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Particulate matter-bound organic compounds: levels, mutagenicity, and health risks

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ABSTRACT

Increased industrialization and consumption of fossil fuels in the Metropolitan Region of São Paulo (MRSP), Brazil, have caused a growth of the particulate matter emissions to the atmosphere and an increase in population health problems. Particulate and gaseous phase samples were collected in different short campaigns (2015, 2016, and 2017) near an urban-industrial area. Organic carbon (OC), elemental carbon (EC), polycyclic aromatic hydrocarbons (PAH), and its derivatives (nitro and oxy-PAH), *n*-alkanes, hopanes, and pesticides were determined. The *Salmonella*/microsome test confirmed the mutagenic activity of these samples. Among PAH, benzo(a)pyrene was detected as one of the most abundant compounds. Benzo(a)pyrene equivalent concentrations for PAH and nitro-PAH, and

the associated risk of lung cancer showed values above those recommended in the literature. The profile of *n*-alkanes confirmed the predominance of anthropogenic sources. Pesticide concentrations and estimated risks, such as the daily inhalation exposure and hazard quotient, suggest that exposure to these compounds in this area may be dangerous to human health.

1. Introduction

The increase in morbidity and mortality in urban centers has been related to many human activities, including those that cause air pollution (Forman and Finch, 2018; Vallero, 2014).

Once inhaled, atmospheric particulate matter (PM) can reach different levels of the respiratory tract, causing irritations, allergies, infections, and diseases, and contributing to increased acute cardiovascular morbidity, such as myocardial infarction and diseases such as diabetes (Weichenthal et al., 2016). According to the American Cancer Society, each 10 $\mu g \, m^{-3}$ increase in fine particulate ($PM_{2.5}$), was linked with a 6% rise in all-cause mortality, with a 9% intensification in the risk of cardiopulmonary mortality, and a 14% increase in the risk of lung cancer (Pope et al., 2002).

Brazilian legislation recommends exposure limits for PM (CONAMA, 2018), which are less restrictive than those recommended by the World Health Organization (WHO, 2005). Focusing on these limits, it is always necessary to investigate the sources of pollution, the composition of the PM, and the health effects. In this context, the use of mutagenicity assays combined with chemical characterization in studies relating to the effects of exposure to PM are useful tools.

50   Incomplete combustion processes release several compounds into the

51   atmosphere, especially polycyclic aromatic hydrocarbons (PAH), which can be

52   carcinogenic, mutagenic, and endocrine disruptors (Kim et al., 2013; Vasconcellos

53   et al., 2011). When emitted, PAH can be adsorbed by the suspended particles and

54   can react with oxidizing species to produce nitro and oxy derivatives, which can

55   present even higher mutagenicity and/or carcinogenicity than their precursors

56   (Andersson and Achten, 2015; Karavalakis et al., 2010).

57   Emission marker species, such as n-alkanes and hopanes, are efficient to

58   assess the contribution of emission sources (Andreou and Rapsomanikis, 2009).

59   However, harmful effects of these compounds on humans are not clarify(Alves et al.,

60   2016). Due to their stability in atmospheric conditions, hopanes have been used as

61   markers for the use of oil and lubricating oils in motor vehicles (Kleeman et al. 2008;

62   Alves et al. 2017). n-Alkanes have also been used as tracers of anthropogenic and

63   natural emissions. Their low reactivity and volatility make them interesting tracers for

64   atmospheric transport and particle origin (Simoneit et al., 2004; Vasconcellos et al.,

65   2010).

66   Other less studied pollutants in the particulate matter are pesticides. These

67   are chemical compounds often used in agriculture to eliminate pests, and unwanted

68   plants. They are also used in public health to eliminate vectors of diseases, such as

69   mosquitoes (World Helath Organization WHO, 2018). Brazil is the world's largest

70   consumer of pesticides, and over 526 different active products are used, from which

71   30% of them are banned in the European Union and United States of America

72   (ANVISA, 2017; Bombardi, 2017; Yera et al., 2020). The literature shows that the

73   intensive use of pesticides has contaminated different environmental compartments,

74   and several studies correlate their exposure to the increase in diseases in rural and

75   urban areas (Liu et al., 2018; Mostafalou and Abdollahi, 2013).

76   To assess the impact of pollutants on biological matrices, some tests are

77   documented in the literature (Roubicek et al., 2011; Zeiger, 2019). The

78   *Salmonella/*microsome mutagenicity test, also known as the Ames test, is a short-

79   term *in vitro* test that detects the mutagenic properties of a wide spectrum of

80   chemicals through reverse mutation of *Salmonella enterica* ser. Typhimurium

81   strains. Auxotrophic mutant strains for histidine, an essential amino acid, are used

82   in this assay. These strains have mutations in specific genes that prevent the

83   synthesis of this amino acid, so they can only grow in a culture medium

84   supplemented with histidine. However, the attack of chemical substances on specific

85   sites of bacterial DNA can produce new mutations that re-establish biochemical

86   processes and reverse the auxotrophy of bacteria (Ames et al., 1975; Ishikawa et

87   al., 2016; Kado et al., 1983).

88   Most studies aimed to access anthropogenic contribution to atmospheric

89   pollution are conducted at sites influenced by urban-industrialized emissions or by

90   agricultural emissions. However, the present work joined these both kinds of

91   emissions, since the sampling site is exposed to vehicular and petrochemical

92   emissions together with pesticides, used for the control of domestic pests in the

93   region, or produced in the industrial complex, or transported from other regions.

94   According to Word Health Organization (WHO), among deaths relating to air

95   pollution per year, more than 90% of these deaths occur in low- and middle-income

96   countries, 25% are caused by stroke, 43% by chronic obstructive pulmonary disease

97   and 29% by lung cancer (WHO, 2018). As industrial activities contribute to air quality

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98 degradation, it is important to address this issue, and identify new ways to improve

99 the situation.

100   The current study aimed to determine the chemical profile and the toxic

101 potential of PM samples collected between 2015 and 2017 in an area near an

102 industrial complex located in the Metropolitan Region of São Paulo (MRSP), Brazil.

103

104 2. Methodology

105 2.1. Sampling and site characterization

106   In this study (2015-2017 campaigns), aerosols were collected at a sampling

107 station of the Environmental Agency of the São Paulo State (CETESB) (23º 38'

108 44.44" S and 46º 29' 35.71" W). This site is located approximately 500 m from a

109 complex that shelters fourteen industrial facilities of different chemical segments. All

110 industries cover an area of 3.7 million square meters. Approximately 80,000 people

111 live around this area.

112   A high-volume sampler (Thermo Fisher Scientific) (flow rate of 280 L min^{-1})

113 was used to collect total suspended particles (TSP), in quartz fiber filters, and

114 gaseous phase, in polyurethane foams (PUF), which is placed below the filter

115 section. This equipment allowed the simultaneously sampling of PM and gaseous

116 phase for 2015 and 2016 campaigns. In 2017, another high-volume sampler (flow

117 rate of 1.13 m^3 min^{-1}) was used to collect only fine particles (PM$_{2.5}$). For all the

118 campaigns, twenty-four-hour samples were collected. Filters were previously heated

119 in an oven at 600°C for 6 hours and were wrapped in aluminum foil and stored in the

120 fridge after each sampling. The PM concentrations were determined by weighing the

121 filters before and after sampling. The foams, which gaseous phase were collected,

122 were pre-cleaned in ultrasonic bath for 20 min for three times with 80 mL of acetone

123 (adapted from Boonyatumanond et al., 2007). Table 1 shows the season, period,

124 and PM size for each sampling campaign.

125

126 **Table 1 -** *Season and period of sampling, and PM size for samples collected around the industrial*

127 *complex in MRSP.*

Year	Season	PM size	Period
2015	Spring	TSP + PUF	October 16th – November 20th
2016	Winter	TSP + PUF	July 26th – August 15th
2017	Winter	PM2.5	June 9th – July 12th

128

129

130 **2.2. Sample extraction and preparation**

131 **2.2.1. PAH and derivatives, *n*-alkanes and hopanes**

132 For PAH, derivatives, and *n*-alkanes, the methodology is described by Caumo

133 et al. (2018). Briefly, the samples were extracted in an ultrasonic bath with organic

134 solvent (dichloromethane for PM and acetone for PUF samples). Then, the extracts

135 were concentrated by rotary evaporation and fractionated in a glass column with

136 silica and alumina to separate the organic classes. The first fraction containing *n*-

137 alkanes and hopanes was eluted with hexane; the second fraction comprising PAH

138 and their derivatives was obtained by eluting a mixture of hexane and

139 dichloromethane (1:1).

140 The aliphatic fractions were determined in a gas chromatograph coupled to a

141 mass spectrometer (GC/MS - Thermo Scientific), according to the following

142   conditions: splitless mode, column ZB – 5MS (60 m × 0.25 mm × 0.25 μm), flow of

143   1.2 mL min^{-1} of helium, as the carrier gas. The heating program was 60ºC for 1 min;

144   60ºC until 150ºC (10ºC min^{-1}); from 150ºC to 290ºC (5ºC min^{-1}); 290ºC for 27 min.

145   The interface and ion source temperatures were 290ºC; MS operated at scan mode,

146   from 33 to 800 m/z (Alves et al., 2016). Standard solutions of *n*-alkanes (Sigma–

147   Aldrich) and a 17β(H),21β(H)-hopane solution (Fluka) were used to calibrate the

148   GC/MS. Tetracosane-d50 and 1-chlorohexadecane (Aldrich) were used as internal

149   standards. Fourteen hopanes were identified: tetracyclic terpane (5 Cyc Terp), (R +

150   S) tetracyclic terpane (5 Cyc Terp (R+S)), 18α(H)-22,29,30-trisnorneohopane

151   ($C_{27}T_s$), 17α(H)-22,29,30-trisnorhopane ($C_{27}T_m$), 17α(H),21β(H)-30-norhopane

152   ($C_{29}αβ$), 17β(H),21α(H)-30-norhopane ($C_{29}βα$), 17α(H)-diahopane ($C_{30}D$),

153   17β(H)21α(H)-30-norhopane ($C_{29}βα$ M), 17α(H),21β(H)-hopane ($C_{30}αβ$),

154   17β(H),21α(H)-hopane ($C_{30}βαM$), 17α(H),21β(H)-22S-homohopane ($C_{31}αβS$),

155   17α(H),21β(H)-22R-homohopane ($C_{31}αβR$), 17α(H),21β(H)-22S-bishomohopane

156   ($C_{32}αβS$) and 17α(H),21β(H)-22R-bishomohopane ($C_{32}αβR$). The relative response

157   factors were determined individually for the majority of compounds. Compound

158   identification was based on the GC-MS spectra libraries (Wiley and NIST), co-

159   injection with authentic standards and analysis of fragmentation patterns.

160   Quantification was performed by both single ion monitoring (SIM) and total ion

161   chromatogram (TIC) analysis, in two separate GC runs.

162   The qualitative and quantitative analyses for PAH and derivatives were

163   conducted in a GC/MS (Agilent model 7820A) with a column VF – 5ms (30 m × 0.25

164   mm × 0.25 μm) in splitless mode and helium as carrier gas at 1.3 mL min^{-1}. The

165   temperature programme was the following: 80°C for 4 min; increasing until 200°C

166   (20°C min^{-1}); from 200 to 270°C (4°C min^{-1}) and until 280°C (5°C min^{-1}) (de Oliveira

167   Alves et al., 2015). The temperature of the transfer line was 280°C and the ion source

168   was at 200°C. MS worked in SIM mode, according to m/z of each compound. EPA

169   525 PAH Mix A (48953-U, Sigma), benzo(e)pyrene (442475, Sigma), and coronene

170   (27830, Fluka) were used to obtain calibration curves with correlation coefficients

171   (R^2) greater than 0.99. The methodology identified fifteen PAH: fluorene (FLU),

172   phenanthrene (PHE), anthracene (ANT), fluoranthene (FLT), pyrene (PYR),

173   benz(a)anthracene (BaA), chrysene (Chr, benzo(b)fluoranthene (BbF),

174   benzo(k)fluoranthene (BkF), benzo(e)pyrene (BeP), benzo(a)pyrene (BaP),

175   indene(1,2,3-c,d)pyrene (InD), dibenz(a,h)anthracene (DBA) and

176   benzo(g,h,i)pyrene (BgP) and coronene (COR).

177   For nitro and oxy-PAH, the same CG/MS was used in splitless mode to

178   determine all compounds simultaneously, based on the methodology developed by

179   Santos et al. (2016). A VF–1ms (30 m × 0.25 mm × 0.25 µm) was used. The oven

180   temperature program was the following: started at 70ºC, kept for 2 min, then it was

181   raised to 200ºC at 30ºC min^{-1} and held for 5 min; lastly, the temperature was

182   increased to 330ºC at 5ºC min^{-1} and maintained for 0.67 min. The carrier gas was

183   helium with a flow rate of 1.0 mL min^{-1}. The injector was operated at 310ºC. The ion

184   source temperature was 250ºC, and the GC–MS transfer line temperature was at

185   280ºC. A Standard Reference Material (SRM) with 16 nitro-PAHs (NIST-2265) and

186   individual standards (Sigma-Aldrich) were used to determine the retention time of

187   each compound and the respective calibration curve to quantify these compounds

188   in the samples (R^2 >0.99). The nitro-PAH identified and quantified were as follows:

189   9-nitroanthracene (9-NAnt), 9-nitrophenanthrene (9-NPhe), 3-nitrophenanthrene (3-

NPhe), 2-nitrophenanthrene (2-NPhe), 2-nitroanthracene (2-NAnt), 9-nitroanthracene (9-NAnt), 2-nitrofluorene (2-NFlu), 3-nitrofluoranthene (3-NFlt), 2-nitrofluoranthene (2-NFlt), 2-nitropyrene (2-NPyr), 1-nitropyrene (1-NPyr), 4-nitropyrene (4-NPyr), 7- nitrobenz(a)anthracene (7-NBaA), 6-nitrochrysene (6-NChr), 3-nitrobenzanthrone (3-NBaT) and 6-nitrobenzo(a)pyrene (6-NBaP). For oxy-PAH determination, standards of 9-fluorenone (9-Fluo), 9,10-anthraquinone (9,10-Antq), 2-methylanthraquinone (2-MAntq), benzo(a)anthracene-7,12-dione (BAntd) and 1,4-benzoquinone (1,4-Bzq) (Sigma-Aldrich) were applied in calibrations curves (R^2 > 0.99).

2.2.2 Pesticides

The pesticide analysis was performed for the PM samples (TSP and PM2.5), since PUF samples were extracted and fractionated for PAH and derivatives, n-alkanes and hopanes. The determination of pesticides was carried out with a method recently developed in collaboration with the Federal University of Bahia and was based on a previous methodology (Yera et al., 2020). As the extraction of PAH and derivatives, n-alkanes and hopanes was previously carried out, it was more feasible to adopt this innovative method for pesticides. Solvent and time reductions were key to develop the methodology.

The technique consists of separating an area of 4.15 cm^2 from the filter, then, transfer it to a micro extractor device of borosilicate glass chamber (Whatman Mini-UniPrep G2), with a mixture of dichloromethane/acetonitrile (500 µL - 82%:18%). The samples were sonicated for 23 min, and the extracts were filtered. The analysis was carried out using a gas chromatograph coupled to a mass spectrometer (GC–

MS 7820A Agilent Technologies). Separation of the compounds occurred in a NST-5MS gas capillary column (30 m × 0.25 mm × 0.25 μm). The GC temperature program was: initial 90ºC, hold 5 min; rate 25°C min^{-1}, until 180ºC; increasing 5°C min^{-1} to 280ºC, hold 3 min; raised at 10°C min^{-1} to 300°C, hold 3 min. Splitless injection conditions were adopted, and the mass spectrometer was operated in the electron impact mode (EI) using a voltage of 70eV. The total run time analysis was 36.6 min. The pesticides determined were: diuron, λ-cyhalothrin, kresoxim-methyl, permethrin (isomers mix), atrazine, malathion, heptachlor, heptachlor-epoxide, α-endosulfan, β-endosulfan, and ethion (Sigma-Aldrich). A mix standard solution containing all pesticides was prepared in dichloromethane and acetonitrile (82%:18%). Matrix-matching calibration curves were prepared for each compound, adding variables volumes of the mix solution in extracts of blank filters. All calibration curves presented suitable determination coefficient values (R^2 >0.99).

2.2.3 Quality assurance/quality control (QA/QC)

The analytical standards and solvents used in the present study were of analytical grades and purchased from well-reputed suppliers such as SigmaAldrich. Blanks and spiked blanks were processed along with the samples for all organic groups analyzed. Spiked matrices (fiber filters and in polyurethane foams) with certified standard solution were analyzed to evaluate method accuracy and ensure that any procedural contamination accounted for. PAH, oxy and nitro-PAH, and pesticides fractions were stored using amber glassware to avoid photodegradation.

Detection limit (DL) and quantification limit (QL) were calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3:1 and 10:1,

238   respectively, for each compounds analyzed and was previously published (Caumo,

239   2020).

240

241   **2.3. Elemental and organic carbon**

242   Elemental carbon (EC), organic carbon (OC) were determined using a

243   thermal-optical transmission technique (Alves et al. 2016). The equipment is

244   composed of a quartz tube, a pulsed laser, and a non-dispersive infrared CO_2

245   analyzer. Two 9 mm punches of each filter are placed inside the quartz tube. The

246   oven was first heated up until 600ºC in a nitrogen atmosphere to evaporate the

247   organic fraction. After that, the oven was heated up to 850ºC in a N_2 atmosphere

248   with 4% of oxygen to vaporize the EC. The CO_2 released from the volatilization and

249   oxidation of different carbon fractions under controlled heating is measured by a non-

250   dispersive infrared (NDIR) analyzer. The blackening of the filter is monitored using

251   a laser beam and a photodetector, which enables separating the EC formed by

252   pyrolysis.

253

254   **2.4. Mutagenicity**

255   The extraction step consisted of three 20-minutes cycles in an ultrasonic bath

256   using 80 mL of methylene chloride (DCM). The volume of the extract was reduced

257   to 3 mL on a rotary vacuum evaporator and filtered using polycarbonate membranes

258   (Nucleopore™). The material was then dried with a stream of pure nitrogen gas.

259   The total extracts were tested for mutagenicity using using the microsuspension

260   protocol of the *Salmonella*/microsome test (Kado et al., 1983) with *Salmonella*

261   Typhimurium strain TA98 (*his*D3052, *rfa*, Δ*bio*, Δ*uvrB*, pKM101), with and without *in*

262 *vitro* metabolic activation (S9 – Moltox Inc., Boone, NC) . Different doses were

263 tested, varying between 5 µg and 240 µg equivalent of particulate matter per plate;

264 the analyzes were performed in duplicate, in the tests, negative control was dimethyl

265 sulfoxide (DMSO) and as a positive control for TA98-S9, 4-nitro-*o*-quinoline (4NQO)

266 with a concentration equal to 0.5 µg / plate and for TA98 + S9, 2-aminoanthracene

267 (2AA) at 2.5 µg / plate.

268   The extracts were resuspended in DMSO. At the time of testing, 50 µL of strain

269 suspension, 50 µL of 0.015 M sodium phosphate buffer, or 50 µL of S9 mix (S9 +

270 cofactors), and 5 µL of the particulate matter extract were added to a tube and

271 incubated at 37°C for 90 minutes. After incubation, 2 mL of agar was added, and the

272 mixture was transferred to a Petri dish (diameter = 90 mm) containing an appropriate

273 culture medium. Colony counting was performed after 66 hours of incubation at 37ºC

274 using an automatic counter.

275   The data were analyzed by the SALANAL software, kindly provided by Integrated

276 Laboratory Systems, Research Triangle Park, NC., using Bernstein statistical model,

277 (Bernstein et al., 1982). They were considered positive when the analysis of variance

278 (ANOVA) showed significant differences between the tested doses and the negative

279 control. The dose/response relationship was statistically significant. The

280 mutagenicity ratio (RM - mean number of revertants in the test plates divided by the

281 average of the spontaneous reversals of the control plates) was higher than or equal

282 to 2.0. When only one of the criteria was met, the sample was considered to have

283 evidence of mutagenicity (Maselli et al., 2019).

284

285

2.5. Risks assessment and inhalation exposure

In order to assess the health risks and exposure to inhalation of pollutants, some calculations to evaluate the risk assessment were conducted for PAH and pesticides separately, which are described below.

Through the PAH determination, the equivalent carcinogenicity of PAH relative to benzo(a)pyrene (BaP_{Eq}) was calculated. This is achieved by multiplying the concentration of the eight PAH classified as carcinogenic species by the carcinogenic equivalent factors (TEF) for each compound (Nisbet and Lagoy, 1992). BaP_{Eq} was calculated as follows:

BaP_{Eq} = [BaA] x 0.1 + [Chr] x 0.01 + [BbF] x 0.1 + [BkF] x 0.1 + [BaP] x 1 + [InP] x 0.1 + [DBA] x 5 + [BgP] x 0.01   (1)

This parameter can also be calculated considering the nitro-PAH toxicity ($BaP_{Eq\ Nitro-PAH}$). It was determined from relative potency factors (RPF) of four nitro-PAH (de Oliveira Galvão et al., 2017).

$BaP_{Eq\ Nitro-PAH}$ = [1-NPyr]x0.10 + [4-NPyr]x0.10 + [6-NChr]x10 + [2-Nflt]x0.01   (2)

The lifetime lung cancer risk (LCR), a parameter to estimate the hazard of exposure to humans, was obtained by the multiplication of the BaP_{Eq} or $BaP_{Eq\ Nitro-PAH}$ by the unit risk (UR) (Callén et al., 2014). The UR value for lung cancer, according to the WHO, for a lifetime exposure to BaP, is 8.7×10^{-5} $(ng\ m^{-3})^{-1}$. This

13

309   value means an incidence of 87 cases of lung cancer per 100,000 people subjected

310   to chronic exposure to 1 ng m^{-3} BaP along 70 years of their lifetime (WHO, 2000).

311

312   **LCR** = UR x BaP$_{Eq}$ (3)

313

314   For pesticides determination, the equations proposed by Coscollà and Yusà

315   (2016) calculate the daily inhalation exposure (DIE) (Eq. 4), the risk assessment (Eq.

316   5) and the cancer risk (Eq. 6) three different populations: infants (6 months to 1.5

317   years), children (1.5 to 6 years) and adults (> 12 years).

318

319   **DIE** (mg kg^{-1}day^{-1}) = Σ (C x IR$_{inh}$ x ED) / BW   (4)

320   The DIE refers to the amount of pesticides that a given population is daily

321   exposed to; C (mg m^{-3}) is the total concentration (gas + particulate phase) of each

322   pesticide; IR$_{inh}$ (m^3 h^{-1}) is the inhalation rate per hour. The IR$_{inh}$ applied was 8 m^3

323   day^{-1} for infants, 10 m^3 day^{-1} for children and 20 m^3 day^{-1} for adults; ED is the

324   exposure duration for the three groups of individuals; 24 hours of exposure were

325   considered; BW is the body weight (kg), which was assumed as 10 kg for infants, 15

326   kg for children and 70 kg for adults.

327   The total concentration of pesticides is the sum of the concentrations in the

328   gaseous and particulate phases. In the present study, only pesticides in the

329   particulate matter were determined. The corresponding gas-phase concentrations

330   were estimated using the K$_{oa}$ model (Harner and Bidleman, 1998). The K$_{oa}$ model

331   uses equations 5 and 6 to calculate the potential distribution of pesticides in the

332   particulate phase.

$$\Phi = (K_p \, C_{TSP}) / (1 + K_p \, C_{TSP}) \tag{5}$$

$$\mathrm{Log} \, K_p = \log K_{oa} + \log f_{OM} - 11.91 \tag{6}$$

Φ is the fraction of pesticides in the particulate phase. K_p is the gas/particle partition coefficient. C_{TSP} ($\mu g \, m^{-3}$) is the concentration of total suspended particles in the air.

The risk assessment was calculated using the Hazard Quotient (HQ) as a descriptor of the risk.

$$\mathbf{HQ} = DIE \, / \, HBRV \tag{7}$$

HBRV represents the Health-Based Reference Values. It was calculated using the AOEL, defined as the Acceptable Operator Exposure Levels for a chronic inhalation exposure, considering the absorption of the compound, the body weight and the days of exposure (Coscollà and Yusà, 2016). AOEL values for the studied compounds are tabulated in (Yera et al., 2020). HQ values higher than 1 mean that the populations are more exposed to the pesticides than the operator (person who works directly with the compounds).

The cancer risk for pesticides exposure was calculated by Equation 8. It refers to pesticides classified by the Environmental Protection Agency as "likely to be carcinogenic to humans" and/or "possible human carcinogen".

$$\mathbf{Cancer \, risk} = DIE \, x \, PF \tag{8}$$

356   PF (potency factor) is the factor that estimates the potency of the carcinogenicity.

357   In general, these values range from 10^{-3} to 10^{-1}. Some values of PF for specific

358   pesticides were not found, and 0.1 was assumed for all pesticides (the situation with

359   major potency).

360

361   **3. Results**

362   **3.1.   PM, OC and EC concentrations**

363   Wind roses (Figure 1) were generated by WRPLOT software (Lakes

364   Environmental). For the first (2015), second (2016) and third (2017) sampling

365   periods, the software used 864, 528 and 720 observations, respectively. The

366   industrial complex covers an area from southeast to northeast in the wind roses.

367   During the 2015 campaign, which showed the highest TSP concentration (Table 2),

368   the dominant wind was from the south, where an important road is located. The 2016

369   and 2017 samples had a higher contribution from winds coming mostly from the

370   southeast and northeast, where the petrochemical complex is situated. These

371   findings may highlight the differences in composition and concentration of

372   compounds determined in this study, in order to better understand the contribution

373   to risk assessment of a predominant vehicular emission scenario (2015 campaign)

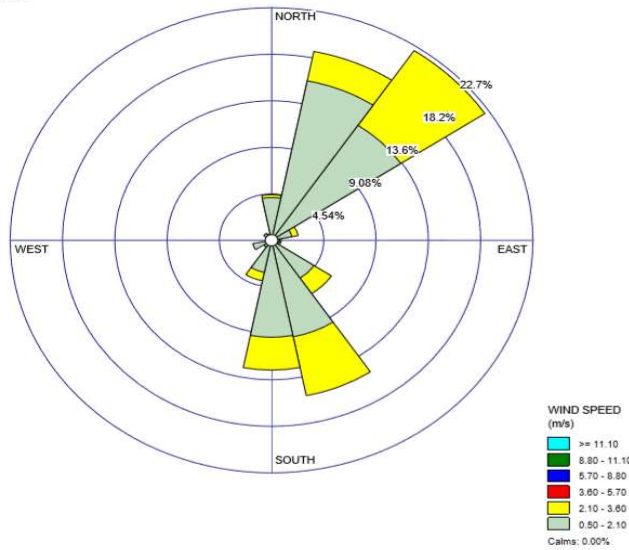374   versus a majority industrial emission scenario (2016 campaign and 2017).

375

2015
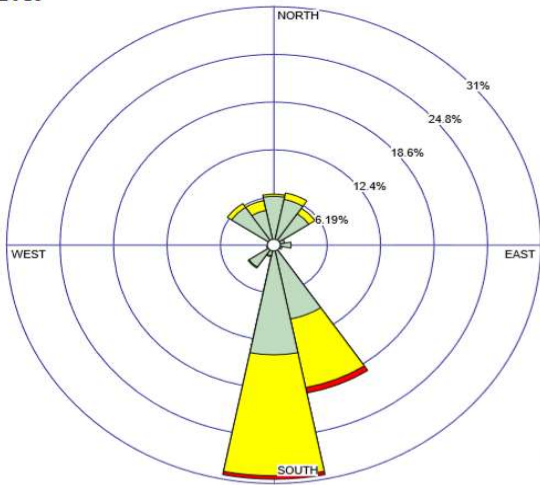
2016
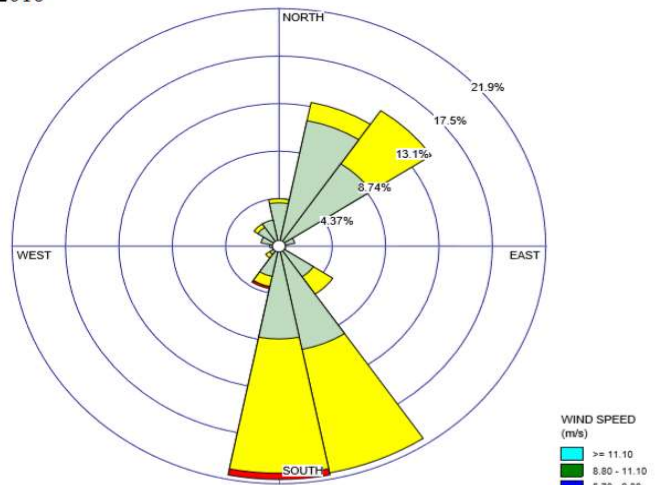
2017

376

377   *Figure 1 - Wind roses for the three sampling campaigns*

378   Table 2 shows the mean PM concentrations for each campaign. The

379   samplings took out one or two months for each year, so these concentrations values

380   could be not representative for the current year of the campaign. However, these

381 results were relevant to evaluate the risk of exposure to the population living around

382 the petrochemical complex.

383   In São Paulo, winter is often the season with the highest levels of air pollutants

384 in São Paulo due to thermal inversion layers that provide unfavorable conditions for

385 the dispersion of pollutants (Pérez-Martínez et al., 2017). The TSP samples

386 collected in 2016, during the winter season, presented a lower concentration range

387 $(17 - 55 \ \mu g \ m^{-3})$ than the TSP campaign conducted during the spring, in 2015 (26 –

388 99 μg m-3), which may be attributed to the predominance of vehicle emissions

389 (Figure 1). These two ranges were comparable to that obtained previously in a study

390 conducted 6 km far from the major smokestack of a petrochemical area in the South

391 region of Brazil $(8 \ to \ 92 \ \mu g \ m^{-3})$ (Coronas et al., 2008). There is no limit value

392 recommended by the Brazilian legislation.

393   The $PM_{2.5}$ mean concentration $(21 \ \mu g \ m^{-3}$, Table 2) was below the level

394 recommended by WHO $(25 \ \mu g \ m^{-3})$ (WHO, 2005). A previous study conducted in

395 winter in an industrial area in Ostrava (Czech Republic) (Mikuška et al., 2015), which

396 is included among the worst air quality regions in the European Union, presented

397 higher $PM_{2.5}$ variation $(33 - 288 \ \mu g \ m^{-3})$ than the current study. Besides that, PM_{10}

398 concentration at Houtem, an industrialised city in Belgium, ranged between 13 and

399 27 μg m^{-3} (mean = 18 μg m^{-3}) (Maenhaut et al., 2012)$^.$ These lower results may result

400 from a tighter air quality legislation in the Europe Union (Fuzzi et al., 2016)

401

402

403 **Table 2** - *PM, OC, and EC concentrations (μg m^{-3}) for each year of sampling.*

Year	PM size	PM		OC		EC	
		Mean	Range	Mean	Range	Mean	Range
2015	TSP	49	26 – 99	9	3 – 21	4	1 – 9
2016	TSP	39	17 – 55	6	2 – 12	3	1 – 5
2017	PM$_{2.5}$	21	7 – 48	0.8	0.3 – 2	0.3	0.03 – 1

404

405   OC and EC followed the same pattern as the PM concentrations. TSP

406   samples collected in 2015 showed the highest values (OC = 9 μg m^{-3} and EC = 4 μg

407   m^{-3}). On average, OC and EC accounted for TSP mass fractions of 15-18% and 8%,

408   respectively. The fine aerosol samples presented the lowest values between three

409   samplings (OC = 0.8 μg m^{-3} and EC = 0.3 μg m^{-3}, Table 2). EC is emitted directly by

410   sources such as fossil fuel or biomass burning and is an important component that

411   absorbs light in the atmosphere. On the other hand, OC may be emitted by primary

412   sources or secondarily formed in the atmosphere through photochemical reactions

413   (Brito et al., 2013).

414   The OC/EC ratio is applied as an indicator of carbonaceous aerosol sources,

415   especially to separate primary and secondary sources contributing to PM (Pio et al.,

416   2011). OC/EC ratios in the present work were 2.2, 2.0, and 2.7 for 2015, 2016, and

417   2017, respectively. According to the literature, values between 2 and 5 are often

418   found in urban background areas, where a higher contribution of secondary aerosol

419   formation is observed. Values lower than 1 are found in roadway tunnels due to fresh

420   traffic emissions (Castro et al., 1999; Na et al., 2004; Pio et al., 2011).

3.2. PAH and derivatives

PAHs have been investigated over the years, and many studies have stressed the importance of these compounds, even in low concentrations, due to their toxicological behavior in different biological assays (He et al., 2012; Ravindra et al., 2008a). Besides that, the BaP equivalent carcinogenic concentration (BaP_{Eq}) is a useful tool to estimate the toxic fraction of these compounds (Pereira et al., 2017; Schoeny and Poirier, 1993; Zhang et al., 2012).

The percentage contribution of each PAH used to calculate BaP_{Eq} to the total PAH concentration is showed in Figure 2. BaP, a compound carcinogenic to humans (IARC, 2012), presented the highest concentration for fine particles, in 2017 (23 ng m^{-3}), when winds contribution came mostly from the petrochemical complex (Figure 1), while the lowest value was registered for PUF samples collected in 2015 (0.7 ng m^{-3}), when dominant wind was from a road. According to He et al. (2012), BaP might affect embryonic neuron development as well as neuron cell survival and recovery.

DBA, a marker for stationary emissions (Ravindra et al., 2008b), showed a higher share in PM samples than in PUF samples (Figure 2). The total PAH concentration was higher in the fine fraction (2017, 80 ng m^{-3}) than in TSP (2016, 35 ng m^{-3} and 2015, 5 ng m^{-3}). This finding confirms the wind roses (Figure 1) since 2017 showed a high influence of winds from the petrochemical area.

The physicochemical behavior of gaseous and particulate PAH plays an important role in their distribution in the atmosphere, since the low molecular weight compounds (LMW: PAH with three or four aromatic rings) have been found frequently to shift the gas–particle partitioning due to the seasonal change in ambient temperature (Kishida et al., 2011).

445   For gaseous phase compounds collected in 2015 and 2016, the LMW-PAH

446   were dominant. Among the eight PAH adopted in BaP_{Eq} calculation BaA showed a

447   mean of 0.1 and 1 ng m^{-3} for 2015 and 2016, respectively, whereas mean values of

448   0.2 and 4 ng m^{-3} were obtained for Chr in 2015 and 2016. Despite the 2015 campaign

449   showing the higher PM mean concentration (Table 2) and a dominant wind from a

450   roadway, BaA and Chr were higher in 2016 samples, when the influence from the

451   petrochemical region was dominant (Figure 1).

452   Other LMW-PAH, associated with fossil fuel emissions (Karavalakis et al.,

453   2010), such as anthracene and phenanthrene, presented the same behavior as that

454   observed for BaA and Chr: higher mean concentration in 2016 (mean = 20 ng m^{-3})

455   compared to 2015 (mean = 1 ng m^{-3}). Total PAH concentrations in the gaseous

456   phase presented large differences, with low concentration (2 ng m^{-3}) in 2015

457   samples, and a much higher mean concentration (52 ng m^{-3}) in 2016. Although the

458   same sources are considered, meteorological conditions and wind roses might

459   explain the difference in concentrations between these years, since the 2015

460   campaign was conducted in the springtime, with predominant wind from a road, while

461   the 2016 campaign was carried out in wintertime, with dominant winds blowing from
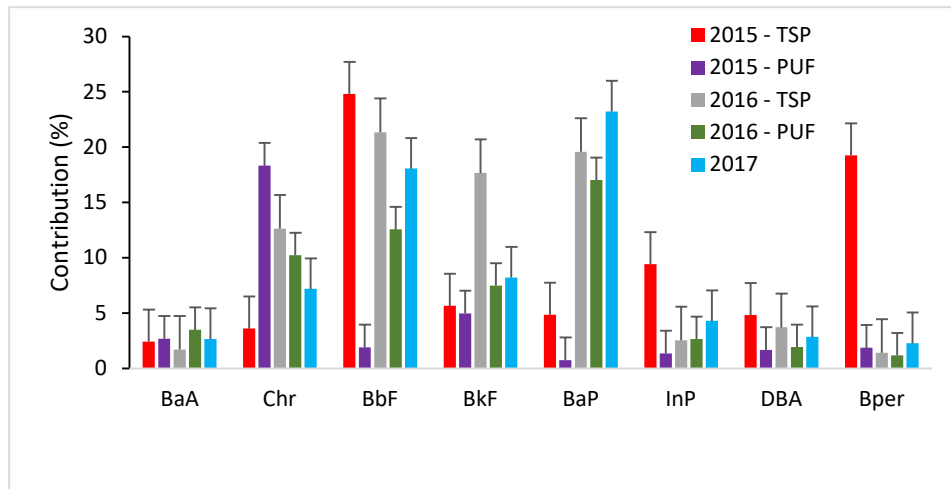
462   the petrochemical area.

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Figure 2- *Percentage e contribution of each PAH used to calculate BaP$_{Eq}$ to the total PAH concentration in PM samples.*

With respect to nitro-PAH, for the 2015 campaign, fourteen compounds were determined in TSP and PUF samples, while in 2016 and 2017, only seven nitro-PAH could be quantified. TSP samples presented the highest concentration for 4-nitropyrene (0.3 ng m^{-3}), in 2015. This compound was reported as more carcinogenic in newborn mouse assays than its precursor, BaP (OEHHA, 2011). 6-Nitrochrysene (1 ng m^{-3}) was the nitro-PAH most abundant in TSP samples collected in 2016. It is known to raise DNA adducts in lung and liver cells (Delclos et al., 1989).

In the gaseous phase, the most predominant nitro-PAH was 7-nitrobenzo(a)anthracene (0.2 ng m^{-3}), observed in samples collected in 2015 during spring. In 2016 winter samples, nitro-PAH in the gaseous phase were higher than in the particulate phase. The most abundant in PUF samples was 2-nitrofluorene (7 ng m^{-3}). This compound developed forestomach tumors in rat assays (OEHHA, 2011).

For PM$_{2.5}$ samples, nitro-PAH presented significant concentrations of 2-nitrofluorene (2 ng m^{-3}), when winds blew predominantly from the petrochemical

22

complex. In a previous study conducted in Porto Alegre, southern Brazil, in an urban site surrounded by different industries, such as oil refinery and steel mills, the 2-nitrofluorene concentration was much lower (0.5 ng m^{-3}) than that of the present study (Teixeira et al. 2011).

1-Nitropyrene, a compound known to be mutagenic (IARC, 2016), was determined in the three campaigns. 6-Nitrobenzo(a)pyrene and 6-nitrochrysene, which showed significant concentrations in 2016 (TSP + PUF = 5 ng m^{-3} and 3 ng m^{-3}, respectively) and 2017 (= 1 ng m^{-3} and 1.5 ng m^{-3}), are considered markers of diesel-burning (Guttenplan et al., 2007). 2-NFlt and 2-NPyr, formed by photochemical reactions and considered mutagenic (Ciccioli et al., 1996), were identified in several samples.

Table 3 shows the mean and range concentration of the various pollutants determined. The total concentration of nitro-PAH in both TSP and PUF samples was higher in 2016 (7 ng m^{-3}) than in 2015 (1 ng m^{-3}). Meteorological factors may contribute to the variability of these species in the atmosphere. As already stated, the 2015 sampling campaign occurred in the spring, a wet season in Brazil, while the 2016 campaign happened in the winter, dry season when pollution events are more observed. Wind roses (2015) indicated winds coming mainly from major avenues (vehicular emissions), whilst in 2016, winds blew mostly from the industrial area.

Total nitro-PAH concentrations ranged between 1 and 24 ng m^{-3} for the 2017 campaign. In a work conducted in the Araraquara region, countryside of São Paulo, during winter, the nitro-PAH concentration was from 1 to 15 ng m^{-3} (Souza et al., 2014). In a work conducted in São Paulo city, nitro-PAH mean concentration was

510   much lower (479 pg m^{-3}) (Vasconcellos et al., 2008) than that of the 2017 campaign

511   (6 ng m^{-3}).

512

513   **Table 3 –** Mean and range concentration of nitro-PAH, oxy-PAH, *n*-alkanes and hopanes in samples
514   collected around the industrial complex in 2015, 2016 and 2017.

		∑nitro-PAH	∑oxy-PAH	∑*n*-alkanes	∑hopanes
2015	Mean	1	2	716	6
TSP+PUF	Range	0.4 – 9	0.2 – 4	40 – 963	1 – 12
2016	Mean	7	9	586	5
TSP + PUF	Range	0.5 - 31	1 – 11	20 – 942	2 – 6.5
2017	Mean	6	1	190	5
PM$_{2.5}$	Range	1 – 24	0.2 – 3	50 – 360	0.4 – 22

515

516   Four oxy-PAH were determined in 2015 and 2016, while three compounds

517   were quantified in 2017. 9,10-Anthraquinone, which can be either directly emitted or

518   produced *in situ* (Souza et al., 2014), was predominant in 2015 samples (TSP + PUF

519   = 0.7 ng m^{-3}). Benzo(a)anthracene-7,12-dione, attributed to secondary formation

520   (Huang et al., 2014), was also present in this campaign (TSP + PUF = 0.5 ng m^{-3}).

521   In 2016, 2-methylanthraquinone, which was proved to be toxic to aquatic organisms

522   (Gori et al., 2009), was dominant in both phases (TSP + PUF = 2 ng m^{-3}). This value

523   is comparable to those found in Queensway Road Tunnel in Birmingham (1.7 ng m^{-}

524   ^{3}) (Keyte et al., 2016).

525   For fine particles collected in 2017, 2-methylanthraquinone (0.4 ng m^{-3})

526   showed the highest abundance, followed by 9-fluorenone (0.3 ng m^{-3}). This

527 compound has been identified as a marker of diesel combustion (Nocun and

528 Schantz, 2013), and it is formed from the oxidation of fluorene, a PAH with low

529 molecular weight, usually prevalent in the gas phase. In a previous study conducted

530 at a French alpine valley, 9-fluorenone mean concentration for PM_{10} was 4.1 ng m^{-3}

531 (Albinet et al., 2008).

532 For 2015, the sum of oxy-PAH was equal to 2 ng m^{-3}, while for 2016, the total

533 concentration was 9 ng m^{-3}. For $PM_{2.5,}$ the total concentration was 1 ng m^{-3}. Large

534 differences in the concentrations are observed in the samples despite the same

535 emission sources. Meteorological conditions can affect the concentrations,

536 especially radiation, which intervenes to the secondary formation of compounds.

537 In a study conducted in Mount Tai, China, with a large impact of biomass

538 burning, the total concentration of oxy-PAH (4 ng m^{-3}) for fine particles was four times

539 higher than that of the current study.

540 Toxic equivalent factors were used to calculate BaP_{Eq} in an attempt to

541 evaluate how dangerous the inhalation of PAH is to humans in this region (Nisbet

542 and Lagoy, 1992). According to IARC (2010), values higher than 1.0 ng m^{-3} represent

543 carcinogenic risks to humans. Mean values of 21 ng m^{-3} (2017), 22 ng m^{-3} (2016,

544 TSP + PUF) and 2.1 ng m^{-3} (2015, TSP + PUF) were obtained. Although the total

545 concentration of PAH have fluctuated considerably over the years, all campaings

546 presented potential carcinogenic risks to human health. The maximum value in this

547 work was up to thirty times higher than the results obtained for aerosols collected in

548 an industrial area located in Rio Grande do Sul, Brazil (0.64 ng m^{-3}) (Agudelo-

549 Castañeda and Teixeira, 2014).

550   The lifetime lung cancer risk based on BaP_{Eq} (LCR - BaP_{Eq}) is a parameter to

551   evaluate the incidence of lung cancer through chronic exposure. This value is

552   obtained by multiplying BaP_{Eq} and a unit risk (UR) (Callén et al., 2014) of 8.7×10^{-5}

553   ($ng\ m^{-3}$). Results of this study are 18×10^{-5} for 2015, i.e., 18 cases of lung cancer

554   per 100,000 people by chronic exposure; for 2016 it was 197×10^{-5}, (197 cases of

555   lung cancer per 100,000 people); in 2017, this value was 159×10^{-5} (159 cases of

556   lung cancer per 100,000 people).

557   The BaP_{Eq} was also estimated for nitro-PAH ($BaP_{Eq\ Nitro-PAH}$). Considering

558   TSP and PUF samples, the $BaP_{Eq\ Nitro-PAH}$ was $1\ ng\ m^{-3}$ for 2015 and $16\ ng\ m^{-3}$ for

559   2016. For fine particles, $BaP_{Eq\ Nitro-PAH}$ showed a high value over $14\ ng\ m^{-3}$. These

560   values exceed that one found for PM_{10} samples collected at an Amazonian site

561   during the biomass burning period ($0.8\ ng\ m^{-3}$, de Oliveira Galvão et al. 2017). In

562   addition, in the 2016 and 2017 campaigns, when the high values were observed, the

563   winds were predominant from the petrochemical area.

564   For the lung cancer risk (LCR) related to nitro-PAH, the values were 11×10^{-5}

565   (2015), 140×10^{-5} (2016) and 122×10^{-5} (2017), which mean 11, 140 and 122 cases

566   of lung cancer per 100, 000 people.

567

568   **3.3.   Aliphatic compounds**

569   The *n*-alkane mean concentrations are shown in Table 3. The total

570   concentration for 2015 samples, for which the homologous series ranged from C_{11}

571   to C_{31}, was $716\ ng\ m^{-3}$ (PM + PUF). In 2016, homologues from C_{11} to C_{32} presented

572   total mean concentrations of $586\ ng\ m^{-3}$ (PM + PUF). For $PM_{2.5}$, the total *n*-alkane

573   concentration was $190\ ng\ m^{-3}$. A study conducted in the Czech Republic (Mikuška

574 et al. 2015), in one of the most polluted areas in Europe, the total concentration for

575 $PM_{2.5}$ samples was 307 ng m^{-3}.

576   *n*-Alkanes can penetrate tissues, causing damages in humans (Alves et al.,

577 2016). The homologues lower than C_{25} have been related to anthropogenic sources

578 (Simoneit et al., 1995). In 2015 samples, the sum of *n*-alkanes lower than C_{25}

579 accounted for 75% (TSP + PUF) of total *n*-alkanes. In 2016, this contribution was

580 higher, representing 86% for TSP and PUF. In 2017 samples, homogues lower than

581 C_{25} contributed to 40%. These percentages highlighted the importance of the

582 compounds emitted by anthropogenic sources.

583   The carbon preference index (CPI), a parameter to infer the anthropogenic

584 versus biogenic contribution in the samples, was calculated using the ratio between

585 *n*-alkanes with odd carbon numbers and even carbon numbers (from C_{18} to C_{33}).

586 Values close to one have been associated with incomplete combustion of fossil fuel,

587 while values far beyond one are attributed to biogenic emissions (Oros and Simoneit,

588 2001). In this study, the CPI values were around 1 for all samples (Table 4), and

589 C_{max} was below C_{25} for the 2015 and 2016 campaigns, indicating a higher

590 contribution of anthropogenic emissions (Simoneit et al., 1995). With the purpose of

591 evaluating the biogenic contribution for *n*-alkanes, the percentage of wax *n*-alkanes

592 (WNA) has been adopted (Simoneit et al., 1990). WNA values were calculated

593 following the equations:

594

595          $WNA = C_n [(C_{n-1} + C_{n+1}) / 2]$ (9)

596          $\%WNA = \% (\sum WNA / \sum NA)$ (10)

597

598 **Table 4** – CPI values, WNA contribution, and C_{max} for each year of sampling.

|	TSP		PUF		PM$_{2.5}$
	2015	**2016**	**2015**	**2016**	**2017**
CPI	1.3	0.9	1.2	0.8	0.9
%WNA	8	9	4	-	-
C$_{max}$	C$_{11}$	C$_{15}$	C$_{19}$	C$_{20}$	C$_{30}$

599

600   Hopanes, which are emitted by the high boiling fraction of crude petroleum,

601 are not easily degradable and have been used as markers to identify emissions

602 related to petrochemical processes. Besides that, the stereochemical configurations

603 of hopanes are helpful to determine the geological maturity of the petroleum (Alves

604 et al. 2016). This class of compounds showed a lower contribution among all the

605 classes identified in the present study. Figure 3 shows the mean concentration of

606 hopanes for the three years. The highest concentrations were found in samples

607 collected in 2015 (TSP + PUF=15 ng m^{-3}, Table 4). A much higher concentration (45

608 ng m^{-3}) was found for PM$_{10}$ in a previous study conducted in a road tunnel with

609 intense traffic located in São Paulo city (Alves et al., 2017). Tunnels present poor air

610 circulation, high traffic, and high PM concentrations are expected.

611   The 17β(H),21β(H) configurations are found in hopanes emitted by immature

612 petroleum, 17β(H),21α(H) are moderately mature, and 17α(H),21β(H) are

613 completely mature (Bost et al., 2001; Omar et al., 2001). In the tree sampling

614 campaigns, the highest concentrations were observed for the 17α(H),21β(H)

615 configuration, indicating that the same source that used completely mature

616 petroleum was dominant in the region over the campaigns. Zakaria et al. (2000)

617   reported that a $(C_{31}-C_{32})/C_{30}$ ratio from 0.41 to 2.30 is linked to crude oil emissions.

618   In this work, this ratio varied between 0.6 and 1.7 for all samples in the particulate

619   and gaseous phases.
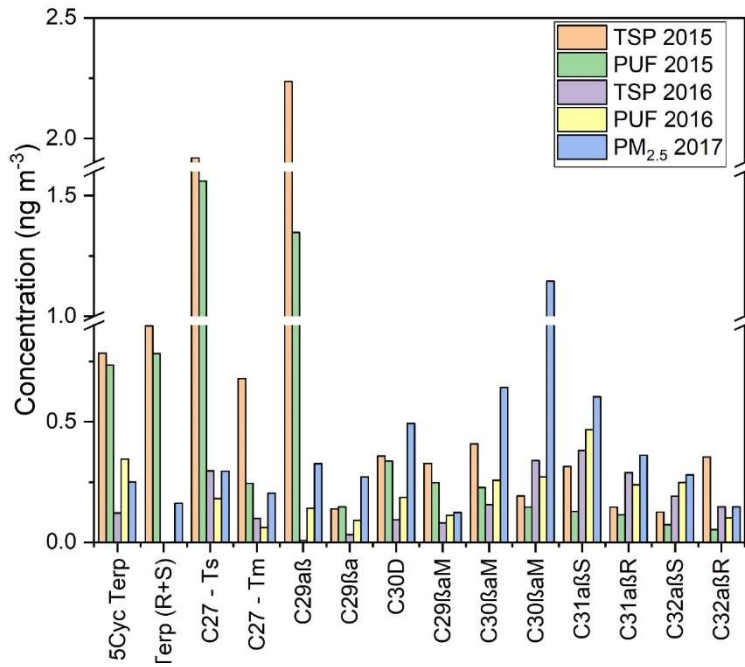
620

621   **Figure 3 –** Average concentration of hopanes determined in this study between 2015 and

622   2017.

623   Another parameter adopted by previous studies is the $C29\alpha\beta/C30\alpha\beta$ ratio,

624   which indicates the origin of petroleum. In 2015 this ratio was 5.6, while for 2016 and

625   2017, the values were lower (0.6 and 0.5, respectively) for PM samples. Han et al.

626   (2015) documented values of $C29\alpha\beta/C30\alpha\beta$ between 0.4 and 0.6 for vehicle

627   exhausts. Ratios higher than 1 were previously described for residential and

628   industrial coal burning (Boonyatumanond et al., 2007; Zakaria et al., 2000).

629   Residential heating is rare in Brazil; the input from other different sources should be

630   taken into account.

3.4.   Pesticides

Eleven pesticides were determined in PM samples between 2015 and 2017. This analysis can highlight relevant information about the use and behavior of pesticides in the petrochemical area. Some compounds such as heptachlor, α and β endosulfan, classified as persistent organic pollutants (POP) (Stockholm Convention, 2017), were banned some years ago in Brazil (ANVISA, 2017), , sugesting atmospheric transport from other regions. Many of these POPs evaporate in hot climates, travel long distances through the atmosphere and settle in other receptor locations. Other compounds, such as diuron, atrazine, and permethrin, can give an indication of the frequency of use of these classes of compounds. Table 5 shows the detection frequency, ranges, and average concentrations for each pesticide.

Table 5 - Detection frequency and concentration range of the pesticides determined.

	Detection frequency (%)			Concentration range (pg m^{-3})		
Pesticides	**2015**	**2016**	**2017**	**2015**	**2016**	**2017**
Diuron	100	100	-	<LQ - 65	<LQ - 61	-
Atrazine	13	-	100	ND - 40	-	<LQ - 52
Heptachlor	87	67	100	<LD - 56	<LD - <LQ	<LQ - 54
Malathion	100	100	92	74 - 360	35 - 161	ND - 537
Heptachlor epoxide	-	-	50	-	-	<LD - <LQ

| Kresoxim methyl	-	-	8	-	-	45
Ethion	-	7	83	-	25	<LD - 750
β-endosulfan	93	73	67	ND - 62	ND - 53	ND - 534
λ-cyhalothrin	-	-	17	-	-	ND - 84
Permethrin I	93	40	100	ND - 464	ND - 67	35 - 518
Permethrin II	93	40	100	ND - 536	ND - 81	25 - 625

645   <LD: below limit of detection; <LQ: below limit of quantification; ND: not detected

646   The average concentrations of pesticides found across the sampling years

647 are showen in Figure 4. Permethrin I and permethrin II were detected in all samples,

648 showing similar concentrations in 2015 and 2017. These compounds are isomers

649 and principal components of permethrin formulation, a well-known pesticide. This

650 formulation is generally used for the control of domestic pests. Thus, it is frequently

651 found in higher concentrations in urban areas. In recent years, Brazil has presented

652 outbreaks of dengue, zika, chikungunya, and yellow fever. According to the

653 Government of São Paulo State (2018), SPMR presented a growing number of

654 cases of dengue (100,000 cases reported), which resulted in a massive application

655 of insecticides composed by permethrin to mitigate the situation that could explain

656 these high concentrations of the isomers in the area.

657   Malathion showed high detection frequency and concentration in samples,

658 presenting mean concentrations of 165 pg m^{-3}, 91 pg m^{-3}, and 215 pg m^{-3} in 2015,

659 2016, and 2017, respectively. This compound has been used for mosquito control,

660 such as permethrin. An increase in the applications of some insecticides to fight the

661 vector was observed.
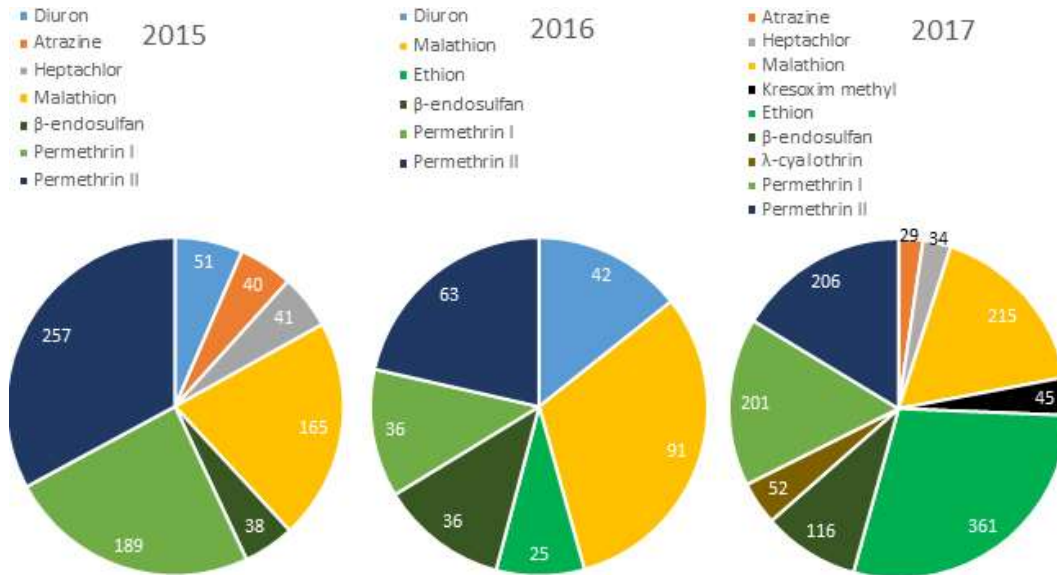
662

663 **Figure 4 -** Average concentrations (pg m^{-3}) of pesticides found in 2015, 2016 and 2017.

664   Heptachlor and endosulfan were banned in the European Union, some years

665 ago. Endosulfan is composed of α and β isomers and was banned in Brazil, in 2013.

666 These compounds have low vapor pressures. For this reason, they are usually

667 detected in the gaseous phase (Weber et al., 2010). α-Endosulfan was not detected

668 in the collected samples. On the other hand, β-endosulfan was found with high

669 frequencies. In 2017, this compound reached a maximum concentration of 116 pg

670 m^{-3}.

671   Heptachlor was used in Brazil for wood preservation until 2016 (ANVISA,

672 2017). Generally, the presence of this compound is accompanied by heptachlor

673 epoxide, one of its major degradation products (Secretary of Health of São Paulo

674 State, 2003). Samples collected in 2015 showed the highest concentration of

675 heptachlor (56 pg m^{-3}), while heptachlor epoxide was not detected in this campaign,

676 suggesting a recent application of the precursor pesticide. In 2016, heptachlor and

677 heptachlor epoxide were not detected in the samples.

678   In the 2017 campaign, when heptachlor was no longer being used, the

679 lowest concentration was found (19 pg m^{-3}), and differently from previous years,

680 heptachlor epoxide was detected in 50% of the samples. However, it was below the

681 quantification limit. The detection of heptachlor and heptachlor epoxide does not

682 point to illegal use. In contrast, the concentrations of the degradation product have

683 growth, whereas the heptachlor concentrations decreased over the years.

684   The Daily Inhalation Exposure (DIE) for infants, children, and adults was

685 calculated to determine the risk of exposure through inhalation of concentrations

686 found at the petrochemical site. There are no standard values to establish a safe

687 limit of exposure. Still, it is possible to infer from these results the compound to which

688 inhabitants in the study area are mostly exposed.

689   In 2015, the highest DIE values were related to malathion: 1×10^{-4} mg kg^{-}

690 ^{1}day^{-1} for infants, 9×10^{-5} mg kg^{-1}day^{-1} for children and 4×10^{-5} mg kg^{-} ^{1}day^{-1} for

691 adults. These results are attributed to the high malathion concentrations found in the

692 samples. In addition, considering the high vapor pressure of this compound ($4 \times 10^{-}$

693 ^{5} mmHg at 25° C) (Sergi, 2019), malathion concentration in the gaseous phase can

694 be much higher than those observed in PM samples. On the other hand, permethrin,

695 which also showed similar concentrations to those of malathion, presented the

696 lowest DIE values: 1×10^{-7} mg kg^{-1}day^{-1} for infants, 8×10^{-8} mg kg^{-1}day $^{-1}$ for children

697   and 4 x 10^{-8} mg kg^{-1}day^{-1} for adults. The vapor pressure of permethrin (2 x 10^{-8}

698   mmHg at 25° C) (Toynton et al., 2009) indicates that its distribution is predominantly

699   in the particulate phase. The gaseous phase contribution tends to be insignificant for

700   permethrin.

701   Previous studies highlighted the harmful effects of malathion on human

702   health, related to acute and chronic exposure, mutagenicity, as well as

703   carcinogenicity. Moreover, the most critical tumors in humans are classified as non-

704   Hodgkin lymphomas, with a positive association with malathion exposure (Sergi,

705   2019). Permethrin is considered a low toxicity compound for mammalian (Toynton

706   et al., 2009). However, it is classified by EPA as likely to be carcinogenic for humans

707   (US EPA, 2017).

708   In 2016, DIE values were lower than those observed in the previous year.

709   The highest values for this campaign correspond to malathion, being 9 x 10^{-6} mg kg^{-}

710   ^{1}day^{-1} for infants, 8 x 10^{-6} mg kg^{-1}day^{-1} for children and 3 x 10^{-6} mg kg^{-1}day^{-1} for

711   adults. While lowest values correspond to ethion, 4 x 10^{-9} mg kg^{-1}day^{-1} for infants, 3

712   x 10^{-9} mg kg^{-1}day^{-1} for children and 1 x 10^{-9} mg kg^{-1}day^{-1} for adults.

713   In other cities, DIE associated with malathion showed lower results than

714   those obtained in the present study: 5 x10^{-8} mg kg^{-1}day^{-1} in Salvador, an urban town

715   in northern Brazil with the influence of crop areas (Nascimento et al., 2018), and 6 x

716   10^{-7} mg kg^{-1}day^{-1} in Valencia, Spain, a region strongly influenced by agricultural

717   activity (López et al., 2017).

718   In the 2017 samples, heptachlor presented the highest levels of DIE: 2×10^{-4}

719   mg kg^{-1} day^{-1} for infants, 1×10^{-4} mg kg^{-1}day^{-1} for children and 6×10^{-5} mg kg^{-1}day^{-1}

720   for adults. These results were higher than those reported for 2015 and 2016. The

721   lowest DIE values were obtained for λ-cyhalothrin: 2×10^{-8} mg kg^{-1}day^{-1} for infants,

722   1×10^{-8} mg kg^{-1} day^{-1} for children and 6×10^{-9} mg kg^{-1}day^{-1} for adults.

723   The Hazard Quotient, HQ$_{AOEL}$, measures the daily inhalation value versus the

724   highest amount of chronic exposure to an operator (US EPA, 2009) during a labor

725   day . According to the literature, values higher than 1 indicate a risk to human health

726   (Coscollà and Yusà, 2016). Comparing to results relating to the presented work,

727   HQ$_{AOEL}$ was calculated for each campaign, even with a 24 hours of the sampling

728   period. The highest value of HQ$_{AOEL}$ in 2015 was for malathion (3×10^{-3} in infants)

729   and the lowest for permethrin II (2×10^{-6} in infants). In 2016, lowest values were

730   registered, ranging from 7×10^{-4} for β-endosulfan to 8×10^{-7} for permethrin I, both

731   for infants. The highest values of HQ$_{AOEL}$ in the three years were observed in 2017

732   when the value for β-endosulfan reached 0.02 for infants. All the values found in this

733   work are below 1.0, indicating that there is no significant risk to human health by

734   inhalation exposure to these compounds.

735   Cancer risk was calculated for pesticides. Table 6 shows the values obtained

736   for infants for all campaigns. Pesticides classified by the EPA as "likely to be

737   carcinogenic to humans" and "possibly carcinogen to humans" showed that high

738   concentrations of these compounds increase cancer risk in the population (Table 5).

739   The maximum acceptable value for cancer risk is 1×10^{-6} (Yusà et al., 2014).

740   Considering compounds classified as likely carcinogenic and possible

741   carcinogenic, values above the recommended were observed for heptachlor. For

742 2015, the values were 1×10^{-5} in infants, 8×10^{-6} in children and 3×10^{-6} in adults,

743 while in 2107, the values surpassed those of 2015, being 2×10^{-5}, 1×10^{-5} and $6 \times$

744 10^{-6} in infants, children, and adults, respectively. Malathion, a compound classified

745 as "suggestive evidence of carcinogenicity, but not sufficient to assess human

746 carcinogenic potential" (US EPA, 2017), presented in 2015 and 2017 similar results

747 to those observed for heptachlor. The values for 2015 were 1×10^{-5} in infants, $9 \times$

748 10^{-6} in children, and 4×10^{-6} in adults. Another compound that presented higher

749 values in 2017 was β-endosulfan (5×10^{-6} in infants), but this compound is classified

750 as "not likely to be carcinogenic to humans" (US EPA, 2017). Therefore, their

751 concentrations should not influence the risk of cancer. An environment strongly

752 impacted by vehicle emissions and near a large industrial park can be harmful to

753 human health.

754

755 **Table 6 -** Cancer classification and cancer risk for pesticides in 2015, 2016, and 2017.

Pesticides	Classification*	2015	2016	2017
Diuron	Likely Carcinogenic	2×10^{-7}	3×10^{-8}	-
Heptachlor	Possible	1×10^{-5}	-	2×10^{-5}
Malathion	No Evidence	1×10^{-5}	9×10^{-7}	8×10^{-6}
Ethion	Evidence	-	5×10^{-9}	4×10^{-7}
β-endosulfan	Not Carcinogenic	2×10^{-7}	1×10^{-7}	5×10^{-6}
Atrazine	Not Carcinogenic	2×10^{-8}	-	4×10^{-8}
Kresoxim-methyl	Likely Carcinogenic	-	-	1×10^{-8}

λ-cyhalothrin	Not Classifiable	-	-	8×10^{-9}
Permethrin I	Likely Carcinogenic	1×10^{-7}	1×10^{-8}	2×10^{-7}
Permethrin II	Likely Carcinogenic	2×10^{-7}	1×10^{-8}	2×10^{-7}

*Not Carcinogenic (Not likely to be carcinogenic to humans), Likely Carcinogenic (Likely to be carcinogenic to humans), No Evidence (Suggestive evidence of carcinogenicity, but not sufficient to assess human carcinogenic potential), Evidence (Evidence of no carcinogenicity in humans), Possible (Possible human carcinogen), Not Classifiable (Not Classifiable as a Human Carcinogen).

3.5. Mutagenicity

The *Salmonella*/microsome test was used to investigate the mutagenicity of the PM collected in this urban-industrial area. After extraction, the extract mass obtained was as follows: 2361 µg from the filters collected in 2015 (TSP); 2253 µg from those collected in 2016 (TSP) and 1302 µg from those filters collected in 2017 ($PM_{2.5}$). The selected strain was *Salmonella* Typhimurium TA98 that detects frameshift mutations. This strain was found to detect the majority of environmental mutagens (Maselli et al., 2019; Umbuzeiro et al., 2008). The tests were conducted in the presence and absence of metabolic activation in vitro, to verify the mutagenic potencies of compounds and/or their bioproducts (Maselli et al., 2019; Umbuzeiro et al., 2008).

Table 7 showed the number of revertants induced by the extracts of PM without S9 mix (-S9) and with S9 mix (+S9) in the present study and other cities, expressed in revertants per cubic meter. The organic extracts of TSP and $PM_{2.5}$ showed

mutagenic activity under test conditions. The strain TA98 of *Salmonella* Typhimurium in the presence of metabolic activation (+S9) responded more weakly than in the absence (-S9), demonstrating the presence of compounds capable of interacting directly on the genetic material. The occurrence of mutagenic compounds in air particles with direct action on the genetic material was widely demonstrated in urban samples collected in different cities, showing the contribution mainly of nitro-PAH and oxy-PAH (Feretti et al., 2019; Ishikawa et al., 2016).

Table 7 - *Mutagenic activity (rev m^{-3}) for the sites analysed in this study and comparison with other sites that used Salmonella/microsome test in micro-suspension*

Site	*Salmonella* Typhimurium (rev m^{-3})		References
	TA98-S9	**TA98+S9**	
CAP2015	5.7	5.4	This work
CAP2016	5.5	4.7	
CAP2017	3.7	3.0	
São Paulo, Brazil	88	19	(Umbuzeiro et al., 2008)
Rio de Janeiro, Brazil	5.7	n.d.	(Ramos De Rainho et al., 2013)
Brescia, Italy	1.2	1.4	(Feretti et al., 2019)

Limeira, Brazil	160.0	18.0	(Maselli et al., 2019)
Zelzate, Belgium	17.0	15.5	(Du Four et al., 2005)

788   n.d. = mutagenicity not detected under test conditions

789   In general, the highest mutagenic potencies were obtained in the absence

790   of S9 (-S9) for the three campaigns. It means that polar fractions of the samples,

791   which typically contain nitro compounds (e.g., nitro-PAH), were more mutagenic.

792   These compounds have shown high mutagenic influences in the absence of S9

793   (Umbuzeiro et al., 2014) than the less polar fraction, which contains unsubstituted

794   PAH and are reactive with DNA only in the presence of S9.

795   Likewise, these results are in accordance with the chemical composition of

796   the samples since the decreases of the mutagenic response observed in the

797   presence of S9 for all campaigns are a typical of PM with high concentrations of

798   nitroaromatic compounds (Claxton et al., 2004), confirming the greater mutagenic

799   activity observed in the assays without metabolic activation. Umbuzeiro and

800   collaborators (2008 and 2014) demonstrated the high contribution of nitroaromatics

801   to the total mutagenic activity registered with the TA98 strain of *S*. Typhimurium,

802   reinforcing the inference of this study that nitro-PAH are important contributors to the

803   mutagenicity detected in the analysed extracts.

804   The samples collected in the three campaigns showed similar results to those

805   of urban sites with intense vehicular traffic, such as Rio de Janeiro, Brazil (Ramos

De Rainho et al., 2013). In contrast, the values were lower than those in samples collected in Limeira, city in the countryside of São Paulo, which was impacted by heavy traffic, industrial emissions, and biomass burning associated with ethanol production (Maselli et al., 2019) and São Paulo city (Table 7) (Umbuzeiro et al., 2008), because, at that time, a massive aerosol contribution of sugarcane burning from rural cities reached the metropolitan area summing to vehicular and industrial emissions. According to the literature, the predictability of a compound being positive for the *Salmonella*/microsome test and also for a carcinogenicity test is approximately 90% (Zeiger, 2019). Another study, conducted in Zelzate, Belgium, showed higher results to the test with the absence of S9 (Table 3). This sampling site was located near a petrochemical factory in the Flanders region (Du Four et al., 2005).

The tested PM samples demonstrated their ability to induce genetic damage. Both the TSP particles and the fine particles showed evidence for mutagenic activity. The detected effects were constant over time and did not show significant variability in relation to the different seasons. The emission sources did not change, whilst the profile of the pollutants showed interesting differences.

4. Conclusion

Atmospheric particulate matter collected in three campaigns in a site with strong anthropogenic influence was characterized. TSP samples collected in 2015 presented the highest PM, OC and EC concentrations, and the wind roses indicating

828 strong contribution from roadway and traffic. The 2016 and 2017 samples, collected

829 during winter, presented low PM values comparing to 2015 samples. Twenty-three

830 percent of the fine particles samples (2017) presented PM concentrations above that

831 recommended by WHO.

832   The 2017 samples showed the highest total PAH concentration, including

833 benzo(a)pyrene, as well as high levels of the pesticide ethion, the wind rose for this

834 campaign showed that the sampling site was under the influence of prevailing winds

835 blowing predominantly from the industrial area. The 2016 campaign presented the

836 maximum for nitro and oxy-PAH total concentrations. 9-Fluorenone and 1-

837 nitropyrene, markers of diesel combustion, were detected in several samples.

838   Regarding exposure risk for PAH (BaP_{Eq} and LCR) and nitro-PAH ($BaP_{Eq\ Nitro\text{-}}$

839 $_{PAH}$ and LCR), samples from the three campaigns presented much higher values

840 than those showed in different sites, evidencing the hazardous to human health

841 related to aerosol inhalation in this region.

842   The predominance of homologues below C_{25} for the *n*-alkanes, low carbon

843 preference indexes, and low percentage of biogenic compounds, stressed a major

844 input from anthropogenic sources for these aliphatic compounds. The

845 stereochemical configurations of hopanes suggest the same source over the years,

846 in which the emissions were associated with completely mature petroleum.

847 Compounds such as permethrin and malathion, also used in the control of domestic

848 pests, presented the highest concentrations in all campaigns due to their extensive

849 use in this area for mosquitoes' control. 2017 was the year for which the highest

850 values of daily inhalation exposure, hazard quotient, and cancer risk were obtained,

41

851 with malathion and heptachlor being the ones that contributed most to these results,

852 suggesting that exposure to these compounds can be dangerous.

853   The results of the *Salmonella*/microsome test in the absence of metabolic

854 activation suggest that some compounds in the samples are directly mutagenic.

855   Even though the remarkable contribution of petrochemical industries and oil

856 refineries to air pollution worldwide, there is a lack of scientific knowledge about the

857 potential adverse health effects for the population living in their neighborhood.

858 Therefore, studies on the atmospheric chemical composition around a petrochemical

859 complex can present a tremendous impact in an attempt to reduce the levels of air

860 pollutants and, consequently, reduce the risks to human health. Thus, the present

861 study contributed to this purpose, expanding the field understanding.

862

863 **Declarations**

864 **Ethics approval and consent to participate**

865 Not applicable.

866

867 **Consent for publication**

868 Not applicable.

869

870 **Availability of data and materials**

871 All data generated or analyzed during this study are included in this article

872

873 **Competing interests**

874 The authors declare that they have no competing interest.

Authors' contributions

Author Sofia Caumo conducted Organic carbon (OC), elemental carbon (EC), polycyclic aromatic hydrocarbons (PAH), and its derivatives (nitro and oxy-PAH), *n*-alkanes, and hopanes laboratory analyses, wrote the article, calculated the risk, and did statistical analyses. Author Aleinnys Year conducted pesticides laboratory analyses, wrote the article, and calculated the risk for pesticides. Author Professor Author Ana Vicente supported Sofia on *n*-alkanes, and hopanes analyses reviewed the calculations, and revised the manuscript. Author Célia Alves supported Sofia on OC, EC, *n*-alkanes, and hopanes analyses critically reviewed these analyses, and revised the manuscript. Deborah A. Roubicek conducted the Salmonella/microsome

43

899 analyses, calculated the risk, and revised the manuscript. Pérola Vasconcellos

900 designed and supervised the work, participated in calculations, and the text

901 correction. All authors read and approved the final version of the manuscript. Direct

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911 **5. References**

912 Agudelo-Castañeda, D.M., Teixeira, E.C., 2014. Seasonal changes, identification

913   and source apportionment of PAH in PM1.0. Atmospheric Environment 96,

914   186–200. https://doi.org/10.1016/j.atmosenv.2014.07.030

915 Albinet, A., Leoz-Garziandia, E., Budzinski, H., Villenave, E., Jaffrezo, J.L., 2008.

916   Nitrated and oxygenated derivatives of polycyclic aromatic hydrocarbons in the

917   ambient air of two French alpine valleys. Part 1: Concentrations, sources and

918   gas/particle partitioning. Atmospheric Environment 42, 43–54.

919   https://doi.org/10.1016/j.atmosenv.2007.10.009

920 Alves, C.A., Oliveira, C.C., Martins, N., Mirante, F., Caseiro, A., Pio, C., Matos, M.,

921   Silva, H.F., Oliveira, C.C., Camões, F., 2016. Road tunnel, roadside, and

922   urban background measurements of aliphatic compounds in size-segregated

923   particulate matter. Atmospheric Research 168, 139–148.

924   https://doi.org/10.1016/j.atmosres.2015.09.007

925 Alves, C.A., Vicente, A.M., Rocha, S., Vasconcellos, P., 2017. Hopanoid

926   hydrocarbons in PM10 from road tunnels in São Paulo, Brazil. Air Quality,

927   Atmosphere & Health. https://doi.org/10.1007/s11869-017-0462-3

928 Ames, B.N., Joyce, M., Yamasaki, E., 1975. Methods for Detecting Carcinogens

929   and Mutagens With. Mutation Research 31, 347–363.

930 Andersson, J.T., Achten, C., 2015. Time to Say Goodbye to the 16 EPA PAHs?

931   Toward an Up-to-Date Use of PACs for Environmental Purposes. Polycyclic

932   Aromatic Compounds 35, 330–354.

933   https://doi.org/10.1080/10406638.2014.991042

934 Andreou, G., Rapsomanikis, S., 2009. Origins of n-alkanes, carbonyl compounds

935   and molecular biomarkers in atmospheric fine and coarse particles of Athens,

936   Greece. Science of the Total Environment 407, 5750–5760.

937   https://doi.org/10.1016/j.scitotenv.2009.07.019

938 ANVISA, 2017. Listas de ingredientes ativos com uso autorizado e banidos no

939   Brasil. URL http://portal.anvisa.gov.br/rss/-

940   /asset_publisher/Zk4q6UQCj9Pn/content/consulta-publica-sobre-avaliacao-

941   toxicologi-1/219201?inheritRedirect=false (accessed 8.13.19).

942 Bernstein, L., Kaldor, J., McCann, J., Pike, M.C., 1982. An empirical approach to

943   the statistical analysis of mutagenesis data from the Salmonella test. Mutation

944   Research 97, 267–281. https://doi.org/0165-1161/82/0000-0000

945 Bombardi, L.M., 2017. Geografia do uso de Agrotóxicos no Brasil e Conexões com

946   a União Européia, 1st ed. FFLCH-USP, São Paulo.

947 Boonyatumanond, R., Murakami, M., Wattayakorn, G., Togo, A., Takada, H., 2007.

948   Sources of polycyclic aromatic hydrocarbons (PAHs) in street dust in a tropical

949   Asian mega-city, Bangkok, Thailand. Science of the Total Environment 384,

950   420–432. https://doi.org/10.1016/j.scitotenv.2007.06.046

951 Bost, F.D., Frontera-Suau, R., McDonald, T.J., Peters, K.E., Morris, P.J., 2001.

952   Aerobic biodegradation of hopanes and norhopanes in Venezuelan crude oils.

953   Organic Geochemistry 32, 105–114. https://doi.org/10.1016/S0146-

954   6380(00)00147-9

955 Brito, J., Rizzo, L. V, Herckes, P., Vasconcellos, P.C., Caumo, S.E.S., Fornaro, A.,

956   Ynoue, R.Y., Artaxo, P., Andrade, M.F., 2013. Physical–chemical

957   characterization of the particulate matter inside two road tunnels in the São

958   Paulo Metropolitan Area. Atmos. Chem. Phys 13, 12199–12213.

959   https://doi.org/10.5194/acp-13-12199-2013

960 Callén, M.S., Iturmendi, A., López, J.M., 2014. Source apportionment of

961   atmospheric PM2.5-bound polycyclic aromatic hydrocarbons by a PMF

962   receptor model. Assessment of potential risk for human health. Environmental

963   Pollution 195, 167–177. https://doi.org/10.1016/j.envpol.2014.08.025

964 Castro, L.M., Pio, C.A., Harrison, R.M., Smith, D.J.T., 1999. Carbonaceous aerosol

965   in urban and rural European atmospheres: Estimation of secondary organic

966   carbon concentrations. Atmospheric Environment 33, 2771–2781.

967   https://doi.org/10.1016/S1352-2310(98)00331-8

968 Caumo, S., 2020. Caracterização química e determinação do potencial oxidativo

969   em material particulado atmosférico coletado perto de uma área industrial na

970   região metropolitana de São Paulo. Universidade de São Paulo.

971 Caumo, S., Vicente, A., Custódio, D., Alves, C., Vasconcellos, P., 2018. Organic

972   compounds in particulate and gaseous phase collected in the neighbourhood

973   of an industrial complex in São Paulo (Brazil). Air Quality, Atmosphere and

974   Health 11, 271–283. https://doi.org/10.1007/s11869-017-0531-7

975 Ciccioli, P., Cecinato, A., Brancaleoni, E., Frattoni, M., Zacchei, P., Miguel, A.H.,

976   Vasconcellos, P.D.C., 1996. Formation and transport of 2-nitrofluoranthene

977   and 2-nitropyrene of photochemical origin in the troposphere. Journal of

978   Geophysical Research 101, 19567. https://doi.org/10.1029/95JD02118

979 Claxton, L.D., Matthews, P.P., Warren, S.H., 2004. The genotoxicity of ambient

980   outdoor air, a review: Salmonella mutagenicity. Mutation Research - Reviews

981   in Mutation Research 567, 347–399.

982   https://doi.org/10.1016/j.mrrev.2004.08.002

983 CONAMA, 2018. RESOLUÇÃO Nº 491 ("Padrões Nacionais de Qualidade do Ar").

984 Coronas, M.V., Horn, R.C., Ducatti, A., Rocha, J.V., Vargas, V.M.F., 2008.

985   Mutagenic activity of airborne particulate matter in a petrochemical industrial

986   area. Genetic Toxicology and Environmental Mutagenesis 650, 196–201.

987   https://doi.org/10.1016/j.mrgentox.2007.12.002

988 Coscollà, C., Yusà, V., 2016. Pesticides and Agricultural Air Quality.

989   Comprehensive Analytical Chemistry 73, 423–490.

990   https://doi.org/10.1016/BS.COAC.2016.04.012

991 de Oliveira Alves, N., Brito, J., Caumo, S., Arana, A., de Souza Hacon, S., Artaxo,

992   P., Hillamo, R., Teinilä, K., Batistuzzo de Medeiros, S.R., de Castro

993   Vasconcellos, P., 2015. Biomass burning in the Amazon region: Aerosol

994   source apportionment and associated health risk assessment. Atmospheric

995   Environment 120. https://doi.org/10.1016/j.atmosenv.2015.08.059

996 de Oliveira Galvão, M.F., de Oliveira Alves, N., Ferreira, P.A., Caumo, S., de

997   Castro Vasconcellos, P., Artaxo, P., de Souza Hacon, S., Roubicek, D.A.,

998   Batistuzzo de Medeiros, S.R., 2017. Biomass burning particles in the Brazilian

999   Amazon region: Mutagenic effects of nitro and oxy-PAHs and assessment of

1000   health risks. Environmental Pollution 1–11.

1001   https://doi.org/10.1016/j.envpol.2017.09.068

1002 Delclos, K.B., El-Bayoumy, K., Casciano, D.A., Walker, R.P., Kadlubar, F.F.,

1003   Hecht, S.S., Shivapurkar, N., Mandal, S., Stoner, G.D., 1989. Metabolic

1004   activation of 6-nitrochrysene in explants of human bronchus and in isolated rat

1005   hepatocytes. Cancer Research 49, 2909–2913.

1006 Du Four, V.A., Janssen, C.R., Brits, E., Van Larebeke, N., 2005. Genotoxic and

1007   mutagenic activity of environmental air samples from different rural, urban and

1008   industrial sites in Flanders, Belgium. Mutation Research - Genetic Toxicology

1009   and Environmental Mutagenesis 588, 106–117.

1010   https://doi.org/10.1016/j.mrgentox.2005.09.007

1011 Feretti, D., Pedrazzani, R., Ceretti, E., Dal Grande, M., Zerbini, I., Viola, G.C.V.,

1012   Gelatti, U., Donato, F., Zani, C., 2019. "Risk is in the air": Polycyclic aromatic

1013   hydrocarbons, metals and mutagenicity of atmospheric particulate matter in a

1014   town of Northern Italy (Respira study). Mutation Research - Genetic

1015   Toxicology and Environmental Mutagenesis 842, 35–49.

1016   https://doi.org/10.1016/j.mrgentox.2018.11.002

1017 Forman, H.J., Finch, C.E., 2018. A critical review of assays for hazardous

1018   components of air pollution. Free Radical Biology and Medicine 117, 202–217.

1019   https://doi.org/10.1016/j.freeradbiomed.2018.01.030

1020   Fuzzi, S., Gilardoni, S., Kokhanovsky, A.A., Di Nicolantonio, W., Mukai, S., Sano,

1021   I., Nakata, M., Tomasi, C., Lanconelli, C., 2016. Aerosol and Air Quality.

1022   Atmospheric Aerosols 553–596. https://doi.org/10.1002/9783527336449.ch9

1023   Gori, G., Carrieri, M., Scapellato, M.L., Parvoli, G., Ferrara, D., Rella, R., Sturaro,

1024   A., Bartolucci, G.B., 2009. 2-Methylanthraquinone as a marker of occupational

1025   exposure to teak wood dust in boatyards. Annals of Occupational Hygiene 53,

1026   27–32. https://doi.org/10.1093/annhyg/men069

1027   Guttenplan, J.B., Zhao, Z.L., Kosinska, W., Norman, R.G., Krzeminski, J., Sun,

1028   Y.W., Amin, S., El-Bayoumy, K., 2007. Comparative mutational profiles of the

1029   environmental mammary carcinogen, 6-nitrochrysene and its metabolites in a

1030   lacI mammary epithelial cell line. Carcinogenesis 28, 2391–2397.

1031   https://doi.org/10.1093/carcin/bgm142

1032   Han, F., Cao, J., Peng, L., Bai, H., Hu, D., Mu, L., Liu, X., 2015. Characteristics of

1033   hopanoid hydrocarbons in ambient PM10 and motor vehicle emissions and

1034   coal ash in Taiyuan, China. Environmental Geochemistry and Health 37, 813–

1035   829. https://doi.org/10.1007/s10653-015-9763-3

1036   Harner, T., Bidleman, T.F., 1998. Octanol-air partition coefficient for describing

1037   particle/gas partitioning of aromatic compounds in urban air. Environmental

1038   Science and Technology 32, 1494–1502. https://doi.org/10.1021/es970890r

1039   He, C., Wang, C., Zhou, Y., Li, J., Zuo, Z., 2012. Embryonic exposure to

1040   benzo(a)pyrene influences neural development and function in rockfish

1041   (Sebastiscus marmoratus). NeuroToxicology 33, 758–762.

1042   https://doi.org/10.1016/j.neuro.2012.01.002

Huang, B., Liu, M., Bi, X., Chaemfa, C., Ren, Z., Wang, X., Sheng, G., Fu, J., 2014. Phase distribution, sources and risk assessment of PAHs, NPAHs and OPAHs in a rural site of Pearl River Delta region, China. Atmospheric Pollution Research 5, 210–218. https://doi.org/10.5094/apr.2014.026

IARC, 2016. Outdoor Air Pollution: Volume 109, IARC monographs.

IARC, 2012. Agents Classified by the IARC Monographs , Volumes 1 – 104. IARC Monographs 7, 1–25.

IARC, 2010. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans.

Ishikawa, S., Kanemaru, Y., Nara, H., Erami, K., Nagata, Y., 2016. Assessing the mutagenic activities of smoke from different cigarettes in direct exposure experiments using the modified Ames Salmonella assay. Mutation Research - Genetic Toxicology and Environmental Mutagenesis 803–804, 13–21. https://doi.org/10.1016/j.mrgentox.2016.04.008

Kado, N.Y., Langley, D., Eisenstadt, E., 1983. A simple modification of the Salmonella liquid-incubation assay. Mutation Research 121, 25–32. https://doi.org/0165-7992/83

Karavalakis, G., Fontaras, G., Ampatzoglou, D., Kousoulidou, M., Stournas, S., Samaras, Z., Bakeas, E., 2010. Effects of low concentration biodiesel blends application on modern passenger cars. Part 3: Impact on PAH, nitro-PAH, and oxy-PAH emissions. Environmental Pollution.

Keyte, I.J., Albinet, A., Harrison, R.M., 2016. On-road traffic emissions of polycyclic aromatic hydrocarbons and their oxy- and nitro- derivative compounds measured in road tunnel environments. Science of the Total Environment

1067   566–567, 1131–1142. https://doi.org/10.1016/j.scitotenv.2016.05.152

1068   Kim, K.-H., Jahan, S.A., Kabir, E., Brown, R.J.C., 2013. A review of airborne

1069   polycyclic aromatic hydrocarbons (PAHs) and their human health effects.

1070   Environment international 60, 71–80.

1071   https://doi.org/10.1016/j.envint.2013.07.019

1072   Kishida, M., Nishikawa, A., Fujimori, K., Shibutani, Y., 2011. Gas-particle

1073   concentrations of atmospheric polycyclic aromatic hydrocarbons at an urban

1074   and a residential site in Osaka, Japan: Effect of the formation of

1075   atmospherically stable layer on their temporal change. Journal of Hazardous

1076   Materials 192, 1340–1349. https://doi.org/10.1016/j.jhazmat.2011.06.046

1077   Kleeman, M.J., Riddle, S.G., Robert, M. A., Jakober, C. A., 2008. Lubricating oil

1078   and fuel contributions to particulate matter emissions from light-duty gasoline

1079   and heavy-duty diesel vehicles. Environmental science & technology 42, 235–

1080   42. https://doi.org/10.1021/es071054c

1081   Liu, L., Tang, J., Zhong, G., Zhen, X., Pan, X., Tian, C., 2018. Spatial distribution

1082   and seasonal variation of four current-use pesticides (CUPs) in air and surface

1083   water of the Bohai Sea, China. Science of the Total Environment 621, 516–

1084   523. https://doi.org/10.1016/j.scitotenv.2017.11.282

1085   López, A., Yusà, V., Muñoz, A., Vera, T., Borràs, E., Ródenas, M., Coscollà, C.,

1086   2017. Risk assessment of airborne pesticides in a Mediterranean region of

1087   Spain. Science of the Total Environment 574, 724–734.

1088   https://doi.org/10.1016/j.scitotenv.2016.08.149

1089   Maenhaut, W., Vermeylen, R., Claeys, M., Vercauteren, J., Matheeussen, C.,

1090   Roekens, E., 2012. Assessment of the contribution from wood burning to the

1091   PM10 aerosol in Flanders, Belgium. The Science of the total environment 437,

1092   226–36. https://doi.org/10.1016/j.scitotenv.2012.08.015

1093 Maselli, B.S., Giron, M.C.G., Lim, H., Bergvall, C., Westerholm, R., Dreij, K.,

1094   Watanabe, T., Cardoso, A.A., Umbuzeiro, G.A., Kummrow, F., 2019.

1095   Comparative mutagenic activity of atmospheric particulate matter from limeira,

1096   stockholm, and kyoto. Environmental and Molecular Mutagenesis 60, 607–

1097   616. https://doi.org/10.1002/em.22293

1098 Mikuška, P., Křůmal, K., Večeřa, Z., 2015. Characterization of organic compounds

1099   in the PM2.5 aerosols in winter in an industrial urban area. Atmospheric

1100   Environment 105, 97–108. https://doi.org/10.1016/j.atmosenv.2015.01.028

1101 Mostafalou, S., Abdollahi, M., 2013. Pesticides and human chronic diseases:

1102   Evidences, mechanisms, and perspectives. Toxicology and Applied

1103   Pharmacology 268, 157–177. https://doi.org/10.1016/j.taap.2013.01.025

1104 Na, K., Sawant, A. A., Song, C., Cocker, D.R., 2004. Primary and secondary

1105   carbonaceous species in the atmosphere of Western Riverside County,

1106   California. Atmospheric Environment 38, 1345–1355.

1107   https://doi.org/10.1016/j.atmosenv.2003.11.023

1108 Nascimento, M.M., da Rocha, G.O., de Andrade, J.B., 2018. A rapid low-

1109   consuming solvent extraction procedure for simultaneous determination of 34

1110   multiclass pesticides associated to respirable atmospheric particulate matter

1111   (PM2.5) by GC–MS. Microchemical Journal 139, 424–436.

1112   https://doi.org/10.1016/j.microc.2018.03.023

1113 Nisbet, I.C.T., Lagoy, P.K., 1992. Toxic Equivalency Factors (TEFs) for Polycyclic

1114   Aromatic Hydrocarbons (PAHs). Regulatory toxicology and pharmacology :

1115 RTP 16, 290–300.

1116 Nocun, M.S., Schantz, M.M., 2013. Determination of selected oxygenated

1117 polycyclic aromatic hydrocarbons (oxy-PAHs) in diesel and air particulate

1118 matter standard reference materials (SRMs). Analytical and Bioanalytical

1119 Chemistry 405, 5583–5593. https://doi.org/10.1007/s00216-013-6957-3

1120 OEHHA, 2011. Chemical-specific Summaries of the Information Used to Derive

1121 Unit Risk and Cancer Potency Values. (Appendix B).

1122 Omar, N.Y.M.J., Abas, M.R. Bin, Ketuly, K.A., Tahir, N.M., 2001. Heavy molecular-

1123 weight organic compounds in the atmosphere : The hopanes 7, 203–208.

1124 Oros, D.R., Simoneit, B.R.T., 2001. Identification and emission factors of molecular

1125 tracers in organic aerosols from biomass burning Part 1. Temperate climate

1126 conifers, Applied Geochemistry. https://doi.org/10.1016/S0883-

1127 2927(01)00021-X

1128 Pereira, G.M., De Oliveira Alves, N., Caumo, S.E.S., Soares, S., Teinila, K.,

1129 Custódio, D., Hillamo, R., Alves, C., Vasconcellos, P.C., 2017. Chemical

1130 composition of aerosol in São Paulo, Brazil: influence of the transport of

1131 pollutants. Air Quality, Atmosphere and Health 10, 457–468.

1132 https://doi.org/10.1007/s11869-016-0437-9

1133 Pérez-Martínez, P.J., de Fátima Andrade, M., de Miranda, R.M., 2017. Heavy truck

1134 restrictions and air quality implications in São Paulo, Brazil. Journal of

1135 Environmental Management. https://doi.org/10.1016/j.jenvman.2017.07.022

1136 Pio, C., Cerqueira, M., Harrison, R.M., Nunes, T., Mirante, F., Alves, C., Oliveira,

1137 C., Sanchez de la Campa, A., Artíñano, B., Matos, M., 2011. OC/EC ratio

1138 observations in Europe: Re-thinking the approach for apportionment between

1139 primary and secondary organic carbon. Atmospheric Environment 45, 6121–

1140 6132. https://doi.org/10.1016/j.atmosenv.2011.08.045

1141 Pope, A., Burnett, R., Thun, M., EE, C., D, K., I, K., GD, T., 2002. Long-term

1142 Exposure to Fine Particulate Air Pollution. Jama 287, 1192.

1143 https://doi.org/10.1001/jama.287.9.1132

1144 Ramos De Rainho, C., Machado Corea, S., Mazzei, J.L., Aiub, C.A.F.,

1145 Felzenszwalb, I., 2013. Genotoxicity of polycyclic aromatic hydrocarbons and

1146 nitro-derived in respirable airborne particulate matter collected from urban

1147 areas of Rio de Janeiro (Brazil). BioMed Research International 2013, 9.

1148 https://doi.org/10.1155/2013/765352

1149 Ravindra, K., Sokhi, R., Van Grieken, R., 2008a. Atmospheric polycyclic aromatic

1150 hydrocarbons: Source attribution, emission factors and regulation.

1151 Atmospheric Environment 42, 2895–2921.

1152 https://doi.org/10.1016/j.atmosenv.2007.12.010

1153 Ravindra, K., Wauters, E., Van Grieken, R., 2008b. Variation in particulate PAHs

1154 levels and their relation with the transboundary movement of the air masses.

1155 Science of the Total Environment 396, 100–110.

1156 https://doi.org/10.1016/j.scitotenv.2008.02.018

1157 Roubicek, D.A., Mazzini, F., Soares, C.M., Rech, C.M., 2011. Use of different

1158 Salmonella/microsome assay protocols to evaluate the mutagenicity of air

1159 samples from São Paulo metropolitan area, Brazil. Toxicology Letters P1160,

1160 S60.

1161 Santos, A.G., Regis, A.C.D., da Rocha, G.O., Bezerra, M. de A., de Jesus, R.M.,

1162 de Andrade, J.B., 2016. A simple, comprehensive, and miniaturized solvent

1163   extraction method for determination of particulate-phase polycyclic aromatic

1164   compounds in air. Journal of Chromatography A 1435, 6–17.

1165   https://doi.org/10.1016/j.chroma.2016.01.018

1166   Schoeny, R., Poirier, K., 1993. Provisional Guidance for Quantitative Risk

1167   Assessment of Polycyclic Aromatic Hydrocarbons 1980.

1168   https://doi.org/EPA/600/R-93/089 (NTIS PB94116571)

1169   Sergi, C.M., 2019. Malathion: An Insecticide, in: Reference Module in Earth

1170   Systems and Environmental Sciences. Elsevier. https://doi.org/10.1016/B978-

1171   0-12-409548-9.11317-X

1172   Simoneit, B.R.T., Abas, M.R. Bin, Cass, G.R., Rogge, W.F., Mazurek, M.A.,

1173   Standleys, L.J., Hildemann, M., 1995. Natural Organic Compounds as Tracers

1174   for Biomass Combustion in Aerosols.

1175   Simoneit, B.R.T., Cardoso, J.N., Robinson, N., 1990. An assessment of the origin

1176   and composition of higher molecular weight organic matter in aerosols over

1177   Amazonia. Chemosphere 21, 1285–1301. https://doi.org/10.1016/0045-

1178   6535(90)90145-J

1179   Simoneit, B.R.T., Kobayashi, M., Mochida, M., Kawamura, K., Lee, M., Lim, H.J.,

1180   Turpin, B.J., Komazaki, Y., 2004. Composition and major sources of organic

1181   compounds of aerosol particulate matter sampled during the ACE-Asia

1182   campaign. Journal of Geophysical Research D: Atmospheres 109, 1–22.

1183   https://doi.org/10.1029/2004JD004598

1184   Souza, K.F., Carvalho, L.R.F., Allen, A.G., Cardoso, A.A., 2014. Diurnal and

1185   nocturnal measurements of PAH, nitro-PAH, and oxy-PAH compounds in

1186   atmospheric particulate matter of a sugar cane burning region. Atmospheric

1187   Environment 83, 193–201. https://doi.org/10.1016/j.atmosenv.2013.11.007

1188   Teixeira, E.C., Garcia, K.O., Meincke, L., Leal, K.A., 2011. Study of nitro-polycyclic

1189   aromatic hydrocarbons in fine and coarse atmospheric particles. Atmospheric

1190   Research 101, 631–639. https://doi.org/10.1016/j.atmosres.2011.04.010

1191   Toynton, K., Luukinen, B., Buhl, K., Stone, D., 2009. Permethrin Technical fact

1192   sheet. Oregon Sate University Report.

1193   Umbuzeiro, G. de A., Franco, A., Magalhães, D., de Castro, F.J.V., Kummrow, F.,

1194   Rech, C.M., Carvalho, L.R.F., Vasconcellos, P. de C., 2008. A Preliminary

1195   Characterization of the Mutagenicity of Atmospheric Particulate Matter

1196   Collected During Sugar Cane Harvesting Using the Salmonella / Microsome

1197   Microsuspension Assay. Environmental and Molecular Mutagenesis 000.

1198   https://doi.org/10.1002/em

1199   Umbuzeiro, G. de A., Kummrow, F., Morales, D.A., Alves, D.K.M., Lim, H., Jarvis,

1200   I.W.H., Bergvall, C., Westerholm, R., Stenius, U., Dreij, K., 2014. Sensitivity of

1201   Salmonella YG5161 for Detecting PAH-Associated Mutagenicity in Air

1202   Particulate Matter. Environmental and Molecular Mutagenesis 55, 510–517.

1203   https://doi.org/10.1002/em

1204   US EPA, 2017. Chemicals Evaluated for Carcinogenic Potential Annual Cancer

1205   Report 2017. Annual Cancer Report 2017, US Environmental Protection

1206   Agency Office of Pesticide Programs.

1207   US EPA, 2009. Risk Assessment Guidance for Superfund Volume I: Human Health

1208   Evaluation Manual (Part F, Supplemental Guidance for Inhalation Risk

1209   Assessment). Office of Superfund Remediation and Technology Innovation

1210   Environmental Protection Agency I, 1–68. https://doi.org/EPA-540-R-070-002

1211   Vallero, D., 2014. Air Pollutant Hazards. Fundamentals of Air Pollution 197–214.

1212   https://doi.org/10.1016/b978-0-12-401733-7.00007-4

1213   Vasconcellos, P.C., Sanchez-Ccoyllo, O., Balducci, C., Mabilia, R., Cecinato, A.,

1214   2008. Occurrence and Concentration Levels of Nitro-PAH in the Air of Three

1215   Brazilian Cities Experiencing Different Emission Impacts. Water Air Soil Pollut

1216   190, 87–94. https://doi.org/10.1007/s11270-007-9582-y

1217   Vasconcellos, P.C., Souza, D.Z., Magalhães, D., Da Rocha, G.O., 2011. Seasonal

1218   variation of n-alkanes and polycyclic aromatic hydrocarbon concentrations in

1219   PM10 samples collected at urban sites of São Paulo State, Brazil. Water, Air,

1220   and Soil Pollution 222, 325–336. https://doi.org/10.1007/s11270-011-0827-4

1221   Vasconcellos, P.C., Souza, D.Z., Sanchez-Ccoylo, O., Bustillos, J.O. V, Lee, H.,

1222   Santos, F.C., Nascimento, K.H., Araújo, M.P., Saarnio, K., Teinila, K., Hillamo,

1223   R., Teinilä, K., Hillamo, R., 2010. Determination of anthropogenic and biogenic

1224   compounds on atmospheric aerosol collected in urban, biomass burning and

1225   forest areas in São Paulo, Brazil. Science of the Total Environment 408,

1226   5836–5844. https://doi.org/10.1016/j.scitotenv.2010.08.012

1227   Weber, J., Halsall, C.J., Muir, D., Teixeira, C., Small, J., Solomon, K., Hermanson,

1228   M., Hung, H., Bidleman, T., 2010. Endosulfan, a global pesticide: A review of

1229   its fate in the environment and occurrence in the Arctic. Science of the Total

1230   Environment 408, 2966–2984. https://doi.org/10.1016/j.scitotenv.2009.10.077

1231   Weichenthal, S.A., Lavigne, E., Evans, G.J., Godri Pollitt, K.J., Burnett, R.T., 2016.

1232   Fine particulate matter and emergency room visits for respiratory illness: Effect

1233   modification by oxidative potential. American Journal of Respiratory and

1234   Critical Care Medicine 194, 577–586. https://doi.org/10.1164/rccm.201512-

1235   2434OC

1236   WHO, World Helath Organization, 2018. Pesticides, WHO. World Health

1237   Organization.

1238   WHO, World Health Organisation, 2018. 9 Out of 10 People Worldwide Breathe

1239   Polluted Air But More Countries Are Taking Action.

1240   WHO, World Health Organisation, 2005. Air quality guidelines for particulate

1241   matter, ozone, nitrogen dioxide and sulfur dioxide.

1242   WHO, World Health Organisation, 2000. Air Quality Guidelines, WHO Regional

1243   Publications, European Series.

1244   Yera, A., Nascimento, M., da Rocha, G., de Andrade, J., Vasconcellos, P., 2020.

1245   Occurrence of Pesticides Associated to Atmospheric Aerosols: Hazard and

1246   Cancer Risk Assessments. Journal of the Brazilian Chemical Society 31,

1247   1317–1326. https://doi.org/10.21577/0103-5053.20200017

1248   Yusà, V., Coscollà, C., Millet, M., 2014. New screening approach for risk

1249   assessment of pesticides in ambient air. Atmospheric Environment 96, 322–

1250   330. https://doi.org/10.1016/j.atmosenv.2014.07.047

1251   Zakaria, M.P., Horinouchi, A., Tsutsumi, S., Takada, H., Tanabe, S., Ismail, A.,

1252   2000. Oil Pollution in the Straits of Malacca , Malaysia : Application of

1253   Molecular Markers for Source Identification. Environmental Science &

1254   Technology 34, 1189–1196.

1255   Zeiger, E., 2019. The test that changed the world: The Ames test and the

1256   regulation of chemicals. Mutation Research - Genetic Toxicology and

1257   Environmental Mutagenesis 841, 43–48.

1258   https://doi.org/10.1016/j.mrgentox.2019.05.007

1259   Zhang, Y., Guo, C.S., Xu, J., Tian, Y.Z., Shi, G.L., Feng, Y.C., 2012. Potential

1260   source contributions and risk assessment of PAHs in sediments from Taihu

1261   Lake, China: Comparison of three receptor models. Water Research 46,

1262   3065–3073. https://doi.org/10.1016/j.watres.2012.03.006

1263