S., Holland, N., Bolognesi, C., Fenech, M.F., 2014. Commentary: critical questions, misconceptions and a road map for improving the use of the lymphocyte cytokinesis-block micronucleus assay for in vivo biomonitoring of human exposure to genotoxic chemicals—a HUMN project perspective. Mutat. Res. 759, 49–58. https://doi.org/10.1016/j.mrrev.2013.12.001.

León-Mejía, G., Silva, L.F.O., Civeira, M.S., Oliveira, M.L.S., Machado, M., Villela, I.V., Hartmann, A., Premoli, S., Corrêa, D.S., Da Silva, J., Henriques, J.A.P., 2016. Cytotoxicity and genotoxicity induced by coal and coal fly ash particles samples in V79 cells. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-016-7623-z.

Lloyd, S.M., Lopez, M., El-Zein, R., 2013. Cytokinesis-blocked micronucleus cytome assay and spectral karyotyping as methods for identifying chromosome damage in a lung cancer case-control population. Genes, Chromosom. Cancer 47. https://doi.org/10.1002/gcc.22065 n/a-n/a.

Malhi, Y., Roberts, J.T., Betts, R.A., Killeen, T.J., Li, W., Nobre, C.A., 2008. Climate change, deforestation, and the fate of the Amazon. Science 319, 169–172. https://doi.org/10.1126/science.1146961.

Maron, D.M., Ames, B.N., 1983. Revised methods for the Salmonella mutagenicity test. Mutat. Res. 113, 173–215. https://doi.org/10.1016/0165-1161(83)90010-9.

Marvin, C.H., Hewitt, L.M., 2007. Analytical methods in bioassay-directed investigations of mutagenicity of air particulate material. Mutat. Res. - Rev. Mutat. Res. 636, 4–35. https://doi.org/10.1016/j.mrrev.2006.05.001.

Mirante, F., Alves, C., Pio, C., Pindado, O., Perez, R., Revuelta, M.A., Artiñano, B., 2013. Organic composition of size segregated atmospheric particulate matter, during summer and winter sampling campaigns at representative sites in Madrid. Spain. Atmos. Res. 132–133, 345–361. https://doi.org/10.1016/j.atmosres.2013.07.005.

Mortelmans, K., Zeiger, E., 2000. The Ames Salmonella/microsome mutagenicity assay. Mutat. Res. 455, 29–60. https://doi.org/10.1016/S0027-5107(00)00064-6.

Mosmann, T., 1983. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 65, 55–63.

Mutlu, E., Warren, S.H., Matthews, P.P., King, C., Linak, W.P., Kooter, I.M., Schmid, J.E., Ross, J.A., Gilmour, M.I., DeMarini, D.M., 2013. Bioassay-directed fractionation and sub-fractionation for mutagenicity and chemical analysis of diesel exhaust particles. Environ. Mol. Mutagen 54, 719–736. https://doi.org/10.1002/em.21812.

Nemmar, A., Holme, J.A., Rosas, I., Schwarze, P.E., Alfaro-Moreno, E., 2013. Recent advances in particulate matter and nanoparticle toxicology: a review of the in vivo and in vitro studies. Biomed. Res. Int. 2013 https://doi.org/10.1155/2013/279371.

Office of Environmental Health Hazard Assessment (OEHHA), 1994. Benzo[a]pyrene as a Toxic Air Contaminant. In: Part B. Health Effects of Benzo[a]pyrene. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Air Toxicology and Epidemiology Section, Berkeley, CA.

Office of Environmental Health Hazard Assessment (OEHHA), 2005. Air Toxics Hot Spots Program Risk Assessment Guidelines. In: Part II. Technical Support Document for Describing Available Cancer Potency Factors. California Environmental Protection Agency, Office of Environmental Health Hazard Assessment, Air Toxicology and Epidemiology Section, Oakland, CA.

Office of Environmental Health Hazard Assessment (OEHHA), 2011. Chemical-specific Summaries of the Information Used to Derive Unit Risk and Cancer Potency Values. (Appendix B). http://www.oehha.ca.gov/air/hot_spots/tsd052909.html.

Organisation for Economic Cooperation and Development (OECD), 2016. OECD library, test No. 487: in vitro mammalian cell micronucleus test. In: OECD Guidelines for the Testing of Chemicals, Section 4. Health Effects, pp. 2074–5788. https://doi.org/10.1787/20745788 ISSN.

Palacio, I.C., Oliveira, I.F., Franklin, R.L., Barros, S.B.M., Roubicek, D.A., 2016. Evaluating the mutagenicity of the water-soluble fraction of air particulate matter: a comparison of two extraction strategies. Chemosphere 158, 124–130. https://doi.org/10.1016/j.chemosphere.2016.05.058.

Pereira, T.S., Beltrami, L.S., Rocha, J.A.V., Broto, F.P., Comellas, L.R., Salvadori, D.M.F., Vargas, V.M.F., 2013. Toxicogenetic monitoring in urban cities exposed to different airborne contaminants. Ecotoxicol. Environ. Saf. 90, 174–182. https://doi.org/10.1016/j.ecoenv.2012.12.029.

Raaschou-Nielsen, O., Andersen, Z.J., Beelen, R., Samoli, E., Stafoggia, M., Weinmayr, G., Hoffmann, B., et al., 2013. Air pollution and lung cancer incidence in 17 european cohorts: prospective analyses from the european study of cohorts for air pollution effects (ESCAPE). Lancet Oncol. 14, 813–822. https://doi.org/10.1016/S1470-2045(13)70279-1.

Reddington, C.L., Butt, E.W., Ridley, D.A., Artaxo, P., Morgan, W.T., Coe, H., Spracklen, D.V., 2015. Air quality and human health improvements from reductions in deforestation-related fire in Brazil. Nat. Geosci. 8, 768–771. https://doi.org/10.1038/ngeo2535.

Roubicek, D.A., Gutiérrez-Castillo, M.E., Sordo, M., Cebrián-García, M.E., Ostrosky-Wegman, P., 2007. Micronuclei induced by airborne particulate matter from Mexico City. Mutat. Res. - Genet. Toxicol. Environ. Mutagen 631, 9–15. https://doi.org/10.1016/j.mrgentox.2007.04.001.

Salmeen, I., Durisin, A.M., Prater, T.J., Riley, T.R., Schuetzle, D., 1982. Contribution of l-nitropyrene to direct-acting Ames assay mutagenicities of diesel particulate extracts. Mutat. Res. 104, 17–23.

Shang, Y., Fan, L., Feng, J., Lv, S., Wu, M., Li, B., Zang, Y.S., 2013. Genotoxic and inflammatory effects of organic extracts from traffic-related particulate matter in human lung epithelial A549 cells: the role of quinones. Toxicol. Vitr. 27, 922–931. https://doi.org/10.1016/j.tiv.2013.01.008.

Shang, Y., Zhang, L., Jiang, Y., Li, Y., Lu, P., 2014. Airborne quinones induce cytotoxicity and DNA damage in human lung epithelial A549 cells: the role of reactive oxygen species. Chemosphere 100, 42–49. https://doi.org/10.1016/j.chemosphere.2013.12.079.

Shen, G., Chen, Y., Du, W., Lin, N., Wang, X., Cheng, H., Liu, J., Xue, C., Liu, G., Zeng, E.Y., Xing, B., Tao, S., 2016. Exposure and size distribution of nitrated and oxygenated polycyclic aromatic hydrocarbons among the population using different household fuels. Environ. Pollut. 216, 1–8. https://doi.org/10.1016/j.envpol.2016.07.002.

Sisenando, H., de Medeiros, S., Artaxo, P., Saldiva, P.H., de Souza Hacon, S., 2012. Micronucleus frequency in children exposed to biomass burning in the Brazilian Legal Amazon region: a control case study. BMC Oral Health 12, 6. https://

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

doi.org/10.1186/1472-6831-12-6.

Thybaud, V., Aardema, M., Clements, J., Dearfield, K., Galloway, S., Hayashi, M., Jacobson-Kram, D., Kirkland, D., MacGregor, J.T., Marzin, D., Ohyama, W., Schuler, M., Suzuki, H., Zeiger, E., 2007. Strategy for genotoxicity testing: hazard identification and risk assessment in relation to in vitro testing. Mutat. Res. Toxicol. Environ. Mutagen 627, 41–58. https://doi.org/10.1016/j.mrgentox.2006.10.003.

Tokiwa, H., Nakagawa, R., Morita, K., Ohnishi, Y., 1981. Mutagenicity of nitro derivatives induced by exposure of aromatic compounds to nitrogen dioxide. Mutat. Res. Mutagen. Relat. Subj 85, 195–205.

Tomaz, S., Shahpoury, P., Jaffrezo, J.-L., Lammel, G., Perraudin, E., Villenave, E., Albinet, A., 2016. One-year study of polycyclic aromatic compounds at an urban site in Grenoble (France): seasonal variations, gas/particle partitioning and cancer risk estimation. Sci. Total Environ. 565, 1071–1083. https://doi.org/10.1016/j.scitotenv.2016.05.137.

Umbuzeiro, G.A., Magalhães, D., de Castro, F.J.V., Kummrow, F., Rech, C.M., Rothschild Franco de Carvalho, L., de Castro Vasconcellos, P., 2008a. A preliminary characterization of the mutagenicity of atmospheric particulate matter collected during sugar cane harvesting using the Salmonella/microsome microsuspension assay. Environ. Mol. Mutagen 49, 249–255. https://doi.org/10.1002/em.20378.

Umbuzeiro, G.A., Franco, A., Martins, M.H., Kummrow, F., Carvalho, L., Schmeiser, H.H., Leykauf, J., Stiborova, M., Claxton, L.D., 2008b. Mutagenicity and DNA adduct formation of PAH, nitro-PAH, and oxy-PAH fractions of atmospheric particulate matter from São Paulo, Brazil. Mutat. Res. - Genet. Toxicol. Environ. Mutagen 652, 72–80. https://doi.org/10.1016/j.mrgentox.2007.12.007.

Umbuzeiro, G.A., Kummrow, F., Alexandre Morales, D., Alves, D.K., Lim, H., Jarvis, I.W.H., Bergvall, C., Westerholm, R., Stenius, U., Dreij, K., 2014. Sensitivity of Salmonella YG5161 for detecting PAH-associated mutagenicity in air particulate matter. Environ. Mol. Mutagen 55, 510–517. https://doi.org/10.1002/em.21861.

Valle-Hernández, B.L., Mugica-Alvarez, V., Salinas-Talavera, E., Amador-Muñoz, O., Murillo-Tovar, M.A., Villalobos-Pietrini, R., De Vizcaya-Ruíz, A., 2010. Temporal variation of nitro-polycyclic aromatic hydrocarbons in PM_{10} and $PM_{2.5}$ collected in Northern Mexico City. Sci. Total Environ. 408, 5429–5438. https://doi.org/10.1016/j.scitotenv.2010.07.065.

Wang, T., Xia, Z., Wu, M., Zhang, Q., Sun, S., Yin, J., Zhou, Y., Yang, H., 2016. Pollution characteristics, sources and lung cancer risk of atmospheric polycyclic aromatic hydrocarbons in a new urban district of Nanjing. China. J. Environ. Sci. 1–11. https://doi.org/10.1016/j.jes.2016.06.025.

Wei, S., Huang, B., Liu, M., Bi, X., Ren, Z., Sheng, G., Fu, J., 2012. Characterization of PM2.5-bound nitrated and oxygenated PAHs in two industrial sites of South China. Atmos. Res. 109–110, 76–83. https://doi.org/10.1016/j.atmosres.2012.01.009.

Wickramasinghe, A.P., Karunaratne, D.G.G.P., Sivakanesan, R., 2011. PM10-bound polycyclic aromatic hydrocarbons: concentrations, source characterization and estimating their risk in urban, suburban and rural areas in Kandy, Sri Lanka. Atmos. Environ. 45, 2642–2650. https://doi.org/10.1016/j.atmosenv.2011.02.067.

World Health Organization (WHO), 2000. Air Quality Guidelines for Europe, second ed. WHO Regional Publications, European Series, No. 91. World Health Organization Regional Office for Europe, Ed. WHO, Copenhagen, Denmark.

World Health Organization (WHO), 2005. Air Quality Guidelines Global Update 2005. Report on a Working Group Meeting, Bonn, Germany, October 18–20, 2005. World Health Organization, Regional Office for Europe, Copenhagen, Denmark.

Wultsch, G., Nersesyan, A., Kundi, M., Wagner, K.-H., Ferk, F., Jakse, R., Knasmueller, S., 2015. Impact of exposure to wood dust on genotoxicity and cytotoxicity in exfoliated buccal and nasal cells. Mutagenesis 30, 701–709. https://doi.org/10.1093/mutage/gev034.

Xin, L., Wang, J., Guo, S., Wu, Y., Li, X., Deng, H., Kuang, D., Xiao, W., Wu, T., Guo, H., 2014. Organic extracts of coke oven emissions can induce genetic damage in metabolically competent HepG2 cells. Environ. Toxicol. Pharmacol. 37, 946–953. https://doi.org/10.1016/j.etap.2014.03.012.

Zhai, Q., Duan, H., Wang, Y., Huang, C., Niu, Y., Dai, Y., Bin, P., Liu, Q., Chen, W., Ma, J., Zheng, Y., 2012. Genetic damage induced by organic extract of coke oven emissions on human bronchial epithelial cells. Toxicol. Vitr. 26, 752–758. https://doi.org/10.1016/j.tiv.2012.04.001.

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