



**Universidade do Estado do Rio de Janeiro**  
**Centro Biomédico**  
**Instituto de Biologia Roberto Alcantara Gomes**

Claudia Ramos de Rainho Ribeiro

**Material particulado respirável presente no ar da cidade do Rio de Janeiro:  
estudo geneotóxico**

Rio de Janeiro

2016

Claudia Ramos de Rainho Ribeiro

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geneotóxico**

Tese apresentada, como requisito parcial  
para obtenção do título de Doutor, ao  
Programa de Pós-Graduação em  
Biociências, da Universidade do Estado  
do Rio de Janeiro.

Orientador: Prof. Dr. Israel Felzenszwalb

Coorientadora: Prof.<sup>a</sup> Dr<sup>a</sup>. Claudia Alessandra Fortes Aiub

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Aprovada em: 10/03/2016

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Rio de Janeiro  
2016

## **DEDICATÓRIA**

A minha família.

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A força mais potente do mundo? A fé

*Madre Teresa de Calcutá*

## RESUMO

Ribeiro, Claudia Ramos de Rainho. **Material particulado respirável presente no ar da cidade do Rio de Janeiro:** estudo geneotóxico. 2016. 127 f. Tese (Doutorado em Biociências) –Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2016.

Material particulado (MP2,5) pode estar associado a diversos compostos orgânicos como os hidrocarbonetos policíclicos aromáticos (HPA). Estudos recentes mostram que os HPA podem não ser os predominantes mutágeneos presentes no poluentes atmosféricos, e que compostos nitroaromáticos, aminas aromáticas e acetonas, podem ser potentes mutágeneos. Biomarcadores genéticos são ferramentas úteis para a avaliação inicial da exposição à poluição ambiental e ocupacional. No presente trabalho nós comparamos os níveis de HPA, Nitro-HPA e mutageneicidade, e investigamos os efeitos geneotóxicos em trabalhadores expostos a estes poluentes. As amostras ambientais foram coletadas em dois períodos: (I) Julho à Outubro de 2010 e (II) Novembro de 2010 à Maio de 2011 nos três pontos da cidade do Rio de Janeiro – (1) Campus UERJ; (2) Avenida Brasil e (3) Túnel Rebouças. O biomonitoramento humano foi realizado utilizando amostras de linfócitos e células da mucosa oral no ensaio de micronúcleo (MN). Ensaio de PCR foi realizado para avaliar o polimorfismo em genes responsáveis pela expressão de enzimas de metabolização de fase I (CYP1A1), e de fase II (GSTM1 e GSTT1). Altas concentrações de HPA e Nitro-HPA foram detectadas no ponto 3 em ambos os períodos. Nitroarenos e dinitroarenos foram detectados nos três pontos no período I. No período II, a presença de dinitroarenos foi detectada somente nos pontos 2 e 3. As frequências de MN em linfócitos e em células da mucosa oral apresentaram aumento significativo em indivíduos do grupo exposição (trabalhadores do túnel Rebouças), em comparação aos indivíduos do grupo controle. As informações geradas neste estudo mostram que diferentes níveis de HPA e nitroderivados contribuem para a mutageneicidade. A alta frequência de MN detectada nos indivíduos do grupo exposição, pode estar relacionada a exposição ocupacional aos poluentes aderidos ao MP2,5. O aumento significativo na concentração de 1-HP e 2-NAP ( $\mu\text{mol/mol creatinina}$ ) foi detectado em amostras de urina de indivíduos do grupo exposição. Polimorfismo m2 (CYP1A1\*2B) foi detectado somente em um indivíduo do grupo exposição. Nenhum polimorfismo do gene CYP1A1 foi detectado em amostras do grupo controle. Deleções dos genes GSTM1 e GSTT1 foram detectadas em 100% das amostras dos indivíduos do grupo controle. No grupo exposição foram detectadas deleções em 72,74% das amostras para GSTM1 e em 86,37% das amostras para GSTT1. Estes resultados demonstram que este indivíduo exposto a poluição ocupacional no Túnel Rebouças, pode apresentar intensificação da metabolização de poluentes como os HPA, e ter dificuldades na desintoxicação dos metabólitos destes poluentes.

Palavras-chave: Material particulado. Hidrocarbonetos policíclicos aromáticos. Nitroderivados. Biomonitoramento humano.

## ABSTRACT

Ribeiro, Claudia Ramos de Rainho. **Respirable particulate matter in the air of the city of Rio de Janeiro:** a geneotoxic study. 2016. 127 f. Tese (Doutorado em Biociências) –Instituto de Biologia Roberto Alcantara Gomes, Universidade do Estado do Rio de Janeiro, Rio de Janeiro, 2016.

Particulate matter (PM2.5) can be associated with various organic compounds such as polycyclic aromatic hydrocarbons (PAH). Recent studies show that in addition to PAH, nitroaromatic compounds, aromatic amines and ketones can also be considered potent mutagens. Genetic biomarkers are useful tools for the initial evaluation of human exposure to environmental and occupational pollution. In the present work we: (i) compare the levels of PAH, nitro-PAH and mutagenicity levels of PM2.5 samples from three points of the city of Rio de Janeiro, UERJ Campus, Brasil Avenue and Rebouças Tunnel and (ii) investigate in workers exposed to these pollutants induced geneotoxic effects. Air samples were collected in two periods: July to October 2010 and November 2010 to May 2011. The human biomonitoring was conducted using lymphocytes samples and cells of the oral mucosa in the micronucleus test (MN). Polymerase Chain Reaction assay was performed to evaluate polymorphism in genes responsible for the expression of metabolism enzymes of phase I (CYP1A1) and phase II (GSTM1 and GSTT1). High concentrations of PAH and nitro-PAH were detected at Rebouças Tunnel site in both periods. Nitroarene and dinitroarenes were detected in all sites in the period July to October 2010. In the period November 2010 to May 2011, the presence of dinitroarenes was detected only in Brasil Avenue and Rebouças Tunnel. MN frequency in lymphocytes and in oral mucosal cells showed a significant increase in the exposure group (Rebouças Tunnel workers). The data generated in this study show that different levels of PAH, and nitroderivatives contribute to mutagenicity induction. The high frequency of MN detected in individuals of exposure group may be related to occupational exposure to pollutants adhered to PM2.5. The significant increase in the concentration of HP-1 and 2-NAP (mol / mol creatinine) was detected in urine samples from exposure group. Polymorphism m2 (CYP1A1\*2B) was detected only in an exposure group individual. No polymorphism of the gene CYP1A1 was detected in the control group samples. Deletions of the GSTM1 and GSTT1 genes were detected in 100% of samples from control group. Deletions were detected in 72.74% of the samples for GSTM1 and 86.37% of the samples for GSTT1 in exposure group. These results demonstrate that this individual exposed to occupational pollution at Rebouças Tunnel, may have intensified metabolism of pollutants such as PAH, and present difficulties in detoxification of metabolites of these pollutants.

Keywords: Particulate matter. Polycyclic aromatic hydrocarbons. Nitroderivates. Human biomonitoring.

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## LISTA DE ABREVIATURAS E SIGLAS

ABNT	Associação Brasileira de Normas Técnicas
AGV	Amostrador de Grande Volume
CAAE	Certificado de Apresentação para Apreciação Ética
CAS	<i>Chemical Abstracts Service</i>
CETESB	Companhia Ambiental do Estado de São Paulo
CET-RIO	Companhia de Engenharia e Tráfego do Rio de Janeiro
CIEP	Centro Integrado de Educação Pública
CONAMA	Conselho Nacional do Meio Ambiente
CONEP	Comissão Nacional de Ética em Pesquisa
CG/DEM	Cromatografia em fase gasosa de alta resolução com detecção por espectrometria de massas
CtB	Citocalasina B
CYP	Citocromos P450
DNA	Ácido desoxirribonucleico
GIT	Gerência de Informações de Tráfego
GST	Glutationa-S-transferase
HPLC	<i>High performance liquid chromatography</i>
IARC	<i>International Agency for Research on Cancer</i>
I.M.	Índice de mutageneicidade
INMET	Instituto Nacional de Meteorologia
MN	Micronúcleo
MOE	Matéria orgânica extraída
MP	Material Particulado
OMS	Organização Mundial de Saúde
PCR	Reação em cadeia da polimerase
SALANAL	<i>Salmonella Assay Analysis</i>
UERJ	Universidade do Estado do Rio de Janeiro
USEPA	<i>United States Environmental Protection Agency</i>

## LISTA DE SÍMBOLOS

HPA	Hidrocarbonetos Policíclicos Aromáticos
Nitro-HPA	Nitro-Hidrocarbonetos Policíclicos Aromáticos
Oxi-HPA	Oxi-Hidrocarbonetos Policíclicos Aromáticos
EDTA	Ácido etilenodiamino tetra-acético
SO <sub>2</sub>	Dióxido de Enxofre
NO <sub>3</sub>	Óxido Nítrico
HCl	Ácido Clorídrico
KCl	Cloreto de Potássio
NaCl	Cloreto de Sódio
CO <sub>2</sub>	Dióxido de Carbono
rpm	Rotações por minuto
MgCl <sub>2</sub>	Cloreto de Magnésio
1-HP	1-hidroxipireno
2-NAP	2 naftol

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## INTRODUÇÃO

Poluição atmosférica é toda e qualquer forma de matéria ou energia com intensidade e em quantidade, concentração, tempo ou características em desacordo com os níveis estabelecidos em legislação, e que tornem ou possam tornar o ar impróprio, nocivo ou ofensivo à saúde, inconveniente ao bem-estar público, danoso aos materiais, à fauna e à flora ou prejudicial à segurança, ao uso e gozo da propriedade e às atividades normais da comunidade (CONAMA, 1990).

A partir da descoberta do fogo, o homem pré-histórico começou a contribuir de forma atuante, e não consciente, para a deterioração da qualidade do ar (Lima et al., 2012). Na Revolução Industrial, a poluição do ar realmente passou a ser considerada um problema ligado à saúde pública, quando começaram a ser adotadas técnicas baseadas na queima de grandes quantidades de carvão, lenha e, posteriormente, óleo combustível (Lima et al., 2012). No final do século XVIII, foram assinadas as primeiras leis de qualidade do ar na Inglaterra. A poluição do ar tem sido, desde a primeira metade do século XX, um grave problema nos centros urbanos industrializados, com a presença cada vez maior dos automóveis, que vieram a somar-se às indústrias, como fontes poluidoras (Lima et al., 2012).

A relação entre efeitos à saúde e poluição atmosférica foi estabelecida a partir de episódios agudos de contaminação do ar e estudos sobre a ocorrência do excesso de milhares de mortes registradas em Londres, em 1948 e 1952. Atualmente, estudos epidemiológicos demonstram a associação entre exposição aos poluentes atmosféricos e o aumento da incidência de doenças cardiovasculares, mortes prematuras e doenças respiratórias e câncer de pulmão (CETESB, 2015).

Pontos na cidade do Rio de Janeiro apresentam altas concentrações de material particulado respirável, Hidrocarbonetos Policíclicos Aromáticos, mutageneicidade e clastogeneicidade em amostras de extratos orgânicos (Rainho et al., 2013a,b). O presente estudo compara a influencia sazonal na atividade mutagênica induzida por material particulado respirável, e realiza uma avaliação geneotóxica quanto a possível dano induzido por estes poluentes, na população de trabalhadores do túnel Rebouças.

## 1 MATERIAL PARTICULADO

O Material Particulado (MP) constitui uma mistura heterogênea de compostos sólidos (partículas) e líquidos (gotículas), composta por ácidos, compostos orgânicos, metais e poeira (Agência de Proteção Ambiental dos Estados Unidos da América - USEPA, 2011).

As principais fontes de emissão de MP para a atmosfera são: veículos automotores, processos industriais, queima de biomassa, suspensão de poeira do solo. O MP pode também se formar na atmosfera a partir de gases como dióxido de enxofre ( $\text{SO}_2$ ), óxidos de nitrogênio ( $\text{NO}_x$ ) e compostos orgânicos voláteis, os quais são emitidos principalmente em atividades de combustão, transformando-se em partículas como resultado de reações químicas no ar (Companhia Ambiental do Estado de São Paulo CETESB, 2011).

Estas partículas são classificadas de acordo com o seu diâmetro aerodinâmico ( $\mu\text{m}$ ): grossas (MP10), finas (MP2,5), ultrafinas (MP0,1), e nanopartículas (Quadro 1) (Kittelson *et al.*, 2004; Mazzoli-Rocha *et al.*, 2010). Em geral, MP10 são depositadas nas vias aéreas e podem ser removidas junto com o muco e por isso são chamadas de partículas inaláveis. O MP2,5, MP0,1 e as nanopartículas são conhecidos como partículas respiráveis, já que ocorre deposição dessas nos alvéolos pulmonares (Tao *et al.*, 2003; Mazzoli-Rocha *et al.*, 2010). Estudos epidemiológicos demonstram a associação entre exposição ao MP respirável e o aumento da incidência de doenças cardíacas (Pope *et al.*, 2002), mortes prematuras e doenças respiratórias como: asma (Etzel, 2003; Ko *et al.*, 2007a), obstruções pulmonares (Mayer e Newman, 2001; Ko *et al.*, 2007b), infecções respiratórias e câncer de pulmão (Pope *et al.*, 2002; Landen *et al.*, 2007; Mazzoli-Rocha *et al.*, 2010).

Quadro 1 - Classificação das partículas de acordo com o seu diâmetro aerodinâmico.

Material Particulado (MP)	Diâmetro Aerodinâmico ( $\mu\text{m}$ )
Partículas grossas (MP10)	$\leq 10$
Partículas finas (MP2,5)	$\leq 2,5$
Partículas ultrafinas (MP0,1)	$\leq 0,1$
Nanopartículas	$\leq 0,05$

Fonte: Mazzoli-Rocha *et. al.*, (2010).

Devido à associação da emissão de MP com inúmeros malefícios à saúde, principais instituições como a Organização Mundial de Saúde (OMS) e a Agência de Proteção Ambiental dos Estados Unidos da América (USEPA) determinam valores máximos para a concentração deste poluente. Como legislação nacional temos a resolução do Conselho Nacional do Meio Ambiente (CONAMA) N° 003 de 1990, que determina Padrões Nacionais de Qualidade do Ar (Quadro 2).

Quadro 2 - Valores limites legais para a concentração de MP10 e MP2,5 em  $\mu\text{g}/\text{m}^3$  para coletas de 24 h.

Agências	Concentração Limite para MP10	Concentração Limite para MP2,5
	( $\mu\text{g}/\text{m}^3$ )	( $\mu\text{g}/\text{m}^3$ )
OMS <sup>a</sup>	50	25
USEPA <sup>b</sup>	150	35
CONAMA N° 003/1990 <sup>c</sup>	150	-

Legenda: (-) Valor não determinado pela legislação nacional.

Fonte: <sup>a</sup>OMS, 2005; <sup>b</sup> USEPA, 2011; <sup>c</sup> CONAMA N° 003/1990.

Estudos realizados no período de Julho à Outubro de 2010, no túnel Rebouças, detectaram altas concentrações de MP2,5 ( $83\text{-}132 \mu\text{g}/\text{m}^3$ ) (Rainho *et al.*, 2013a,b). Estas concentrações estão acima do limite diário estabelecido pela OMS ( $25 \mu\text{g}/\text{m}^3$ ), podendo oferecer risco à saúde dos trabalhadores do túnel e da população que utiliza esta via.

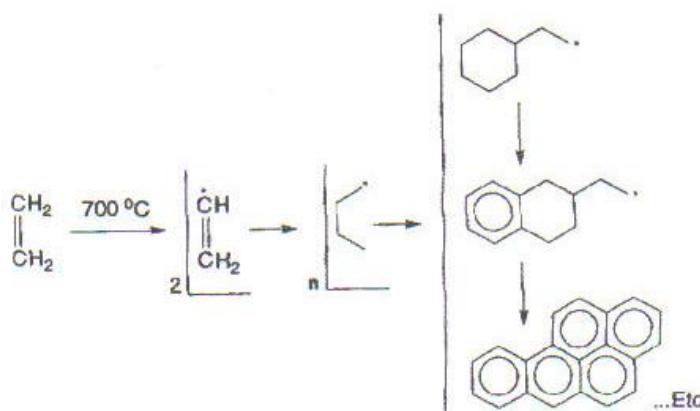
## 1.1 Hidrocarbonetos Policíclicos Aromáticos (HPA) e Derivados

Um dos poluentes constituintes do MP relacionado à atividade mutagênica e geneotóxica são os Hidrocarbonetos Policíclicos Aromáticos (HPA) e seus derivados como os Nitro-Hidrocarbonetos Policíclicos Aromáticos (Nitro-HPA) e os Oxi-Hidrocarbonetos Policíclicos Aromáticos OXI-HPA. Os HPA estão presentes no ambiente em concentrações muito baixas ( $\text{ng}/\text{m}^3$  ou  $\mu\text{g}/\text{m}^3$ ). São substâncias orgânicas com dois ou

mais anéis aromáticos formados por átomos de carbono e hidrogênio, pouco solúveis em água, baixa pressão de vapor e alto ponto de fusão e ebulição. Podem apresentar grande número de estruturas e diferentes isômeros (Nardocci, 2010).

Os HPA são formados em processos de combustão incompleta, a altas temperaturas e, deste modo, são essencialmente emitidos por todos os tipos de combustão. A formação pirolítica de HPA é bastante complexa e variável, dependendo de fatores como pressão e temperatura. O esquema mecanístico mais aceito para esta reação envolve a polimerização via radicais livres, em várias etapas, até a formação de núcleos aromáticos condensados (Figura 1) (Selkirk, 1980; Atkinson *et al.*, 1987; Lopes e Andrade, 1996).

Figura 1 - Esquema representativo da formação de HPA por meio de pirólise



Fonte: adaptado de Lopes e Andrade, 1996.

Em áreas urbanas, se destaca a emissão veicular como fonte de HPA e derivados. Estas emissões dependem de vários fatores como o tipo de combustível, velocidade do veículo, temperatura do ambiente, catalisadores e ajuste de motor (Abrantes *et al.*, 2004; Abrantes *et al.*, 2009; Nardocci, 2010).

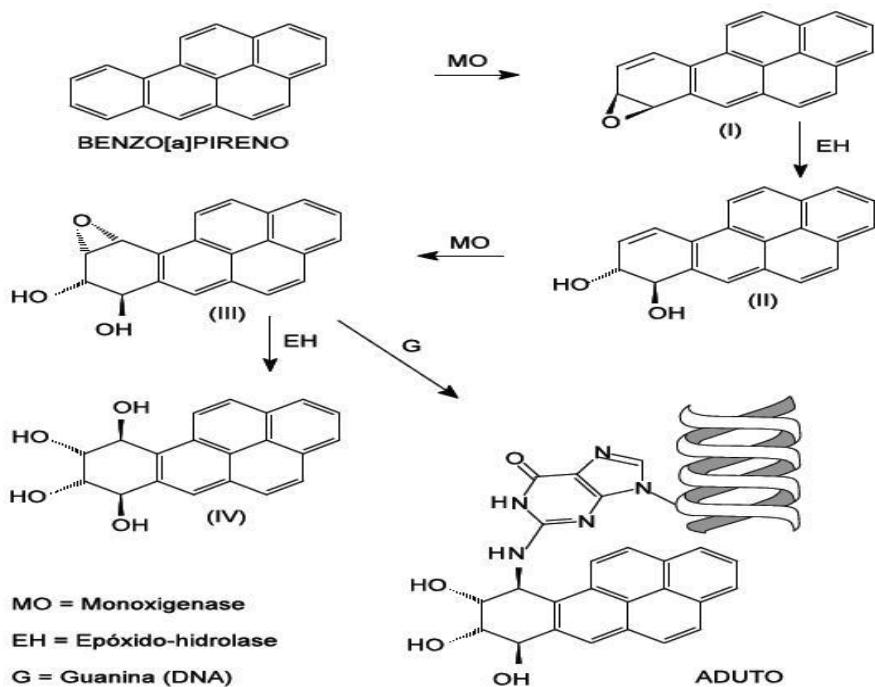
Uma vez emitidos para a atmosfera os principais mecanismos de remoção são a deposição e as transformações fotoquímicas. A eficiência da remoção depende das fases (particulada ou gasosa). O particionamento dos HPA entre gás e partículas depende, por sua vez, da temperatura do ambiente, da umidade relativa, das concentrações de HPA e da composição química dos aerossóis (Nardocci, 2010).

Os Nitro-HPA e OXI-HPA são também formados em processo de combustão incompleta e emitidos para a atmosfera por diversas fontes, incluindo também a emissão de

motores à diesel. Os Nitro-HPA são presumivelmente formados no ambiente através da reação entre HPA e óxidos de nitrogênio e/ou ácido nítrico. Os HPA podem ser nitrados, nitrosados ou oxidados como resultado destas reações. Após transformação metabólica os HPA são eficazes agentees cancerígenos e/ou mutagênicos, sendo estes os principais campos de investigação de suas atividades biológicas. Ao contrário dos HPA, os derivados nitrados são agentes diretamente mutagênicos (Lopes e Andrade, 1996).

Os HPA são lipossolúveis e rapidamente absorvidos pelos pulmões, intestinos e pele de animais experimentais. Esses compostos são rapidamente absorvidos pelo trato respiratório, sendo que os maiores níveis podem ser encontrados no fígado (Meire *et al.*, 2007; Pereira, 2008). Uma importante propriedade dos HPA é a sua biotransformação, ativando produtos intermediários eletrofílicos que podem se ligar covalentemente a alvos nucleofílicos no DNA, formando adutos e induzindo mutações (Pereira, 2008). A biotransformação dos HPA envolve uma série de enzimas que catalisam reações de oxidação, redução e hidrólise (oxigenase de função mista, citocromo P 450, NADPH-citocromo-c-redutase) e de enzimas que catalisam reações de conjugação (sulfotransferase, epóxido hidrolase, glutation-S-transferase e UDP-glicotransferase). Estas enzimas estão distribuídas em todos os tecidos orgânicos (Pereira Netto *et al.*, 2000). Monoxigenases dependentes do citocromo P 450 (CYP1A) são responsáveis pela oxidação enzimática dos HPA. Elas agem principalmente sobre a região de elevada densidade eletrônica formando óxidos de arenos (epóxidos) que podem espontaneamente formar fenois ou, por ação das epóxido hidrolases, produzirem di-hidroídeos vicinais. Destes fenois, alguns são oxidados a quinonas e outros podem sofrer nova epoxidação levando à formação de epóxidos secundários (di-hidroídeos epóxidos). O carbono benzílico dos dihidroídeos epóxidos é capaz de reagir com as bases nucleofílicas do DNA, notadamente a guanina e, eventualmente, iniciar um processo mutagênico (Pereira Netto *et al.*, 2000) (Figura 2).

Figura 2 - Representação esquemática simplificada do metabolismo de um HPA



Fonte: adaptado de Lopes e Andrade, 1996.

Devido à atividade mutagênica dessas substâncias, a USEPA (1996) indicou 16 HPA prioritários para a pesquisa em compartimentos ambientais (Quadro 3).

Quadro 3 - HPA prioritários para pesquisa em compartimentos ambientais e sua classificação quanto à atividade carcinogênica

Substâncias	CAS	IARC	ABNT
Naftaleno	91-20-3	2B	NM
Acenaftileno	208-96-8	-	NM
Acenafteno	83-32-9	3	NM
Fluoreno	86-73-7	3	NM
Fenanreno	85-01-8	3	NM
Antraceno	120-12-7	3	NM
Fluoranteno	206-44-0	3	CP
Pireno	129-00-0	3	NM
Benzo[a]antraceno	56-55-3	2B	CP
Criseno	218-01-9	2B	CP
Benzo[b]fluoranteno	205-99-2	2B	CP
Benzo[k]fluoranteno	207-08-9	2B	NM
Benzo[a]pireno	50-32-8	1	CP
Indeno[1,2,3-cd]pireno	193-39-5	2B	CP
Dibenzo[a,h]antraceno	53-70-3	2A	CP
Benzo[g,h,i]perileno	191-24-2	3	NM

Legenda: CAS: Chemical Abstract Number; IARC, 2011: International Ageneecy for Research on Cancer; ABNT: Associação Brasileira de Normas Técnicas (NBR 10.004); 1 = carcinógeno para humanos; 2A = provável carcinógeno para humanos – limitada evidência em humanos e suficiente em animais; 2B = provável carcinógeno para humanos – limitada evidência em humanos e insuficiente em animais; 3 = não carcinógenos para humanos; CP = Confere periculosidade; NM = não mencionado.

Fonte: USEPA, 1996

Em estudos por nós realizados no período de Agosto à Outubro de 2010 em três pontos da cidade do Rio de Janeiro, detectamos as maiores concentrações, no túnel Rebouças, para os seis HPA estudados: fenantreno, fluoranteno, pireno, benzo[a]antraceno, criseno e benzo[a]pireno (Rainho *et al.*, 2013a,b). Além disso, foi detectada presença de atividade mutagênica através do ensaio de *Salmonella/microssoma*, utilizando linhagens de *Salmonella typhimurium* (TA98 e derivadas específicas na detecção de nitrocompostos), e clastogênicidade em células meristemáticas de *Allium cepa* para os extratos orgânicos de MP2,5 proveniente do túnel Rebouças. Estes danos podem estar relacionados à presença de HPA e Nitro-HPA nestas amostras (Rainho *et al.*, 2013b).

Vários estudos de monitoramento sazonal de poluentes são realizados em todo mundo. Isto porque, diferentes condições climáticas podem favorecer o acúmulo ou a dispersão destes poluentes. No quadro 4 estão presentes as variações de concentrações de HPA em ng/m<sup>3</sup> nas estações de verão e inverno em diferentes países.

Quadro 4 - Monitoramento sazonal da concentração de HPA em diferentes países

HPA (ng/m <sup>3</sup> )	Polônia <sup>a</sup>		China <sup>b</sup>		Índia <sup>c</sup>		México <sup>d</sup>	
	Verão	Inverno	Verão	Inverno	Verão	Inverno	Verão	Inverno
Fenantreno	1,36	3,77	2,80	88,50	14,21	47,91	0,05	0,08
Antraceno	0,10	0,84	3,20	37,10	n.d.	2,29	n.a.	n.a.
Fluoranteno	0,96	8,74	0,30	5,60	8,37	30,48	0,10	0,10
Pireno	0,60	8,36	1,50	78,00	n.d.	9,98	0,25	0,35
Benzo(a)antraceno	0,55	8,21	2,40	27,00	2,68	31,34	0,44	0,22
Criseno	0,82	5,26	2,30	27,00	2,43	32,92	0,27	0,24
Benzo(b)fluoranteno	0,97	4,96	8,70	9,30	n.d.	34,95	0,14	0,14
Benzo(k) fluoranteno	0,29	1,71	4,70	6,10	5,52	18,68	0,12	0,09
Benzo(a)pireno	0,96	10,82	3,40	19,80	3,55	28,02	0,15	0,14
Dibenzo(ah) antraceno	0,16	1,17	0,30	3,20	n.a.	n.a.	0,27	0,10
Benzo(g.h.i)perileno	0,44	4,49	2,50	14,80	4,27	21,57	n.a.	n.a.
Indeno(1,2,3-c,d)pireno	0,31	2,94	5,10	16,30	n.a.	n.a.	0,09	0,07

Legenda: ; n.a. não avaliado; n.d. não detectado.

Fonte: <sup>a</sup>Zaciera *et al.*, 2012 ; <sup>b</sup>Li *et al.*, 2011; <sup>c</sup>Mugica *et al.*, 2010; <sup>d</sup>Masih *et al.*, 2012.

## 1.2 Biomonitoramento Humano

O biomonitoramento humano é utilizado para determinar a exposição humana a poluentes ambientais, e/ou a susceptibilidade genética, e os potenciais efeitos adversos na saúde associados a essa exposição. Este biomonitoramento vem sendo utilizado há mais de meio século na indústria como estratégia para monitorar a saúde dos trabalhadores (Kuno *et al.*, 2009). Porém, nos últimos vinte anos é crescente a sua utilização como uma ferramenta para políticas em saúde ambiental (Kuno *et al.*, 2009). Mais do que as medidas realizadas no ambiente externo, ela fornece informações sobre a “poluição individual”. O biomonitoramento humano não apenas fornece informações valiosas sobre a exposição e seus possíveis efeitos na saúde, mas também é de grande utilidade na identificação preventiva de riscos, e serve de base para a adoção e avaliação de políticas ambientais (Kuno *et al.*, 2009).

Trabalhadores podem estar expostos a contaminantes liberados por diversas fontes. Na exposição ocupacional, a intensidade da exposição depende, entre outros fatores, da concentração do agente tóxico, da duração diária da exposição ao longo da vida profissional, da frequência da exposição pelo trabalhador, das condições de temperatura, umidade e ventilação (Salgado, 1989; Kuno *et al.*, 2009). Normalmente, as concentrações às quais os trabalhadores estão expostos são mais elevadas do que na exposição da população geral. A avaliação da exposição humana a contaminantes presentes no ambiente de trabalho é estimada por biomonitoramento de substância química ou seu metabólito na população exposta, combinadas com o monitoramento periódico dos contaminantes em amostras coletadas no ambiente (Kuno *et al.*, 2009).

A seleção de metodologias sensíveis para biomonitoramento humano é uma estratégia importante na definição de riscos populacionais à exposição de contaminantes ambientais (Pereira, 2008). A utilização de técnicas citogenéticas em populações expostas a potenciais mutagênicos é um eficiente sistema de advertência para doenças genéticas (Kassie *et al.*, 2000; Pereira, 2008).

No presente estudo trabalhadores do túnel Rebouças, na cidade do Rio de Janeiro, foram submetidos ao biomonitoramento através de técnicas citogenéticas para a avaliação de possível dano induzido por material particulado respirável. Todos os procedimentos foram submetidos e aprovados pela Comissão Nacional de Ética em Pesquisa - CONEP (CAAE N° 27402014.6.0000.5259).

### 1.3 Micronúcleo – MN

Micronúcleo (MN) se constitui em uma pequena massa nuclear delimitada por membrana e separada do núcleo principal (Salvadori *et al.*, 2003). Os MN são formados durante a telófase da mitose ou meiose, quando o envelope nuclear é reconstituído ao redor dos cromossomos das células filhas (Salvadori *et al.*, 2003). São resultantes de fragmentos cromossômicos acênicos ou de cromossomos inteiros que não foram incluídos no núcleo principal. Assim sendo, o MN representa perda de cromatina em consequência de dano cromossômico estrutural ou dano no aparelho mitótico (Salvadori *et al.*, 2003).

O ensaio de MN é um biomarcador que permite a avaliação de efeitos clastogênicos e aneugênicos em vários tipos de células, uma vez que são detectados durante a interfase (Pastor *et al.*, 2003). A utilização de bloqueadores do ciclo celular, como a citocalasina B (CtB), confere grande sensibilidade e confiabilidade ao ensaio de MN em linfócitos humanos, demonstrando ser uma ferramenta eficaz na detecção de dano citogenético em várias populações (Bolognesi *et al.*, 1993; da Silva Augusto *et al.*, 1997; Joksić *et al.*, 1997; Falck *et al.*, 1999; Pastor *et al.*, 2003). A (CtB) é um inibidor da polimerização da proteína actina requerida para a formação do anel de microfilamentos que induzem a contração do citoplasma e a clivagem da célula em duas células filhas (Carter, 1967; Salvadori *et al.*, 2003). Por esse motivo a utilização da CtB em testes de micronúcleo *in vitro* leva ao bloqueio da citocinese, mas não da divisão celular, resultando em um acúmulo de células binucleadas (Fenech e Morley, 1985; Salvadori *et al.*, 2003).

Além disso, MN podem ser avaliados em diferentes tipos celulares que não necessitam de divisão *in vitro*, como as células epiteliais. Assim, a análise de MN em células da mucosa oral tem demonstrado ser um método sensível para o biomonitoramento genético em populações humanas (Sarto *et al.*, 1990; Karahalil *et al.*, 1999; Pastor *et al.*, 2003).

### 1.4 Polimorfismos em genes de metabolização relacionados ao câncer de pulmão

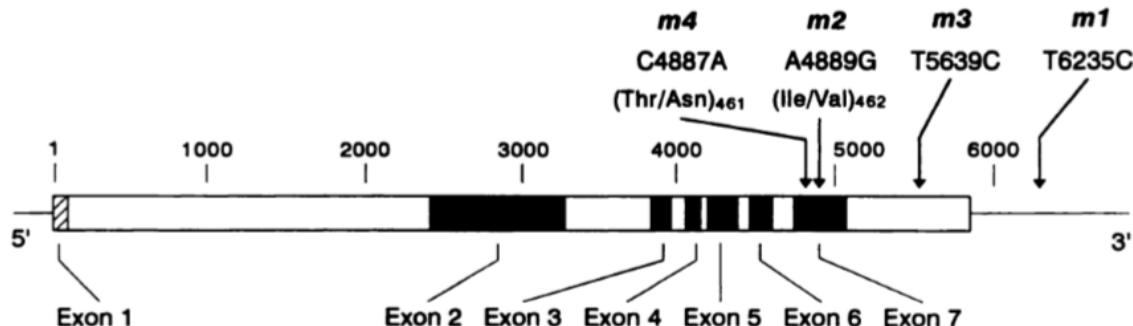
Polimorfismos em genes que expressam enzimas metabolizadoras de fase I (CYPs) e de fase II (GST) são associados com susceptibilidade ao câncer (Li *et al.*, 2014). Especificamente polimorfismos em citocromo P450 1A1 (CYP1A1) e glutationa-S-

transferase (GSTM1 e GSTT1) estão associados ao câncer de pulmão (Shi et al., 2008; Li et al., 2014; Zhang et al., 2014).

Enzimas do citocromos P450 (CYP) e glutationas-tranferases (GST), são responsáveis por biotransformarem pré-carcinógenos em compostos mais hidrossolúveis e, portanto passíveis de serem excretados. Os produtos gerados são extremamente eletrofílicos e irão reagir com centros nucleofílicos das células, dentre eles regiões do DNA, levando a formação de adutos. De um modo geral a formação de agentees eletrofílicos a partir de carcinógenos ambientais é catalisada pelas enzimas CYP (enzimas de fase I) e neutralizadas pelas GST (enzimas de fase II) (Ribeiro Pinto e Felzenszwab, 2003).

CYP1A1 é uma das principais isoformas envolvidas na ativação de carcinógenos ambientais (ativam HPA presentes na fumaça do cigarro, poluição atmosférica e dieta) (Ribeiro Pinto e Felzenszwab, 2003). CYP1A1 pode apresentar quatro variações polimórficas: m1, m2, m3 e m4, também conhecidas como CYP1A1\*1A, CYP1A1\*2B, CYP1A1\*3 e CYP1A1\*4, no exon 7 (Figura 3) (Cascorbi et al., 1996).

Figura 3 - Localização das mutações no gene CYP1A1



Fonte: adaptado de Cascorbi et al., 1996.

As GST compõem uma superfamília de enzimas com funções diversas, que estão envolvidas na desintoxicação de muitas substâncias químicas, incluindo algumas carcinogênicas, como o benzeno e outros HPA (Rohr et al., 2004). A reação enzimática catalisada por GST inibe a reatividade das substâncias eletrofílicas com componentes celulares, e resulta na produção do conjugado com glutationa, o que reduz a citotoxicidade (Rohr et al., 2004). Altos níveis de atividade de GST podem levar a uma proteção tecidual na

exposição a carcinogênicos (Wilkinson e Clapper 1997, Rohr et al., 2004). Existem em torno de 20 isoformas de GST expressas em seres humanos, sendo que as formas mais estudadas são a GSTM1 e a GSTT1 (Lang e Pelkonen, 1999; Ribeiro Pinto e Felzenszwalb, 2003). Os genes GSTM1, localizados no cromossomo 1p13.1, e GSTT1, localizados no cromossomo 22q11.2, apresentam polimorfismos de ausência, o que resulta na falta das proteínas ativas (Pemble et al., 1994, Tan et al., 1995, Xu et al., 1998, Sprenger et al., 2000, Rohr et al., 2004).

## 1.5 Pontos de estudo

### 1.5.1 Campus da Universidade do Estado do Rio de Janeiro –UERJ (ponto 1)

O Campus da Universidade do Estado do Rio de Janeiro –UERJ está localizado no bairro do Maracanã (Figura 3). Atualmente, este bairro faz parte da IX Região Administrativa (RA IX) (Vila Isabel), que abrange os bairros de Vila Isabel, Andaraí e Grajaú. Possui uma área territorial de aproximadamente 1,67 km<sup>2</sup>. Apresenta uma população em torno de 28 mil habitantes, vivendo em uma área totalmente urbanizada, com ruas asfaltadas, rios canalizados e rede de esgoto implantada em toda a região. Quanto ao uso do solo, podemos classificar este bairro como residencial, com grande número de imóveis ([http://www.enapet.ufsc.br/anais/Transformacoes\\_e\\_Retransformacoes\\_no\\_Bairro\\_Maracana.pdf](http://www.enapet.ufsc.br/anais/Transformacoes_e_Retransformacoes_no_Bairro_Maracana.pdf)).

### 1.5.2 Avenida Brasil (ponto 2)

A Avenida Brasil é uma via federal, sob administração Municipal, a partir do Decreto n.º 18.512, de 11/02/93, publicado no D.O. RJ, Parte 1, de 12/02/93, página 3, e integra a BR-101, interligando a Ponte Presidente Costa e Silva (Rio-Niterói), BR-101, Rodovia Washington Luís (Rio/Petrópolis), BR-040, Rodovia Presidente Dutra (Rio-São Paulo), BR-116 e Rodovia Rio-Santos, Br-101. Possui o seu quilômetro zero na Ponte dos Suspiros (Rodoviária Novo Rio) e o seu quilômetro final (km 58), na Av. João XXIII (Santa Cruz).

É constituída em cada sentido de uma pista central de trânsito rápido, com quatro faixas e com velocidades máximas permitidas de 90 km/h nas duas faixas centrais e de 80 km/h na faixa seletiva (faixa exclusiva para ônibus devidamente autorizados) e da faixa da direita (faixa para caminhões), e de uma pista lateral de trânsito local, constituída por 2 faixas, com velocidade máxima de 60 km/h, no trecho, compreendido entre o acesso da Ponte Rio-Niterói (Caju), até a Estrada de Camboatá. A partir de Guadalupe até Santa Cruz, a Av. Brasil é composta por 3 faixas de rolamento por sentido, e a velocidade máxima permitida é de 90 km/h nas duas faixas da esquerda e de 80 km/h na faixa da direita.

Segundo estudos técnicos realizados pela Gerência de Informações de Tráfego (GIT) da Companhia de Engenharia de Tráfego do Rio de Janeiro (CET- Rio), a Avenida Brasil apresenta volume veicular diário de 47969 à 237817 no trajeto na altura da Rua Ricardo Machado (São Cristóvão) à altura da Estrada do Tingui (Campo Grande) em dias úteis ([www.rio.rj.gov.br/cetrio](http://www.rio.rj.gov.br/cetrio)).

Ao longo da Avenida existem várias unidades escolares públicas e privadas, um importante hospital de emergência (Hospital Geral de Bonsucesso), moradias e comércio. Sofrem com a poluição, além da população local, os comerciantes e os motoristas de ônibus que possuem jornadas de trabalho exaustivas, percorrendo em alguns casos a totalidade da via, mais de uma vez ao dia.

#### 1.5.3 Túnel Rebouças (ponto 3)

Túnel Rebouças foi inaugurado em 03 de outubro de 1967, ligando as zonas Norte e Sul, pelo então Governador do Estado da Guanabara, Sr. Negrão de Lima. Projetado para um volume de 76 mil veículos/dia, com duas faixas de rolamento e acostamento, possui 2.040 metros de galeria contínua entre os bairros da Lagoa e Cosme Velho e mais 772 metros na galeria Cosme Velho ao bairro do Rio Comprido, totalizando 2.840 metros (Figura 4). O volume diário é de 190 mil veículos/dia, em três faixas de rolamento sem acostamento.

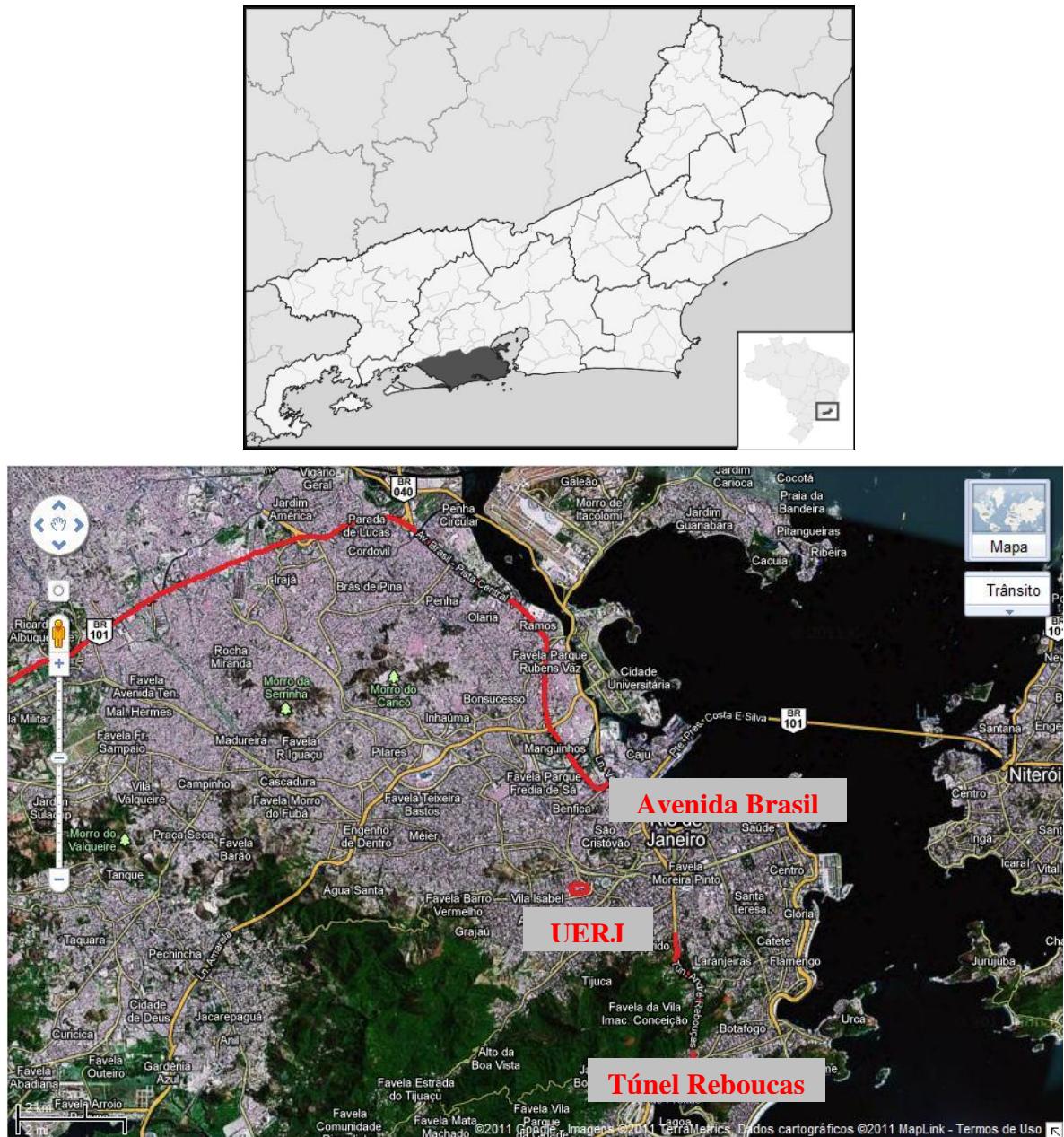
Do ano de 1967 até novembro de 1994, o Túnel Rebouças esteve sob a jurisdição da Fundação do Departamento de Estrada e Rodagens do Estado do Rio de Janeiro (FUNDER-RJ). Com a publicação do Decreto n.º 20.903 de 28/11/94, do então Governador do Estado do Rio de Janeiro, Ilmo. Sr. Nilo Batista, no Diário Oficial do Estado do Rio de Janeiro, n.º 224, em 29/11/94, na parte I, pag. 3, o "Sistema Rebouças" passou para a jurisdição do Município

do Rio de Janeiro, sendo Prefeito o Ilmo. Sr. César Maia. No mês de dezembro de 1994, a Administração do Túnel Rebouças foi feita em co-gestão pela Prefeitura e a FUNDERJ, sendo que a partir do mês de janeiro de 1995, exclusivamente pela Prefeitura. Foi implantado em dezembro de 2000, o sistema de monitoramento das galerias através de câmeras num total de 32, sendo 16 em cada uma das galerias, que funcionam 24 h, inclusive gravando o que acontece no interior das galerias.

A ligação entre a Lagoa Rodrigo de Freitas e São Cristóvão é coordenada pela 1ª Divisão de Vias Especiais, a qual monitora o Viaduto Saint Hilaire, Túnel André Rebouças (Galeria L1) Lagoa/Cosme Velho, Viaduto José de Alencar, Túnel André Rebouças (Galeria R1) Cosme Velho/Rio Comprido, Av. Vital Brasil (acesso á Av. Paulo de Frontin), Av. Engeneheiro Freyssinet (elevado sobre a Av. Paulo de Frontin), Rampa de descida para a Praça da Bandeira e para a Av. Francisco Bicalho, Elevado Prof. Engeneheiro Rufino de Almeida Pizarro, Rampa de descida para a Rua Afonso Cavalcante, Viaduto Engeneheiro Paulo de Souza Reis, Rampa de descida para o Campo de São Cristóvão, Campo de São Cristóvão, Rampa de subida para o Elevado Professor Eng.º Rufino de Almeida Pizarro, Rampa de descida para a Leopoldina, Rampa de subida para o Avenida Engeneheiro Freyssinet, Túnel Antônio Rebouças (Galeria R2) Rio Comprido/Cosme Velho, Túnel Antônio Rebouças (Galeria L2) Cosme Velho/Lagoa, Viaduto Engeneheiro Vital Bandeira de Mello ([www.rio.rj.gov.br/cetrio](http://www.rio.rj.gov.br/cetrio)).

Além dos motoristas de automotores estão expostos à poluição neste ambiente trabalhadores da CET-Rio que atuam na manutenção do Túnel.

Figura 4 - Imagem de satélite dos três pontos monitorados (em vermelho) localizados no Rio de Janeiro



Fonte: googlemaps

## 1.6 Justificativa

Devido as altas concentrações de MP2,5, HPA, mutageneicidade e clastogeneicidade detectados em extratos orgânicos de MP2,5 em estudos anteriores (Rainho *et al.*, 2013a,b), faz-se necessário a comparação da influencia da variação sazonal na atividade mutagênica induzida por MP2,5, e uma avaliação geneetóxica quanto a possível dano induzido por estes poluentes, na população de trabalhadores do túnel Rebouças.

## 2 OBJETIVOS

### 2.1 Objetivo geral

Comparar a influencia da variação sazonal na atividade mutagênica induzida por MP2,5, em amostras coletadas entre Novembro 2010 e Maio 2011, com resultados obtidos em estudos anteriores. Realizar um biomonitoramento geneetóxico da população de trabalhadores do Túnel Rebouças exposta ao material particulado respirável (MP 2,5).

### 2.2 Objetivos específicos

- Determinar a concentração de MP2,5 utilizando Amostrador de Grande Volume.
- Identificar e quantificar os HPA e Nitro HPA presentes nas amostras de MP2,5 através da técnica de cromatografia em fase gasosa utilizando um detector de espectrômetro de massas (CG/DEM).
- Comparar a influencia da variação sazonal na atividade mutagênica induzida por MP2,5.
- Realizar um questionário para a obtenção do estilo de vida de cada participante.
- Determinar as concentrações dos metabólitos 1-hidroxipireno (1-HP) e 2-naftol (2-NAP) nas amostras de urina.
- Determinar o índice de micronúcleos em células da mucosa oral de trabalhadores do Túnel Rebouças.
- Determinar o índice de micronúcleos em linfócitos de trabalhadores do Túnel Rebouças.
- Avaliar quanto ao polimorfismo nos genes CYP1A1 (CYP1A1\*2B, CYP1A1\*3 e CYP1A1\*4), GSTM1 e GSTT1.

### 3 MATERIAIS E MÉTODO

#### 3.1 Coleta e Extração do Material Particulado

As coletas de material particulado MP2,5 foram realizadas em filtro de fibra de vidro (E558 X 10IN, 254 x 203 mm - Energética Indústria e Comércio Ltda) utilizando Amostrador de Grande Volume (Energética Indústria e Comércio Ltda, AVG MP2,5, 1,13 m<sup>3</sup>/min) (Figura 5). Este equipamento foi instalado na UERJ (ponto 1), nas dependências do Instituto de Biologia Roberto Alcantara Gomes. Na Avenida Brasil, (ponto 2), o AGV foi instalado nas dependências do CIEP Leonel de Moura Brizola, localizado no bairro de Ramos. No Túnel Rebouças (ponto 3), o AGV foi instalado no desvio presente na segunda galeria sentido zona sul. Os pontos foram escolhido com base na diferença de tráfego, sendo o ponto 1 (UERJ) estabelecido como ponto controle.

As coletas foram realizadas por 24 h (6 h/dia/semana) nos pontos 1 e 2, e 6 h (1h/dia/semana) no ponto 3. Os diferentes tempos de coletas foram estabelecidos com a finalidade de saturar os filtros nos diferentes ambientes. As amostras foram coletadas semanalmente de novembro de 2010 a maio 2011 (período II). Antes e após as coletas, os filtros foram pesados e estabilizados (45% de umidade) com a finalidade de expressar a quantidade de material particulado em µg/m<sup>3</sup> de ar amostrado. Após estes procedimentos os filtros foram reunidos e submetidos a extração (Ducatti e Vargas, 2003).

Metade de cada filtro contendo material particulado foi submetido a extração por sonicação de três ciclos de 10 min utilizando o solvente diclorometano (DCM, CAS. 75-09-2, Tedia Brasil, Brasil, pureza 99,9%), na proporção 1 mL:1 mg (Vargas *et al.*, 1998). O DCM possibilita a extração de componentes com características moderadamente polares e apolares, resultando na obtenção de extrato contendo pontenciais substâncias com atividades mutagênicas (Claxton *et al.*, 2004; Coronas *et al.*, 2009). Após a sonicação, o extrato foi reduzido a 15 mL em rotaevaporador à 37°C. A massa de matéria orgânica extraída (MOE) foi comparada com a metade do volume de ar amostrado (MOE em µg/m<sup>3</sup>). Antes do ensaio de mutagenecidade os extratos foram filtrados em membrana de Teflon de 0,5 µm, secos a 4°C e suspensos em 5 µL de dimetilsufóxido (DMSO, CAS. 67-68-5, Synth, Brasil, pureza 99,9%) (Vargas *et al.*, 1998).

Figura 5 - Amostrador de Grande Volume – AGV MP2,5 instalado na Avenida Brasil.



Fonte: A autora, 2010.

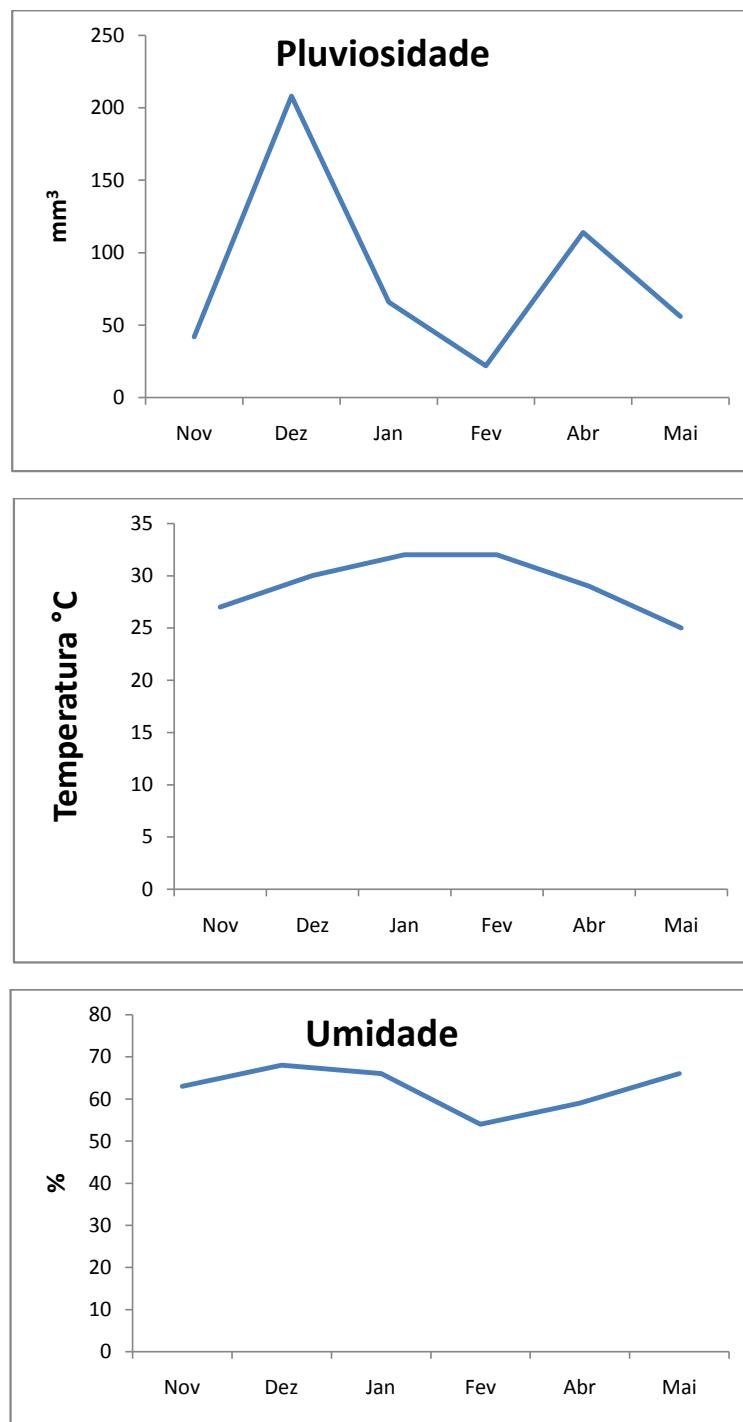
### 3.2 Fatores físicos

Dados de fatores físicos como: temperatura, pluviosidade e umidade foram obtidos através da base metereológica do Instituto Nacional de Metereologia localizada no aeroporto Internacional do Galeão na cidade do Rio de Janeiro ([www.inmet.gov.br](http://www.inmet.gov.br)).

Para a obtenção de dados de fatores físicos no Túnel Rebouças foi utilizado o equipamento Higro-termográfico (Modelo NSIIQ Série 1102901).

O Gráfico 1 mostra os dados de fatores físicos da cidade do Rio de Janeiro no período de novembro de 2010 à maio de 2011 (período II).

Gráfico 1 - Dados de fatores físicos da cidade do Rio de Janeiro no período II.



Fonte: INMET

### 3.3 Análise de HPA

A determinação qualitativa e quantitativa dos 6 HPA prioritários estabelecidos pela USEPA foi realizada no Laboratório de Tecnologia Ambiental (UERJ – Campus Regional de Resende – RJ) pelo Prof. Dr. Sérgio Machado Corrêa, com protocolo adaptado da Califórnia Air Resource Board – SOP MLD 144 (USA). As amostras foram analisadas por cromatografia gasosa de alta resolução com detecção por espectrometria de massas (CG/DEM). A separação cromatográfica foi realizada em uma coluna de fase estacionária VF-5MS (30 m x 0,25 mm x 0,25 µm, Varian) usando He Ultra Puro (5,0) como gás de arraste (99,999 %, Linde Gases Ltda) a 1,6 mL/min em cromatógrafo de fase gasosa Varian 450-GC. Os extratos orgânicos (1-3 µL) foram injetados em injetor tipo “split/splitless” (modelo Varian 1177 S/SL) a temperatura de 300°C e sem variação de fluxo (modo “splitless”), mantendo a vazão de purga a 3 mL/min e abrindo a divisão de fluxo após 1 min. A programação do CG é descrita a seguir: temperatura inicial da coluna 70°C, isoterma por 4 min, taxa de aquecimento de 10°C/min até a temperatura final de 300°C. As substâncias separadas foram detectadas, identificadas e quantificadas em espectrômetro de massa acoplado Varian MS 220 com “Ion Trap” a 250°C, transferido a 280°C e coletado a 40°C.

Curvas de calibração externa foram contruídas com injeção de mistura de padrões nas concentrações de 10 a 250 pg/µL. Os resultados foram calculados em ng/m<sup>3</sup> de material particulado. Os limites de quantificação (LQ) foram calculados a partir da curva de calibração. Para fenantreno, fluoranteno, pireno, benzo[a]antraceno e criseno, LQ foi 0,05 ng/m<sup>3</sup>, e para o benzo[a]pireno, 0,12 ng/m<sup>3</sup>. O limite de detecção (LD) foi estimado como 3-5 x sinal de ruído.

A identificação de cada HPA foi realizada contra padrões SUPELCO (Supelco 48755-U KIT PAH610-S) monitorando os íons em modo SIS (fragmentos característicos de cada íon).

### 3.4 Análise de Nitro-HPA

A determinação qualitativa e quantitativa dos Nitro-HPA foi realizada no Laboratório de Tecnologia Ambiental (UERJ – Campus Regional de Resende – RJ) pelo Prof. Dr. Sérgio Machado Corrêa. Nitro - HPA foram identificados e quantificados por cromatografia em fase gasosa utilizando um detector de espectrômetro de massas (CG/DEM), utilizando cromatógrafo de fase gasosa Varian 450-GC com uma temperatura programável, iniciando a 75°C durante 0,2 min e depois aquecida a 200°C/min até 340°C. Nitro - HPA foram identificados por similaridade de massa e por tempo de retenção dos componentes do kit comercial padrão (Supelco, PAH610-S). A quantificação foi baseada em cinco pontos de calibração, em duplicata, que foram construídos a partir de cada padrão para todos os analitos alvo, que variam de 25 a 400 ppb. A separação cromatográfica foi realizada em uma coluna de fase estacionária VF-5MS (30 m x 0,25 mm x 0,25 µm, Varian) usando He Ultra Puro (5,0) como gás de arraste (99,999%, Linde Gases Ltda) a 1,2 mL/min em cromatógrafo de fase gasosa Varian 450-GC. A programação do CG é descrita a seguir: temperatura inicial da coluna 70°C, durante 12 s, taxa de aquecimento de 10°C/min até a temperatura final de 300°C. Este procedimento foi realizado para a análise dos treze Nitro-HPA : nitro-naftaleno, nitro-acenaftileno, nitro-acenafteno, nitro-fluoreno, nitro-fenantreno, nitro-antraceno, nitro-fluoranteno, nitro-pireno, nitro-benzo[a]antraceno, criseno-nitro, nitro-benzo[b]fluoranteno, nitro-benzo[k] fluoranteno e nitro-benzo[a]pireno. Os limites de quantificação foram determinados a partir do ponto mínimo das curvas de calibração. Os limites de detecção foram determinados a partir de concentrações de Nitro-HPA , o que resultou em uma relação de 3:1 de sinal de ruído. Os resultados foram expressos em ng/m<sup>3</sup>.

### 3.5 *Salmonella/microssoma* e Teste de Citotoxicidade

Os extratos orgânicos foram avaliados quanto à mutageneicidade através do teste *Salmonella/microsoma* (Maron e Ames, 1983), pelo método de microsuspenção (Kado *et al.*, 1986). Este método é o mais utilizado em avaliações mutagênicas de amostras ambientais, já que possui maior sensibilidade. Três modificações são responsáveis pela sua maior sensibilidade: uma maior concentração de células na cultura bacteriana de *Salmonella*

*typhimurium* ( $10^{10}$  células/mL), um menor volume de tampão fosfato 0,2 M pH 7,4 ou fração de metabolização S9 mix 4% (100 µL) e um maior tempo de pré-incubação (90 min). Nos ensaios foram utilizadas linhagens de *Salmonella typhimurium* TA98 e derivadas: YG1021 e YG1024 (Quadro 5) (Watanabe *et al.*, 1989). Os testes foram realizados na presença e na ausência de metabolização (fração S9 mix 4%). Cinco concentrações de cada amostra 10-50 µg/placa foram testadas em triplicata. Um volume de 5 µL de amostra, além de 100 µL de tampão fosfato 0,2 M pH 7,4 ou S9 mix 4%, além de 100 µL de cultura bacteriana foram pré-incubadas por 90 min à 37°C. Todos os ensaios foram realizados na ausência de luz amarela e na presença do controle negativo (DMSO, 5 µg/placa) e dos controles positivos (4-nitroquinolina óxido—4NQO, 0,5 µg/placa, CAS. 56-57-5 e 2-aminofluoreno—2AF, 1 µg/placa, CAS. 153-78-6, (ambos da Sigma, St. Louis, MO). Após a pré-incubação as placas foram mantidas a 37°C por 72 h, e depois as colônias revertentes foram contadas. Os resultados dos diferentes ensaios foram analisados utilizando o programa estatístico SALANAL (*Salmonella Assay Analysis*, versão 1.0, Integrated Laboratory Systems, Research Triangle Park, North Carolina, USA) (Bernstein *et al.*, 1982). Este programa permite avaliar a relação dose resposta de cada ensaio e seu enquadramento em diferentes modelos de regressão. Através desta análise pode ser obtido o cálculo e a significância estatística de: análise de variância entre as médias do número de revertentes nas diferentes doses testadas (teste F); ajuste ao modelo mais aceitável e o cálculo da positividade da curva dose resposta. A inclinação da reta na parte linear da curva dose resposta fornece a estimativa do número de revertentes induzidos por unidade de amostra. O resultado foi considerado positivo quando o índice de mutagenecidade (número de revertentes das amostras / número de revertentes do controle negativo) foi igual ou superior a 2,0 (I.M.  $\geq$  2,0), com curva dose resposta reproduzível e significante pela análise de regressão (Pereira, 2008). Os resultados positivos foram expressos em número de revertentes por unidade de massa de extrato e revertentes/m<sup>3</sup> (rev/m<sup>3</sup>), (rev/µg X MOE em µg/m<sup>3</sup>) (Coronas *et al.*, 2009).

No ensaio de citotoxicidade, uma alíquota de 10 µL da suspensão utilizada no ensaio de mutagenecidade foi diluída de forma seriada em NaCl 0,9% (100-200 células). Ao final, as diluições foram vertidas em placas contendo meio nutriente e incubadas a 37°C por 24 h. Após este período as colônias foram contadas e o resultado foi considerado citotóxico quando obteve-se um número de colônias < 60% em relação ao controle negativo (Vargas *et al.*, 1998).

Quadro 5 - Características genotípicas relevantes das linhagens de *Salmonella typhimurium* utilizadas nos ensaios de mutagenicidade e de citotoxicidade.

Linhagem	Genotípico
TA98	<i>hisD3052</i> (pKM101)
YG1021	TA98 (pYG216): superprodução de nitroreductase
YG1024	TA98 (pYG219): superprodução de <i>O</i> -acetiltransferase

Fonte: Watanabe et al., 1989.

### 3.6 Seleção dos grupos de exposição e controle

O grupo de exposição foi formado por quinze trabalhadores do túnel Rebouças do sexo masculino com idade entre 25 e 64 anos. Estes trabalhadores foram submetidos a um questionário para o fornecimento das seguintes informações: duração da jornada de trabalho, uso de medicamentos, consumo de bebidas alcoólicas, tabagismo, dieta e exposição à radiação (Apêndice I) (Pereira, 2008). Durante a jornada de trabalho foram realizadas coletas de material biológico como: urina, células da mucosa oral e sangue periférico. Ao final da coleta, os materiais foram refrigerados e transportados para o laboratório de Mutagênese Ambiental. Os mesmos procedimentos foram realizados com voluntários do grupo controle, formado por onze servidores da Universidade do Estado do Rio de Janeiro, do sexo masculino com idade entre 30 e 60 anos. Todos os procedimentos foram submetidos e aprovados pela Comissão Nacional de Ética em Pesquisa - CONEP (CAAE N° 27402014.6.0000.5259).

### 3.7 Análise dos metabolitos de HPA: 1-hidroxipireno (1-HP) e 2 naftol (2-NAP)

Para análise dos metabólitos de HPA foram utilizados 1,50 mL de amostra de urina diluída com 100 mL de tampão acetato de sódio 0,2 M pH 5,0 e 10 µL β-glucoronidase/arylsulfatase (Merck) para promover a hidrólise enzimática. A solução foi incubada a 37°C por 18 h com agitação de 200 rpm. Um cartucho de SiO<sub>2</sub>-C18 (Supelco Supelclean ENVI-18 SPE de 100 mg) foi preparado pela passagem de 5 mL de metanol grau

HPLC e 5 mL de água bidestilada. Tomou-se a amostra preparada utilizando uma seringa de vidro e passou-se todo o volume pelo cartucho ENVI-18 lentamente para a retenção das Móculas orgânicas. Em seguida passou-se pelo cartucho 5 mL de água bidestilada para a remoção dos compostos solúveis em água. Em seguida passou-se pelo cartucho 1,5 mL de acetonitrila grau HPLC para um frasco de 2 mL.

As análises químicas foram realizadas em um cromatógrafo de fase líquida de alta performance (HPLC) marca Perkin Elmer series 200 com detector de ultravioleta. Empregou-se um volume de injeção de 30 µL e uma fase móvel com 50% de acetonitrila e 50% água bidestilada. A vazão empregada foi de 1,5 mL/min durante toda a análise. A coluna de separação foi uma C18 de 250 mm com 4,6 mm de diâmetro externo e 5,0 µm de partícula (Supelcosil-LC18), operando a 40°C. O detector de ultravioleta operou com um comprimento de excitação de 240 nm e de emissão de 370 nm.

As curvas de calibração foram confeccionadas com padrões de 2-naftol (Sigma) entre 20 e 100 ng/mL e de 1-hidroxipireno (Sigma) entre 50 e 400 ng/mL. Os coeficientes de determinação foram de 0,99 para ambos compostos. Os padrões de calibração foram preparados em uma urina de controle com os mesmos procedimentos das amostras.

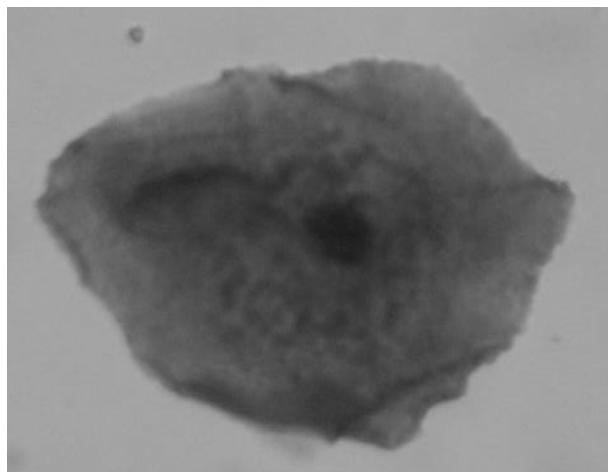
### 3.8 Teste de micronúcleo em células da mucosa oral

As células da mucosa oral foram coletadas com auxílio de um *swab*, o material foi colocado em tubo de centrífuga com NaCl 0,9%. O *swab* foi retirado e a solução centrifugada, durante dez min, a 1000 rpm. Após a centrifugação, o sobrenadante foi retirado deixando-se 0,5 mL de sedimento e solução. Em seguida, foi adicionado 12 mL de solução fixadora de metanol:ácido acético (3:1). A solução foi colocada no freezer durante trinta min. Depois, a solução foi centrifugada novamente e o sobrenadante foi retirado deixando 0,5 mL da solução. Adicionou-se 8 mL de fixador e em seguida a solução foi centrifugada. Após a centrifugação, o sobrenadante foi dispensado deixando o mínimo de solução.

O sedimento foi suspenso com auxílio de uma pipeta Pasteur, sendo transferidos para uma lâmina pré-aquecida a 37°C três gotas da suspensão. Após este procedimento o material presente na lâmina secou por uma noite. Na manhã seguinte, realizou-se a hidrólise com HCl 5N e corou-se com reagente de Shiff por uma hora, e em seguida com “fast-green” por 10 min (Tolbert *et al.*, 1992; Côrrea *et al.*, 2009).

A análise das células foi realizada em microscópio óptico, binocular, com objetiva de 100X. Foram analisadas 2000 células por indivíduo, considerando-se somente as células não fragmentadas e não sobrepostas. Os critérios utilizados para a identificação de um micronúcleo foram os estabelecidos por Picker e Fox (1986): (a) o micronúcleo deverá ter um contorno regular, redondo ou oval e estar dentro do citoplasma de uma célula; (b) o micronúcleo deve ser Feulgenee-positivo e de intensidade igual ou menor, a mesma textura e refração do núcleo principal; (c) o micronúcleo deve ser menor que o núcleo principal, isto é, seu diâmetro deve ser 1/3 do diâmetro do núcleo principal; (d) estar no mesmo plano de foco; e (e) o micronúcleo deverá estar claramente separado do núcleo principal. Serão registrados até três micronúcleos por célula, sendo que micronúcleos questionáveis não serão registrados.

Figura 6 - Célula da mucosa oral



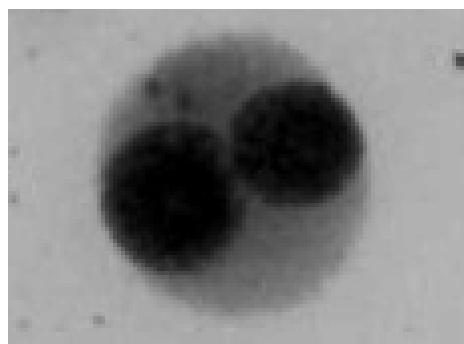
Fonte: A autora, 2013.

### 3.9 Teste de Micronúcleo em Linfócito

Para o preparo da cultura de linfócito foram utilizados 500 µL do sangue periférico coletados em sistema a vácuo em tubos com heparina. Este volume foi adicionado a 5 mL de meio RPMI + 500 µL de soro fetal bovino + 100 µL de fitohemaglutinina A 10 µl/mL, e foi incubado a 37°C em estufa de 5% de CO<sub>2</sub> por 44 h. Após a incubação, adicionou-se Citocalasina B (CtB) na concentração de 6 µg/mL, e foi incubado em estufa de CO<sub>2</sub> por mais 28 h.

No final das 72 h, a cultura foi transferida para um tubo de centrifuga, e o material foi centrifugado a 800 rpm, por 5 min. O sobrenadante foi descartado, e foi adicionado 5 mL de solução hipotônica gelada (KCl 0,075M). A suspensão foi centrifugada novamente a 800 rpm por 5 min. Após a centrifugação, descartou-se o sobrenadante, e foi adicionado 5 mL de fixador (metanol/ ácido acético 5:1 v/v). Este procedimento foi repetido por mais duas vezes. Ao final, o sobrenadante foi descartado, deixando 1 mL de suspensão de células no tubo. Para o preparo das lâminas, as mesmas foram aquecidas previamente, onde foram colocadas duas gotas da suspensão, que secaram a temperatura ambiente. O material foi corado com solução Giemsa 5% por 7 min. A análise das células foi realizada em microscópio óptico, binocular, com objetiva de 100X. Foram analisadas 2000 células binucleadas por indivíduo, considerando-se somente as células não fragmentadas e não sobrepostas (Salvadori *et al.*, 2003).

Figura 7 - Linfócito binucleado com dois micronúcleos



Fonte: A autora, 2013.

### 3.10 Análise estatística

O teste *t* de Student foi utilizado para avaliar a significância estatística dos resultados obtidos nos diferentes ensaios. Os pareamentos foram realizados entre os indivíduos expostos e de controle de acordo com a idade ( $\pm 5$  anos) (Au e Ribeiro, 2003). Comparações entre os resultados dos testes de micronúcleo e os dados obtidos no questionário foram realizadas através do teste de Correlação de Pearson com significância de 0,05, utilizando o programa estatístico SPSS/PC.

### 3.11 Extração de DNA

Para extração do DNA foi utilizado o KIT Pure Link® Geneomic DNA. Neste procedimento foram utilizados 200  $\mu$ L de sangue periférico coletado à vácuo em tubos com EDTA. Adicionou-se a amostra 20  $\mu$ L de proteinase K + 20  $\mu$ L RNase A, e incubou-se a temperatura ambiente por 2 min. Foi adicionado 200  $\mu$ L de tampão de lise Pure Link® Geneomic e incubou-se a 55°C por 10 min. Ao final da incubação foi adicionado 200  $\mu$ L de etanol. A solução foi homogeneizada e aguardou-se por 5 s. Após este tempo, 640  $\mu$ L da solução foi centrifugada a 10.000 x g/1 min. Após a centrifugação, o DNA foi lavado com 500  $\mu$ L de tampão 1 e centrifugado novamente a 10.000 x g/ 1 min. Após este procedimento foi adicionado 500  $\mu$ L de tampão 2 e centrifugado a 10.000 x g/ 3 min. O DNA foi eluído em 100  $\mu$ L de tampão de eluição Pure Link® Geneomic, e incubou-se a temperatura ambiente por 1 min. Após este tempo, o material foi centrifugado por um minuto. O DNA purificado foi armazenado à 4°C até o momento do ensaio.

### 3.12 Reação em cadeia da polimerase – PCR

Variantes polimórficas do gene CYP1A1 foram caracterizadas por PCR segundo Cascorbi et al., 1996. Todos os “primers” foram obtidos da New England Biolabs (Distribuído

por Uniscience do Brasil); e a reação de PCR foi realizada no termociclador (Biorad Thermal cycler-MyCycler).

Para a determinação da variante m3 (CYP1A1<sup>\*3</sup>), um fragmento de DNA 899-bp foi amplificado utilizando 1 unidade de Taq polimerase, 0,5 µM/L de primers M3F 5'-GGCTGAGCAATCTGACCCTA-3' e P80 5'-TAGGAGTCTTGTCTCATGCCT-3', 0,2 mM/L de dinucleotídeo trifosfato e 1,5 mM/L de MgCl<sub>2</sub> em um total de 25 µL. As condições utilizadas para o PCR foram: 5 min à 95°C, seguido de 35 ciclos de 0,5 min à 94°C, 1 min à 63°C, e 1 min à 72°C. Os produtos de PCR foram digeridos com 0,5 unidades da enzima MspI, gerando pequenos fragmentos em caso de mutação. Os fragmentos foram corados com Diamond e avaliados em gel de agarose 1,5%

As variantes polimórficas m2 e m4, (CYP1A1<sup>\*2B</sup> e CYP1A1<sup>\*4</sup>) foram avaliadas utilizando primer de 204-bp M2F 5'-CTGTCTCCCTCTGGTTACAGGAAGC-3' e M2R 5'-TTCCACCCGTTGCAGCAGGATAGCC-3', e os reageneetes descritos acima. As seguintes condições para o PCR foram utilizadas: 5 min a 95°C seguido de 35 ciclos de 0,5 min à 94°C, 0,5 min à 63°C, e 0,5 min à 72°C. Para a digestão dos produtos de m2 foi utilizada a enzima de restrição BsrDI. Para m4 (CYP1A1<sup>\*4</sup>) foi utilizado o mesmo primer de 204-bp, porém para a digestão foi utilizada a enzima de restrição BsaI. Os fragmentos foram corados com Diamond e avaliados em gel de agarose 1,5%

Para a análise polimórfica dos geneees de fase II GSTM1 e GSTT1 foi utilizado o ensaio de PCR, como descrito em Sherif et al., 1996. Para as amplificações foram utilizados 50 ng de DNA, em 25 µL contendo 0,5 mM de primer GSTM1 5' GAACTC CCTGAAAAGCTAAAGC-3' e 5' GTTGGGCTCAAATATACGGTGG-3'; e primer GSTT1 5'-TTCCTTACTGGTCCTCACATCTC-3' e 5'-TCACCGGATCATGGCCAGCA-3', 0,2 mM de dinucleotídeo trifosfato e 1,5 mM/L de MgCl<sub>2</sub>. As seguintes condições para o PCR foram utilizadas: 5 min a 95°C seguidos de 35 ciclos de 2 min à 94°C, 1 min à 59°C, e 1 min à 72°C. Os geneees foram avaliados em Diamond e gel de agarose 1,5%.

## 4 RESULTADOS

Na Tabela 1 são apresentados o volume de ar amostrado ( $m^3$ ), a concentração de MP2,5 e a concentração de MOE ( $\mu g/m^3$ ) para as amostragens dos três pontos.

Tabela 1 - Características das coletas realizadas nos três pontos de estudo de novembro de 2010 a maio de 2011

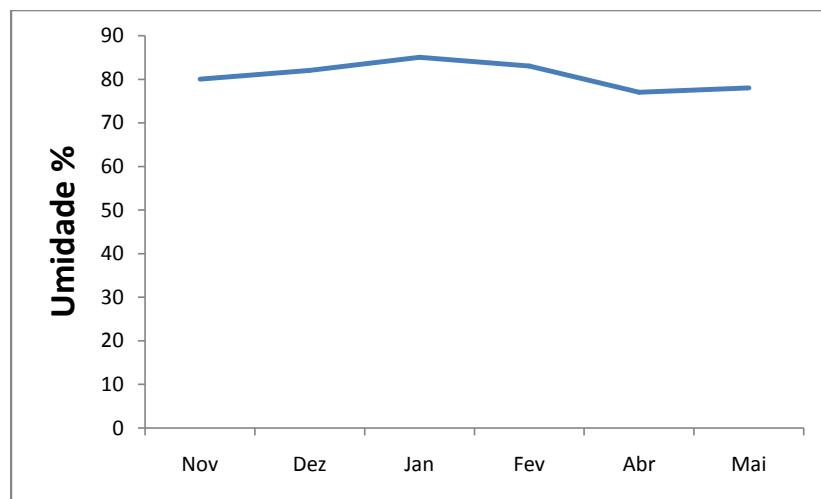
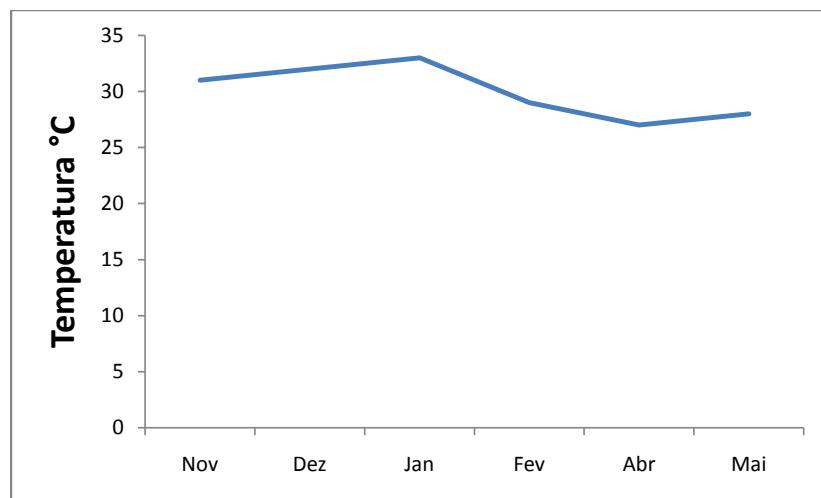
Ponto	Mês	Volume de ar ( $m^3$ ) ± D.P.	MP 2,5 ( $\mu g/m^3$ ) ± D.P.	MOE ( $\mu g/m^3$ )
1	Novembro	1567 ± 112	25 ± 15	8,62
	Dezembro	1538 ± 25	15 ± 7	1,46
	Janeiro	1568 ± 50	17 ± 2	13,89
	Fevereiro	1538 ± 12	14 ± 8	0,35
	Abril	1530 ± 11	19 ± 4	0,68
	Maio	1524 ± 66	34 ± 22	4,19
2	Novembro	1552 ± 9	31 ± 8	8,70
	Dezembro	1536 ± 39	25 ± 9	3,90
	Janeiro	1568 ± 50	28 ± 12	6,22
	Fevereiro	1580 ± 38	27 ± 10	10,92
	Abril	1519 ± 31	38 ± 20	3,21
	Maio	1490 ± 49	27 ± 11	10,73
3	Novembro	379 ± 5	74 ± 24	29,66
	Dezembro	442 ± 10	70 ± 50	49,20
	Janeiro	397 ± 16	68 ± 20	54,85
	Fevereiro	473 ± 67	62 ± 25	1,58
	Abril	415 ± 458	141 ± 44	3,49
	Maio	458 ± 47	54 ± 27	3,16

Legenda: 1- UERJ; 2 - Av. Brasil; 3 - Túnel Rebouças. D.P. - Desvio Padrão. MOE - Matéria Orgânica Extraída. Não houve coleta em março: período de manutenção do equipamento.

As maiores concentrações de MP<sub>2,5</sub> foram detectadas no ponto 3 ( $54 - 141 \mu\text{g}/\text{m}^3$ ), seguida pelo ponto 2 ( $27 - 38 \mu\text{g}/\text{m}^3$ ) e ponto 1 ( $25$  e  $34 \mu\text{g}/\text{m}^3$ ) (Tabela 1).

O Gráfico 2 mostra os dados de fatores físicos do Túnel Rebouças no período de novembro de 2010 à maio de 2011 (período II).

Gráfico 2 - Dados de fatores físicos do Túnel Rebouças no período II

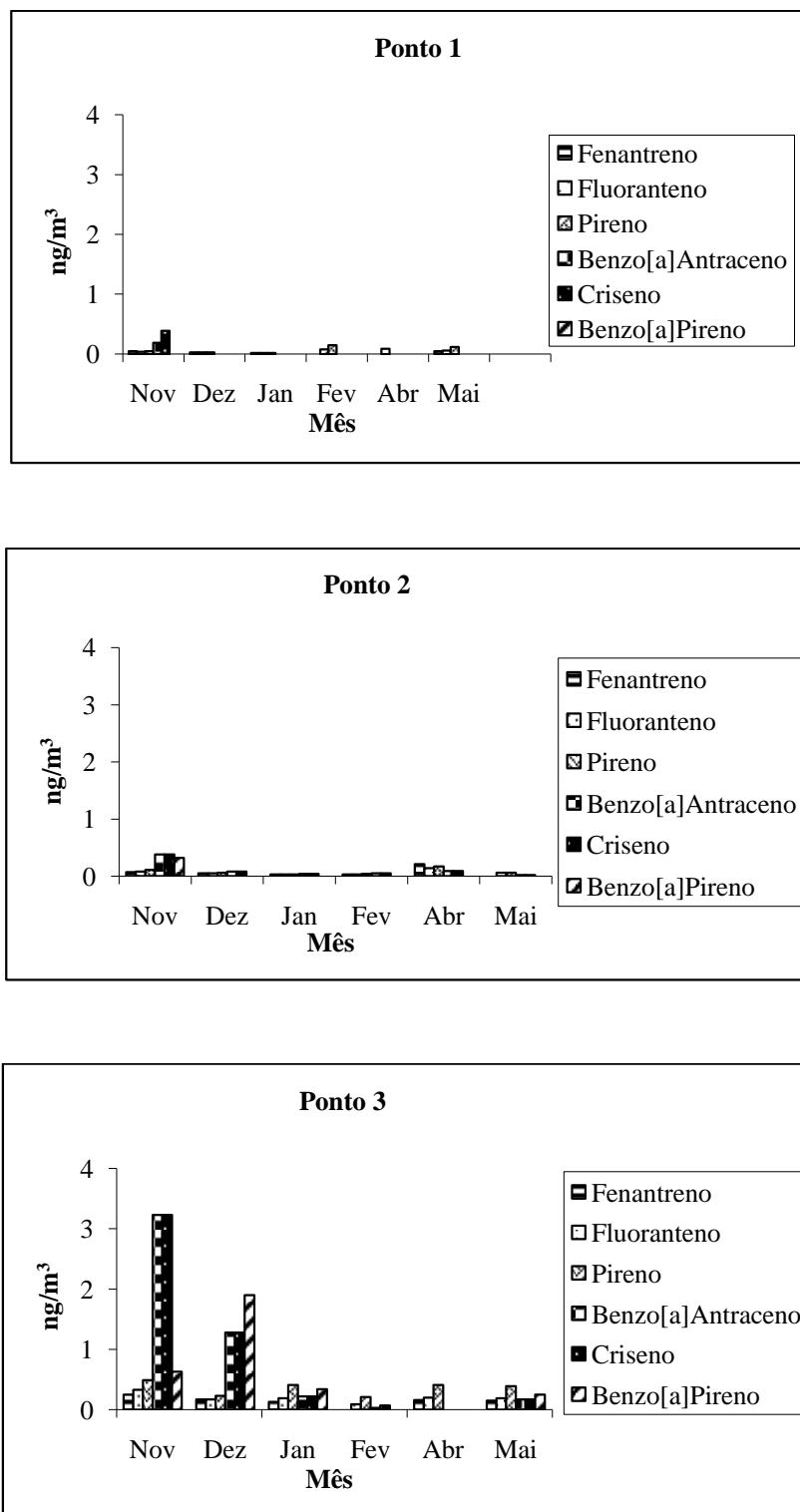


Fonte: A autora, 2011.

Os fatores físicos temperatura e umidade apresentaram pequenas variações entre os meses de estudos (Gráfico 2).

O Gráfico 3 mostra as concentrações dos HPA identificados e quantificados em  $\text{ng}/\text{m}^3$  para os três pontos de estudo durante o período II.

Gráfico 3 - Concentração dos HPA em ng/m<sup>3</sup> nos três pontos de estudo



Fonte: A autora, 2011.

As maiores concentrações de HPA foram detectadas no ponto 3. Os seguintes HPA foram predominantes: benzo[a]antraceno ( $3,23 \text{ ng/m}^3$ ) e criseno ( $3,23 \text{ ng/m}^3$ ) (Novembro). No mês de dezembro, benzo[a]pireno ( $1,90 \text{ ng/m}^3$ ) foi predominante para o mesmo ponto.

O Gráfico 4 mostra as concentrações dos Nitro-HPA identificados e quantificados em ng/m<sup>3</sup> para os três pontos de estudo.

Gráfico 4 - Concentração dos Nitro-HPA em  $\text{ng}/\text{m}^3$  nos três pontos de estudo (contina)

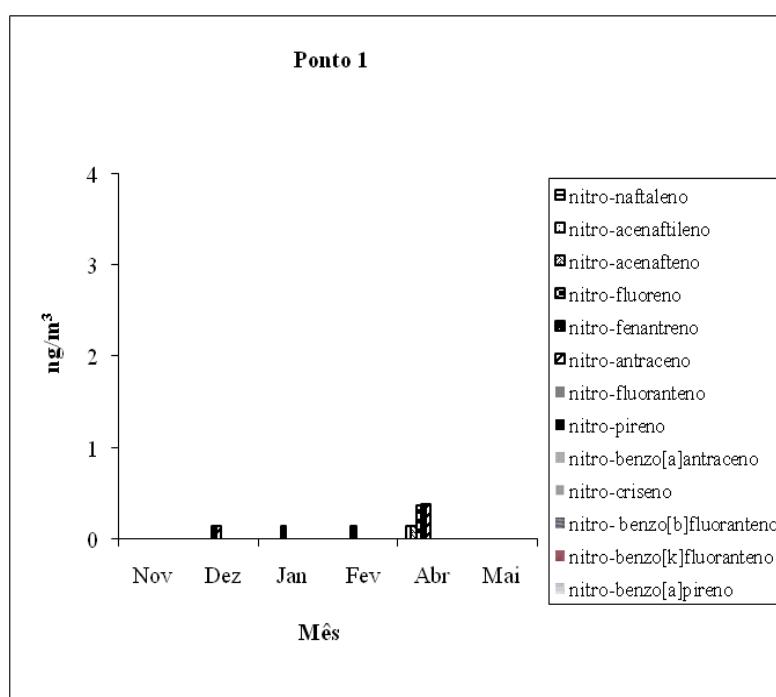
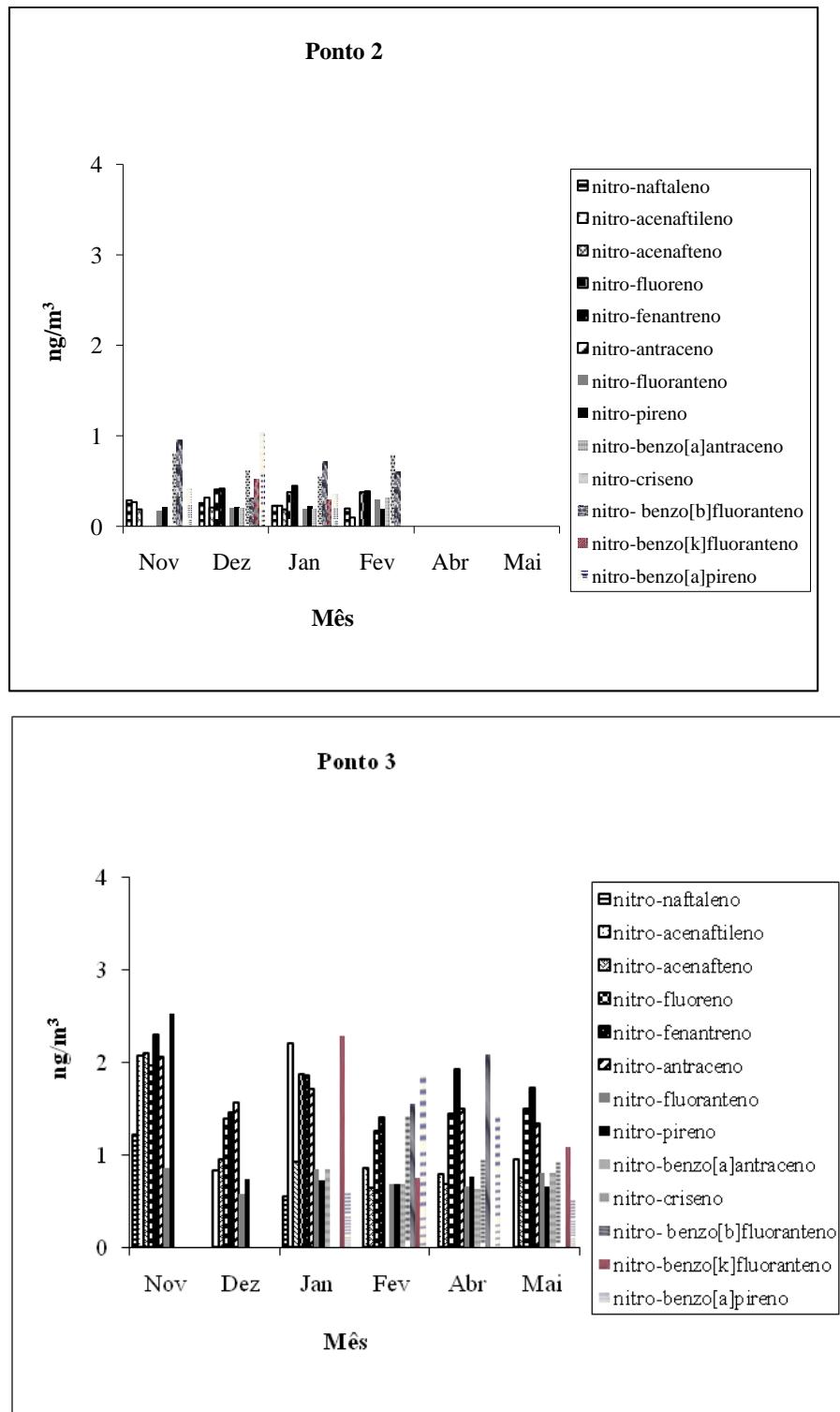


Gráfico 4 - Concentração dos Nitro-HPA em ng/m<sup>3</sup> nos três pontos de estudo (conclusão)



Fonte: A autora, 2011.

As maiores concentrações de Nitro-HPA foram detectadas no ponto 3, em todos os meses de avaliação. Os Nitro-HPA predominantes no ponto 3 : (Novembro) nitro-pireno (2,52 ng/m<sup>3</sup>), nitro-fenantreno (2,30 ng/m<sup>3</sup>), nitro-acenafteno (2,10 ng/m<sup>3</sup>), nitro-acenaftileno (2,07 ng/m<sup>3</sup>), nitro-antraceno (2,06 ng/m<sup>3</sup>) e nitro-fluoreno (1,97 ng/m<sup>3</sup>); (Dezembro) nitro-antraceno (1,56 ng/m<sup>3</sup>), nitro-fenantreno (1,46 ng/m<sup>3</sup>) e nitro-fluoreno (1,39 ng/m<sup>3</sup>); (Janeiro) nitro-benzo[k]fluoranteno (2,28 ng/m<sup>3</sup>), nitro- acenaftileno (2,20 ng/m<sup>3</sup>), nitro-fluoreno (1,87 ng/m<sup>3</sup>), nitro- fenantreno (1,86 ng/m<sup>3</sup>), e nitro-antraceno (1,71 ng/m<sup>3</sup>); (Fevereiro) nitro-benzo[a]pireno (1,86 ng/m<sup>3</sup>), nitro- benzo[b]fluoranteno (1,55 ng/m<sup>3</sup>), e nitro-criseno (1,48 ng/m<sup>3</sup>); (Abril) nitro- benzo[b]fluoranteno (2,08 ng/m<sup>3</sup>), e nitro-fenantreno (1,93 ng/m<sup>3</sup>); (Maio) nitro-phenantreno (1,73 ng/m<sup>3</sup>), nitro-fluoreno (1,93 ng/m<sup>3</sup>), and nitro-antraceno (1,34 ng/m<sup>3</sup>).

Nitro-HPA predominantes no ponto 2: nitro-criseno (0,57 – 0,84 ng/m<sup>3</sup>) e nitro-benzo[b]fluoranteno (0,61 – 0,96 ng/m<sup>3</sup>) nos meses de novembro, janeiro e fevereiro. Nitro-chriseno (0,65 ng/m<sup>3</sup>) e nitro-benzo[a]pireno (1,04 ng/m<sup>3</sup>) no mês de dezembro. Não foram detectados Nitro-HPA nos meses de abril e maio.

Nitro-HPA predominantes no ponto 1: nitro-fenantreno (0,14 ng/m<sup>3</sup>) and nitro-antraceno (0,15 ng/m<sup>3</sup>) em dezembro; nitro-fenantreno (0,14 ng/m<sup>3</sup>) em janeiro; nitro-fenantreno (0,14 ng/m<sup>3</sup>) em fevereiro; nitro-fenantreno (0,39 ng/m<sup>3</sup>), nitro-antraceno (0,39 ng/m<sup>3</sup>) e nitro-fluoreno (0,37 ng/m<sup>3</sup>) em abril. Não foram detectados Nitro-HPA nos meses de novembro e maio.

A Tabela 2 apresenta os resultados de mutageneicidade induzidos pelos extratos orgânicos dos três pontos de monitoramento em rev/m<sup>3</sup> durante o período II.

Nenhuma amostra apresentou efeito citotóxico.

Tabela 2 - Atividade mutagênica em rev/m<sup>3</sup> induzida pelos extratos orgânicos de MP2,5, coletados nos três pontos de estudo

Ponto	Mês	TA98		YG1021		YG1024	
		-S9	+S9	-S9	+S9	-S9	+S9
1	Novembro	9,10 ± 1,30	n.d. <sup>a</sup>	9,10 ± 1,50	7,00 ± 1,80	n.d. <sup>a</sup>	n.d. <sup>a</sup>
	Dezembro	1,50 ± 0,40	n.d. <sup>a</sup>	3,10 ± 0,40	1,20 ± 0,20	n.d. <sup>a</sup>	n.d. <sup>a</sup>
	Janeiro	n.d. <sup>a</sup>					
	Fevereiro	n.d. <sup>a</sup>					
	Abril	0,10 ± 0,01	n.d. <sup>a</sup>	0,20 ± 0,10	0,80 ± 0,10	n.d. <sup>a</sup>	n.d. <sup>a</sup>
	Maio	n.d. <sup>a</sup>					
2	Novembro	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>	19,20 ± 1,70	4,70 ± 0,60
	Dezembro	2,10 ± 0,30	n.d. <sup>a</sup>	4,10 ± 1,00	3,50 ± 0,90	13,60 ± 1,90	4,80 ± 0,80
	Janeiro	4,40 ± 0,70	n.d. <sup>a</sup>	2,80 ± 0,40	n.d. <sup>a</sup>	16,00 ± 1,40	3,40 ± 0,60
	Fevereiro	4,60 ± 1,00	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>	3,90 ± 1,10	n.d. <sup>a</sup>
	Abril	4,80 ± 0,60	n.d. <sup>a</sup>	2,50 ± 0,20	0,30 ± 0,10	2,80 ± 0,60	2,20 ± 0,40
	Maio	18,80 ± 3,30	n.d. <sup>a</sup>	21,70 ± 3,40	16,60 ± 2,60	30,90 ± 1,80	10,60 ± 1,10
3	Novembro	44,20 ± 24,30	49,80 ± 7,10	45,40 ± 13,60	34,70 ± 7,70	83,00 ± 5,00	8,60 ± 3,90
	Dezembro	n.d. <sup>a</sup>	19,20 ± 4,90	n.d. <sup>a</sup>	n.d. <sup>a</sup>	51,70 ± 5,40	n.d. <sup>a</sup>
	Janeiro	n.d. <sup>a</sup>	n.d. <sup>a</sup>	20,30 ± 4,90	n.d. <sup>a</sup>	21,90 ± 6,00	n.d. <sup>a</sup>
	Fevereiro	n.d. <sup>a</sup>	n.d. <sup>a</sup>	2,50 ± 0,50	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>
	Abril	17,00 ± 2,40	n.d. <sup>a</sup>	12,30 ± 2,40	n.d. <sup>a</sup>	8,20 ± 1,30	n.d. <sup>a</sup>
	Maio	n.d. <sup>a</sup>	5,80 ± 1,30	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>

Legenda: 1 – UERJ; 2 – Av. Brasil; 3 - Túnel Rebouças. n.d.<sup>a</sup> – não detectado. Negativo: DMSO para ensaio de mutageneicidade sem S9 mix: TA98, (18 ± 8); YG1021, (28 ± 4); YG1024, (17 ± 2). Para o ensaio de mutageneicidade com S9 mix: TA98, (38 ± 7); YG1021, (32 ± 8); YG1024, (20 ± 7). Controles Positivos para ensaio de mutageneicidade sem S9 mix: 4-nitroquinolina óxido (0,5 µg/placa) para TA98, (42 ± 8); YG1021, (60 ± 2); YG1024, (101 ± 37). Para ensaio de mutageneicidade com S9 mix: 2-aminofluoreno (1 µg/placa) para TA98, (134 ± 4); YG1021, (687 ± 61); YG1024, (67 ± 13).

Fonte: A autora, 2011.

Resposta mutagênica para TA98 em presença de ativação metabólica foi detectada somente no ponto 3, nos meses de novembro, dezembro e maio (Tabela 2).

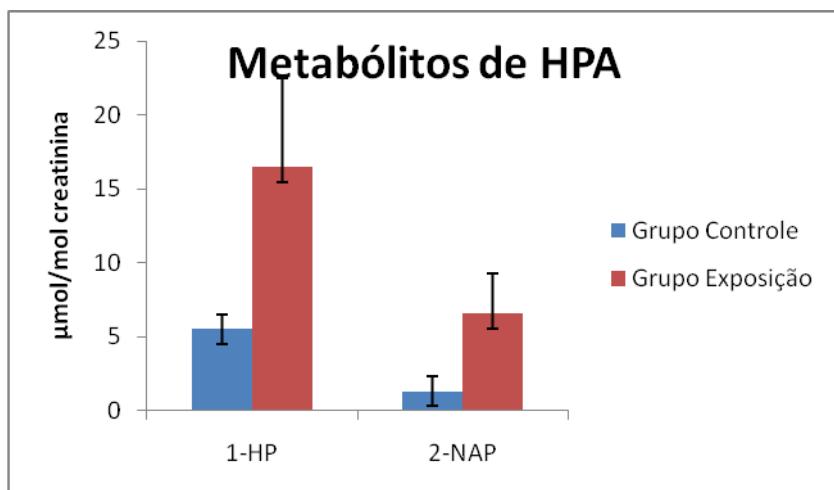
Resposta mutagênica para TA98 na ausência de metabolização foi detectada no ponto 1, nos meses de novembro, dezembro e abril; no ponto 2 nos meses de dezembro, janeiro, fevereiro, abril e maio; e no ponto 3 nos meses de novembro e abril (Tabela 2).

A presença de nitroareenos foi detectada nos pontos: 1 (Novembro, Dezembro e Abril), 2 (Dezembro e Maio) e 3 (Novembro, Janeiro e Fevereiro).

Dinitroareenos foram detectados no ponto 2 (Novembro, Dezembro, Janeiro e Maio), e no ponto 3 (Novembro, Dezembro e Janeiro).

O Gráfico 5 apresenta os resultados da dosagem de metabólitos de HPA em amostras de urina de indivíduos dos grupos controle e exposição.

Gráfico 5 - Concentração de metabólitos de HPA ( $\mu\text{M/M}$  creatinina) em amostras de urina de indivíduos dos grupos controle e exposição



Legenda: 1-HP = 1-hidroxipireno; 2-NAP = 2-naftol.  
Fonte: A autora, 2013.

O aumento significativo na concentração de 1-HP e 2-NAP ( $\mu\text{M/M}$  creatinina) foi detectado em amostras de urina de indivíduos do grupo exposição (Gráfico 5).

A Tabela 3 apresenta as médias e os desvios de micronúcleos em células da mucosa oral e de linfócitos, dos indivíduos dos grupos controle e exposição.

Tabela 3 - Médias e desvios de micronúcleos presentes em células da mucosa oral e de linfócitos, dos indivíduos dos grupos controle e exposição.

	Grupo Controle (Média±DP)	Grupo Exposição (Média±DP)
MN oral	1,19 ± 0,49	10,82 ± 4,90*
MN linfócito	1,71 ± 0,52	4,42 ± 2,78*

Legenda: DP. Desvio Padrão,\* Teste t de Sudent  $p<0,05$ .

Fonte: A autora, 2013.

O aumento significativo na frequência de micronúcleo foi detectado nas células da mucosa oral, e em linfócitos binucleados dos indivíduos do grupo de exposição.

Na Tabela 4 estão presentes as informações sobre o estilo de vida dos indivíduos dos grupos controle e exposição.

Tabela 4 - Principais características dos grupos de estudo.

	Grupo Controle	Grupo Exposição
N	11	15
Idade	44 ± 12	41 ± 10
Exposição a raio X	2	6
Uso de medicamentos	4	4
Ingestão de álcool	6	5
Consumo de defumados	7	10
Consumo de frituras	9	15
Consumo de frutas	10	12
Consumo de verduras	10	14

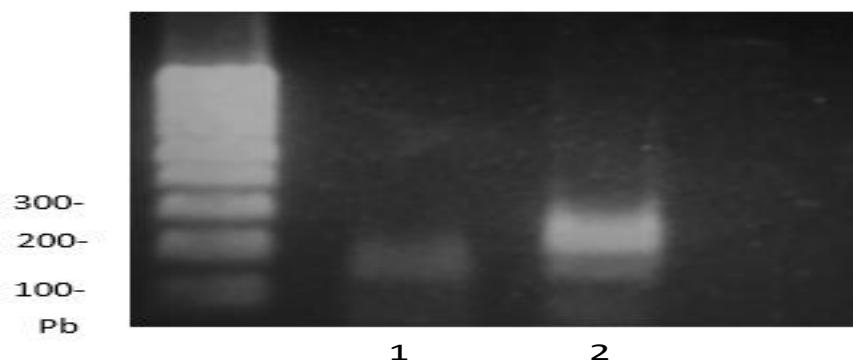
Legenda: Exposição a raio X no período de 12 meses; Ingestão eventual de álcool; Consumo regular de defumados, frituras, frutas e verduras.

Fonte: A autora, 2013.

O teste de correlação detectou correlação positiva (0,787) entre o fator ingestão de álcool, e a freqüência MN oral para o grupo controle. Nenhum dos fatores mencionados nos questionários (Tabela 4) apresentou correlação com as frequências de MN detectadas para o grupo exposição.

As figuras 8 e 9 apresentam os resultados de polimorfismos nos genes de metabolização I (CYP1A1) e II (GSTM1 e GSTT1), respectivamente.

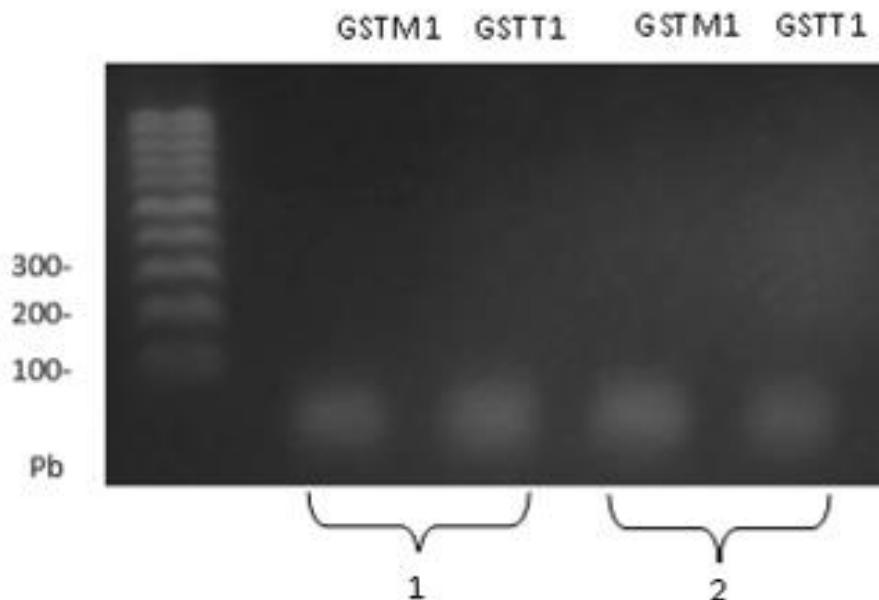
Figura 8 - Gel de agarose 1,5% apresenta polimorfismo CYP1A1<sup>\*</sup>2B em indivíduo do grupo exposição (2)



Fonte: A autora, 2015.

Polimorfismo m2 (CYP1A1<sup>\*</sup>2B) foi detectado somente em um indivíduo do grupo exposição. Nenhum polimorfismo do gene CYP1A1 foi detectado em amostras do grupo controle.

Figura 9 - Gel de agarose 1,5% apresenta polimorfismos nos geneses GSTM1 e GSTT1 em três individuos do grupo exposição



Fonte: A autora, 2015.

Deleções dos geneses GSTM1 e GSTT1 foram detectadas em 100% das amostras dos indivíduos do grupo controle. No grupo exposição foram detectadas deleções em 72,74% das amostras para GSTM1 e em 86,37% das amostras para GSTT1.

## 5 DISCUSSÃO

Altas concentrações de MP2,5 foram detectadas nos três pontos durante o período II ( ponto 3: 54-141  $\mu\text{g}/\text{m}^3$ ; ponto 2: 27-38  $\mu\text{g}/\text{m}^3$ ; ponto 1: 25 e 34  $\mu\text{g}/\text{m}^3$ ). Estas concentrações estão acima em relação à concentração limite diária estabelecida pela OMS (25  $\mu\text{g}/\text{m}^3$ ) (OMS, 2006). O tráfego é a maior fonte de MP em áreas urbanas (Weijers *et al.*, 2004; Gertler, 2005; Onat e Stakeeva, 2013). Na cidade do Rio de Janeiro, a frota de veículos triplicou nos últimos vinte anos, e os altos valores de MP2,5 podem estar relacionados a este fato. (Rainho *et al.*, 2013b).

Os pontos 2 e 3 são áreas da cidade que apresentam intenso tráfego, e como consequência apresentaram as maiores concentrações de MP2,5. O ponto 3, túnel Rebouças, além de apresentar tráfego intenso, apresenta ventilação inadequada, o que pode reduzir a dispersão de poluentes.

Altas concentrações de MP2,5 também foram detectadas no período I ( ponto 3: 94 - 132  $\mu\text{g}/\text{m}^3$ ; ponto 2: 26-60  $\mu\text{g}/\text{m}^3$ ; ponto 1: 30-36  $\mu\text{g}/\text{m}^3$ ) (Rainho *et al.*, 2013a,b). Quando comparamos os dois períodos (entre Julho e Outubro de 2010, e entre Novembro de 2010 e Maio de 2011), nós podemos observar um aumento nos valores de MP2,5 durante o período I. Em estudo realizado durante Agosto de 2010 e Março de 2011 em Porto Alegre, foram detectados altos valores de MP2,5 no mês de Agosto (De Brito *et al.*, 2013). O aumento dos valores de MP2,5 nesta época do ano pode estar relacionado ao inverno. Por outro lado, a redução dos níveis de MP2,5 no ponto 3, durante os meses de Janeiro e Fevereiro de 2011 (período II), pode estar relacionada as férias escolares, causando e diminuição da circulação veículos neste ponto.

HPA são formados através de combustão incompleta de material orgânico, e podem ser introduzido no ambiente por diversas fontes. Segundo Reisen e Arey (2005), a principal fonte de HPA em áreas urbanas é a eliminação de gasolina e diesel por veículos automotores (Pereira *et al.*, 2010). Altas concentrações de HPA foram detectadas no ponto 3. Benzo[a]antraceno, criseno e benzo[a]pireno apresentaram as maiores concentrações no período II, durante os meses de novembro e dezembro no ponto 3. Estes resultados podem estar relacionado a emissão veicular de gasolina e diesel e a ventilação restrita neste ponto (Cadle *et al.*, 1999; He *et al.*, 2006). Estes HPA detectados são classificados de acordo com a Agência Internacional de Pesquisa do Câncer (IARC, 2005), de acordo com o grau de

carcinogêneicidade: benzo[a]pireno como carcinogênico para humanos (Grupo 1), benzo[a]antraceno e criseno como possíveis carcinogênicos para humanos (Grupo 2B).

Apesar do ponto 2 apresentar tráfego intenso, esta região apresenta vários fatores de dispersão como vento e chuva, que podem explicar as baixas concentrações de HPA quando comparadas ao ponto 3. Quando analisamos as concentrações de HPA nos dois períodos, podemos observar valores semelhantes nos pontos 1 e 2 (Rainho *et al.*, 2013b). Porém no período II, benzo[a]antraceno e criseno foram detectados somente no mês de novembro no ponto 1, e benzo[a]pireno foi detectado somente no mesmo mês no ponto 2. As altas temperaturas presentes neste período podem levar a decomposição fotoquímica, favorecendo a dispersão destes HPA. Alguns estudos que monitoraram os níveis de HPA durante o ano, detectaram a redução destes poluentes, principalmente durante o verão (Piekarska e Karpińska-Smulikowska, 2007; Dallarosa *et al.*, 2008; Zacierka *et al.*, 2012).

Nitro-HPA estão presentes na atmosfera provenientes de fontes primárias, tais como as emissões veiculares (Nielsen, 1984; Bamford e Baker, 2003; Teixeira *et al.*, 2011). Estes poluentes também podem ser formados na atmosfera através de reações entre HPA e radicais hidroxil (OH) durante o dia e reações entre HPA e nitrato ( $\text{NO}_3^-$ ) durante a noite (Atkinson e Arey, 1994; Söderström *et al.*, 2005; Teixeira *et al.*, 2011). Os Nitro-HPA são  $2 \cdot 10^5$  vezes mais mutagênicos e 10 vezes mais cancerígenos que os precursores HPA (Durant *et al.*, 1996; Teixeira *et al.*, 2011).

No período I não foram realizadas análises de Nitro-HPA, o que impede a comparação com os resultados obtidos no período II. O ponto 3 apresentou as maiores concentrações de Nitro-HPA. Este resultado pode estar relacionado com a emissão veicular e a restrita ventilação. As concentrações de Nitro-HPA detectadas no ponto 3 são de 3 à 21 vezes maiores do que as concentrações detectadas em outros túneis: Allegheny Mountain (0,12 ng/m<sup>3</sup> 1-nitropireno) (Gorse *et al.*, 1983); Baltimore Harbour (0,34 ng/m<sup>3</sup> 1-nitropireno e 0,29 ng/m<sup>3</sup> 9-Nitroantraceno) (Benner, 1988) e Queensway (0,56 ng/m<sup>3</sup> 1-nitropireno e 0,36 ng/m<sup>3</sup> 9-Nitroantraceno) (Dimashki *et al.*, 2000).

Assim como para os HPA, o ponto 1 também apresentou as menores concentrações para os Nitro-HPA. Este resultado pode estar relacionado as menores emissões veiculares neste ponto. O ponto 2 apresentou os maiores valores de Nitro-HPA, especialmente durante os meses mais quentes do ano. Concentrações similares de Nitro-HPA foram detectadas durante o verão na região metropolitana de Porto Alegre (Teixeira *et al.*, 2011). Estes resultados podem estar relacionados a interação entre os HPA e os nitro compostos resultantes da poluição fotoquímica.

Altos valores de rev/m<sup>3</sup> foram detectados para todas as linhagens no período I (Rainho *et al.*, 2013a,b) comparado ao período II. O período I apresentou baixos índices pluviométricos característica do inverno na cidade do Rio de Janeiro. Esta característica sazonal pode levar ao acúmulo de poluentes e consequente aumento no número de rev/m<sup>3</sup>. O aumento da resposta mutagênica durante o inverno foi detectado em alguns estudos que avaliam a mutagenecidade induzida por MP sazonalmente (Piekarska e Karpińska-Smulikowska, 2007; De Brito *et al.*, 2013; Pereira *et al.*, 2013).

O decréscimo nos valores de rev/m<sup>3</sup> foram detectados nos meses chuvosos (Dezembro 2010 à Fevereiro de 2011 – Período II). A chuva pode remover as partículas da atmosfera. Porém, isto não significa a eliminação do risco de exposição aos contaminantes que estão associados as partículas que se mantém no ambiente (Amador-Munoz *et al.*, 2001; Rinaldi *et al.*, 2012; Pereira *et al.*, 2013).

No período I, resposta mutagênica para TA98 em presença de ativação metabólica foi detectada nos três pontos (Rainho *et al.*, 2013a,b). Porém, no período II, a resposta mutagênica para TA98 em presença de ativação metabólica foi detectada somente para o ponto 3. Estes resultados podem ser atribuídos a presença de pró-mutágenos, como os HPA, resultantes de tráfego intenso.

Respostas mutagênica para TA98 na ausência de ativação metabólica foram detectadas nos três pontos durante os períodos I (Rainho *et al.*, 2013a) e II. Estes resultados podem indicar a presença de mutágenos diretos nos extratos orgânicos de MP2,5. Alguns autores relacionam a mutagenecidade por mudança no quadro de leitura a presença de nitrocompostos, em amostras de MP, associando esta capacidade aos derivados de HPA, como os mono e dinitro HPA (Claxton *et al.*, 2004; Vargas *et al.*, 1998; De Brito *et al.*, 2013).

A contribuição de nitrocompostos para atividade mutagênia foi investigada através do ensaio de *Salmonella/microsoma* utilizando as linhagens específicas YG1021 (pYG216) e YG1024 (pYG219), que superproduzem enzimas com alta sensibilidade na detecção de nitrocompostos como nitroarenos, dinitroarenos, hidroxilaminas e aminas aromáticas, respectivamente (Watanabe *et al.*, 1989). No período I, nitroarenos e dinitroarenos foram detectados em todos os pontos (Rainho *et al.*, 2013a,b). Durante o período II, a presença de nitroarenos foram detectadas em todos os pontos. Porém, os dinitroarenos foram detectados somente nos pontos 2 e 3. Nitroarenos e dinitroarenos resultam da combustão do diesel, e podem ser produzidos na atmosfera através da interação dos HPA com poluentes encontrados na poluição fotoquímica (Pitts, 1985; Sato *et al.*, 1995). Estudos realizados na região de Porto Alegre detectaram respostas similares, durante o verão, quando é marcado a atividade

mutagênica induzida por mono e dinitroareenos presentes em diferentes tipos de partículas tais como: PTS (Partículas Totais em Suspensão), MP10 e MP2,5 (Vargas *et al.*, 2011; De Brito *et al.*, 2013).

Estudos demonstram que a exposição ocupacional à poluentes como os HPA pode induzir a formação de danos no material genético (Liu *et al.*, 2010; Singaravelu e Sellappa, 2013), aumento na ocorrência de doenças respiratórias (Cavelier *et al.*, 1978; Singaravelu e Sellappa, 2013) e cânceres (Bosetti *et al.*, 2007; Singaravelu e Sellappa, 2013). No presente trabalho foi realizada uma investigação quanto à alteração citogenética, em trabalhadores do túnel Rebouças expostos à poluição atmosférica.

Dos 50 trabalhadores do túnel Rebouças, 15 aceitaram participar deste trabalho. Nossos resultados apresentaram um aumento significativo na concentração de 1-HP e 2-NAP em amostras de urina do grupo de exposição. Estudos que avaliam exposição ocupacional detectaram altas concentrações de 1-HP em trabalhadores que atuam em: forno de coque e produção de grafite (Buchet *et al.*, 1992; Ferreira *et al.*, 1994); liquefação de carvão (Quinlan *et al.*, 1995); pavimentação de rodovias (Levin *et al.*, 1995) e produção de alumínio (Schoket *et al.*, 1999). A mesma observação foi feita em estudos que monitoraram a concentração de 2-NAP em amostras de urina de trabalhadores que atuam: na produção de carvão vegetal (Kato *et al.*, 2004; Kim *et al.*, 2004) e na inspeção de emissão (Kim *et al.*, 2004). Na urina são excretados metabólitos de HPA com baixo peso molecular, como naftaleno e o pireno. Vários estudos de monitoramento ocupacional utilizam a determinação de 1-HP e 2-NAP como marcadores eficazes para exposição de HPA (Hansen *et al.*, 2008; Wilhelm *et al.*, 2008). Avaliações com seres humanos expostos ao naftaleno têm atraído cada vez mais interesse em saúde ambiental, uma vez que este HPA mais volátil foi classificado como um possível carcinógeno humano por agências internacionais (IARC, 2002; USEPA, 2002; Wilhelm *et al.*, 2008). Em geral, os estudos de monitoramento ocupacional apontam diferenças entre os grupos controle e de populações expostas, o que sugere uma ligação provável entre a classe do agente monitorado e os danos detectados por biomarcadores (DeMarini, 2013).

As amostras de células da mucosa oral e de linfócitos dos trabalhadores do túnel Rebouças apresentaram um aumento significativo na frequência de MN. Além disso, este resultado não apresentou correlação com nenhum dos dados fornecidos no questionário. Estes aumentos nas frequências de MN nos dois tipos celulares utilizados no presente estudo podem estar relacionados à exposição aos poluentes presentes neste ambiente de trabalho. Estudos que investigam a exposição humana aos poluentes apontam para o aumento da frequência de MN em células da mucosa oral, em trabalhadores expostos aos HPA (Singaravelu e Sellappa,

2013), metais pesados (Letaj *et al.*, 2012) e ao ozônio (Chen *et al.*, 2006). Este aumento também é observado em estudos que utilizam o ensaio de MN em linfócitos humanos (Zhao *et al.*, 1998; Chen *et al.*, 2006; Ravimanickam e Bhaskar, 2013). O teste de correlação detectou correlação positiva entre o fator ingestão de álcool e a freqüência MN oral para o grupo controle. O consumo de álcool pode aumentar o número de micronúcleos (Dittberner *et al.*, 1997; Singaravelu e Sellappa, 2013).

Polimorfismos em genes que codificam enzimas de metabolização de fase I e II estão associados com suscetibilidade ao câncer de pulmão (Shi *et al.*, 2008; Ji *et al.*, 2012; Li *et al.*, 2014; Zhang *et al.*, 2014; Zhao *et al.*, 2014). A forte relação entre polimorfismo m2 (CYP1A1\*2B) e o câncer de pulmão foi encontrado primeiramente na população japonesa (Kawaji *et al.*, 1993; Cascorbi *et al.*, 1996). Em caucasianos, porém, a frequência do alelo m2 (CYP1A1\*2B) é rara (Cascorbi *et al.*, 1996). O mesmo resultado foi detectado para o genótipo de brasileiros não negros (Cascorbi *et al.*, 1996). Nossos resultados detectaram polimorfismo m2 (CYP1A1\*2B) em um indivíduo do grupo exposição. Este mesmo indivíduo apresentou deleções dos genes de fase II GSTM1 e GSTT1. Estes resultados demonstram que este indivíduo exposto a poluição ocupacional no Túnel Rebouças, pode apresentar intensificação da metabolização de poluentes como os HPA, e ter dificuldades na desintoxicação dos metabólitos destes poluentes.

## CONCLUSÃO

Altas concentrações de MP2,5 detectadas na Avenida Brasil e no Túnel Rebouças podem estar relacionadas ao intenso tráfego. O Túnel Rebouças apresentou as maiores concentrações de HPA e Nitro-HPA. Estes resultados podem estar relacionados a emissão veicular e a restrita ventilação. A Avenida Brasil apresenta tráfego intenso. Porém, esta região apresenta vários fatores de dispersão como vento e chuva, que podem explicar as baixas concentrações de HPA quando comparadas ao túnel Rebouças.

Altos valores de rev/m<sup>3</sup> detectados para todas as linhagens no período I podem estar relacionados aos baixos índices pluviométricos, característica sazonal deste período. Respostas mutagênicas para TA98 em presença de ativação metabólica detectadas nos períodos I e II podem ser atribuídas à presença de pró-mutágenos, como os HPA, resultantes de tráfego intenso. Nitroarenos e dinitroarenos detectados nos períodos I e II resultam da combustão do diesel, e podem ser produzidos na atmosfera através da interação dos HPA com poluentes encontrados na poluição fotoquímica.

Altas concentrações de 1-HP e 2-NAP em amostras de urina do grupo de exposição podem ser atribuídos à exposição aos poluentes presentes no Túnel Rebouças.

Aumentos significativos nas frequências de MN em linfócitos e em células da mucosa oral demonstram que a exposição ocupacional à poluentes, presentes no Túnel Rebouças, pode induzir a formação de danos no material genético. Indivíduo exposto a poluição ocupacional no Túnel Rebouças, pode apresentar intensificação da metabolização de poluentes como os HPA, e ter dificuldades na desintoxicação dos metabólitos destes poluentes. Esta combinação de resultados aliados a exposição ocupacional aponta a suscetibilidade ao câncer de pulmão.

## REFERÊNCIAS

- Abrantes, R., Assunção, J. V., Pesquero, C. R. Emission of polycyclic aromatic hydrocarbons from light-duty diesel vehicles exhaust. *Atmos. Environ.* 2004, 38, 1631-1640.
- Abrantes, R., Assunção, J. V., Pesquero, C. R., Bruns, R. E., Nóbrega, R. P. Emission of polycyclic aromatic hydrocarbons from gasohol and ethanol vehicles. *Atmos. Environ.* 2009, 43, 648-654.
- Amador-Munoz, O., Delgado-Rodriguez, A., Villalobos-Pietrini, R., Ortiz-Martello, R., Díaz-González, G., Bravo-Cabrera, J. L., Gómez-Arroyo, S. Partículas suspendidas hidrocarburos aromáticos policíclicos y mutageneicidad en el suroeste de la Ciudad de México. *Rev. Int. Cont. Ambiental*, 2001, 17, 193–204.
- Atkinson, R., Arey, J., Zielinska, B., Winer, A. M. In Polynuclear Aromatic Hydrocarbons: Measurements, Means and Metabolism; Cooke, M., Loening, K., Joy, M., Eds., Battelle Press: Columbus, 1987; p. 69.
- Atkinson, R., Arey, J. Atmospheric chemistry of gas phase polycyclic aromatic hydrocarbons: formation of atmospheric mutagens. *Environ. Health Perspect.* 1994, 102, 117–126.
- Au, W. W., Ribeiro, L. R. Estratégias para a condução de estudos em monitoramento genotóxico em populações humanas. In: Mutagênese Ambiental. Canoas: Editora ULBRA. 2003.
- Bamford, H. A., Baker, J. E. Nitro-polycyclic aromatic hydrocarbon concentrations and sources in urban and suburban atmospheres of the Mid-Atlantic region. *Atmos. Environ.* 2003, 37, 2077–2091.
- Benner, Jr. B. A. Mobile sources of polycyclic aromatic hydrocarbons (PAH) and nitro-PAH: a roadway tunnel study, USA, University of Maryland, 1988.
- Bernstein, L., Kaldor, J., McCann, J. e Pike, M.C., An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test. *Mutat. Res.* 1982, 97, 267–281.
- Bolognesi, C., Parrini, M., Bonassi, S., Ianello, G., Salanitto, A. Cytogenetic analysis of a human population occupationally exposed to pesticides. *Mutat. Res.* 1993, 285, 239–249.

Bosetti, C., Boffetta, P., La Vecchia, C. Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to 2005. Ann. Oncol. 2007, 18, 431–446.

Buchet , J. P., Geneenart, J. P., Mercado-Calderon, F., Delavignette, J. P., Cupers, L., Lauwerys, R. Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. Br J Ind Med 1992, 49, 761–768.

Cadle, S. H., Mulawa, P. A., Hunsanger, E. C. et al., Composition of light-duty motor vehicle exhaust particulate matter in the Denver, Colorado area. Environ. Science Technol. 1999, 33, 2328–2339.

Carter, S. B. Effects of cytochalasin on mammalian cells. Nature. 1967, 213, 261-264.

Cascorbi, I., Brockmöller, J., Roots, I. A C4887A Polymorphism in Exon 7 of Human CYP1A1: Population Frequency, Mutation Linkages, and Impact on Lung Cancer Susceptibility. Cancer Research. 1996, 56, 4965-4969.

Cavelier, C., Pham, Q.T., Mur, J. M. et al. Etude clinique et fonctionnelle respiratoire chez les ouvriers noyauteurs de fonderie utilisant le procédé Ashland. Archives des Maladies Professionnelles de Médecine du Travail et de Sécurité Sociale 1978, 39, 607-15.

CETESB - Companhia Ambiental do Estado de São Paulo, 2011. Qualidade do Ar. <http://www.cetesb.sp.gov.br/ar/Informacoes-Basicas/21-Poluentes>. Acesso em 31 de dezembro de 2011.

CETESB - Companhia Ambiental do Estado de São Paulo, 2015. Qualidade do Ar. <http://ar.cetesb.sp.gov.br/Histórico>. Acesso em 13 de novembro de 2015.

Cet-Rio-Companhia de Engenharia e Tráfego do Rio de Janeiro <http://www.rio.rj.gov.br/web/cetrio>. Acesso em 31 de dezembro de 2011.

Chen, C., Arjomandi, M., Qin, H., Balmes, J., Tager, I., Holland, N. Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone. Mutagenesis. 2006, 21, 131–137.

Claxton, L.D., Matthews, P. P., Warren S. H. The genotoxicity of ambient outdoor air, a review: *Salmonella* mutagenicity. Mutat. Res. 2004, 567, 347–399.

CONAMA. Conselho Nacional do Meio Ambiente –. Resolução CONAMA nº 03 de 28 de junho de 1990. Dispõe sobre os padrões de qualidade do ar, previsto no PRONAR. Brasília: DOU, seção 1, de 22 de agosto de 1990: 15937-15939.

Coronas, M. V., Pereira, T. S., Rocha, J. A. V., Lemos, A. T., Fachel, J. M. G., Salvadori, D. M. F. et al. Geneetic biomonitoring of urban population exposed to mutagenic airborne pollutants. *Environ. Int.* 2009, 35, 1023–1029.

Corrêa, N. S., Bassan, J. S., da Cunha, C. J., Fernández, R. R., Bachettini, P. S., Garcias, G. L., Martino-Roth, M. G. Monitoramento da ação genotoxicidade em trabalhadores de sapatarias através do teste de micronúcleos, Pelotas, Rio Grande do Sul. *Ciência e Saúde Coletiva*, 2009, 14, 2251-2260.

Dallarosa, J., Teixeira, E. C., Meira, L., Wiegand, F. Study of the chemical elements and polycyclic aromatic hydrocarbons in atmospheric particles of PM10 and PM2.5 in the urban and rural areas of South Brazil. *Atmos. Res.* 2008, 89, 76–92.

Da Silva Augusto, L. G., Lieber, S. R., Ruiz, M. A., de Souza, C. A. Micronucleus monitoring to assess human occupational exposure to organochlorides. *Environ. Monit. Mutagen.* 1997, 29, 46–52.

De Brito, K. C. T., de Lemos, C. T., Rocha, J. A. V., Mielli, A. C., Matzenbacher, C., Vargas, V. M. F. Comparative genotoxicity of airborne particulate matter (PM2.5) using *Salmonella*, plants and mammalian cells. *Ecotoxicol. Environ. Saf.* 2013, 94, 14–20.

DeMarini, D. M. Genotoxicity biomarkers associated with exposure to traffic and near-road atmospheres: a review. *Mutagenesis* 2013, 28, 485–505.

Dimashki, M., Harrad, S., Harrison, R. M. Measurements of nitro-PAH in the atmospheres of two cities. *Atmos. Environ.* 2000, 34, 2459-2469.

Dittberner, U., Schmetzer, B., Gölzer, P. et al. Genotoxic effects of 2-trans-hexenal in human buccal mucosa cells in vivo. *Mut. Res.* 1997, 390, 161-5.

Ducatti, A., Vargas, V. M. F. Mutagenic activity of airborne particulate matter as an indicative measure of atmospheric pollution. *Mutat. Res.* 2003, 540, 67-77.

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

Etzel, R. A. How environmental exposure influence the development and exacerbation of asthma. *Pediatrics*. 2003, 112, 233-9.

Falck, G. C. M., Hirvonen, A., Scarpato, R., Saarikoski, S. T., Migliore, L., Norppa, H. Micronuclei in blood lymphocytes and geneetic polymorphism for *GSTM1*, *GSTT1* and *NAT2* in pesticide-exposed greenhouse workers. *Mutat. Res.* 1999, 441, 225–237.

Fenech, M., Morley, A. Solutions to the kinetic problem in the micronucleus test. *Cytobios*. 1985, 43, 233-246.

Ferreira, M., Buchet, J. P., Burrión, J. B., Moro, J., Cupers, L., Delavignette, J. P., Jacques, J., Lauwerys, R. Determinants of urinary thioethers, D-glutaric acid and mutagenicity after exposure to polycyclic aromatic hydro-carbons assessed by air monitoring and measurement of 1-hydroxypyrene in urine: a cross-sectional study in workers of coke and graphite-electrode-producing plants. *Int Arch Occup Environ Health* 1994, 65, 329–338.

Gertler, A.W. Diesel vs. gasoline emissions: does PM from diesel or gasoline vehicles dominate in the US? *Atmos. Environ.* 2005, 39, 2349–2355.

Google Maps. Imagem de satélite da Avenida Brasil, Túnel Rebouças e do Campus UERJ. <http://maps.google.com.br/> Acesso em 31 de dezembro de 2011.

Gorse Jr., R. A., Riley, T. L., Ferris, F. C., Pero, A. M., Skewes, L. M. 1-nitropyrene concentration and bacterial mutagenicity in on-road vehicle particulate emissions. *Environ. Science Technol.* 1983, 17, 198-202.

Hansen, A. M., Mathiesen, L., Pedersen, M., Knudsen, L.E. Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies—A review. *Int J Hyg Environ Health* 2008, 211, 471 – 503.

He, L. Y., Hu, M., Huang, X. F., Zhang, Y. H., Yu, B. D., Liu, D. Q. Chemical characterization of fine particles from on-road vehicles in the Wutong tunnel in Shenzhen China. *Chemosphere*, 2006, 62, 1565–1573.

IARC (International Ageneecy for Research on Cancer). IARC Monographs on the Evaluation of Carcinogeneic Risks to Humans: some Traditional Herbal Medicines, some Mycotoxins, Naphthalene and Styrene, 82. IARC, 2002, Lyon.

IARC, Agência Internacional para Pesquisa sobre o Câncer, monografias sobre a avaliação dos riscos cancerígenos para os seres humanos. Alguns hidrocarbonetos aromáticos não-heterocíclicos e alguns riscos relacionados, vol. 92, 1–18, Lyon, 2005.

Ji, Y. N., Wang, Q., Lin, X. Q., et al. CYP1A1 MspI polymorphisms and lung cancer risk: an up dated meta-analysis involving 20, 209 subjects. *Cytokine*. 2012, 59, 324–34.

Joksić, G., Vidaković, A., Spasojević-Tišma, V. Cytogenetic monitoring of pesticide sprayers. *Environ. Res.* 1997, 75, 113–118.

Kado, N.Y., Guirguis, C., Guirguis, N., Flessel, P., Chan, R.C., Chang, K. et al. Mutagenicity of fine (<2.5 $\mu$ M) airborne particles: diurnal variation in community air determined by a *Salmonella* micro preincubation (microsuspension) procedure. *Environ. Mutagen.* 1986, 8, 53-66.

Karahalil, B., Karakaya, A. E., Burgaz, S. The micronucleus assay in exfoliated buccal cells: application to occupational exposure to polycyclic aromatic hydrocarbons. *Mutat. Res.* 1999, 442, 29–35.

Kassie, F., Parzefall, W., Knasmüller, S. Single cell gel electrophoresis assay: a new technique for human biomonitoring studies. *Mutat. Res.* 2000, 463, 13-31.

Kato, M., Loomis, D., Brooks, L. M., Gattas, G. F. J., Gomes, L., Carvalho, A. B., Rego, M. A. V., DeMarini, D. M. Urinary Biomarkers in Charcoal Workers Exposed to Wood Smoke in Bahia State, Brazil. *Cancer Epidemiol Biomarkers Prev* 2004, 213, 1005-1012.

Kawajiri, K., Nakachi, K., Imai, K., Watanabe, J., Hayashi, S., The CYP1A1 gene and cancer susceptibility. *Crit. Rev. Oncol. Hematol.* 1993, 14, 77-87.

Kim, M. K., Oh, S., Lee, J. H., Im, H., Ryu, Y. M., Oh, E., Lee, J., Lee, E., Sul, D. Evaluation of biological monitoring markers using genomic and proteomic analysis for automobile emission inspectors and waste incinerating workers exposed to polycyclic aromatic hydrocarbons or 2,3,7,8-tetrachlorodibenz-p-dioxins. *Exp M Med* 2004, 36, 396–410.

Kittelson, D. B., Watts, W. F., Johnson, J. P. Nanoparticle emissions on Minnesota highways. *Atmos. Environ.* 2004, 38, 9-19.

Ko, F. W. S., Tam, W., Wong, T. W., Lai, C. K. W., Wong G. W. K. Leung, T. F., et al. Effects of air pollution on asthma hospitalization rates in different age groups in Hong Kong. *Clin. Exp. Allergy*. 2007a, 37, 1312-9.

Ko, F. W. S., Tam, W., Wong, T. W., Chan, D. P. S., Tung, A. H., Lai, C. K. W., et al. Temporal relationship between air pollutants and hospital admissions for chronic obstructive pulmonary disease in Hong Kong. *Thorax*. 2007b, 62, 779-84.

Kuno, R., Roquetti, M. H., Umbuzeiro, G. A. Indicadores Biológicos de Exposição: Ocupacional x Ambiental. *INTERFACEHS*, 2009, 4, 1-13.

Landen, F., Hart, J. E., Smith, T. J., Davis, M. E., Garshick, E. Cause-specific mortality in the unionized U. S. Trucking industry. *Environ. Health Perspect.* 2007, 115, 1192-6.

Lang, M.A., Pelkonen, O. Metabolism of xenobiotics and chemical carcinogenesis. In: Metabolic polymorphisms and susceptibility to cancer (P. Vineis, N. Malats, M. A. Lang, A. D'Errico, N. Caporaso, J. Cuzick, P. Boffetta), 1999, IARC, Lyon, FR.

Letaj, K., Elezaj, I., Selimi, Q., Kurteshi, K. The effects of environmental pollution with heavy metals in frequency of micronuclei in epithelial buccal cells of human population in mitrovica. *Journal of Chemical Health Risks* 2012, 2, 1- 4.

Levin, J.O., Rhén, M., Sikström, E. Occupational PAH exposure: urinary 1-hydroxypyrene levels of coke oven workers, aluminium smelter pot-room workers, road pavers, and occupational non-exposed persons in Sweden. *Sci Total Environ* 1995, 163, 169 177.

Li, W., Qiang Song, L., Tan, J., Combined effects of CYP1A1 MspI and GSTM1 geneetic polymorphisms on risk of lung cancer: an updated meta-analysis. *Tumor Biol* 2014, 35, 9281– 9290.

Lima, Y. L., Farias, F. F., Lima, A. M. L., Elisa, M., Poluição Atmosférica e Clima: refletindo sobre os padrões de qualidade do ar no Brasil. *Revista Geonorte* 2012, 2, 555 – 564.

Liu, H. H., Lin, M. H., Chan, C. I. et al. Oxidative damage in foundry workers occupationally co-exposed to PAHs and metals. *Int. J. Hyg. Environ. Health* 2010, 213, 93–98.

Lopes, W. A., Andrade, J. B. Fontes, formação, reatividade e quantificação de hidrocarbonetos policíclicos aromáticos (HPA) na atmosfera. *Química Nova*. 1996, 19, 497- 516.

Maron, D.M., Ames B. N. Revised method for *Salmonella* mutagenicity test. *Mutat. Res.* 1983, 113, 175-215.

Mayer, A. S., Newman, L. S. Geneetic and environmental modulation of chronic obstructive pulmonary disease. *Respir. Physiol.* 2001, 128, 3-11.

Mazzoli-Rocha, F., Fernandes, S., Einicker-Lamas, M., Zin, W. A. Roles of oxidative stress in signaling and inflammation induced by particulate matter. *Cell Biol. Toxicol.* 2010, 26, 481-498.

Meire, R. O., Azevedo, A., Torres, J. P. M. Aspectos ecotoxicológicos de hidrocarbonetos policíclicos aromáticos. *Oecol. Bras.* 2007, 11, 188-201.

Nardocci, A. C. Avaliação probabilística de risco da exposição aos hidrocarbonetos policíclicos aromáticos (HPA) para a população da cidade de São Paulo. São Paulo. Tese de Livre Docência, 2010 – Faculdade de Saúde Pública da USP.

Nielsen, T. Reactivity of polycyclic aromatic hydrocarbons towards nitrating species. *Environ. Science Technol.* 1984, 18, 157–163.

Onat, B., Stakeeva, B. Personal exposure of commuters in public transport to PM2.5 and fine particle counts. *Atmos. Poll. Res.* 2013, 4, 329-335.

Organização Mundial de Saúde. Diretrizes da qualidade do ar: Atualização Global 2005. Material particulado, ozônio, dióxido de nitrogênio e dióxido de enxofre. Escritório Regional da OMS para a Europa, Copenhaga, Dinamarca, 2005.

Organização Mundial de Saúde. Health Risks of Particulate Matter From Long-Range Transboundary Air Pollution, Copenhagenee, 99 páginas, 2006.

Pastor, S., Creus, A., Parrón, T., Cebulska-Wasilewska, A., Siffel, C., Piperakis, S., Marcos, R. Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. *Mutageneesis* 2003, 18, 249–258.

Pemble, S., Schroeder, K. R., Spence, S. R., Meyer, D. J., Hallier, E., Bolt, H. M., Ketterer, B., Taylor, J. B., Human glutathione S-transferase theta (GSTT1): cDNA cloning and characterization of geneetic polymorphism. *Biochemical Journal* 1994, 300, 271-276.

Pereira Netto, A. D., Moreira, J. C., Dias, A. E. X. O., Arbillia, G., Ferreira, L. F. V., Oliveira, A. S., et al. Avaliação da contaminação humana por hidrocarbonetos policíclicos aromáticos (HPA) e seus derivados nitrados (NHPA): uma ravisão metodológica. *Química Nova.* 2000, 23, 765-773.

Pereira, T. S. Biomonitoramento de populações humanas através de avaliação de genotoxicidade em áreas sujeitas a risco ecotoxicológico. Tese. Porto Alegre. Universidade Federal do Rio Grande do Sul. 221 p. 2008.

Pereira, T. S., Gotor, G. N., Beltrami L. S., et al. *Salmonella* mutagenicity assessment of airborne particulate matter collected from urban areas of Rio Grande do Sul State, Brazil, differing in anthropogenic influences and polycyclic aromatic hydrocarbon levels. *Mutat. Res.* 2010, 702, 78–85.

Pereira, T. S., Beltrami, L. S., Rocha, J. A. V., Broto, F. P., Comellas, L. R., Salvadori, D. M. F., Vargas, V. M. F. Toxicogenetic monitoring in urban cities exposed to different airborne contaminants. *Ecotoxicol. Environ. Saf.* 2013, 90, 174–182.

Picker, J. D., Fox, D. P. Do curried foods produce micronuclei in buccal epithelial cells? *Mutat. Res.* 1986;171:185-188.

Piekarska, K., Karpińska-Smulikowska, J. Mutagenic Activity of Environmental Air Samples from the Area of Wrocław, Poland. *Pol. J. Environ. Stud.* 2007, 16, 745-752.

Pitts Jr., J. N. On the trail of atmospheric mutagens and carcinogens: a combined chemical/microbiological approach. *American Zoo.* 1985, 25, 415–431.

Pope, C.A., Burnett, R.T., Thun, M.J., Calle, E.E., Krewski, D., Ito, K., Thurston, G.D. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *J. American Med. Ass.* 2002, 287, 1132–1141.

Quinlan, R., Kowalczyk, G., Gardiner, K., Calvert, I. A., Hale, K. A., Walton, S.T. Polycyclic aromatic hydrocarbon exposure in coal liquefaction workers: the value of urinary 1-hydroxypyrene excretion in the development of occupational hygiene control strategies. *Ann Occup Hyg* 1995, 39, 329–346.

Rainho, C. R., Velho, A. M. A., Corrêa, S. M., Mazzei, J. L., Aiub, C. A. F., Felzenszwalb, I. Prediction of health risk due to polycyclic aromatic hydrocarbons present in urban air in Rio de Janeiro, Brazil. *Genee. M. Res.* 2013a, 12, 3992-4002.

Rainho, C. R., Corrêa, S. M., Mazzei, J. L., Aiub, C. A. F., Felzenszwalb, I. Genotoxicity of Polycyclic Aromatic Hydrocarbons and Nitro-Derived in Respirable Airborne Particulate Matter Collected from Urban Areas of Rio de Janeiro (Brazil). *BioMed Res. Inter.* 2013b, 2013, 1-9.

Ravimanickam, T., Bhaskar, A. A study on the micronuclei induction in traffic Police population in Tiruchirappalli, Tamilnadu, India. *Int. J. Pure Appl. Zool.* 2013, 1, 135-138.

Reisen, F., Arey, J. Atmospheric reactions influence seasonal PAH and nitro-PAH concentrations in the Los Angeles Basin. *Environ. Science Technol.* 2005, 39, 64–73.

Ribeiro Pinto, L. F., Felzenszwalb, I. Genotoxicidade do câncer humano. In: *Mutagênese Ambiental*. Canoas: Editora ULBRA. 2003.

Rinaldi, M. C. S., Domingos, M., Dias, A. P. L, Esposito, J. B. N., Pagliuso, J. D. Leaves of *Lolium multiflorum* “Lema” and tropical tree species as biomonitoring of polycyclic aromatic hydrocarbons. *Ecotoxicol. Environ. Saf.* 2012, 79, 139–147.

Rohr, P., Cañedo, A. D., Paskulin, G., Schüller, I., Nardi, N. B., Kvitko, K., Análise dos polimorfismos GSTM1 e GSTT1 em pacientes que desenvolveram leucemias agudas. *Brazilian Journal of Biosciences*, 2004, 2, 143-150.

Salgado, P. E. T., Fernicola, N. A. G. G. Noções básicas de toxicologia ocupacional. São Paulo: Opas; Ed. Unesp, 1989.

Salvadori, D. M., Ribeiro, L. R., Fenech, M. Teste do micronúcleo em células humanas in vitro. In: *Mutagênese Ambiental*. Canoas: Editora ULBRA. 2003.

Sato, M. I. Z., Umbuzeiro, G.A., Coimbrão, C. A. et al. Mutagenicity of airborne particulate organic material from urban and industrial areas of São Paulo, Brazil. *Mutat. Res.* 1995, 335, 317–330.

Sarto, F., Tomanin, R., Giacomelli, L., Canova, A., Raimondi, F., Ghiootto, C., Fiorentino, M. V. Evaluation of chromosomal aberrations in lymphocytes and micronuclei in lymphocytes, oral mucosa and hair root cells of patients under antiblastic therapy. *Mutat. Res.* 1990, 228, 157–169.

Schoket, B., Poirier, M. C., Mayer, G., Török, G., Kolozsi-Ringelhann, A., Bognár, G., Bigbee, W. L., Vincze, I. Biomonitoring of human genotoxicity induced by complex occupational exposures. *Mutat Res* 1999, 445, 193–203.

Shi, X., Zhou, S., Wang, Z., et al. CYP1A1 and GSTM1 polymorphisms and lung cancer risk in Chinese populations: a meta-analysis. *Lung Cancer*. 2008, 59, 155–63.

Singaravelu, S. R., Sellappa, S. Assessment of Genotoxicity in exfoliated buccal epithelial cells of foundry workers occupationally exposed to polycyclic aromatic hydrocarbons. *Asian J. Pharm. Clin. Res.* 2013, 6, 339-342.

Söderström, H., Hajslova, J., Kocourek, V., Siegmund, B., Kocan, A., Obiedzinski, M.W., Tysklind, M., Bergqvist, P. PAHs and nitrated PAHs in air of Five European countries determined using SPMDs as passive samplers. *Atmos. Environ.* 2005, 39, 1627–1640.

Sprenger, R., Schlageneehafer, R., Kerb, R., Bruhn, C., BrockMler, J., Roots, I., Brinkmann, U. Characterization of glutathione S-transferase GSTT1 deletion: discrimination of all geneotypes by polymerase chain reaction indicates a trimodular geneotypephenotype correlation. *Pharmacogenetics*. 2000, 10, 557-565.

Tan, K. L., Webb, G. C., Baker, R. T., Board, P. G., Mecular cloning of a cDNA and chromosomal localization of a human theta-class glutathione S-transferase geneee (GSTT2) to chromosome 22. *Geneomics*. 1995, 25, 381-387.

Tao, F., Gonzalez-Flecha, B., Kobzik, L. Reactive oxygene species in pulmonary inflammation by ambient particulates. *Free Radic. Biol. Med.* 2003, 35, 327-40.

Teixeira, E. C., Garcia, K. O., Meincke, L., Leal, K. A. Study of nitro-polycyclic aromatic hydrocarbons in fine and coarse atmospheric particles. *Atmos. Res.* 2011, 101, 631–639.

Tolbert, P., Shy, C., Allen, J. Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res* 1992; 271, 69–77.

US Environmental Protection Ageneecy. Estimating Carcinogeneic Potency for Mixtures of Polycyclic Organic Matter (POM) for the 1996 National-Scale Assessment. Appendix H- 3, Washington, DC, 1996.

USEPA, United States Environmental Protection Ageneecy. Health effects support document for naphthalene, external review draft. EPA 822-R-02-031, 2002; Washington DC.

US Environmental Protection Ageneecy. Air Pollutants.  
<http://www.epa.gov/air/airpollutants.html> Acesso em 31 de dezembro de 2011.

Vargas, V. M. F., Horn, R. C., Guidobono, R. R., Mittelstaed, A. B., Azevedo. I. G. Mutageneic activity of airborne particulate matter from the urban area of Porto Alegre, Brazil. *Genee. M. Biol.* 1998, 21, 247- 253.

Vargas, V. M. F., Brito, K. C. T., Coronas, M. V. *Geneetic biomarkers applied to environmental air quality: ecological and human health aspects*. In:Mazzeo,N. A. (Ed.), *Air Quality Monitoring*, [www.intechPen.com/books/](http://www.intechPen.com/books/) air-quality-monitoring-assessment-and-management, 2011.

Watanabe, M., Ishidate, M., Nohmi, T. A sensitive method for detection of mutagenic nitroarenes: construction of nitroreductases-overproducing derivatives of *Salmonella typhimurium* strains TA98 and TA100. Mutat. Res. 1989, 216, 211–220.

Weijers, E. P., Khlystov, A. Y., Kos, G. P. A., Erisman, J. W. Variability of particulate matter concentrations along roads and motorways determined by a moving measurement unit. Atmos. Environ. 2004, 38, 2993–3002.

Wilhelm, M., Hardt, J., Schulz, C., Angerer, J. On behalf of the Human Biomonitoring Commission of the German Federal Environment Agency. New reference value and the background exposure for the PAH metabolites 1-hydroxypyrene and 1- and 2-naphthol in urine of the general population in Germany: Basis for validation of human biomonitoring data in environmental medicine. Int J Hyg Environ Health 2008, 211, 447 – 453.

Wilkinson, J., Clapper, M. L. Detoxification enzymes and chemoprevention. Proceeding Society Experimental Biological Medicine, 1997, 216, 192-200.

Xu, S., Wang, Y., Roe, B., Pearson, W. R., Characterization of human class mu glutathione S-transferase gene cluster and the GSTM1 deletion. Journal Biological Chemistry, 1998, 273, 3517-3527.

Zaciera, M., Kurek, J., Dzwonek, L., Feist, B., Jedrzejczak, A. Seasonal variability of PAHs and NITRO-PAHs concentrations in total suspended particulate matter in ambient air of cities of Silesian Voivodeship. Environ. Protec. Engineering 2012, 38, 45-50.

Zhang Q, Jin H, Wang L, et al. Lung cancer risk and genetic variants in East Asians: a meta-analysis. Tumour Biol. 2014. doi:10.1007/s13277-014-1671-0.

Zhao, X., Niu, J., Wang, Y., Yan, C., Wang, X., Wang, J. Genotoxicity and chronic health effects of automobile exhaust: a study on the traffic policemen in the city of Lanzhou. Mutat. Res. 1998, 415, 185–190.

Zhao, Y., Zeng, J., Zhang, Y., et al. GSTM1 polymorphism and lung cancer risk among East Asian populations: a meta-analysis. Tumour Biol. 2014. doi:10.1007/s13277-014-1832-1.

**APENDICE A – Questinário do Laboratório de Mutagênese Ambiental - LABMUT**



**Universidade do Estado do Rio de Janeiro – UERJ  
Instituto de Biologia Roberto Alcantara Gomes – IBRAG  
Laboratório de Mutagênese Ambiental - LABMUT**

Número de Registro \_\_\_\_\_

Nome: \_\_\_\_\_

Profissão: \_\_\_\_\_

Idade: \_\_\_\_ anos

Local de Trabalho: \_\_\_\_\_

Carga horária de trabalho/dia: \_\_\_\_\_

Tempo de Serviço: \_\_\_\_\_

Data das últimas férias: \_\_\_ / \_\_\_ / \_\_\_

Hábitos:

- Fuma? ( ) sim ( ) não há quanto tempo? \_\_\_\_\_

Quantos cigarros por dia? \_\_\_\_\_

Tipo: ( ) cachimbo ( ) charuto ( ) palha ( ) papel com filtro

( ) outros \_\_\_\_\_

- Já fumou ( ) sim ( ) não

Há quanto tempo deixou de fumar? \_\_\_\_\_

Quantos cigarros por dia? \_\_\_\_\_

Durante quanto tempo fumou? \_\_\_\_\_

Tipo: ( ) cachimbo ( ) charuto ( ) palha ( ) papel com filtro ( ) outros

- Convive diariamente com fumantes? ( ) sim ( ) não

- Consome bebida alcoólica? ( ) sim ( ) não ( ) eventualmente

Há quanto tempo? \_\_\_\_\_

Tipo: ( ) cachaça ( ) cerveja ( ) whisky ( ) vodka ( ) vinho ( ) outras

Quantidade por dia (número de copos por dia)?

Já bebeu? ( ) sim ( ) não Há quanto tempo deixou?

Tipo: ( ) cachaça ( ) cerveja ( ) whisky ( ) vodka ( ) vinho ( ) outras

Quantidade por dia (número de copos por dia)?

Tipo: ( ) cachaça ( ) cerveja ( ) whisky ( ) vodka ( ) vinho ( ) outras

- Já usou drogas? ( ) sim ( ) não

Tipo?

Quantidade?

- Já foi exposto à radiação? ( ) sim ( ) não

Número de raio-X: \_\_\_\_\_

Quantas vezes foi exposto nos últimos 12 meses? \_\_\_\_\_

- Usa algum tipo de medicamento? ( ) sim ( ) não

Quais?

( ) Antibiótico ( ) Antiinflamatório ( ) Vitaminas ( ) Xarope

( ) Outros \_\_\_\_\_

- Frequência por dia? \_\_\_\_\_

- Há quanto tempo deixou? \_\_\_\_\_

- Consome fritura? ( ) sim ( ) não

Quantas vezes na semana? \_\_\_\_\_

- Consome alimentos defumados ( ) sim ( ) não

Quantas vezes na semana? \_\_\_\_\_

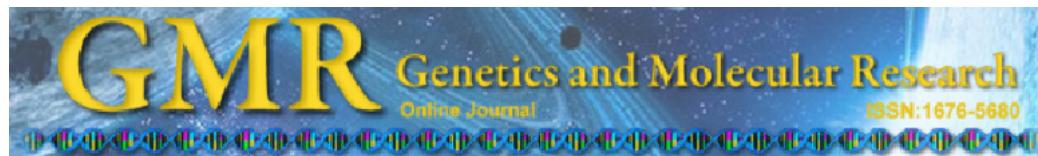
- Consome frutas? ( ) sim ( ) não

Quantas vezes na semana? \_\_\_\_\_

- Consome verduras? ( ) sim ( ) não

Quantas vezes na semana? \_\_\_\_\_

**APENDICE B** - Prediction of health risk due to polycyclic aromatic hydrocarbons present in urban air in Rio de Janeiro, Brazil



## Prediction of health risk due to polycyclic aromatic hydrocarbons present in urban air in Rio de Janeiro, Brazil

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**ABSTRACT.** Risk assessment can provide a comprehensive estimate of potential effects of contaminants under specific, well-defined, and well-described circumstances, providing quantitative relationships between exposure and effects to identify and to define areas of concern. We investigated the mutagenic activity of particulate matter in air samples collected from three sites in Rio de Janeiro city. Samples were collected using a high-volume sampler at Avenida Brasil, at Campus of Universidade do Estado do Rio de Janeiro, and at Rebouças Tunnel. Six polycyclic aromatic hydrocarbons were quantified by gas chromatography/mass spectrometry. *Salmonella typhimurium* TA98 and the derivative strains TA98/1.8-DNP<sub>6</sub>, YG1021, and YG1024,

commonly used in mutagenicity assays, were treated (10-50 µg/plate), with and without exogenous metabolism. The highest values for the polycyclic aromatic hydrocarbons were detected at Rebouças Tunnel. For chrysene, as an example, the concentration was nearly 200 times higher than that established by the US Environmental Protection Agency. Frequent traffic jams can place bus drivers who go through the Rebouças Tunnel at risk of exposure to up to 0.69 ng/m<sup>3</sup> benzo(a)pyrene. Independent of exogenous metabolism, mutagenicity was detected in strains YG1021 and YG1024 at all the sites, suggesting nitro and amino derivatives of polycyclic aromatic hydrocarbons. Rebouças Tunnel air samples gave the highest values for rev/µg and rev/m<sup>3</sup>. This could be due to the fact that the long, enclosed passageway through a mountain restricts ventilation. The cancer risk estimate in this study was 10<sup>-3</sup> for the benzo(a)pyrene, at the two sites, indicating a high risk.

**Key words:** Respirable particulate matter; PAHs; Risk assessment; *Salmonella*/microsome assay; Mutagenicity

## INTRODUCTION

The risk assessment process involves the characterization of toxicities and estimation of possible adverse outcomes from specific chemical exposures (Environment Canada, 1997). The US Environmental Protection Agency (USEPA Draft Cancer Risk Assessment Guidelines, 1996) defines risk characterization as the step in the risk assessment process that integrates hazard identification, dose-response assessment, and exposure assessment, using a combination of qualitative and quantitative information.

The World Health Organization (WHO, 2005) considers air pollution to be an environmental exposure situation that can affect human health, where it is implicated in acute respiratory infections, cancer, and chronic respiratory and cardiovascular diseases. Studies around the world have consistently demonstrated that particulate matter (PM) with an aerodynamic diameter <10 µm (PM10) and, more recently, <2.5 µm (PM2.5), poses a significant threat to human health (Vinitketkumnuen et al., 2002) as it can penetrate deep into alveolar sacs in the lungs. It has been suggested that fine particles from automotive emissions are responsible for a 3% rise in mortality rate for every 10 µg/m<sup>3</sup> increase, while fine coal combustion emissions account for only 1%, and fine crystal aerosols have no discernible effect (Laden et al., 2000).

Urban airborne PM is a complex variable mixture containing many different chemical species (USEPA, 1996; Cassoni et al., 2004). Ambient air genotoxins can originate from fuel combustion (motor vehicle exhausts, central heating, and power generation), waste incineration, and industrial processes, and are also formed by atmospheric reactions (Claxton and Woodall Jr., 2007; Umbuzeiro et al., 2008). Studies on organic extracts of urban PM have proven their genotoxicity (Vinitketkumnuen et al., 2002; Cassoni et al., 2004), revealing the risk it poses to exposed populations. Generally, the mutagenicity of airborne combustion particles is primarily attributed to polycyclic aromatic hydrocarbons (PAHs), but recent reviews have demonstrated that these compounds are not the most predominant class of mutagens in airborne particulate matter, although they significantly contribute to mutagenicity. A wide

range of aromatic compounds, such as nitroarenes, are found in ambient air and are present in emissions from direct sources or may be products of atmospheric reactions in the presence of NO<sub>2</sub> and NO<sub>3</sub> radicals (Coronas et al., 2009).

The objective of the present study was to make a risk assessment of the PM samples collected at three sites (Avenida Brasil, Rebouças Tunnel, and Campus of Universidade do Estado do Rio de Janeiro) in Rio de Janeiro between April and July 2010 for mutagenic activity using a *Salmonella*/microsome assay, as described by Kado et al. (1983).

## MATERIAL AND METHODS

### Sampling sites

The samples were collected at three sites in Rio de Janeiro: Avenida Brasil (site 1), Campus of Universidade do Estado do Rio de Janeiro (site 2), and Rebouças Tunnel (site 3) between April and July 2010. Site 1 has heavy traffic (~250,000 vehicles/day) and is the city's biggest highway, spanning 58 km in length and crossing 27 neighborhoods. Site 2, with little traffic, is located in a residential area in the city's north zone. Site 3 has heavy traffic (~190,000 vehicles/day). It connects the north and south zones of the city and is 2.8 km long.

### Sampling of airborne particulate matter and extraction of organic compounds

Airborne PM2.5 samples were collected on fiberglass filters (E558 X 10IN, 254 mm x 203 mm) using a high-volume collector (Energética Indústria e Comércio Ltda., AVG MP 2.5, 1.13 m<sup>3</sup>/min) for 24 h at Avenida Brasil and Universidade Federal do Rio de Janeiro, and 6 h in Rebouças Tunnel. Samples were collected each week from April to July 2010.

Half of each filter was extracted with dichloromethane (CASRN. 75-09-2, TediaBrazil, Brazil, purity 99.9%) at 40°C by sonicating for three rounds of 10 min each (Vargas et al., 1998). The extracts were concentrated to 15 mL in a rotary evaporator and filtered on a Teflon membrane (0.5 µm). The concentration of extractable organic matter (EOM, in µg/m<sup>3</sup>) was calculated. Prior to bioassays, the organic extract was dried at 4°C and resuspended in dimethyl sulfoxide (DMSO, CASRN. 67-68-5, Synth, Brazil, purity 99.9%) (Vargas et al., 1998).

### Analysis of PAHs

PAHs were quantified by gas chromatography/mass spectrometry (GC/MS). They were identified and quantified using a Varian system consisting of a GC (450-GC) with a split/splitless injector 1177S/SL (kept at 300°C) coupled to a mass spectrometer detector (MS 220). The ion trap (250°C), manifold (280°C), and transfer line (280°C) were maintained at constant temperatures. PAHs were identified by mass similarity and by the retention time of the components in a commercial standard kit (Supelco, PAH610-S).

Quantification was based on five calibration points, which were constructed from each standard for all the target analytes, ranging from 10 to 250 pg/µL. Injections (2.0 µL) were splitless, with the split opened after 0.5 min, and helium was used as the carrier gas. A VF-5MS column (30 m x 0.25 mm x 0.25 µm) was employed. The column and septum purge flows were set at 1.6 and 3 mL/min, respectively. The oven temperature program was as follows:

70°C for 4 min and 70-300°C at 10°C/min. These conditions were designed for the analysis of six PAHs: phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, and benzo(a)pyrene. The limits of quantification were determined from the minimum point in the calibration curves. Limits of detection were determined from PAH concentrations, which resulted in a signal-to-noise ratio of 3:1. The results were expressed in ng/m<sup>3</sup>.

### **Salmonella/microsome assay**

The organic extracts were assayed for mutagenicity using the microsuspension version (Kado et al., 1986) of the *Salmonella*/microsome assay (Maron and Ames, 1983). *Salmonella typhimurium* TA98 (frameshift strain) and the derivative strains YG1021 (nitroreductase-overproducing), TA98/1.8-DNP<sub>6</sub> (O-acetyltransferase deficient) and YG1024 (O-acetyltransferase-overproducing) were used with and without metabolic activation (S9 mix fraction). Five concentrations of each sample (10, 20, 30, 40, and 50 µg/plate) were tested in triplicate. The samples were pre-incubated for 90 min. All assays were carried out under yellow light along with a negative control (DMSO solvent, 5 µL/plate) and positive controls (4-nitroquinoline oxide, 0.5 µg/plate, CASRN. 56-57-5 and 2-aminofluorene, 1 µg/plate, CASRN. 153-78-6, Sigma Chemical Company, St. Louis, MO, USA). Plates were incubated in the dark at 37°C for 72 h, after which time revertants were counted. The assay response for mutagenicity was considered positive when the number of revertant colonies in the test was at least twice the number of spontaneous revertants; the responses were expressed in rev/µg and rev/m<sup>3</sup>. In the cytotoxicity test, the solution containing the sample and the bacterial culture (100-200 cells) were plated on nutrient agar plates and incubated at 37°C for 24 h, and the surviving colonies were counted (Vargas et al., 1998).

### **Statistical analysis**

The mutagenic response was considered positive when the number of revertant colonies in the test was at least twice the number of spontaneous revertants and no cytotoxicity was detected (survival rates > 60%). The significant responses were identified by statistical analysis (ANOVA, P ≤ 0.05) (Vargas et al., 1998).

### **Risk assessment**

The risk assessment was calculated for each detected PAH by potency equivalency factors (PEFs) as described in Collins et al. (1998).

## **RESULTS AND DISCUSSION**

### **Airborne PM**

The PM2.5 concentration and EOM of the samples in µg/m<sup>3</sup> are shown in Table 1. WHO (2005) has established guidelines for long-term and short-term PM2.5 concentrations: 10 µg/m<sup>3</sup> (annual mean) and 25 µg/m<sup>3</sup> (24-h mean). Most of our results were above the levels established by WHO (2005; Table 1). A previous study (Godoy et al., 2009) carried

out near site 2 also detected low concentrations of PM2.5 ( $18.10 \mu\text{g}/\text{m}^3$ ), probably due to the fact that this area has low traffic flows and is far from any industry. A previous study done by Instituto Estadual do Ambiente (2009) to monitor PM10 in the metropolitan area of Rio de Janeiro showed a high annual average of this pollutant ( $64 \mu\text{g}/\text{m}^3$ ). WHO (2005) has established a guideline for long-term PM10 concentrations of  $20 \mu\text{g}/\text{m}^3$  (annual mean). PM10 can only penetrate the upper respiratory tract, whereas PM2.5 can penetrate the lungs and cause various diseases (Claxton and Woodall Jr., 2007). It has been demonstrated that for each  $10 \mu\text{g}/\text{m}^3$  increase in PM concentration, the risk of mortality from cardiopulmonary diseases increases 6%, while the risk of mortality from lung cancer rises 8% (Iainistcki et al., 2009). Our results showed high concentrations of PM2.5 at sites 1 and 3. Most of the PM released into the atmosphere is from diesel-powered vehicles (Claxton and Woodall Jr., 2007). In Brazil, the fleet of gasoline-fueled vehicles rose by 8% between 1996 and 2000, while for the same period there was a 60% increase in the number of diesel vehicles (SISAET, Sistema de Informações do Anuário Estatístico dos Transportes, 2011). The composition of diesel and gasoline has been changed (Braun et al., 2003) in a bid to reduce their pollutant emissions. Nevertheless, the PM concentration was found to be high at sites 1 and 3 - Avenida Brasil and Rebouças Tunnel - where the traffic of diesel vehicles is heavy. The concentrations of PM2.5 detected at sites 1 and 3 are in agreement with other studies performed in urban areas: Santiago, Chile ( $33.00 \mu\text{g}/\text{m}^3$ ) (Seguel et al., 2009), Palermo, Italy ( $34.20 \mu\text{g}/\text{m}^3$ ) (Dongarrá et al., 2010), and Hong Kong ( $68.60 \mu\text{g}/\text{m}^3$ ) (Cheng et al., 2010).

**Table 1.** Collection sites, air volume, PM2.5 concentration, and extractable organic matter (EOM) of the samples analyzed.

Site	Month	Air volume ( $\text{m}^3$ ) $\pm$ SD	PM2.5 ( $\mu\text{g}/\text{m}^3$ ) $\pm$ SD	EOM ( $\mu\text{g}/\text{m}^3$ )
Avenida Brasil (1)	April	1545 $\pm$ 1	40 $\pm$ 12	7.76
	May	1521 $\pm$ 22	60 $\pm$ 22	34.51
	June	1523 $\pm$ 9	34 $\pm$ 21	32.99
	July	1518 $\pm$ 1	35 $\pm$ 8	4.93
UERJ (2)	April	1618 $\pm$ 87	14 $\pm$ 7	6.02
	May	1518 $\pm$ 65	21 $\pm$ 9	8.16
	June	1514 $\pm$ 63	35 $\pm$ 28	8.23
	July	1545 $\pm$ 1	36 $\pm$ 15	4.85
Rebouças Tunnel (3)	July	413 $\pm$ 1	83 $\pm$ 24	18.18

PM = particulate matter; UERJ = Universidade do Estado do Rio de Janeiro.

### Analysis of PAHs

At site 1 in April,  $0.07 \text{ ng}/\text{m}^3$  chrysene was detected. In May, the following PAHs were detected: phenanthrene ( $0.07 \text{ ng}/\text{m}^3$ ), fluoranthene ( $0.13 \text{ ng}/\text{m}^3$ ), pyrene ( $0.22 \text{ ng}/\text{m}^3$ ), benzo(a)anthracene ( $0.15 \text{ ng}/\text{m}^3$ ), chrysene ( $0.26 \text{ ng}/\text{m}^3$ ), and benzo(a)pyrene ( $0.74 \text{ ng}/\text{m}^3$ ). In June, none of the PAHs evaluated were detected. In July, the following were detected: pyrene ( $0.07 \text{ ng}/\text{m}^3$ ), benzo(a)anthracene ( $0.08 \text{ ng}/\text{m}^3$ ), chrysene ( $0.17 \text{ ng}/\text{m}^3$ ), and benzo(a)pyrene ( $0.19 \text{ ng}/\text{m}^3$ ). None of the PAHs under study was detected at site 2 during the study period.

At site 3, we detected phenanthrene ( $0.23 \text{ ng}/\text{m}^3$ ), fluoranthene ( $0.42 \text{ ng}/\text{m}^3$ ), pyrene ( $0.53 \text{ ng}/\text{m}^3$ ), benzo(a)anthracene ( $0.80 \text{ ng}/\text{m}^3$ ), chrysene ( $1.78 \text{ ng}/\text{m}^3$ ), and benzo(a)pyrene ( $1.65 \text{ ng}/\text{m}^3$ ).

All PAHs evaluated in the present study are considered a priority in environmental monitoring, but only benzo(a)pyrene is believed to be a human carcinogen (group 1). Chrysene and benzo(a)anthracene are considered to be possibly carcinogenic to humans (group 2B), and the other PAHs evaluated in these study are classified as non-carcinogenic to humans (group 3) (IARC, 2011). By the estimated unit of risk, the chronic dose of benzo(a)pyrene for cancer is 1.10 ng/m<sup>3</sup>, followed by 0.11 ng/m<sup>3</sup> for benzo(a)anthracene, and 0.01 ng/m<sup>3</sup> for chrysene (USEPA, 1996). Benzo(a)pyrene is the most carcinogenic, with the doses of benzo(a)anthracene and chrysene being calculated from benzo(a)pyrene. Benzo(a)pyrene has been identified in environmental studies as having the highest carcinogenic potential, with the capacity to form adducts and produce base substitutions and frameshifts (Fahl et al., 1981) in the DNA chain. Our study detected concentrations above the recommended levels estimated by USEPA, for chrysene (at site 1 - April, May, and July, and at site 3 - July), benzo(a)anthracene (at site 1 - May and at site 3 - July), and benzo(a)pyrene (at site 3 - July). The urban atmosphere in Rio de Janeiro is influenced by several factors, such as the uneven topography, the irregular occupation of space, the presence of open sea, and Guanabara Bay, which result in a complex regime for winds and irregular distribution and dispersion of pollution (Azevedo et al., 1999). A previous study performed from December 1998 to March 1999, close to site 1, reported similar values for benzo(a)pyrene (0.57-0.75 ng/m<sup>3</sup>) (Fernandes et al., 2002). These results indicate that this pollutant is present during different periods of the year at this site. In another study in 1999, at site 3, 0.58 ng/m<sup>3</sup> benzo(a)pyrene was determined, while we detected 1.65 ng/m<sup>3</sup>. This difference may be related to changes in the flow of vehicles, from about 7200 vehicles/day back then (Azevedo et al., 1999) to the present day level of 190,000 vehicles/day (CET-Rio - Companhia de Engenharia de Tráfego do Rio de Janeiro, 2011).

The existence of phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, and benzo(a)pyrene at sites 1 and 3 can be attributed to the heavy traffic and the absence of dispersion factors such as rainfall (especially at site 3). Moreover, the results found for sites 1 and 3 are in agreement with other studies performed in urban areas: Hong Kong (Zheng and Fang, 2000), Santiago (Kavouras et al., 1999) and São Paulo (Bourretta et al., 2005).

### ***Salmonella/microsome assay***

Tables 2 and 3 show the mutagenicity data for the organic extracts from airborne PM in rev/µg and rev/m<sup>3</sup>, respectively.

At site 1, positive responses were observed for TA98 both in the absence (April and May) and in the presence (July) of metabolic activation. A positive response was observed for TA98/1.8-DNP<sub>6</sub> in July in the absence and in the presence of S9 mix. For YG1021, a positive response was observed in April, also in the absence and presence of S9 mix, while a positive response was observed in May, June, and July only in the presence of S9 mix. For YG1024, a positive response was observed in April in the presence of S9 mix, in May in the absence of S9 mix, and in July in the absence and presence of S9 mix.

At site 2, positive responses were observed for TA98 in the presence of metabolic activation in May, and in the absence and presence of metabolic activation in July. For TA98/1.8-DNP<sub>6</sub>, a positive response was observed in the absence of metabolic activation in June and July. For YG1021 a positive response was observed in the presence of S9 mix in June and July. For YG1024, a positive response was observed in April in the absence of S9 mix and in July in the absence and presence of S9 mix.

**Table 2.** Mutagenicity of airborne particulate matter organic extracts in rev/ $\mu$ g.

Site	Month	TA98		TA98/1.8-DNP <sub>6</sub>		YG1021		YG1024	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1	April	2.39 ± 0.05	nd	nd	nd	1.30 ± 0.35	1.18 ± 0.14	nd	3.35 ± 1.48
	May	4.48 ± 1.02	nd	nd	nd	nd	1.86	1.40 ± 0.20	nd
	June	nd	nd	nd	nd	nd	1.77 ± 0.25	nd	nd
	July	nd	4.53 ± 0.14	6.50 ± 0.57	1.53 ± 0.29	nd	3.53 0.08	5.60 ± 1.69	4.73 ± 0.39
2	April	nd	nd	nd	nd	nd	nd	0.90 ± 0.14	nd
	May	nd	2.51 ± 1.40	nd	nd	nd	nd	nd	nd
	June	nd	nd	1.61 ± 0.05	nd	nd	2.55 ± 0.40	nd	nd
	July	2.85	3.28 ± 0.35	2.51 ± 0.84	nd	nd	2.05 ± 0.25	3.40 ± 0.53	1.59 ± 0.51
3	July	3.43 ± 0.67	1.80 ± 0.13	1.93 ± 0.63	nd	3.80 ± 0.57	1.95 ± 0.07	7.50 ± 4.38	5.47 ± 0.29

nd = not detected. Negative control = DMSO for the mutagenicity assay without S9 mix: TA98 (35 ± 5); TA98/1.8-DNP<sub>6</sub> (14 ± 2); YG1021 (36 ± 12); YG1024 (21 ± 3). For the mutagenicity assay with S9 mix: TA98 (59 ± 10); TA98/1.8-DNP<sub>6</sub> (22 ± 2); YG1021 (24 ± 8); YG1024 (44 ± 2). Positive controls for the mutagenicity assay without S9 mix: 0.5  $\mu$ g/plate 4-nitroquinoline oxide for TA98 (948 ± 56); TA98/1.8-DNP<sub>6</sub> (1214 ± 119); YG1021 (953 ± 62); YG1024 (1154 ± 97). For the mutagenicity assay with S9 mix: 1  $\mu$ g/plate 2-aminofluorene for TA98 (184 ± 15); TA98/1.8-DNP<sub>6</sub> (246 ± 47); YG1021 (1216 ± 104); YG1024 (235 ± 70).

At site 3, positive responses were observed for TA98 in the absence and presence of metabolic activation. For TA98/1.8-DNP<sub>6</sub>, a positive response was observed only in the absence of S9 mix. For YG1021 and YG1024, positive responses were observed in the absence and presence of metabolic activation (Table 3).

The mutagenicity observed in the presence of metabolic activation may be associated with the presence of promutagens, such as PAHs. Benzo(a)pyrene was present in the samples from site 1 (May and July) and site 3 (July). The positive response for TA98 in the presence of S9 mix might have been related to this PAH at sites 1 and 3 in July. It is known that benzo(a)pyrene can induce frameshift mutations in DNA at a concentration of 0.5  $\mu$ g/plate for TA98 in the presence of metabolic activation (Aouadene et al., 2008). In our results, we detected benzo(a)pyrene at site 1 (0.05  $\mu$ g/plate) in July, and at site 3 (0.61  $\mu$ g/plate) also in July. Although we detected the presence of benzo(a)pyrene in May at site 1, no mutagenic response related to this PAH was observed.

The positive response observed at the three sites for the O-acetyltransferase strains (TA98/1.8-DNP<sub>6</sub> and YG1024) suggests the presence of amino compounds. The positive response observed at the three sites for the nitroreductase-overproducing strain (YG1021) could be related to the presence of nitro derivatives of PAHs.

At sites 1 and 2 (outside), the highest values for rev/ $\mu$ g and rev/m<sup>3</sup> for different strains (Tables 3 and 4, respectively) were observed in July (winter). In Rio de Janeiro, there is normally little rainfall in winter. The lack of rain in this season favors the accumulation of these pollutants in the atmosphere. A previous study evaluating the mutagenicity of PM10 near site 2 in the winter of 1984 showed a positive response for TA98 in the presence of S9 mix (2.60 rev/ $\mu$ g and 5.98 rev/m<sup>3</sup>) (Miguel et al., 1990). At this site in the same season, we found higher values for TA98 in the presence of S9 mix (3.28 rev/ $\mu$ g and 15.98 rev/m<sup>3</sup>) (Tables 2 and 3, respectively). These data indicate that the PM2.5 particles contained more PAHs/ $\mu$ g than did the PM10 particles (Claxton and Woodall Jr., 2007).

Site 3 (inside) exhibited the highest rev/ $\mu$ g and rev/m<sup>3</sup> values of the three sites. This finding might have been related to the fact that the long enclosed tunnel running through the mountain has limited ventilation and high traffic volume.

**Table 3.** Mutagenicity of airborne particulate matter organic extracts in rev/m<sup>3</sup>.

Site	Month	TA98		TA98/1.8-DNP <sub>6</sub>		YG1021		YG1024	
		-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
1	April	18.50 ± 0.38	nd	nd	nd	10.08 ± 2.74	9.15 ± 1.09	nd	26.00 ± 11.52
	May	154.60 ± 35.14	nd	nd	nd	nd	64.18	48.50 ± 6.36	nd
	June	nd	nd	nd	nd	nd	58.49 ± 8.25	nd	nd
	July	nd	22.32 ± 0.70	32.04 ± 2.78	7.52 ± 1.42	nd	17.41 ± 0.37	27.60 ± 8.36	23.29 ± 1.92
2	April	nd	nd	nd	nd	nd	nd	5.41 ± 0.85	nd
	May	nd	20.48 ± 11.42	nd	nd	nd	nd	nd	nd
	June	nd	nd	13.24 ± 0.45	nd	nd	19.33 ± 2.32	nd	nd
	July	13.82	15.90 ± 1.71	12.20 ± 4.07	nd	nd	9.94 ± 1.20	16.51 ± 2.61	7.73 ± 2.50
3	July	62.41 ± 12.10	32.72 ± 2.40	35.14 ± 11.43	nd	69.08 ± 10.28	35.45 ± 1.28	136.34 ± 79.70	99.50 ± 5.22

nd = not detected. Negative control = DMSO for the mutagenicity assay without S9 mix: TA98 (35 ± 5); TA98/1.8-DNP<sub>6</sub> (14 ± 2); YG1021 (36 ± 12); YG1024 (21 ± 3). For the mutagenicity assay with S9 mix: TA98 (59 ± 10); TA98/1.8-DNP<sub>6</sub> (22 ± 2); YG1021 (24 ± 8); YG1024 (44 ± 2). Positive controls for the mutagenicity assay without S9 mix: 0.5 µg/plate 4-nitroquinoline oxide for TA98 (948 ± 56); TA98/1.8-DNP<sub>6</sub> (1214 ± 119); YG1021 (953 ± 62); YG1024 (1154 ± 97). For the mutagenicity assay with S9 mix: 1 µg/plate 2-aminofluorene for TA98 (184 ± 15); TA98/1.8-DNP<sub>6</sub> (246 ± 47); YG1021 (1216 ± 104); YG1024 (235 ± 70).

**Table 4.** Estimating risk assessment for each polycyclic aromatic hydrocarbon (PAH) in relation to the benzo(a)pyrene in Avenida Brasil and Rebouças Tunnel.

Sites	PAHs	PEF	Individual cancer risk
Avenida Brasil (1)			
April	Chrysene	0.01	7.7 10 <sup>-7</sup>
May	Phenanthrene	0.001	7.7 10 <sup>-8</sup>
	Fluoranthene	0.001	1.4 10 <sup>-7</sup>
	Pyrene	0.001	2.4 10 <sup>-7</sup>
	Benzo(a)anthracene	0.1	1.7 10 <sup>-5</sup>
	Chrysene	0.01	2.9 10 <sup>-6</sup>
	Benzo(a)pyrene	1	0.8 10 <sup>-3</sup>
July	Pyrene	0.001	7.7 10 <sup>-8</sup>
	Benzo(a)anthracene	0.1	8.8 10 <sup>-6</sup>
	Chrysene	0.01	1.9 10 <sup>-6</sup>
	Benzo(a)pyrene	1	0.2 10 <sup>-3</sup>
Rebouças Tunnel (3)	Phenanthrene	0.001	2.5 10 <sup>-7</sup>
	Fluoranthene	0.001	4.6 10 <sup>-7</sup>
	Pyrene	0.001	5.8 10 <sup>-7</sup>
	Benzo(a)anthracene	0.1	8.8 10 <sup>-5</sup>
	Chrysene	0.01	1.9 10 <sup>-5</sup>
	Benzo(a)pyrene	1	1.8 10 <sup>-3</sup>

The detailed derivation of each potency equivalency factors (PEF) can be found in OEHHA (1994).

## Risk assessment

Table 4 shows the risk assessment. In the estimate of individual risk of cancer, values on the order of 10<sup>-8</sup> to 10<sup>-3</sup> were detected at site 1 in April, May and July. At site 3, observed values were on the order of 10<sup>-7</sup> to 10<sup>-3</sup> for the PAHs listed in Table 4.

The evaluation of health effects and quantitative risk could be carried out for every PAH, where estimating the risk of cancer using PEFs is usually measured relative to benzo(a)pyrene, since it is the only PAH for which a complete quantitative risk assessment has been done (Collins et al., 1991; OEHHA, 1994). The use of PEFs for estimating risk from exposure to PAHs is an improvement for those PAHs for which there are reliable collection and measurement techniques. However, there are a large number of PAHs for which PEFs have not yet

been determined and/or for which measurement techniques are unavailable. PEFs are primarily based on chronic internal dosing experiments and skin-painting studies (Collins et al., 1998). Explanations of the derivation of each PEF, the type of data used for the derivation, and the relevant references were presented in a technical report that underwent public and scientific peer review (OEHHA, 1994); the derivations are detailed in that report (Collins et al., 1998).

In this study, the highest risks were detected for benzo(a)anthracene and for benzo(a)pyrene in May at site 1, and for benzo(a)anthracene, chrysene and benzo(a)pyrene at site 3. According to Collins et al. (1998), a risk of about  $10^{-5}$  could lead to notification of the public. These data reflect the risk to which people using these routes are exposed daily. In general, bus drivers who work at sites 1 and 3 spend, respectively, 4 h and 10 min daily on these roads. Considering these data and the PAH concentrations detected, we can extrapolate how much these workers are at risk of PAH exposure. Traffic jams are frequent at site 3, lasting up to 2 h (<http://www.jb.com.br/rio/noticias>) and subjecting bus drivers to a risk of exposure of up to  $0.69 \text{ ng/m}^3$  benzo(a)pyrene. This concentration is above the USEPA limit, and several studies have demonstrated an increase in DNA adducts at this level (Topinka et al., 1997; Lewtas et al., 1997; Kyrtopoulos et al., 2001).

In conclusion, nitro and amino derivatives of PAHs contributed to the mutagenicity detected for PM2.5. Furthermore, the population that uses routes 1 and 3, especially the bus drivers, are more exposed to cancer risk. This study reinforces the importance of using cleaner fuels and having better indoor ventilation. These measures could result in a reduction in diseases related to air pollution caused by PM2.5, and a consequent improvement in quality of life.

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## REFERENCES

- Aouadene A, Di Giorgio C, Sarrazin L, Moreau X, et al. (2008). Evaluation of the genotoxicity of river sediments from industrialized and unaffected areas using a battery of short-term bioassays. *Environ. Mol. Mutagen.* 49: 283-299.
- Azevedo DA, Moreira LS and Siqueira DS (1999). Composition of extractable organic matter in aerosols form urban areas of Rio de Janeiro city, Brazil. *Atmos. Environ.* 33: 4987-5001.
- Bourouteia C, Fortic MC, Taniguchid S, Bícego MC, et al. (2005). A wintertime study of PAHs in fine and coarse aerosols in São Paulo city, Brazil. *Atmos. Environ.* 39: 3799-3811.
- Braun S, Appel L and Schmal M (2003). Pollution generated by internal combustion engine powered diesel- The question of particulate. Current strategies for the reduction and control of emissions and future trends. *New Chemistry* 27: 472-482.
- Cassoni F, Bocchi C, Martino A, Pinto G, et al. (2004). The *Salmonella* mutagenicity of urban airborne particulate matter (PM<sub>2.5</sub>) from eight sites of the Emilia-Romagna regional monitoring network (Italy). *Sci. Total Environ.* 324: 79-90.
- CET-RIO (Companhia de Engenharia de Tráfego do Rio de Janeiro) (2011). Available at [<http://www.rio.rj.gov.br/web/cetrio>]. Accessed December 4, 2011.
- Cheng Y, Lee SC, Ho KF, Chow JC, et al. (2010). Chemically-specified on-road PM(2.5) motor vehicle emission factors in Hong Kong. *Sci. Total Environ.* 408: 1621-1627.
- Claxton LD and Woodall GM Jr (2007). A review of the mutagenicity and rodent carcinogenicity of ambient air. *Mutat. Res.* 636: 36-94.

- Collins JF, Brown JP, Dawson SV and Marty MA (1991). Risk assessment for benzo[a]pyrene. *Regul. Toxicol. Pharmacol.* 13: 170-184.
- Collins JF, Brown JP, Alexeeff GV and Salmon AG (1998). Potency equivalency factors for some polycyclic aromatic hydrocarbons and polycyclic aromatic hydrocarbon derivatives. *Regul. Toxicol. Pharmacol.* 28: 45-54.
- Coronas MV, Pereira TS, Rocha JA, Lemos AT, et al. (2009). Genetic biomonitoring of an urban population exposed to mutagenic airborne pollutants. *Environ. Int.* 35: 1023-1029.
- Dongarrá G, Manno E, Varrica D, Lombardo M, et al. (2010). Study on ambient concentrations of PM10, PM10-2.5, PM2.5 and gaseous pollutants. Trace elements and chemical speciation of atmospheric particulates. *Atmos. Environ.* 44: 5244-5257.
- Environment Canada (1997). Environmental Assessments of Priority Substances Under the Canadian Environmental Protection Act, EPS/2/CC/3E. Chemical Evaluation Division, Commercial Chemicals Evaluation Branch, Environment Canada, Government of Canada, Ottawa.
- Fahl WE, Scarpelli DG and Gill K (1981). Relationship between benzo(a)pyrene-induced DNA base modification and frequency of reverse mutations in mutant strains of *Salmonella typhimurium*. *Cancer Res.* 41: 3400-3406.
- Fernandes MB, Brickus LS, Moreira JC and Cardoso JN (2002). Atmospheric BTX and polyaromatic hydrocarbons in Rio de Janeiro, Brazil. *Chemosphere* 47: 417-425.
- Godoy MLDP, Godoy JM, Luiz Alfredo R, Soluri DS, et al. (2009). Coarse and fine aerosol source apportionment in Rio de Janeiro, Brazil. *Atmos. Environ.* 43: 2366-2374.
- Ianistck M, Dallarosa J, Sauer C, Teixeira CE, et al. (2009). Genotoxic effect of polycyclic aromatic hydrocarbons in the metropolitan area of Porto Alegre, Brazil, evaluated by *Helix aspersa* (Muller, 1774). *Environ. Pollut.* 157: 2037-2042.
- IARC (International Agency for Research on Cancer) (2011). Agents Classified by the IARC Monographs. Volumes 1-102. Available at [<http://monographs.iarc.fr/ENG/Classification/ClassificationsGroupOrder.pdf>]. Accessed September 27, 2011.
- INEA (Instituto Estadual do Ambiente) (2009). Relatório Anual da Qualidade do Ar do Estado do Rio de Janeiro. Available at [[http://www.inea.rj.gov.br/downloads/relatorios/qualidade\\_ar\\_2009.pdf](http://www.inea.rj.gov.br/downloads/relatorios/qualidade_ar_2009.pdf)]. Accessed September 27, 2011.
- Jornal do Brasil (2012). Túnel Rebouças parado; tempo de travessia é de 2h30. Available at [<http://www.jb.com.br/rio/noticias/2010/03/06/tunel-reboucas-parado-tempo-de-travessia-e-de-2h30/>]. Accessed January 4, 2012.
- Kado NY, Langley D and Eisenstadt E (1983). A simple modification of the *Salmonella* liquid-incubation assay. Increased sensitivity for detecting mutagens in human urine. *Mutat. Res.* 121: 25-32.
- Kado NY, Guirguis GN, Flessel CP, Chan RC, et al. (1986). Mutagenicity of fine (less than 2.5 microns) airborne particles: diurnal variation in community air determined by a *Salmonella* micro preincubation (microsuspension) procedure. *Environ. Mutagen.* 8: 53-66.
- Kavouras IG, Lawrence JL, Koutrakis P, Stephanou EG, et al. (1999). Measurement of particulate aliphatic and polynuclear aromatic hydrocarbons in Santiago de Chile: source reconciliation and evaluation of sampling artifacts. *Atmos. Environ.* 33: 4977-4986.
- Kyrtopoulos SA, Georgiadis P, Autrup H, Demopoulos NA, et al. (2001). Biomarkers of genotoxicity of urban air pollution. Overview and descriptive data from a molecular epidemiology study on populations exposed to moderate-to-low levels of polycyclic aromatic hydrocarbons: the AULIS project. *Mutat. Res.* 496: 207-228.
- Laden F, Neas LM, Dockery DW and Schwartz J (2000). Association of fine particulate matter from different sources with daily mortality in six U.S. cities. *Environ. Health Perspect.* 108: 941-947.
- Lewtas J, Walsh D, Williams R and Dobias L (1997). Air pollution exposure-DNA adduct dosimetry in humans and rodents: evidence for non-linearity at high doses. *Mutat. Res.* 378: 51-63.
- Maron DM and Ames BN (1983). Revised method for *Salmonella* mutagenicity test. *Mutat. Res.* 113: 175-215.
- Miguel AG, Daisey JM and Sousa JA (1990). Comparative study of the mutagenic and genotoxic activity associated with inhalable particulate matter in Rio de Janeiro air. *Environ. Mol. Mutagen.* 15: 36-43.
- OEHHA (Office of Environmental Health Hazard Assessment) (1994). Part B. Health Assessment. In: Benzo[a]pyrene as a Toxic Air Contaminant. Air Resources Board and Office of Environmental Health Hazard Assessment. California Environmental Protection Agency (Cal/EPA), Sacramento.
- Seguel AR, Morales SRIGE and Leiva GMA (2009). Estimations of primary and secondary organic carbon formation in PM2.5 aerosols of Santiago City, Chile. *Atmos. Environ.* 43: 2125-2131.
- SISAET (Sistema de Informações do Anuário Estatístico dos Transportes). (2011). Capítulo 5 - Transporte Rodoviário Available at [<http://www.geipot.gov.br/NovaWeb/IndexAnuario.htm>]. Accessed August 28, 2011.
- Topinka J, Binkova B, Mrackova G, Stavkova Z, et al. (1997). DNA adducts in human placenta as related to air pollution and to GSTM1 genotype. *Mutat. Res.* 390: 59-68.
- Umbuzeiro GA, Franco A, Martins MH, Kummrow F, et al. (2008). Mutagenicity and DNA adduct formation of PAH, nitro-PAH, and oxy-PAH fractions of atmospheric particulate matter from São Paulo, Brazil. *Mutat. Res.* 652: 72-80.

- USEPA (US Environmental Protection Agency) (1996). Proposed Guidelines for Carcinogen Risk Assessment, EPA/600/P-92/003C. Office of Research and Development. U.S. Environmental Protection Agency, Washington.
- Vargas VMF, Horn RC, Guidobono RR, Mittelstaed AB, et al. (1998). Mutagenic activity of airborne particulate matter from the urban area of Porto Alegre, Brazil. *Gen. Mol. Biol.* 21: 247-253.
- Vinitketkumnuen U, Kalayanamitra K, Chewonarin T and Kamens R (2002). Particulate matter, PM10 & PM2.5 levels, and airborne mutagenicity in Chiang Mai, Thailand. *Mutat. Res.* 519: 121-131.
- WHO (World Health Organization) (2005). Air Quality Guidelines: Global Update 2005. Particulate Matter, Ozone, Nitrogen Dioxide and Sulfur Dioxide. WHO Regional Office for Europe, Copenhagen.
- Zheng M and Fang M (2000). Particle-associated polycyclic aromatic hydrocarbons in the atmosphere of Hong Kong. *Water Air Soil Pollution* 117: 175-189.

*Research Article*

# **Genotoxicity of Polycyclic Aromatic Hydrocarbons and Nitro-Derived in Respirable Airborne Particulate Matter Collected from Urban Areas of Rio de Janeiro (Brazil)**

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Air pollution toxic effects are mainly attributed to small inhalable particulates with an aerodynamic diameter of less than  $2.5\text{ }\mu\text{m}$  (PM 2.5). Our objective was to investigate mutagenic and clastogenic activity in PM samples collected in Rio de Janeiro. Samples were collected using a high-volume sampler at three sites: with low traffic and (2) and (3) with a heavy traffic. Six polycyclic aromatic hydrocarbons (PAHs) were quantified by gas chromatography/mass spectrometry (GC/MS). *Salmonella typhimurium* TA98 and the derivative strains YG1021 and YG1024 were used in mutagenicity assays in the presence of organic extracts (10–50  $\mu\text{g}/\text{plate}$ ) with and without exogenous metabolism. *Allium cepa* test was performed to evaluate possible cytotoxic and clastogenic activities. The highest PM 2.5  $\mu\text{m}$  ( $132.73\text{ }\mu\text{m}/\text{m}^3$ ) and PAH values ( $1.22\text{ ng}/\text{m}^3$  for benzo(a)pyrene) were detected at site 3. High mutagenic frameshift responses in absence and presence of metabolic activation were detected at site 3. The participation of nitroarenes and dinitroarenes was detected in the total mutagenicity of the extracts studied. The cytotoxic effect and the abnormalities detected by *Allium cepa* test can be attributed to the PAH nitroderivatives in the organic extracts. Evaluation of the genotoxicity of urban airborne particulate matter is important as a basis for decision making by regulatory authorities.

## **1. Introduction**

Epidemiological studies have demonstrated that exposure to urban particulate matter (PM) is associated with several adverse health effects. Long-term exposure to high concentrations of PM increases the risk of lung cancer, respiratory diseases, and arteriosclerosis, whereas short-term exposure peaks can cause exacerbation of several forms of respiratory diseases, including bronchitis and asthma, as well as changes in heart rate variability [1, 2]. Studies conducted in Rio de

Janeiro city showed an association between PM exposure and mortality from lung cancer [3, 4], reduced respiratory capacity amongst children [5], and increased pediatric visits to treat symptoms of bronchial obstruction [6].

The toxic effects of PM are mainly attributed to small inhalable particulates with an aerodynamic diameter of less than  $2.5\text{ }\mu\text{m}$  (PM 2.5). Because of their large specific surface, these particulates can adsorb various organic substances, such as polycyclic aromatic hydrocarbons (PAHs), nitroaromatic hydrocarbons (nitro-PAHs), and oxygenated

aromatic hydrocarbons (oxy-PAHs) [7]. According to [8], the mutagenicity of airborne particulate can result from at least 500 identified compounds from various chemical classes. Mutagenic nonsubstituted PAHs are the most studied and well-known compounds; however, more recently, nitro- and oxy-PAHs have been shown to be very important because of their strong biological activity [9–11].

The *Salmonella/microsome* microsuspension assay has been used for large, multisite, and/or time-series studies, for bioassay-directed fractionation studies, for identifying the presence of specific classes of mutagens, and for site or source-comparisons of relative levels of airborne mutagens [8]. The use of strains of *Salmonella typhimurium* with different metabolic capacities can indicate the class or classes of compounds present in an environmental sample [11–13].

Higher plants are recognized as excellent genetic models to detect environmental mutagens and are frequently used in monitoring studies. Among the plant species, *Allium cepa* has been used to evaluate DNA damages, such as chromosome aberrations and disturbances in the mitotic cycle. Employing the *Allium cepa* as a test system to detect mutagens dates back to the 30s. It has been used to this day to assess a great number of chemical agents, which contributes to its increasing application in environmental monitoring. It is easily handled and has advantages over other short-term tests that require previous preparations of tested samples, as well as the addition of exogenous metabolic system [14].

The objective of the present work was to investigate mutagenic and clastogenic activities in the PM samples collected at three sites in Rio de Janeiro.

## 2. Material and Methods

**2.1. Sampling Sites.** The samples were collected at three sites in Rio de Janeiro: the campus of the Rio de Janeiro State University (site 1), Avenida Brasil (site 2), and Rebouças tunnel (site 3) between August and October 2010 (winter and spring seasons). Site 1, with low traffic, is located in a residential area of the city's north zone. Site 2 has heavy traffic (~250,000 vehicles/day) and is the city's biggest highway, covering 58 km in length and crossing 27 neighborhoods. Site 3 has heavy traffic (~190 000 vehicles/day). It connects the north and south zones of the city and is 2.8 km long [15].

Airborne PM 2.5 samples were collected on fiberglass filters (E558 X 10IN, 254 mm × 203 mm) using a high-volume collector (AVG MP 2.5, 1.13 m<sup>3</sup>/min) for 24 h for site 1 and site 2 and 6 h for site 3. Four monthly samplings were performed for each site of study. The filters were weighed and stabilized before and after sampling (45% humidity) for the determination of particulate concentration, expressed in µg/m<sup>3</sup> units of sampled air [16–18]. At the end of the sampling, the filters were combined to form a pool sample.

**2.2. Extraction of Organic Compounds.** Half of each filter was sonicated in three rounds of 10 min each using dichloromethane (DCM, CASRN. 75-09-2, TediaBrazil, Brazil, purity 99.9%). The extracts were concentrated to 15 mL in a rotating evaporator and filtered in a Teflon

membrane (0.5 µm). The concentration of extractable organic matter (EOM, in µg/m<sup>3</sup>) was calculated. Prior to bioassays, the organic extract was dried at 4°C and resuspended in 5 µL dimethyl sulfoxide (DMSO, CASRN. 67-68-5, Synth, Brazil, purity 99.9%) [16–18].

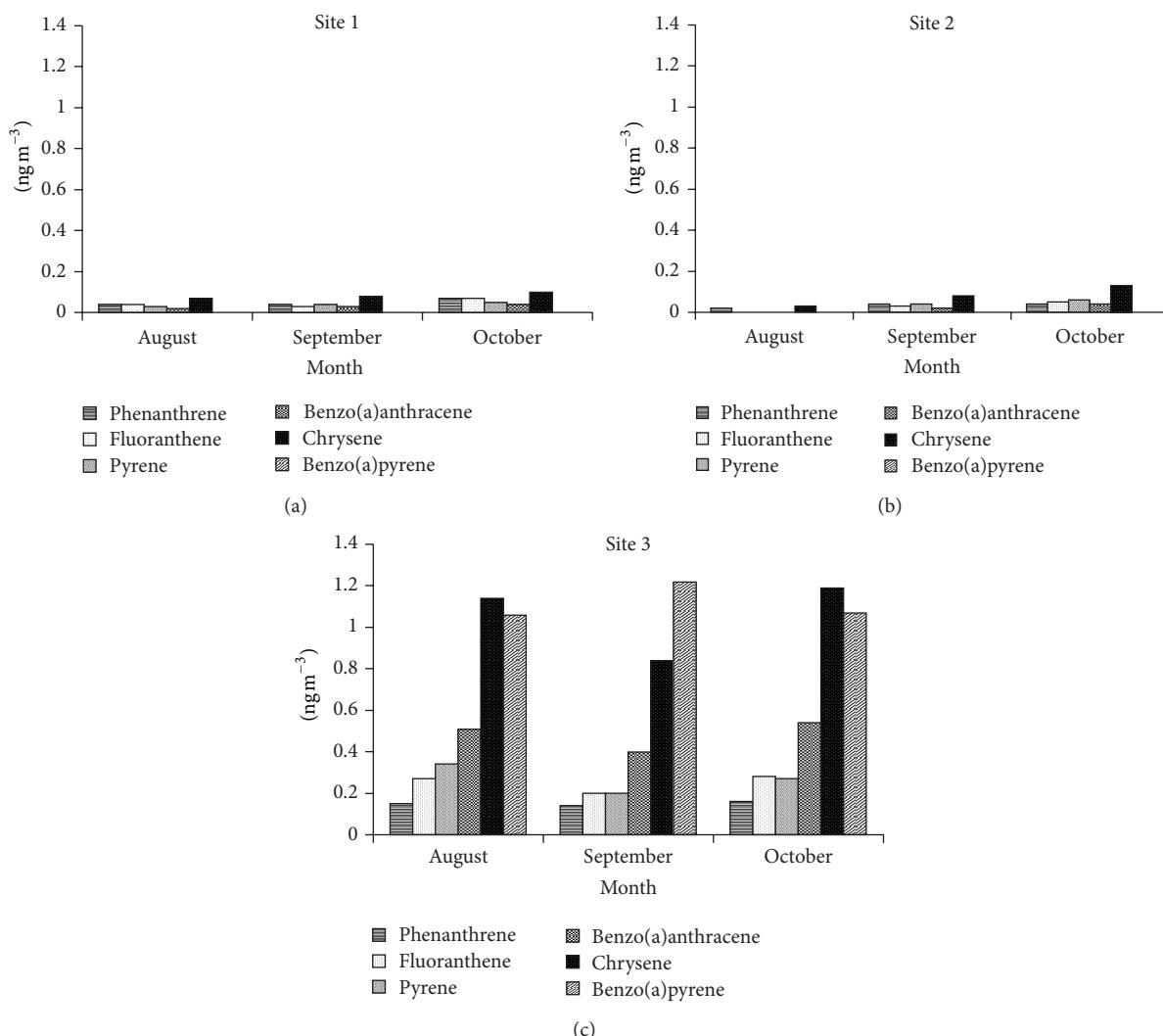
**2.3. Analysis of Polycyclic Aromatic Hydrocarbons (PAHs).** PAHs were identified and quantified by gas chromatography/mass spectrometry (GC/MS), using a Varian system consisting of a gas chromatograph (450-GC) with a split/splitless injector 1177S/SL (kept at 300°C) coupled to the mass spectrometer detector (MS 220). The ion trap (250°C), manifold (280°C), and transfer line (280°C) were maintained at constant temperatures. PAHs were identified by mass similarity and by the retention time of the components in a commercial standard kit (Supelco, PAH610-S). Quantification was based on five calibration points, which were constructed from each standard for all the target analytes, ranging from 10 to 250 pg/µL. Injections (2.0 µL) were splitless, with the split opened after 0.5 min, and helium 5.0 was used as the carrier gas. A VF-5MS column (30 m × 0.25 mm × 0.25 µm) was employed. The column and septum purge flows were set at 1.6 and 3 mL/min, respectively. The oven temperature program was as follows: 70°C for 4 min then heating to 300°C at 10°C/min. This directive was designed for the analysis of the sixteen main priority PAHs, but only six were detected: phenanthrene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, and benzo(a)pyrene. The limits of quantification were determined from the minimum point in the calibration curves. Limits of detection were determined from PAH concentrations, which resulted in a signal-to-noise ratio of 3:1. The results were expressed in ng/m<sup>3</sup> [19].

**2.4. Salmonella/Microsome Assay.** The organic extracts were assayed for mutagenicity using the microsuspension version [20] of the *Salmonella/microsome* assay [21]. *Salmonella typhimurium* TA98 (frameshift strain) and the derivative strains YG1021 (nitroreductase overproducing) and YG1024 (O-acetyltransferase overproducing) [22] were used, with and without metabolic activation (S9 mix fraction). Five concentrations of each sample (10, 20, 30, 40, and 50 µg/plate) were tested in triplicate. The samples were preincubated for 90 min. All assays were carried out under yellow light and in the presence of negative (dimethyl sulfoxide-DMSO solvent, 5 µL/plate) and positive (4-nitroquinoline oxide-4NQO, 0.5 µg/plate, CASRN. 56-57-5 and 2-aminofluorene-2AF, 1 µg/plate, CASRN. 153-78-6 from Sigma Chemical Company, St. Louis, MO, USA) controls. Plates were incubated in the dark at 37°C for 72 h; after which time, revertants were counted. The sample was considered positive when a mutagenesis value of at least twice the negative value, a significant ANOVA ( $P < 0.05$ ), and a positive dose-response rate ( $P < 0.05$ ) were observed. The results of the different assays were analyzed via the SALANAL program (Salmonella Assay Analysis, version 1.0, Integrated Laboratory Systems of Research Triangle Institute, RTP, North Carolina, USA). The choice between linear regression and the Bernstein model [23] was made to allow the elimination of data for doses

TABLE 1: Collection sites, air volume, PM 2.5  $\mu\text{m}$  concentration, and extractable organic matter (EOM) of the samples analyzed.

Site	Month	Air volume ( $\text{m}^3$ ) $\pm$ S.D.	PM 2.5 $\mu\text{m}$ individual values ( $\mu\text{g}/\text{m}^3$ )	PM 2.5 $\mu\text{m}$ ( $\mu\text{g}/\text{m}^3$ ) $\pm$ S.D.	EOM ( $\mu\text{g}/\text{m}^3$ )
1	August	1513 $\pm$ 36	17.51/18.63/13.63/45.09	23.71 $\pm$ 14.41	9.66
	September	1582 $\pm$ 53	23.43/43.83/40.24/14.48	30.49 $\pm$ 13.89	7.92
	October	1623 $\pm$ 148	14.46/13.61/21.73/11.27	15.27 $\pm$ 4.51	5.54
2	August	1541 $\pm$ 1	40.19/44.59/44.01/51.77	45.14 $\pm$ 4.83	7.76
	September	1554 $\pm$ 31	13.76/34.66/41.58/15.20	26.30 $\pm$ 13.94	7.24
	October	1640 $\pm$ 194	22.48/36.47/11.74/25.08	23.94 $\pm$ 10.15	5.48
3	August	430 $\pm$ 67	42.38/168.85/35.60/131.33	94.54 $\pm$ 66.00	20.93
	September	420 $\pm$ 40	68.85/167.33/91.55/61.01	97.18 $\pm$ 48.52	21.44
	October	359 $\pm$ 6	77.15/239.77/75.68/138.33	132.73 $\pm$ 77.09	25.05

1: UERJ; 2: Avenida Brasil; 3: Rebouças tunnel; S.D.: standard deviation; airborne PM 2.5  $\mu\text{m}$  samples were collected for 24 h at sites 1 and 2 and 6 h at site 3. Six-h time filter saturation at site 3.

FIGURE 1: (a)–(c) PAH concentrations in  $\text{ng}/\text{m}^3$  of the three sites. Benzo(a)pyrene not detected at sites 1 and 2.

outside the linear portion of the dose-response curve. Positive results were interpreted as presenting significant mutagenicity. Positive responses were expressed as the number of revertants per volume of air sampled ( $\text{rev}/\text{m}^3$ ), that is,  $\text{rev}/\mu\text{g}$  multiplied by EOM in  $\mu\text{g}/\text{m}^3$ . In the cytotoxicity test, the solution containing the sample and the bacterial culture (100–200 cells) were plated on nutrient agar plates and incubated at  $37^\circ\text{C}$  for 24 h, and the surviving colonies were counted. The sample was considered cytotoxic if the percentage of surviving cells was less than 60% of the negative control at one or more doses [18].

**2.5. Allium cepa.** Onions (*Allium cepa* L.,  $2n = 16$ ) were obtained commercially and were placed in small jars with their basal ends dipped in distilled water. The newly sprouted roots (1–2 cm in length) were treated with organic extract of PM (5; 10; 15; 20 and 25  $\mu\text{g}/\text{mL}$ ) for 24 h. DMSO was used as a negative control and benzo(a)pyrene (10  $\mu\text{g}/\text{mL}$ ) as a positive control. The roots were exposed to treatment with organic extract and the controls for 24 h. Afterwards, the roots were removed from the bulbs and hydrolyzed with HCl 1N : acetic acid 45% (1:9) at  $50^\circ\text{C}$  for 5 min. The hydrolyzed root tips were squashed and stained with 2% aceto-orcein [24, 25]. Cytological abnormalities like micronuclei (MCN), bridges, breaks, and laggards were analyzed. The mitotic index (MI) was calculated for each treatment as the number of dividing cells per 1000 cells.

### 3. Results and Discussion

**3.1. Airborne Particulate Matter.** Table 1 shows the air volume ( $\text{m}^3$ ), PM  $2.5\ \mu\text{m}$  concentration ( $\mu\text{g}/\text{m}^3$ ), and extractable organic matter (EOM) ( $\mu\text{g}/\text{m}^3$ ) of the samples analyzed from the different sites.

The highest average PM  $2.5\ \mu\text{m}$  values were detected at site 3 (94.54 to 132.73  $\mu\text{g}/\text{m}^3$ ), followed by site 2 (26.30; 45.14  $\mu\text{g}/\text{m}^3$ ) and site 1 (30.49  $\mu\text{g}/\text{m}^3$ ) (Table 1). The WHO has suggested that the maximum PM 2.5 concentration without incurring health risks is 25  $\mu\text{g}/\text{m}^3$  (24-hour mean) [26]. It has been demonstrated that for each 10  $\mu\text{g}/\text{m}^3$  increase in PM concentration, the risk of mortality from cardiopulmonary diseases increases 6%, while the risk of mortality from lung cancer rises 8% [27]. High levels of PM 2.5 were also detected in a previous study conducted from April to July 2010 at these same sites. The PM concentrations (site 3: 93  $\mu\text{g}/\text{m}^3$ ; site 2: 34–60  $\mu\text{g}/\text{m}^3$ ; site 1: 25  $\mu\text{g}/\text{m}^3$ ) reveal the persistence of this pollutant [15]. In both studies, the highest concentrations of PM  $2.5\ \mu\text{m}$  were detected at site 3. A study performed in Wutong tunnel in Shenzhen city, China detected PM 2.5 concentrations of 110.8  $\mu\text{g}/\text{m}^3$  [28]. Both these results can be attributed to intense traffic and poor ventilation in tunnels. The implementation of road space rationing measures and improved ventilation could reduce the concentration of PM 2.5 in these tunnels.

In urban areas, high concentrations of PM derive mainly from motor vehicle emissions. In Rio de Janeiro city, vehicles account for 77% of emissions [29]. Despite improvements

in the quality of fuels, high concentrations of PM are still detected in the city. This is due to the increased fleet of vehicles in the city, which over the last twenty years, has tripled from 838,521 to 2,529,432 [30]. Other urban areas with intense traffic also show high levels of PM 2.5: Beijing, China (66  $\mu\text{g}/\text{m}^3$ ) [31], Mexico City (47.20  $\mu\text{g}/\text{m}^3$ ) [32], Palermo, Italy (34.20  $\mu\text{g}/\text{m}^3$ ) [33], and Coimbatore, India (57.93  $\mu\text{g}/\text{m}^3$ ) [34]. The use of clean fuels and improved public transport are the best solutions for this problem, since they would result in lower PM emissions in urban areas.

**3.2. Analysis of Polycyclic Aromatic Hydrocarbons (PAHs).** Figure 1 shows PAH concentrations in  $\text{ng}/\text{m}^3$  at the three sites.

In this study, the six PAHs evaluated are considered a priority in environmental monitoring [35]. PAHs are formed due to incomplete combustion [28]. A lot of effort has been invested in understanding their emissions, dynamic formation mechanisms, and atmospheric behavior. Exhausts from diesel fuel combustion and gasoline engines are a major source of PAH in the atmosphere [28, 36]. Urban traffic is the biggest contributor to PAH emissions in Rio de Janeiro city. Site 3 showed the highest concentrations of PAH; these results are in agreement with results obtained in July of the same year, for this same site [37]. These results too are in agreement with results obtained in other urban areas. In Europe, for example, the concentration of benzo(a)pyrene in high traffic areas varies from 1 to 5  $\text{ng}/\text{m}^3$  [38]. In Sweden, in particular, the concentration of benzo(a)pyrene ranges from 1 to 2  $\text{ng}/\text{m}^3$ . These data have prompted political leaders in this country to stipulate a target concentration limit of 0.1  $\text{ng}/\text{m}^3$  for benzo[a]pyrene [39]. In our results, benzo(a)pyrene was detected only in site 3 at a concentration of between 1.06 and 1.22  $\text{ng}/\text{m}^3$ . The fact that site 3 is enclosed and poorly ventilated may favor the accumulation of this PAH.

Sites 1 and 2 showed the lowest concentrations of the PAH studied. These results could be attributed to the fact that Rio de Janeiro is a coastal city, and the sea serves as an effective dispersant of pollutants [37, 40].

**3.3. *Salmonella*/Microsome Assay.** Table 2 shows the mutagenicity data for the organic extracts from airborne particulate matter in  $\text{rev}/\text{m}^3$ . Cytotoxic effects were not detected for any of the samples analyzed.

Mutagenic frameshift responses in the presence of metabolic activation were detected at sites 1 (August and September), 2 (August), and 3 (August, September, and October) (Table 2). These results can be attributed to the presence of promutagens such as PAH detected in this study. Highest mutagenic frameshift responses in the presence of metabolic activation were detected at site 3; this result may be related to high concentrations of PAH (Figure 1), resulting from intense emission by vehicles in this area of the city. Sites 1 (August) and 2 (August), winter season, showed higher values for  $\text{rev}/\text{m}^3$  than in April and May, autumn season, of the same year [15]. These results suggest that winter season, which is a dry season with low rainfall season, favors the

TABLE 2: Induced mutagenic by airborne particulate matter organic extracts (rev/m<sup>3</sup>).

Site	Month	TA98		YG1021		YG1024	
		-S9	+S9	-S9	+S9	-S9	+S9
1	August	n.d. <sup>a</sup>	15.80 ± 1.40	13.90 ± 0.80	9.50 ± 1.40	38.20 ± 1.60	15.50 ± 1.40
	September	n.d. <sup>a</sup>	13.00 ± 2.10	6.10 ± 1.30	n.d. <sup>a</sup>	15.80 ± 2.50	3.40 ± 0.80
	October	n.d. <sup>a</sup>	n.d. <sup>a</sup>	10.20 ± 1.30	6.40 ± 1.10	2.90 ± 0.60	n.d. <sup>a</sup>
2	August	17.00 ± 1.90	1.50 ± 0.50	17.30 ± 1.80	5.70 ± 1.60	43.40 ± 8.50	10.70 ± 1.80
	September	5.70 ± 0.90	n.d. <sup>a</sup>	13.90 ± 1.30	6.40 ± 1.24	11.70 ± 2.50	6.00 ± 0.70
	October	n.d. <sup>a</sup>	n.d. <sup>a</sup>	4.90 ± 0.70	n.d. <sup>a</sup>	3.80 ± 0.30	n.d. <sup>a</sup>
3	August	39.60 ± 11.90	13.00 ± 2.30	4.40 ± 1.90	3.10 ± 0.80	28.30 ± 3.10	2.30 ± 0.80
	September	56.40 ± 20.60	58.70 ± 11.80	63.00 ± 3.20	26.60 ± 3.90	57.00 ± 6.90	49.30 ± 3.90
	October	9.30 ± 1.80	46.50 ± 4.20	35.40 ± 0.80	18.30 ± 1.80	22.80 ± 2.00	6.00 ± 2.00

1: UERJ; 2: Avenida Brasil; 3: Rebouças tunnel. n.d.<sup>a</sup>: not detected. Negative control: DMSO for the mutagenicity assay without S9 mix was TA98, (28 ± 5); YG1021, (20 ± 2); YG1024, (18 ± 4). DMSO for the mutagenicity assay with S9 mix was TA98, (43 ± 3); YG1021, (35 ± 23); YG1024, (18 ± 5). Positive controls for the mutagenicity assay without S9 mix were 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (853 ± 72); YG1021, (719 ± 75); YG1024, (1021 ± 54). Positive controls for the mutagenicity assay with S9 mix were 2-aminofluorene (1 µg/plate) for TA98, (214 ± 42); YG1021, (150 ± 32); YG1024, (219 ± 81).

TABLE 3: Mitotic index and percentage of mitosis stages and mitotic aberrations in the root tip cells of *A. cepa* treated with organic extract derived at site 1.

Month	µg/mL	Mitotic index	% P.	% M.	% A.	% T.	% T.A.	% MCN	% Bridge	% Break	% Lagging
August	NC	0.03 ± 0.00	1.40	0.90	0.50	1.10	0.07	0.05	0.00	0.00	0.02
	5	0.04 ± 0.01	3.20*	0.80	0.60	0.30	0.12	0.02	0.02	0.00	0.08
	10	0.02 ± 0.00*	0.20*	1.00	0.40	1.00	0.35	0.20	0.10	0.00	0.05
	15	0.01 ± 0.01*	0.03*	0.30	0.10	0.20*	0.55	0.55	0.00	0.00	0.00
	20	0.01 ± 0.01*	0.02*	0.10*	0.03*	0.20*	0.05	0.03	0.00	0.02	0.00
	25	0.00 ± 0.00*	0.00*	0.00*	0.00*	0.00*	0.00	0.00	0.00	0.00	0.00
September	NC	0.02 ± 0.01	0.80	0.60	0.20	0.50	0.15	0.00	0.05	0.05	0.05
	5	0.04 ± 0.02	1.10	1.30	0.80	0.50	0.54	0.04	0.20	0.08	0.22
	10	0.07 ± 0.03	2.00	2.50*	1.50*	1.50	1.30*	0.10	0.40	0.40	0.40
	15	0.01 ± 0.01	0.50	0.30	0.30	1.30	0.60	0.40	0.05	0.05	0.10
	20	0.06 ± 0.05	2.00	2.00	1.00	1.40	1.08*	0.06	0.33	0.26	0.43
	25	0.01 ± 0.01	0.50	0.50	0.10	0.30	0.28	0.03	0.06	0.16	0.03
October	NC	0.02 ± 0.00	1.40	0.40	0.00	0.00	0.10	0.08	0.00	0.02	0.00
	5	0.11 ± 0.05	7.00	0.70	0.40	2.60	0.10	0.00	0.00	0.07	0.03
	10	0.05 ± 0.05	2.70	0.70	0.50	1.60	0.18	0.00	0.03	0.10	0.05
	15	0.06 ± 0.04	2.60	1.30	0.30	2.30	0.29*	0.07	0.05	0.12*	0.05
	20	0.05 ± 0.03	2.50	1.10	0.40	0.80	0.10	0.00	0.02	0.03	0.05
	25	0.06 ± 0.03	3.20	1.00	0.50	1.00	0.31	0.03	0.00	0.18	0.10

P: prophase; M: metaphase; A: anaphase; T: telophase; T.A.: total abnormalities; MCN.: micronucleus; NC.: negative control—DMSO; positive control = benzo(a)pyrene 10 µg/mL (mitotic index = 0.00\*; % T.A. = 0.20; % MCN = 0.16; % bridge = 0.04)\* P ≤ 0.05.

accumulation of pollutants. Similar results were obtained in a study conducted in the city of Rio de Janeiro winter 1984 [41] and July of 2010 [37].

Mutagenic frameshift responses in the absence of metabolic activation were detected at sites 2 (August and September) and 3 (August, September, and October) (Table 2) which indicate the predominance of direct-acting frameshift activity in the airborne particulate material. The occurrence of direct frameshift mutagens adsorbed onto air particulate has been extensively demonstrated in urban samples collected throughout the world, and PAH derivatives, mainly nitro-PAH and oxygenated PAH, can be implicated [42–44]. These compounds may be derived from

the emission of diesel. The highest mutagenic frameshift responses in the absence of metabolic activation values were detected at site 3. These results may be related to the emission of nitrocompounds at this site. Site 3 shows high flow of buses and trucks, contributing to the emission of compounds derived of diesel. Besides, the absence of pollutant dispersion factors may have contributed to the buildup of these pollutants.

The contribution of nitrocompounds to direct mutagenic activity was investigated through the *Salmonella/microsome* assay with specific strains YG1021 and YG1024. At the three assessment sites, the increase in mutagenic activity in overexpressing strains, YG1021 and YG1024 compared to the

TABLE 4: Mitotic index and percentage of mitosis stages and mitotic aberrations in the root tip cells of *A. cepa* treated with organic extract derived at site 2.

Month	$\mu\text{g/mL}$	Mitotic index	% P.	% M.	% A.	% T.	% T.A.	% MCN	% Bridge	% Break	% Lagging
August	NC	0.08 ± 0.03	4.50	1.90	0.40	1.10	0.30	0.18	0.07	0.00	0.05
	5	0.07 ± 0.03	3.20	1.60	0.90*	1.80	0.57	0.25	0.17	0.00	0.15
	10	0.04 ± 0.01*	1.80*	0.80	0.40	0.60	0.41	0.03	0.06	0.16	0.16
	15	0.04 ± 0.01*	0.33*	3.26	0.56	0.36	0.75*	0.03	0.23*	0.26*	0.23
	20	0.04 ± 0.02*	2.53	1.23	0.46	0.76	0.19	0.00	0.00	0.16	0.03
	25	0.04 ± 0.01*	0.10*	1.40	0.90*	1.40	0.70	0.00	0.50*	0.10	0.10
September	NC	0.02 ± 0.01	0.80	0.60	0.20	0.50	0.15	0.00	0.05	0.05	0.05
	5	0.04 ± 0.02	1.10	1.30	0.80	0.50	0.54	0.04	0.20	0.08	0.22
	10	0.07 ± 0.03	2.00	2.50*	1.50*	1.50	1.30*	0.10	0.40	0.40	0.40
	15	0.01 ± 0.01	0.50	0.30	0.30	0.01*	0.60	0.40	0.05	0.05	0.10
	20	0.06 ± 0.05	2.00	2.00	1.00	1.40	1.08*	0.06	0.33	0.26	0.43
	25	0.01 ± 0.01	0.50	0.50	0.10	0.30	0.28	0.03	0.06	0.16	0.03
October	NC	0.05 ± 0.02	3.90	0.50	0.20	0.30	0.30	0.10	0.00	0.10	0.10
	5	0.05 ± 0.01	2.00	1.50	0.70	1.30	0.67	0.00	0.17*	0.30	0.20
	10	0.07 ± 0.01	1.70	1.80	0.80	3.20*	1.05	0.00	0.10	0.75	0.20
	15	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	20	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.03	0.00	0.03	0.00	0.00
	25	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

P.: prophase; M.: metaphase; A.: anaphase; T.: telophase; T.A.: total abnormalities; MCN.: micronucleus; NC.: negative control—DMSO; positive control = benzo(a)pyrene 10  $\mu\text{g/mL}$  (mitotic index = 0.00\*; % T.A. = 0.20; % bridge = 0.10; % break = 0.10) \*P ≤ 0.05.

TABLE 5: Mitotic index and percentage of mitosis stages and mitotic aberrations in the root tip cells of *A. cepa* treated with organic extract derived at site 3.

Month	$\mu\text{g/mL}$	Mitotic index	% P.	% M.	% A.	% T.	% T. A.	% MCN	% Bridge	% Break	% Lagging
August	NC	0.04 ± 0.02	2.60	0.70	0.40	1.00	0.12	0.07	0.00	0.00	0.05
	5	0.03 ± 0.02	0.70	0.40	0.90	0.30	0.30	0.04	0.02	0.00	0.14
	10	0.07 ± 0.03	2.70	2.20	0.90	2.10	0.50*	0.30	0.10	0.00	0.10
	15	0.06 ± 0.04	1.70	2.20	1.10*	1.00	0.26	0.10	0.13	0.00	0.03
	20	0.04 ± 0.02	1.60	1.40	0.70	1.00	0.16	0.10	0.03	0.00	0.03
	25	0.03 ± 0.01	1.30	0.90	0.60	0.60	0.15	0.00	0.00	0.00	0.15
September	NC	0.08 ± 0.06	6.00	1.60	0.90	1.40	0.67	0.10	0.08	0.20	0.42
	5	0.04 ± 0.03	3.10	1.00	0.40	2.10	2.05*	1.63*	0.12	0.22	0.05
	10	0.03 ± 0.03	1.00*	0.80	0.40	0.60	0.78	0.25	0.03	0.20	0.30
	15	0.10 ± 0.10	3.30	3.20	1.20	2.00	1.80	0.00	1.00*	0.60	0.20
	20	0.00 ± 0.00*	0.00*	0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.02 ± 0.01*	1.00*	0.10*	0.00	0.70	0.12	0.00	0.00	0.10	0.02
October	NC	0.08 ± 0.01	5.70	0.70	0.60	1.20	0.30	0.00	0.05	0.05	0.20
	5	0.09 ± 0.01	3.80	1.60	0.80	2.90	0.60	0.00	0.00	0.50*	0.10
	10	0.06 ± 0.05	1.90*	0.70	0.40	2.70	0.28	0.10	0.03	0.05	0.10
	15	0.03 ± 0.01*	0.90*	0.70	0.50	0.80	0.40	0.20	0.10	0.00	0.10
	20	0.00 ± 0.00*	0.00*	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	25	0.01 ± 0.02*	0.80*	0.00	0.03	0.03	0.33	0.00	0.03	0.00	0.00

P.: prophase; M.: metaphase; A.: anaphase; T.: telophase; T.A.: total abnormalities; MCN.: micronucleus; NC.: negative control—DMSO; positive control = benzo(a)pyrene 10  $\mu\text{g/mL}$  (mitotic index = 0.00\*; % T.A. = 0.00; %) \*P ≤ 0.05.

parental TA98, indicates the participation of nitroarenes and dinitroarenes in the total mutagenicity of the extracts studied. The presence of nitroarenes was detected at site 1 (August, September, and October), 2 (August and October), and 3 (September). The presence of dinitroarenes was detected at

site 1 (September and October), 2 (August and October), and 3 (September and October). It is well known that when nitrocompounds are implicated, the mutagenic response in the presence of S9 mix is decreased compared to the situations without S9 mix [8, 11]. This is because mammalian

enzymes reduce nitroarenes all the way to arylamines, and arylamines are not direct-aging mutagens. The mutagenicity of these chemicals can be explained by their reoxidation by cytochrome P450-dependent enzymes to the arylhydroxylamines. However, it must be noted that generally arylamines exhibit a much lower mutagenicity in the presence of S9 mix than the corresponding nitroarenes in the absence of S9 mix [45].

These nitrocompounds result from direct emissions of diesel combustion and can be produced by atmospheric reactions of PAH with gaseous copollutants found in photochemical smog [44, 46]. In Brazil, the presence of nitrocompounds has also been observed in air samples from Rio de Janeiro [15, 37], Porto Alegre [17, 18, 47], and São Paulo [11, 44, 48].

**3.4. Allium cepa.** Tables 3, 4, and 5 show the mitotic index, percentage of mitosis stages, and mitotic aberrations in the root tip cells of *A. cepa* treated with organic extract derived from sites 1, 2, and 3, respectively.

The mitotic root meristems of *Allium cepa* have been used as cytogenetic materials for clastogenicity studies of physical and chemical agents since the early 1930s [49, 50]. In this work, we evaluated organic extracts from different sites of the city of Rio de Janeiro for cytotoxic/proliferation effect and chromosomal abnormalities. A reduced MI in relation to the negative control could indicate a cytotoxic effect, while a higher MI could be indicative of a proliferative effect [51]. In our results, we detected a cytotoxic effect in organic extracts at site 1 (August) (Table 3), site 2 (August) (Table 4), and site 3 (September to October) (Table 5). The positive control used in the test also presented a cytotoxic effect, as observed in the literature [52]. The reduction in the percentage of mitotic cells in certain stages observed at the three sites may indicate the need to disrupt the mitotic cycle in order to possibly repair the damage caused by the components of the organic extracts.

Chromosome aberrations are characterized by changes in either chromosomal structure or the total number of chromosomes, which can occur both spontaneously and as a result of exposure to physical or chemical agents [14]. Structural chromosomal alterations may be induced by several factors, such as DNA breaks, inhibition of DNA synthesis, and replication of altered DNA [14, 53]. Two studies conducted in different regions of Yugoslavia to evaluate the occurrence of chromosomal abnormalities in the particulate matter deposited in the snow using the *Allium cepa* pointed to an increase in chromosomal abnormalities [54, 55]. Our results are in agreement with data from the literature. We detected significantly increased abnormalities, such as a bridge at site 2 (Table 4) and a micronucleus and bridge at site 3 (Table 5). A significant increase in breaks was observed at all three sites. Bridges noticed in the cells were probably formed by the breakage and fusion of chromosomes and chromatids [25]. Micronuclei are the result of damage in the parental cells, that is, either not repaired or wrongly repaired, and are easily observed in daughter cells as a similar structure to the main nucleus, but in a reduced size [14]. Micronucleus, bridge, and break abnormalities are attributed to clastogenic substances,

which indicate that our results may indicate the clastogenic activity of the organic extracts. We call to attention that in some periods, the high concentrations of the extracts lead to a cytotoxic effect that can dissemble a clastogenic effect.

According to [56], a complex mixture of hydrocarbons may present clastogenic and aneugenic activities or even induce cell death in *Allium cepa* genetic material. The authors also suggest that these actions mainly result from the presence of polycyclic aromatic hydrocarbons (PAHs) detected in the tested sample. Furthermore, *Allium cepa* assay has also the ability to detect nitrocompounds [25, 52]; therefore, these results may be related to the presence of these compounds in organic extracts.

The cytotoxic effect and the abnormalities detected in the present study can be attributed to the presence of PAH nitroderivatives in the organic extracts. As a response to the presence of pollutants, *Allium cepa* has a mechanism involving cytochrome P450- (CYP-) dependent mixed function oxidases with particular reference to ethoxresorufin-O-deethylase (EROD) activity [57, 58]. According to this mechanism, ligands like PAH activate the hydrocarbon receptor (AhR), which in its inactive form resides in the cytoplasm in a complex with the molecular chaperones, hsp90 and p23 [59]. CYP1A1 activation may be a result of the weakening of the interactions between AhR and hsp90, thus releasing the AhR to translocate to the nucleus and activate the CYP1A1 gene [58]. This reinforces the ability of this plant species to detect this class of pollutants.

## 4. Conclusion

In conclusion, both PAH and nitroderivatives probably contributed to the detected airborne genotoxicity at different sites of Rio de Janeiro. The information generated in this study shows the importance of simple biological tests such as the *Salmonella/microsome* and *Allium cepa* to better characterize air pollution. Evaluation of the genotoxicity of urban airborne particulate matter performed by these tests is important as a basis for decision making by regulatory authorities.

## Conflict of Interests

There is no conflict of interests.

## Acknowledgments

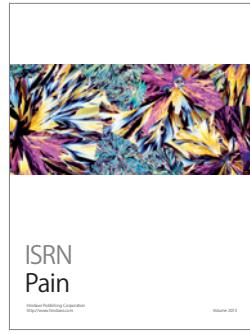
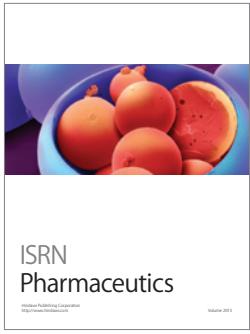
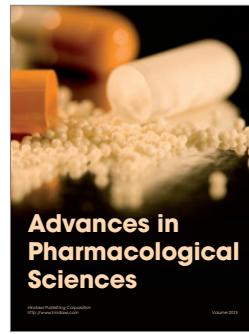
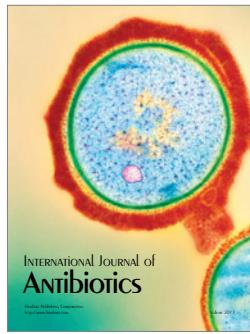
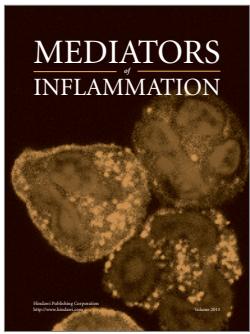
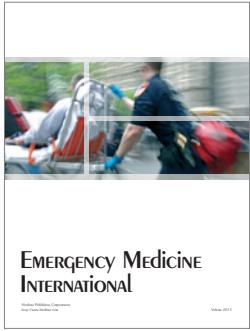
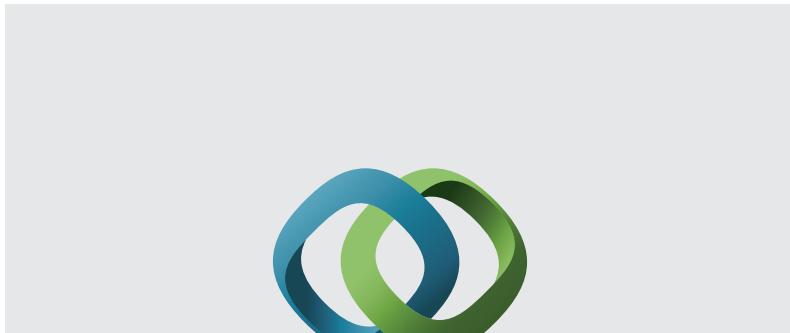
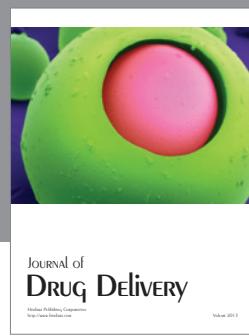
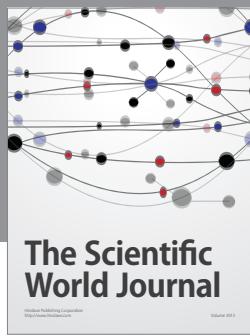
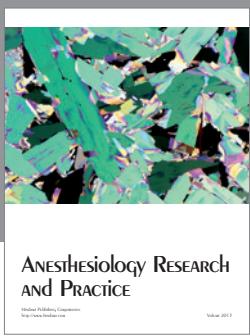
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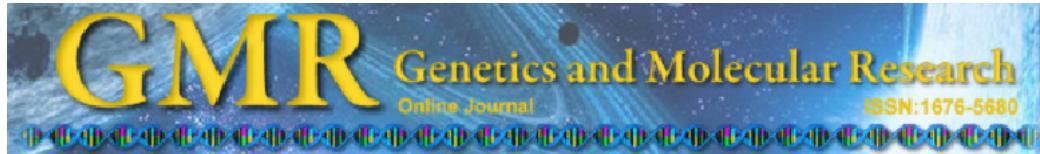
## References

- [1] M. Sørensen, H. Autrup, P. Møller et al., "Linking exposure to environmental pollutants with biological effects," *Mutation Research*, vol. 544, no. 2-3, pp. 255–271, 2003.

- [2] T. M. C. M. de Kok, H. A. L. Driece, J. G. F. Hogervorst, and J. J. Briedé, "Toxicological assessment of ambient and traffic-related particulate matter: a review of recent studies," *Mutation Research*, vol. 613, no. 2-3, pp. 103–122, 2006.
- [3] N. Gouveia, G. A. S. Mendonça, A. P. Leon et al., "Poluição do ar e efeitos na saúde nas populações de duas grandes metrópoles brasileiras," *Epidemiologia e Serviços de Saúde*, vol. 12, pp. 29–240, 2003.
- [4] W. L. Junger, A. P. Leon, and G. A. S. Mendonça, "Associação entre mortalidade diária por câncer de pulmão e poluição do ar no município do Rio de Janeiro: um estudo ecológico de séries temporais," *Revista Brasileira de Cancerologia*, vol. 51, pp. 111–115, 2005.
- [5] H. A. Castro, M. F. Cunha, G. A. S. Mendonça, W. L. Junger, J. C. Cruz, and A. P. Leon, "Efeitos da poluição do ar na função respiratória de escolares, Rio de Janeiro, RJ," *Revista Saúde Pública*, vol. 43, pp. 26–34, 2009.
- [6] M. Moura, W. L. Junger, G. A. E. S. Mendonça, and A. P. de Leon, "Air quality and emergency pediatric care for symptoms of bronchial obstruction categorized by age bracket in Rio de Janeiro, Brazil," *Caderno Saúde Pública*, vol. 25, no. 3, pp. 635–644, 2009.
- [7] S. M. Oh, H. R. Kim, Y. J. Park, S. Y. Lee, and K. H. Chung, "Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells)," *Mutation Research*, vol. 723, no. 2, pp. 142–151, 2011.
- [8] L. D. Claxton, P. P. Matthews, and S. H. Warren, "The genotoxicity of ambient outdoor air, a review: salmonella mutagenicity," *Mutation Research*, vol. 567, no. 2-3, pp. 347–399, 2004.
- [9] T. Enya, H. Suzuki, T. Watanabe, T. Hirayama, and Y. Hisamatsu, "3-Nitrobenzanthrone, a powerful bacterial mutagen and suspected human carcinogen found in diesel exhaust and airborne particulates," *Environmental Science and Technology*, vol. 31, no. 10, pp. 2772–2776, 1997.
- [10] WHO-IPCS INCHEM, "Selected nitro- and nitro-oxy-polycyclic aromatic hydrocarbons," Environmental Health Criteria (EHC) Monograph 229, 2003.
- [11] G. D. A. Umbuzeiro, A. Franco, D. Magalhães et al., "A preliminary characterization of the mutagenicity of atmospheric particulate matter collected during sugar cane harvesting using the salmonella/microsome microsuspension assay," *Environmental and Molecular Mutagenesis*, vol. 49, no. 4, pp. 249–255, 2008.
- [12] D. M. DeMarini, L. R. Brooks, S. H. Warren, T. Kobayashi, M. I. Gilmour, and P. Singh, "Bioassay-directed fractionation and Salmonella mutagenicity of automobile and forklift diesel exhaust particles," *Environmental Health Perspectives*, vol. 112, no. 8, pp. 814–819, 2004.
- [13] C. H. Marvin and L. M. Hewitt, "Analytical methods in bioassay-directed investigations of mutagenicity of air particulate material," *Mutation Research*, vol. 636, no. 1-3, pp. 4–35, 2007.
- [14] D. M. Leme and M. A. Marin-Morales, "Allium cepa test in environmental monitoring: a review on its application," *Mutation Research*, vol. 682, no. 1, pp. 71–81, 2009.
- [15] C. R. Rainho, A. M. A. Velho, C. A. F. Aiub, S. M. Corrêa, J. L. Mazzei, and I. Felzenszwalb, "Evaluation of urban airborne particulate matter (PM 2.5) in the city of Rio de Janeiro (Brazil) by mutagenicity assays," in *Essays on Environmental Studies*, A. Sarin, Ed., pp. 1–8, Athens Institute for Education and Research, Athens, Ga, USA, 2012.
- [16] V. M. F. Vargas, R. C. Horn, R. R. Guidobono, A. B. Mittelstaedt, and I. G. de Azevedo, "Mutagenic activity of airborne particulate matter from the urban area of Porto Alegre, Brazil," *Genetics and Molecular Biology*, vol. 21, no. 2, pp. 247–253, 1998.
- [17] M. V. Coronas, R. C. Horn, A. Ducatti, J. A. V. Rocha, and V. M. F. Vargas, "Mutagenic activity of airborne particulate matter in a petrochemical industrial area," *Mutation Research*, vol. 650, no. 2, pp. 196–201, 2008.
- [18] T. S. Pereira, G. N. Gotor, L. S. Beltrami et al., "Salmonella mutagenicity assessment of airborne particulate matter collected from urban areas of Rio Grande do Sul State, Brazil, differing in anthropogenic influences and polycyclic aromatic hydrocarbon levels," *Mutation Research*, vol. 702, no. 1, pp. 78–85, 2010.
- [19] S. M. Corrêa and G. Arbilla, "A two-year monitoring program of aromatic hydrocarbons in Rio de Janeiro downtown area," *Journal of the Brazilian Chemical Society*, vol. 18, no. 3, pp. 539–543, 2007.
- [20] N. Y. Kado, G. N. Guirguis, C. P. Flessel, R. C. Chan, K. Chang, and J. J. Wesolowski, "Mutagenicity of fine (<2.5 μm) airborne particles: diurnal variation in community air determined by a Salmonella micro preincubation (microsuspension) procedure," *Environmental Mutagenesis*, vol. 8, no. 1, pp. 53–66, 1986.
- [21] D. M. Maron and B. N. Ames, "Revised methods for the Salmonella mutagenicity test," *Mutation Research*, vol. 113, no. 3-4, pp. 173–215, 1983.
- [22] M. Watanabe, M. Ishidate, and T. Nohmi, "A sensitive method for the detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of *Salmonella typhimurium* strains TA98 and TA100," *Mutation Research*, vol. 216, no. 4, pp. 211–220, 1989.
- [23] L. Bernstein, J. Kaldor, J. McCann, and M. C. Pike, "An empirical approach to the statistical analysis of mutagenesis data from the Salmonella test," *Mutation Research*, vol. 97, no. 4, pp. 267–281, 1982.
- [24] G. L. Cabrera and D. M. G. Rodriguez, "Genotoxicity of leachates from a landfill using three bioassays," *Mutation Research*, vol. 426, no. 2, pp. 207–210, 1999.
- [25] C. R. de Rainho, A. Kaezer, C. A. F. Aiub, and I. Felzenszwalb, "Ability of *Allium cepa* L. root tips and *Tradescantia pallida* var. *purpurea* in N-nitrosodiethylamine genotoxicity and mutagenicity evaluation," *Anais da Academia Brasileira de Ciencias*, vol. 82, no. 4, pp. 925–932, 2010.
- [26] World Health Organization, *Guidelines for Air Quality For Europe*, WHO, Geneva, Switzerland, 2nd edition, 2000.
- [27] M. Ianistcki, J. Dallarosa, C. Sauer, C. E. Teixeira, and J. da Silva, "Genotoxic effect of polycyclic aromatic hydrocarbons in the metropolitan area of Porto Alegre, Brazil, evaluated by *Helix aspersa* (Müller, 1774)," *Environmental Pollution*, vol. 157, no. 7, pp. 2037–2042, 2009.
- [28] L. Y. He, M. Hu, X. F. Huang, Y. H. Zhang, B. D. Yu, and D. Q. Liu, "Chemical characterization of fine particles from on-road vehicles in the Wutong tunnel in Shenzhen, China," *Chemosphere*, vol. 62, no. 10, pp. 1565–1573, 2006.
- [29] Instituto Estadual do Ambiente (INEA), "Relatório Anual da Qualidade do Ar do Estado do Rio de Janeiro," 2009, [http://www.inea.rj.gov.br/downloads/relatorios/qualidade\\_ar\\_2009.pdf](http://www.inea.rj.gov.br/downloads/relatorios/qualidade_ar_2009.pdf).
- [30] "Só 14 unidades automáticas medem o ar que o carioca respira," <http://oglobo.globo.com/rio20/so-14-unidades-automaticas-medem-ar-que-carioca-respira-4948672>.
- [31] L. Y. He, M. Hu, X. F. Huang, Y. H. Zhang, and X. Y. Tang, "Seasonal pollution characteristics of organic compounds in

- atmospheric fine particles in Beijing," *Science of the Total Environment*, vol. 359, pp. 167–176, 2006.
- [32] B. L. Valle-Hernández, V. Mugica-Álvarez, E. Salinas-Talavera et al., "Temporal variation of nitro-polycyclic aromatic hydrocarbons in PM10 and PM 2.5 collected in Northern Mexico City," *Science of the Total Environment*, vol. 408, pp. 5429–5438, 2010.
- [33] G. Dongarrà, E. D. Manno, D. Varrica, M. Lombardo, and M. Vultaggio, "Study on ambient concentrations of PM10, PM10-2.5, PM2.5 and gaseous pollutants. Trace elements and chemical speciation of atmospheric particulates," *Atmospheric Environment*, vol. 44, no. 39, pp. 5244–5257, 2010.
- [34] R. Mohanraj, S. Dhanakumar, and G. Solaraj, "Polycyclic aromatic hydrocarbons bound to PM 2.5 in urban Coimbatore, India with emphasis on source apportionment," *The Scientific World Journal*, vol. 2012, Article ID 980843, pp. 1–8, 2012.
- [35] US Environmental Protection Agency, "Estimating Carcinogenic Potency for Mixtures of Polycyclic Organic Matter (POM) for the 1996 National-Scale Assessment," Appendix H-3, Washington, DC, USA, 1996.
- [36] S. H. Cadle, P. A. Mulawa, E. C. Hunsanger et al., "Composition of light-duty motor vehicle exhaust particulate matter in the Denver, Colorado area," *Environmental Science and Technology*, vol. 33, no. 14, pp. 2328–2339, 1999.
- [37] C. R. Rainho, A. M. A. Velho, C. A. F. Aiub, S. M. Corrêa, J. L. Mazzei, and L. Felzenszwalb, "Prediction of health risk due to polycyclic aromatic hydrocarbons present in urban air in Rio de Janeiro, Brazil," *Genetics and Molecular Research*, 2013.
- [38] Agency for Toxic Substances and Disease Registry, *Toxicological Profile for Polycyclic Aromatic Hydrocarbons*, Agency for Toxic Substances and Disease Registry, Atlanta, Ga, USA, 1995.
- [39] C. E. Boström, P. Gerde, A. Hanberg et al., "Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air," *Environmental Health Perspectives*, vol. 110, no. 3, pp. 451–488, 2002.
- [40] M. B. Fernandes, L. S. R. Brickus, J. C. Moreira, and J. N. Cardoso, "Atmospheric BTX and polyaromatic hydrocarbons in Rio de Janeiro, Brazil," *Chemosphere*, vol. 47, no. 4, pp. 417–425, 2002.
- [41] A. G. Miguel, J. M. Daisey, and J. A. Sousa, "Comparative study of the mutagenic and genotoxic activity associated with inhalable particulate matter in Rio de Janeiro air," *Environmental and Molecular Mutagenesis*, vol. 15, no. 1, pp. 36–43, 1990.
- [42] R. Crebelli, S. Fuselli, A. Meneguz et al., "In vitro and in vivo mutagenicity studies with airborne particulate extracts," *Mutation Research*, vol. 204, no. 4, pp. 565–575, 1988.
- [43] M. Adonis and L. Gil, "Mutagenicity of organic extracts from Santiago (Chile) airborne particulate matter," *Mutation Research*, vol. 292, no. 1, pp. 51–61, 1993.
- [44] M. I. Z. Sato, G. A. Umbuzeiro, C. A. Coimbrão et al., "Mutagenicity of airborne particulate organic material from urban and industrial areas of São Paulo, Brazil," *Mutation Research*, vol. 335, pp. 317–330, 1995.
- [45] H. S. Rosenkranz and R. Mermelstein, "Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal," *Mutation Research*, vol. 114, no. 3, pp. 217–267, 1983.
- [46] J. N. Pitts Jr., "On the trail of atmospheric mutagens and carcinogens: a combined chemical/microbiological approach," *American Zoologist*, vol. 25, no. 2, pp. 415–431, 1985.
- [47] V. M. F. Vargas, "Mutagenic activity as a parameter to assess ambient air quality for protection of the environment and human health," *Mutation Research*, vol. 544, no. 2-3, pp. 313–319, 2003.
- [48] B. S. de Martinis, N. Y. Kado, L. R. F. de Carvalho, R. A. Okamoto, and L. A. Gundel, "Genotoxicity of fractionated organic material in airborne particles from São Paulo, Brazil," *Mutation Research*, vol. 446, no. 1, pp. 83–94, 1999.
- [49] T. H. Ma, Z. Xu, C. Xu et al., "The improved *Allium/Vicia* root tip micronucleus assay for clastogenicity of environmental pollutants," *Mutation Research*, vol. 334, no. 2, pp. 185–195, 1995.
- [50] L. D. Claxton and G. M. Woodall, "A review of the mutagenicity and rodent carcinogenicity of ambient air," *Mutation Research*, vol. 636, no. 1–3, pp. 36–94, 2007.
- [51] T. C. C. Fernandes, D. E. C. Mazzeo, and M. A. Marin-Morales, "Mechanism of micronuclei formation in polyploidized cells of *Allium cepa* exposed to trifluralin herbicide," *Pesticide Biochemistry and Physiology*, vol. 88, no. 3, pp. 252–259, 2007.
- [52] G. Fiskesjö and C. Lassen, "Benzo[a]pireno and nitrosoguanidine in the *Allium* test," *Mutation Research*, vol. 97, p. 188, 1982.
- [53] R. J. Albertini, D. Anderson, G. R. Douglas et al., "IPCS guideline for the monitoring of genotoxic effects of carcinogens in humans, International Programme on Chemical Safety," *Mutation Research*, vol. 463, pp. 111–172, 2000.
- [54] K. Al-Sabti, "Allium test for air and water borne pollution control," *Cytobios*, vol. 58, no. 233, pp. 71–78, 1989.
- [55] P. Poli, A. Buschini, F. M. Restivo et al., "Comet assay application in environmental monitoring: DNA damage in human leukocytes and plant cells in comparison with bacterial and yeast tests," *Mutagenesis*, vol. 14, no. 6, pp. 547–556, 1999.
- [56] D. M. Leme, D. D. F. D. Angelis, and M. A. Marin-Morales, "Action mechanisms of petroleum hydrocarbons present in waters impacted by an oil spill on the genetic material of *Allium cepa* root cells," *Aquatic Toxicology*, vol. 88, no. 4, pp. 214–219, 2008.
- [57] M. J. Moore, I. V. Mitrofanov, S. S. Valentini et al., "Cytochrome P4501A expression, chemical contaminants and histopathology in roach, goby and sturgeon and chemical contaminants in sediments from the Caspian Sea, Lake Balkhash and the Ily River Delta, Kazakhstan," *Marine Pollution Bulletin*, vol. 46, no. 1, pp. 107–119, 2003.
- [58] R. A. Fatima and M. Ahmad, "Allium cepa derived EROD as a potential biomarker for the presence of certain pesticides in water," *Chemosphere*, vol. 62, no. 4, pp. 527–537, 2006.
- [59] J. R. Petrusis and G. H. Perdew, "The role of chaperone proteins in the aryl hydrocarbon receptor core complex," *Chemico-Biological Interactions*, vol. 141, no. 1-2, pp. 25–40, 2002.





# **Comparison of the sensitivity of strains of *Salmonella enterica* serovar Typhimurium in the detection of mutagenicity induced by nitroarenes**

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**ABSTRACT.** The use of strains of *Salmonella enterica* serovar Typhimurium with different metabolic capacities can indicate the class or classes of compounds present in an environmental sample and enable the diagnosis of the mutagenic activity of these pollutants adsorbed on particulate matter (PM) in the air. In the present study, the sensitivity of *Salmonella* strains TA98NR, TA98/1,8-DNP<sub>6</sub>, YG1021, and YG1024 to detect nitro compounds adsorbed on samples of PM 2.5 was compared from three sites in Rio de Janeiro city. Samples were collected using a

high-volume sampler at three sites: one with light traffic and two with heavy traffic. The assays were performed in the presence of 10-50 µg/plate organic extracts with and without exogenous metabolism. The YG1021 and YG1024 strains showed the highest rev/m<sup>3</sup> values, confirming their enhanced sensitivity. As YG1024 also demonstrated sensitivity to nitro and amino compounds, we suggest its use in research into environmental contamination.

**Key words:** Sensitivity; *Salmonella enterica* serovar Typhimurium; Nitroarenes; Mutagenicity

## INTRODUCTION

The *Salmonella*/microsome microsuspension assay has been used for large, multisite, and/or time series studies, for bioassay-directed fractionation studies, for identifying the presence of specific classes of mutagens, and for site or source comparisons of relative levels of airborne mutagens (Claxton et al., 2004). The use of strains of *Salmonella enterica* serovar Typhimurium with different metabolic capacities can indicate the class or classes of compounds present in an environmental sample (DeMarini et al., 2004; de Aragão Umbuzeiro et al., 2008). The *Salmonella*/microsome assay identifies the presence of organic compounds and enables diagnosis of the mutagenic activity of these pollutants adsorbed on particulate matter (PM) in the air. The responses are related to the presence of a variety of compounds, including polycyclic aromatic hydrocarbons (PAHs) and nitroarenes (Menck et al., 1974; Ducatti and Vargas, 2003).

Nitroarenes are a class of environmentally hazardous compounds (Watanabe et al., 1990). These nitro compounds have been detected in the extracts of diesel and gasoline emissions, fly ash, cigarette smoke condensates, and home heater emissions (Rosenkranz and Mermelstein, 1983; Watanabe et al., 1990). Typical nitroarenes, such as 2-nitrofluorene, 1-nitropyrene (1-NP), and 1,8-dinitropyrene (1,8-DNP), are potent mutagens (Wang et al., 1980; Watanabe et al., 1990). The contributions of nitroarenes to direct mutagenic activity are commonly investigated through the *Salmonella*/microsome assay with the following *S. enterica* serovar Typhimurium strains: TA98 (frameshift strain) and the derivative strains TA98/1,8-DNP<sub>6</sub> (*O*-acetyltransferase-deficient), TA98NR (nitroreductase-deficient) (Rosenkranz and Mermelstein, 1980), YG1021 (nitroreductase-overproducing), and YG1024 (*O*-acetyltransferase-overproducing) (Watanabe et al., 1989, 1990). Genotoxic evaluation studies of particulate matter using these strains have been conducted previously (Sato et al., 1995; Vargas et al., 1998; Ducatti and Vargas, 2003; de Aragão Umbuzeiro et al., 2008; Coronas et al., 2009; Pereira et al., 2010; Rainho et al., 2012, 2013a,b).

In the present study, comparisons were made of the sensitivity of strains of *S. enterica* serovar Typhimurium to detect nitro compounds adsorbed on samples of PM 2.5 collected at three sites in the city of Rio de Janeiro.

## MATERIAL AND METHODS

### Sampling sites

The samples were collected between August and October 2010 at three sites in Rio

de Janeiro: the campus of Universidade do Estado do Rio de Janeiro (site 1), Avenida Brasil (site 2), and Rebouças tunnel (site 3). Site 1, with light traffic, is located in a residential area of the city northern zone. Site 2 has heavy traffic (~250,000 vehicles/day) and is the city longest expressway, covering 58 km in length and crossing 27 neighborhoods. Site 3 also has heavy traffic (~190,000 vehicles/day) and is a 2.8 km-long tunnel that connects the northern and southern zones of the city (Rainho et al., 2012, 2013a,b).

Airborne PM 2.5 samples were collected on fiberglass filters (E558 X 10IN; 254 x 203 mm) using a high-volume collector (AVG MP 2.5; 1.13 m<sup>3</sup>/min) for 24 h for sites 1 and 2, and for 6 h for site 3. Four monthly samplings were performed at each site. At the end of the sampling period, the filters were combined to form a pooled sample (Rainho et al., 2012, 2013a,b).

### Extraction of organic compounds

Half of each filter was sonicated in three rounds of 10 min each using 99.9% pure dichloromethane (CASRN. 75-09-2; TediaBrazil; Brazil). The extracts were concentrated to 15 mL in a rotating evaporator and filtered through a 0.5 µm Teflon membrane. The concentration of extractable organic matter (EOM) was calculated and expressed as µg/m<sup>3</sup>. Prior to the bioassays, the organic extract was dried at 4°C and resuspended in 5 µL 99.9% pure dimethyl sulfoxide (DMSO; CASRN. 67-68-5; Synth; Brazil) (Vargas et al., 1998).

### Salmonella/microsome assay

The organic extracts were assayed for mutagenicity using the microsuspension version (Kado et al., 1986) of the *Salmonella*/microsome assay (Maron and Ames, 1983). *S. enterica* serovar Typhimurium TA98 (frameshift strain) and the derivative strains TA98/1.8-DNP<sub>6</sub> (*O*-acetyltransferase-deficient) and TA98NR (nitroreductase-deficient) (Rosenkranz and Mermelstein, 1980) were used, with and without metabolic activation (S9 mix fraction). Five concentrations of each sample (10, 20, 30, 40, and 50 µg/plate) were tested in triplicate. The samples were pre-incubated for 90 min. All assays were carried out under yellow light and in the presence of negative (5 µL/plate DMSO solvent) and positive (0.5 µg/plate 4-nitroquinoline oxide; CASRN. 56-57-5; Sigma Chemical Company; St. Louis, MO, USA) controls. Plates were incubated in the dark at 37°C for 72 h, after which time revertants were counted. The sample was considered to be positive when the mutagenesis value was at least twice the negative value, and when a significant ANOVA ( $P < 0.05$ ) and a positive dose-response rate ( $P < 0.05$ ) were observed. The results of the different assays were analyzed using the SALANAL program (Salmonella Assay Analysis, version 1.0; Integrated Laboratory Systems; Research Triangle Park, NC, USA). The choice between linear regression and the Bernstein model (Bernstein et al., 1982) was made to allow the elimination of data for doses outside the linear portion of the dose-response curve. Positive results were interpreted as presenting significant mutagenicity. Positive responses were expressed as the number revertants per volume of air sampled (rev/m<sup>3</sup>), i.e., rev/µg multiplied by EOM in µg/m<sup>3</sup>. In the cytotoxicity test, the solutions containing the sample and the bacterial culture (100-200 cells) were plated on nutrient agar plates and incubated at 37°C for 24 h, and the surviving colonies were counted. The sample was considered to be cytotoxic if the percentage of surviving cells was less than 60% of the negative control at one or more doses (Coronas et al., 2009).

## RESULTS AND DISCUSSION

### *Salmonella/microsome assay*

Table 1 shows the mutagenic activity, expressed as rev/m<sup>3</sup>, analyzed using the classic nitroreductase- and *O*-acetyltransferase-deficient derivate strains TA98NR and TA98/1,8-DNP<sub>6</sub>.

**Table 1.** Induced mutagenic by airborne particulate matter organic extracts (rev/m<sup>3</sup>).

Site	Month	TA98		TA98NR		TA98/1,8-DNP <sub>6</sub>	
		-S9	+S9	-S9	+S9	-S9	+S9
1	August	n.d.	15.80 ± 1.40	n.d.	6.50 ± 1.00	6.76 ± 2.12	n.d.
	September	n.d.	13.00 ± 2.10	1.42 ± 0.63	2.50 ± 0.50	n.d.	n.d.
	October	n.d.	n.d.	4.54 ± 0.60	2.20 ± 0.40	n.d.	2.60 ± 0.30
2	August	17.00 ± 1.90	1.50 ± 0.50	9.85 ± 1.70	10.20 ± 1.00	12.26 ± 0.93	4.30 ± 1.70
	September	5.70 ± 0.90	n.d.	7.16 ± 2.67	6.00 ± 0.50	1.44 ± 0.07	n.d.
	October	n.d.	n.d.	n.d.	2.40 ± 0.30	1.80 ± 0.65	n.d.
3	August	39.60 ± 11.90	13.00 ± 2.30	2.30 ± 1.25	7.10 ± 0.80	10.67 ± 2.93	7.70 ± 0.80
	September	56.40 ± 20.60	58.70 ± 11.80	2.78 ± 1.71	4.30 ± 2.80	35.37 ± 6.21	6.90 ± 1.70
	October	9.30 ± 1.80	46.50 ± 4.20	n.d.	5.80 ± 2.50	n.d.	19.00 ± 4.00

1 = Universidade do Estado do Rio de Janeiro; 2 = Avenida Brasil; 3 = Rebouças tunnel. n.d. = not detected. Negative controls: DMSO for the mutagenicity assay without S9 mix were: TA98, (28 ± 5); TA98NR (23 ± 3); TA98/1,8-DNP<sub>6</sub>, (17 ± 2). DMSO for the mutagenicity assay with S9 mix were: TA98, (45 ± 8); TA98NR (33 ± 7); TA98/1,8-DNP6, (25 ± 4). Positive controls for the mutagenicity assay without S9 mix were: 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (853 ± 72); TA98NR (120 ± 2); TA98/1,8-DNP<sub>6</sub>, (114 ± 35). Positive controls for the mutagenicity assay with S9 mix were: 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (198 ± 50); TA98NR (121 ± 1); TA98/1,8-DNP6 (95 ± 33).

The EOM values ranged from 5.54 to 9.66 µg/m<sup>3</sup> at site 1, from 5.48 to 7.76 µg/m<sup>3</sup> at site 2, and from 20.93 to 25.05 µg/m<sup>3</sup> at site 3 in the three months of study, as calculated elsewhere (Rainho et al., 2013b). No cytotoxic effects were detected for any of the samples analyzed.

The contribution of nitro compounds to the mutagenic activity of air samples from urban areas is associated with the presence of PAH derivatives. Among these, the mono- and dinitro-PAHs associated with oxygenated PAH have been correlated with direct frameshift mutagenic activity (Sato et al., 1995; Vargas et al., 1998; Ducatti and Vargas, 2003). In the present study, the contribution of mono- and di-nitroarenes to direct mutagenic activity was investigated through the *Salmonella/microsome* assay with strains TA98NR and TA98/1,8-DNP<sub>6</sub>, respectively. Nitroarenes are dependent upon this ‘classic’ nitroreductase to express their mutagenicity, as evidenced by their greatly decreased activity in the nitroreductase-deficient strain (Rosenkranz and Mermelstein, 1983). However, some nitroarenes express all or a major fraction of their activity even in the absence of the ‘classic’ nitroreductase (e.g., 1,8-DNP). The fact that there is residual activity expressed in TA98NR (the microorganism deficient in the ‘classic’ nitroreductase), and the finding of the full expression of the mutagenicity of other chemicals in TA98NR has led to the conclusion that *S. enterica* serovar Typhimurium may contain additional nitroreductases as well as other specific enzymes. Indeed, it has been possible to construct bacterial strains lacking the enzyme that recognizes 1,8-DNP (e.g., TA98/1,8-DNP<sub>6</sub>) (Rosenkranz and Mermelstein, 1983). The lower mutagenic activity in nitroreductase-deficient and *O*-acetyltransferase-expressing strains, TA98NR and TA98/1,8-DNP<sub>6</sub>, compared to the parental strain TA98, indicated the participation of mono- and di-nitroarenes in the total mutagenicity of the extracts. The presence of mono-nitroarenes was detected at site 2 (August) and site 3 (August and September). A lower mutagenic response was

observed for TA98NR (42% at site 2 and 94-95% at site 3) than was observed for TA98. At site 2 (August and September) and site 3 (August and September), a lower mutagenic response was observed for the TA98/1,8-DNP<sub>6</sub> strain (28-75% at site 2 and 37-73% at site 3) than for TA98, indicating the presence of di-nitroarenes. The extent of the reduction in the mutagenic response detected at sites 2 and 3 can be attributed to the intense traffic in these areas of the city. Studies performed with TA98NR and TA98/1,8-DNP<sub>6</sub> in areas contaminated by vehicular emissions and industrial activity in Rio Grande do Sul State of Brazil showed similar results: a reduction of 10-95% for TA98NR and a reduction of 61-85% for TA98/1,8-DNP<sub>6</sub> (Vargas et al., 1998; Ducatti and Vargas, 2003). Similar results were also detected in the industrial city of Cubatão, São Paulo (Sato et al., 1995). Meanwhile, an absence of mutagenicity was observed at site 1, which is characteristic of residential areas with a low flow of vehicles.

Mutagenic results were detected in the presence of metabolic activation for TA98NR and TA98/1,8-DNP<sub>6</sub> for all sites. However, the decrease in revertants after metabolism was only observed in a few months. The effects of the S9 mix preparations on the mutagenicity of nitroarenes in the *Salmonella* strains revealed a broad spectrum of responses ranging from the absence of mutagenicity to an absolute requirement for mutagenic activation. In some instances, the presence of S9 mix permitted the expression of mutagenicity by nitroarenes even in nitroreductase-deficient microorganisms, indicating the presence of nitroreductase activity in the S9 mix (Rosenkranz and Mermelstein, 1983). However, it must be noted that, in general, arylamines exhibit much lower mutagenicity in the presence of S9 mix than do corresponding nitroarenes in the absence of S9 mix.

Strains YG1021 and YG1024 are also commonly used in environmental studies (Coronas et al., 2009; Pereira et al., 2010; Rainho et al., 2013b). The higher mutagenic activity seen in over-expressing strains YG1021 and YG1024 compared to the parental strain TA98 indicates the participation of nitro and amine compounds, respectively. The extracts from sites 1, 2, and 3 were therefore also tested with strains YG1021 and YG1024, and showed the following results: increase in reversion for YG1021 (6-14% at site 1; 2-144% at site 2; 12-281% at site 3), and increase in reversion for YG1024 (3-38% at site 1; 105-155% at site 2; 1-145% at site 3) (Rainho et al., 2013b). Two studies conducted in different parts of Rio Grande do Sul also detected the presence of nitro and amino compounds through the high activity of strains YG1021 (23-264%) and YG1024 (554-1821%) (Coronas et al., 2009; Pereira et al., 2010). This increase in the percentage of reversal shown by YG1021 and YG1024 is attributed to plasmids pYG216 and pYG219, which increase the sensitivity of these strains to nitro and amino compounds, respectively (Watanabe et al., 1990).

## CONCLUSION

The *Salmonella*/microsome assay has been used for large, multi-site and/or time series studies to identify the presence of specific mutagens and classes of mutagens. All the strains used in the present study are sensitive to nitroarene, although strains YG1021 and YG1024 showed the highest rev/m<sup>3</sup> values, thus confirming their enhanced sensitivity. YG1024 also demonstrated sensitivity to nitro and amino compounds. We recommend the use of strains YG1021 and YG1024 in research into environmental contamination by these pollutants.

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## REFERENCES

- Bernstein L, Kaldor J, McCann J and Pike MC (1982). An empirical approach to the statistical analysis of mutagenesis data from the *Salmonella* test. *Mutat. Res.* 97: 267-281.
- Claxton LD, Matthews PP and Warren SH (2004). The genotoxicity of ambient outdoor air, a review: *Salmonella* mutagenicity. *Mutat. Res.* 567: 347-399.
- Coronas MV, Pereira TS, Rocha JA, Lemos AT, et al. (2009). Genetic biomonitoring of an urban population exposed to mutagenic airborne pollutants. *Environ. Int.* 35: 1023-1029.
- de Aragão Umbuzeiro G, Franco A, Magalhães D, de Castro FJ, et al. (2008). A preliminary characterization of the mutagenicity of atmospheric particulate matter collected during sugar cane harvesting using the *Salmonella*/microsome microsuspension assay. *Environ. Mol. Mutagen.* 49: 249-255.
- DeMarini DM, Brooks LR, Warren SH, Kobayashi T, et al. (2004). Bioassay-directed fractionation and *Salmonella* mutagenicity of automobile and forklift diesel exhaust particles. *Environ. Health Perspect* 112: 814-819.
- Ducatti A and Vargas VM (2003). Mutagenic activity of airborne particulate matter as an indicative measure of atmospheric pollution. *Mutat. Res.* 540: 67-77.
- Kado NY, Guirguis GN, Flessel CP, Chan RC, et al. (1986). Mutagenicity of fine (less than 2.5 microns) airborne particles: diurnal variation in community air determined by a *Salmonella* micro preincubation (microsuspension) procedure. *Environ. Mutagen.* 8: 53-66.
- Maron DM and Ames BN (1983). Revised methods for the *Salmonella* mutagenicity test. *Mutat. Res.* 113: 173-215.
- Menck HR, Casagrande JT and Henderson BE (1974). Industrial air pollution: possible effect on lung cancer. *Science* 183: 210-212.
- Pereira TS, Gotor GN, Beltrami LS, Nolla CG, et al. (2010). *Salmonella* mutagenicity assessment of airborne particulate matter collected from urban areas of Rio Grande do Sul State, Brazil, differing in anthropogenic influences and polycyclic aromatic hydrocarbon levels. *Mutat. Res.* 702: 78-85.
- Rainho CR, Velho AMA, Aiub CAF and Corrêa SM (2012). Evaluation of Urban Airborne Particulate Matter (PM 2.5) in the City of Rio de Janeiro (Brazil) by Mutagenicity Assays. In: Essays on Environmental Studies (Sarin A, ed.). Athens Institute for Education and Research, Athens, 1-8.
- Rainho CR, Velho AMA, Aiub CAF, Corrêa SM, et al. (2013a). Health risk prediction induced by polycyclic aromatic hydrocarbons present in respirable urban airbone in Rio de Janeiro (Brazil). *Genet. Mol. Res.* 12: 3992-4002.
- Rainho CR, Corrêa SM, Mazzei JL, Aiub CAF, et al. (2013b). Genotoxicity of polycyclic aromatic hydrocarbons and nitro-derived in respirable airborne particulate matter collected from urban areas of Rio de Janeiro (Brazil). *BioMed Res. Int.* 2013: 1-9.
- Rosenkranz HS and Mermelstein R (1980). The *Salmonella* Mutagenicity and the *E. coli* Pol A+/Pol A1- Repair Assays: Evaluation of Relevance to Carcinogenesis. In: The Predictive Value of *in vitro* Short-term Screening Tests in the Evaluation of Carcinogenicity (Williams GM, Kroes R, Waaijers HW and Van de Poll KW, eds.). Elsevier, Amsterdam, 5-26.
- Rosenkranz HS and Mermelstein R (1983). Mutagenicity and genotoxicity of nitroarenes. All nitro-containing chemicals were not created equal. *Mutat. Res.* 114: 217-267.
- Sato MI, Valent GU, Coimbrão CA, Coelho MC, et al. (1995). Mutagenicity of airborne particulate organic material from urban and industrial areas of São Paulo, Brazil. *Mutat. Res.* 335: 317-330.
- Vargas VMF, Horn RC, Guidobono RR, Mittelstaedt AB, et al. (1998). Mutagenic activity of airborne particulate matter from the urban area of Porto Alegre, Brazil. *Genet. Mol. Biol.* 21: 247-253.
- Wang CY, Lee MS, King CM and Warner PO (1980). Evidence for nitroaromatics as direct-acting mutagens of airborne particulates. *Chemosphere* 9: 83-87.
- Watanabe M, Ishidate M Jr and Nohmi T (1989). A sensitive method for the detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of *Salmonella typhimurium* strains TA98 and TA100. *Mutat. Res.* 216: 211-220.
- Watanabe M, Ishidate M Jr and Nohmi T (1990). Sensitive method for the detection of mutagenic nitroarenes and aromatic amines: new derivatives of *Salmonella typhimurium* tester strains possessing elevated O-acetyltransferase levels. *Mutat. Res.* 234: 337-348.

## **APENDICE E - Seasonal variations in the level of mutagenicity: an assessment of respirable particulate matter in Rio de Janeiro, Brazil**

# **Seasonal variations in the level of mutagenicity: an assessment of respirable particulate matter in Rio de Janeiro, Brazil**

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## **Abstract**

Respirable particles (PM2.5) can become associated with organic matter containing several compounds such as polycyclic aromatic hydrocarbons (PAHs). Many PAHs have been identified as cancer-inducing chemicals. The mutagenicity of airborne particles is generally associated with PAHs, but recent reviews show that PAHs may not be the predominant mutagens in atmospheric pollution, and that nitroaromatic compounds, aromatic amines and aromatic ketones, often found in moderately polar or highly polar organic fractions, are potent mutagens. Nitro-polycyclicaromatics (nitro-PAHs) are persistent environmental mutagens and can be found in airborne suspended particles from direct sources such as diesel and gasoline exhausts, or may be products of atmospheric reactions in the presence of NO<sub>2</sub> and NO<sub>3</sub> radicals. In the present work we compared PAH levels and mutagenicity using gas chromatography spectrometry and the *Salmonella*/microsome assay on organic extracts of PM2.5 for *Salmonella typhimurium* strain TA98. The samples were collected in two periods: (I) July to October 2010 and (II) November 2010 to May 2011 at three sites in Rio de Janeiro – (1) low urban traffic at the University campus; (2) heavy



urban traffic at Brasil Avenue and (3) Rebouças tunnel. We also performed measurements of nitro-PAH levels for November 2010 to May 2011. For both periods, site 3 showed the highest concentrations of PAHs and nitro-PAHs. Period I showed the higher values of rev/m<sup>3</sup>. Mutagenic frameshift responses in the absence of metabolic activation were detected at all the sites in periods I and II. In the presence of metabolic activation this response was observed for all three sites in period I, but only for site 3 in period II. Nitroarenes and dinitroarenes were detected at all three sites in period I. In period II, the presence of nitroarenes was also detected at all sites, but dinitroarenes were only detected at sites 2 and 3. The information generated in this study shows that different levels of PAHs and nitroderivatives, influenced by seasonal variations in climatic conditions, probably contribute to the detected airborne mutagenicity.

*Keywords:* *seasonal variations, mutagenicity assessment, respirable particulate matter, polycyclic aromatic hydrocarbons, nitro-polycyclicaromatics.*

## 1 Introduction

Respirable particles  $\leq 2.5 \mu\text{m}$  (PM2.5) originating from motor vehicle exhaust fumes are a major source of particulate matter, with potentially serious health impacts [1]. These pollutants can become associated with organic matter containing several compounds, such as polycyclic aromatic hydrocarbons (PAHs) [2]. Many PAHs have been identified as cancer-inducing chemicals [2]. Recent reviews show that PAHs are not the predominant mutagens in atmospheric pollution. Nitroaromatic compounds, aromatic amines and aromatic ketones, often found in moderately polar or highly polar fractions, are potent mutagens [3, 4]. Nitro-polycyclicaromatics (nitro-PAHs) are persistent environmental mutagens and can be found in suspended airborne particles from direct sources such as diesel and gasoline exhaust fumes, or may be products of atmospheric reactions in the presence of NO<sub>2</sub> and NO<sub>3</sub> radicals [4–6].

In Rio de Janeiro, climatic conditions vary during the year, with precipitation occurring mainly between the months of December and March (rainy season) and scarcely at all in July and August (dry season) [7]. Critical air pollutants, such as PAHs and PM, increase during the dry season and decrease during the rainy season [8]. In 2010, we conducted a monitoring study at three sites of the city of Rio de Janeiro: the campus of the Rio de Janeiro State University, Avenida Brasil and Rebouças tunnel [9, 10]. In these studies we detected values for PM2.5 that exceeded the values established by the World Health Organization [11] at all three sites. Furthermore, mutagenicity was detected in the organic extract of PM2.5 for *Salmonella typhimurium* strain TA98 and derivatives with sensitivity to nitro compounds [9, 10].

To study the influence of seasonal variations on the mutagenic activity of PM samples, we analyzed samples collected between November 2010 and May 2011 at the same sites and compared them with the data from our previous studies [9, 10].



## 2 Materials and methods

### 2.1 Sampling sites

The samples were collected at three sites in Rio de Janeiro: the campus of the Rio de Janeiro State University (site 1), Avenida Brasil (site 2) and Rebouças tunnel (site 3) between November 2010 and May 2011 (Period II). Site 1, with low traffic, is located in a residential area of the city's north zone. Site 2 has heavy traffic (~250,000 vehicles/day) and is the city's biggest highway, covering 58 km in length and crossing 27 neighborhoods. Site 3 has heavy traffic (~190,000 vehicles/day). It connects the north and south zones of the city and is 2.8 km long [9, 10].

Airborne PM<sub>2.5</sub> samples were collected on fiberglass filters (E558 X 10IN, 254mm x 203mm) using a high-volume collector (AVG MP 2.5, 1.13 m<sup>3</sup>/min) for 24h at sites 1 and 2, and 6h at site 3. Four monthly samplings were performed for each site. The filters were weighed and stabilized before and after samples (45% humidity) for the determination of particulate concentration, expressed in µg/m<sup>3</sup> units of sampled air [12–14]. At the end of the sampling, the filters were combined to form a pool sample.

### 2.2 Extraction of organic compounds

Half of each filter was sonicated in three rounds of 10 min each using dichloromethane (DCM, CASRN. 75-09-2, Tedia Brazil, Brazil, purity 99.9%). The extracts were concentrated to 15 mL in a rotating evaporator and filtered in a Teflon membrane (0.5 µm). The concentration of extractable organic matter (EOM, in µg/m<sup>3</sup>) was calculated. Prior to bioassays, the organic extract was dried at 4°C and resuspended in 5 µl dimethyl sulfoxide (DMSO, CASRN. 67-68-5, Synth, Brazil, purity 99.9%) [12–14].

### 2.3 Analysis of polycyclic aromatic hydrocarbons (PAHs)

PAHs were identified and quantified by gas chromatography/mass spectrometry (GC/MS) using a Varian system consisting of a gas chromatograph (450-GC) with a split/splitless injector 1177S/SL (kept at 300°C) coupled to the mass spectrometer detector (MS 220). The ion trap (250°C), manifold (280°C) and transfer line (280°C) were maintained at constant temperatures. PAHs were identified by mass similarity and by the retention time of the components in a commercial standard kit (Supelco, PAH610-S). Quantification was based on five calibration points, which were constructed from each standard for all the target analytes, ranging from 10 to 250 pg/µL. Injections (2.0 µL) were splitless, with the split opened after 0.5 min, and helium 5.0 was used as the carrier gas. A VF-5MS column (30 m × 0.25 mm × 0.25 µm) was employed. The column and septum purge flows were set at 1.6 and 3 mL/min, respectively. The oven temperature program was as follows: 70°C for 4 min then heating to 300°C at 10°C/min. This procedure was designed for the analysis of the 16 priority PAHs, but only six were detected: phenanthrene, fluoranthene, pyrene, benzo[a]anthracene, chrysene and benzo[a]pyrene. The limits of quantification

were determined from the minimum point in the calibration curves. Limits of detection were determined from PAH concentrations, which resulted in a signal-to-noise ratio of 3:1. The results were expressed in ng/m<sup>3</sup> [15].

#### **2.4 Analysis of nitro-polycyclic aromatic hydrocarbons (nitro-PAHs)**

Nitro-PAHs were identified and quantified by GC/MS using a Varian system consisting of a gas chromatograph (450-GC) with a Programmed Temperature Vaporization Injector 1079 (PTV) starting at 75°C for 0.2 min then heated at 200°C/min until 340°C. The mass spectrometer detector (MS 220) was operated under the following conditions: ion trap (250°C), manifold (280°C) and transfer line (280°C) were maintained at constant temperatures. Nitro-PAHs were identified by mass similarity and by the retention time of the components in a commercial standard kit (Supelco, PAH610-S). Quantification was based on five calibration points in duplicate, which were constructed from each standard for all the target analytes, ranging from 25 to 400 ppb. Injections (50 µL) were splitless, with the split opened after 0.5 min, and helium 5.0 was used as the carrier gas at a constant flow of 1.2 mL/min. A VF-5MS column (30 m × 0.25 mm × 0.25 µm) was employed. The oven temperature program was as follows: 70°C for 2 min then heating to 210°C at 10°C/min, then heating to 300°C at 20°C/min. This procedure was designed for the analysis of 13 nitro-PAHs: nitro-naphthalene, nitro-acenaphthylene, nitro-acenaphthene, nitro-fluorene, nitro-phenanthrene, nitro-anthracene, nitro-fluoranthene, nitro-pyrene, nitro-benzo[a]anthracene, nitro-chrysene, nitro-benzo[b]fluoranthene, nitro-benzo[k]fluoranthene and nitro-benzo[a]pyrene. The limits of quantification were determined from the minimum point in the calibration curves. Limits of detection were determined from nitro-PAH concentrations, which resulted in a signal-to-noise ratio of 3:1. The results were expressed in ng/m<sup>3</sup>.

#### **2.5 Salmonella/microsome assay**

The organic extracts were assayed for mutagenicity using the microsuspension version [16] of the *Salmonella/microsome* assay [17]. *Salmonella typhimurium* TA98 (frameshift strain) and the derivative strains YG1021 (nitroreductase-overproducing) and YG1024 (*O*-acetyltransferase-overproducing) [18] were used, with and without metabolic activation (S9 mix fraction). Five concentrations of each sample (10, 20, 30, 40 and 50 µg/plate) were tested in triplicate. The samples were pre-incubated for 90 min. All assays were carried out under yellow light and in the presence of negative (dimethyl sulfoxide - DMSO solvent, 5 µL/plate) and positive (4-nitroquinoline oxide, 0.5 µg/plate, CASRN. 56-57-5, and 2-aminofluorene, 1 µg/plate, CASRN. 153-78-6, from Sigma-Aldrich, St. Louis, MO, USA) controls. Plates were incubated in the dark at 37°C for 72h, after which time revertants were counted. The sample was considered positive when a mutagenesis value of at least twice the negative value, a significant ANOVA ( $p < 0.05$ ) and a positive dose-response rate ( $p < 0.05$ ) were observed. The results of the different assays were analyzed via the SALANAL program (Salmonella Assay Analysis, version 1.0, Integrated Laboratory Systems of Research Triangle Institute, RTP, North Carolina, USA).

The choice between linear regression and the Bernstein model [19] was made to allow the elimination of data for doses outside the linear portion of the dose-response curve. Positive results were interpreted as presenting significant mutagenicity, and were expressed as the number of revertants per volume of air sampled ( $\text{rev}/\text{m}^3$ ), i.e.  $\text{rev}/\mu\text{g}$  multiplied by EOM in  $\mu\text{g}/\text{m}^3$ . In the cytotoxicity test, the solution containing the sample and the bacterial culture (100-200 cells) were plated on nutrient agar plates and incubated at 37°C for 24 h and the surviving colonies were counted. The sample was considered cytotoxic if the percentage of surviving cells was less than 60% of the negative control after one or more doses [14].

### 3 Results

#### 3.1 Airborne particulate matter

Table 1 shows the air volume ( $\text{m}^3$ ), PM2.5 concentration ( $\mu\text{g}/\text{m}^3$ ), and extractable organic matter (EOM) ( $\mu\text{g}/\text{m}^3$ ) of the samples analyzed from the different sites between November 2010 and May 2011.

The highest average PM2.5 values were detected at site 3 (54 to 141  $\mu\text{g}/\text{m}^3$ ), followed by site 2 (27 to 38  $\mu\text{g}/\text{m}^3$ ) and site 1 (25 and 34  $\mu\text{g}/\text{m}^3$ ) (Table 1).

Table 1: Collection sites, air volume, PM 2.5  $\mu\text{m}$  concentration and extractable organic matter (EOM) of the samples analyzed.

Site	Month	Air Volume ( $\text{m}^3$ ) $\pm$ S.D.	PM 2.5 $\mu\text{m}$ ( $\mu\text{g}/\text{m}^3$ ) $\pm$ S.D.	EOM ( $\mu\text{g}/\text{m}^3$ )
1	Nov	1567 $\pm$ 112	25 $\pm$ 15	8.62
	Dec	1538 $\pm$ 25	15 $\pm$ 7	1.46
	Jan	1568 $\pm$ 50	17 $\pm$ 2	13.89
	Feb	1538 $\pm$ 12	14 $\pm$ 8	0.35
	Apr	1530 $\pm$ 11	19 $\pm$ 4	0.68
	May	1524 $\pm$ 66	34 $\pm$ 22	4.19
2	Nov	1552 $\pm$ 9	31 $\pm$ 8	8.70
	Dec	1536 $\pm$ 39	25 $\pm$ 9	3.90
	Jan	1568 $\pm$ 50	28 $\pm$ 12	6.22
	Feb	1580 $\pm$ 38	27 $\pm$ 10	10.92
	Apr	1519 $\pm$ 31	38 $\pm$ 20	3.21
	May	1490 $\pm$ 49	27 $\pm$ 11	10.73
3	Nov	379 $\pm$ 5	74 $\pm$ 24	29.66
	Dec	442 $\pm$ 10	70 $\pm$ 50	49.20
	Jan	397 $\pm$ 16	68 $\pm$ 20	54.85
	Feb	473 $\pm$ 67	62 $\pm$ 25	1.58
	Apr	415 $\pm$ 458	141 $\pm$ 44	3.49
	May	458 $\pm$ 47	54 $\pm$ 27	3.16

1 – UERJ; 2 – Avenida Brasil; 3 – Rebouças tunnel. S.D.= standard deviation. Airborne PM 2.5 $\mu\text{m}$  samples were collected for 24h at sites 1 and 2, and 6h at site 3. 6h time filter saturation at site 3. No collections in March.



### 3.2 Analysis of polycyclic aromatic hydrocarbons (PAHs)

Figure 1 shows PAH concentrations, in ng/m<sup>3</sup>, at the three sites during period II.

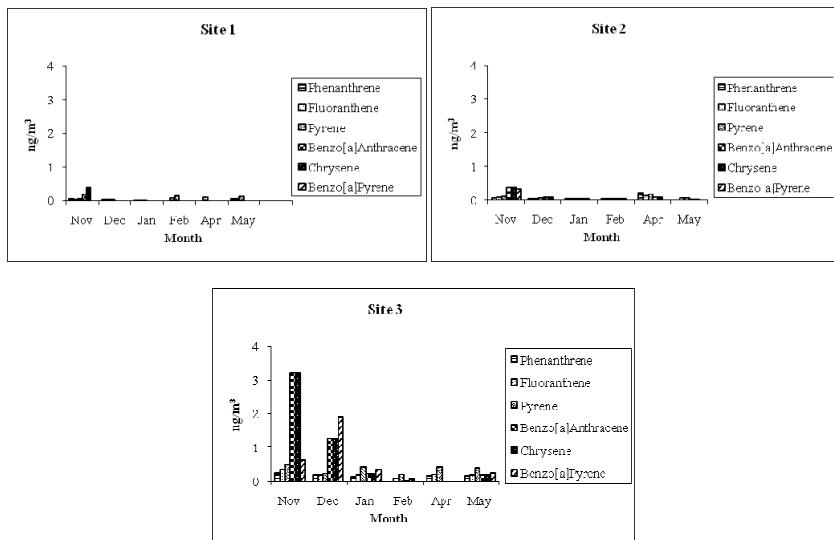


Figure 1: PAH concentrations in ng/m<sup>3</sup> of the three sites.

The highest concentrations of PAHs were at site 3, where benzo[a]anthracene (3.23 ng/m<sup>3</sup>) and chrysene (3.23 ng/m<sup>3</sup>) were predominant in November, and benzo[a]pyrene (1.90 ng/m<sup>3</sup>) was predominant in December.

### 3.3 Analysis of nitro-polycyclic aromatic hydrocarbons (nitro-PAHs)

Figure 2 shows the nitro-PAH concentrations, in ng/m<sup>3</sup>, at the three sites.

Site 3 showed the highest concentrations of nitro-PAHs. The predominant nitro-PAHs at site 3 were: (November) nitro-pyrene (2.52 ng/m<sup>3</sup>), nitro-phenanthrene (2.30 ng/m<sup>3</sup>), nitro-acenaphthene (2.10 ng/m<sup>3</sup>), nitro-acenaphthylene (2.07 ng/m<sup>3</sup>), nitro-anthracene (2.06 ng/m<sup>3</sup>) and nitro-fluorene (1.97 ng/m<sup>3</sup>); (December) nitro-anthracene (1.56 ng/m<sup>3</sup>), nitro-phenanthrene (1.46 ng/m<sup>3</sup>) and nitro-fluorene (1.39 ng/m<sup>3</sup>); (January) nitro-benzo[k]fluoranthene (2.28 ng/m<sup>3</sup>), nitro-acenaphthylene (2.20 ng/m<sup>3</sup>), nitro-fluorene (1.87 ng/m<sup>3</sup>), nitro-phenanthrene (1.86 ng/m<sup>3</sup>) and nitro-anthracene (1.71 ng/m<sup>3</sup>); (February) nitro-benzo[a]pyrene (1.86 ng/m<sup>3</sup>), nitro-benzo[b]fluoranthene (1.55 ng/m<sup>3</sup>) and nitro-chrysene (1.48 ng/m<sup>3</sup>); (April) nitro-benzo[b]fluoranthene (2.08 ng/m<sup>3</sup>) and nitro-phenanthrene (1.93 ng/m<sup>3</sup>); (May) nitro-phenanthrene (1.73 ng/m<sup>3</sup>), nitro-fluorene (1.93 ng/m<sup>3</sup>) and nitro-anthracene (1.34 ng/m<sup>3</sup>).

The predominant nitro-PAHs at site 2 were: (November, January and February) nitro-chrysene (0.57–0.84 ng/m<sup>3</sup>) and nitro-benzo[b]fluoranthene

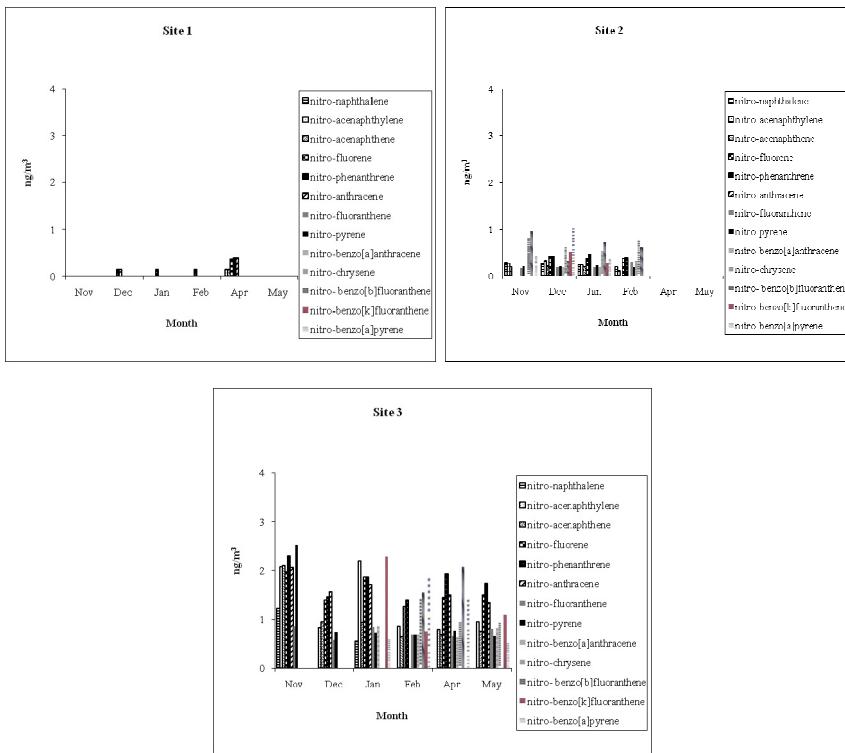


Figure 2: Nitro-PAH concentrations in  $\text{ng}/\text{m}^3$  of the three sites.

(0.61–0.96  $\text{ng}/\text{m}^3$ ); (December) nitro-chrysene (0.65  $\text{ng}/\text{m}^3$ ) and nitro-benzo[a]pyrene (1.04  $\text{ng}/\text{m}^3$ ). No nitro-PAHs were detected at site 2 in April or May.

The predominant nitro-PAHs at site 1 were: (December) nitro-phenanthrene (0.14  $\text{ng}/\text{m}^3$ ) and nitro-anthracene (0.15  $\text{ng}/\text{m}^3$ ); (January) nitro-phenanthrene (0.14  $\text{ng}/\text{m}^3$ ); (February) nitro-phenanthrene (0.14  $\text{ng}/\text{m}^3$ ); (April) nitro-phenanthrene (0.39  $\text{ng}/\text{m}^3$ ), nitro-anthracene (0.39  $\text{ng}/\text{m}^3$ ) and nitro-fluorene (0.37  $\text{ng}/\text{m}^3$ ). No nitro-PAHs were detected at site 1 in November or May.

### 3.4 Salmonella/microsome assay

Table 2 shows the mutagenicity data for the organic extracts from airborne particulate matter in  $\text{rev}/\text{m}^3$  during period II. Cytotoxic effects were not detected for any of the samples analyzed.

Mutagenic frameshift responses in the presence of metabolic activation were detected only at site 3 (November, December and May) (Table 2).

Mutagenic frameshift responses in the absence of metabolic activation were detected at site 1 (November, December and April), site 2 (December, January, February, April and May) and site 3 (November and April) (Table 2).

Table 2: Induced mutagenicity by airborne particulate matter organic extracts (rev/m<sup>3</sup>).

Site	Month	TA98	-S9	+S9	YGI021	-S9	+S9	YGI024	-S9	+S9
1	Nov	9.10 ± 1.30	n.d. <sup>a</sup>	9.10 ± 1.50	7.00 ± 1.80	n.d. <sup>a</sup>				
	Dec	1.50 ± 0.40	n.d. <sup>a</sup>	3.10 ± 0.40	1.20 ± 0.20	n.d. <sup>a</sup>				
	Jan	n.d. <sup>a</sup>								
	Feb	n.d. <sup>a</sup>								
	Apr	0.10 ± 0.01	n.d. <sup>a</sup>	0.20 ± 0.10	0.80 ± 0.10	n.d. <sup>a</sup>				
	May	n.d. <sup>a</sup>								
	Nov	n.d. <sup>a</sup>	19.20 ± 1.70	4.70 ± 0.60						
	Dec	2.10 ± 0.30	n.d. <sup>a</sup>	4.10 ± 1.00	3.50 ± 0.90	13.60 ± 1.90	4.80 ± 0.80			
	Jan	4.40 ± 0.70	n.d. <sup>a</sup>	2.80 ± 0.40	n.d. <sup>a</sup>	16.00 ± 1.40	3.40 ± 0.60			
	Feb	4.60 ± 1.00	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>	3.90 ± 1.10	n.d. <sup>a</sup>			
2	Apr	4.80 ± 0.60	n.d. <sup>a</sup>	2.50 ± 0.20	0.30 ± 0.10	2.80 ± 0.60	2.20 ± 0.40			
	May	18.80 ± 3.30	n.d. <sup>a</sup>	21.70 ± 3.40	16.60 ± 2.60	30.90 ± 1.80	10.60 ± 1.10			
	Nov	44.20 ± 24.30	49.80 ± 7.10	45.40 ± 13.60	34.70 ± 7.70	83.00 ± 5.00	8.60 ± 3.90			
	Dec	n.d. <sup>a</sup>	19.20 ± 4.90	n.d. <sup>a</sup>	n.d. <sup>a</sup>	51.70 ± 5.40	n.d. <sup>a</sup>			
	Jan	n.d. <sup>a</sup>	n.d. <sup>a</sup>	20.30 ± 4.90	n.d. <sup>a</sup>	21.90 ± 6.00	n.d. <sup>a</sup>			
3	Feb	n.d. <sup>a</sup>	n.d. <sup>a</sup>	2.50 ± 0.50	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>			
	Apr	17.00 ± 2.40	n.d. <sup>a</sup>	12.30 ± 2.40	n.d. <sup>a</sup>	8.20 ± 1.30	n.d. <sup>a</sup>			
	May	n.d. <sup>a</sup>	5.80 ± 1.30	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>	n.d. <sup>a</sup>			

1 – UERJ; 2 – Avenida Brasil; 3 – Rebouças tunnel. n.d.<sup>a</sup> – not detected. Negative Control: DMSO for the mutagenicity assay without S9 mix were: TA98, (1.8±8); YGI021, (28±4); YGI024, (17±2). For the mutagenicity assay with S9 mix were: TA98, (38±7); YGI021, (32±8); YGI024, (20±7). Positive Controls for the mutagenicity assay without S9 mix were: 4-nitroquinoline oxide (0.5 µg/plate) for TA98, (42±8); YGI021, (60±2); YGI024, (101±3). For the mutagenicity assay with S9 mix were: 2-aminofluorene (1 µg/plate) for TA98, (134 ± 4); YGI021, (687±61); YGI024, (67±13).



The presence of nitroarenes was detected at site 1 in November, December and April; at site 2 in December and May; and at site 3 in November, January and February. The presence of dinitroarenes and hydroxylamines was detected at site 2 in November, December, January and May, and at site 3 in November, December and January.

## 4 Discussion

The average PM2.5 concentrations at all three sites were higher during period II than recommended by the WHO (site 3: 54–141  $\mu\text{g}/\text{m}^3$ ; site 2: 27–38  $\mu\text{g}/\text{m}^3$ ; site 1: 25 and 34  $\mu\text{g}/\text{m}^3$ ), which recommends a daily mean of up to 25  $\mu\text{g}/\text{m}^3$ [11]. Vehicle emissions are a major source of particles, especially in urban areas [20–22]. The fleet of vehicles in Rio de Janeiro has tripled in the last twenty years, and the high values of PM2.5 can be related to this fact [10]. Sites 2 and 3 are areas of the city that have heavy traffic, and they are the sites that yielded the highest average levels of PM2.5. Site 3 is a tunnel, and besides the heavy traffic it also lacks adequate ventilation, which could hamper the dispersion of pollutants.

High levels of PM2.5 were also detected during period I (site 3: 94–132  $\mu\text{g}/\text{m}^3$ ; site 2: 26–60  $\mu\text{g}/\text{m}^3$ ; site 1: 30–36  $\mu\text{g}/\text{m}^3$ ) at these same sites [9, 10]. When comparing the two periods (July to October 2010 and November 2010 to May 2011), we observed higher PM2.5 values in period I. A study conducted from August 2010 to March 2011 in Porto Alegre, Brazil, detected high concentrations of PM2.5 in August [4]. The increased values of PM at this time of year can be related to the winter weather conditions. The reduction in PM2.5 values at site 3 during January and February 2011 (period II) may be related to the summer school vacations, during which time there are fewer vehicles on the roads.

PAHs are formed from the incomplete combustion of organic matter and may be introduced into the environment from several sources. Their main source in urban areas, according to Reisen and Arey [23], is exhaust gases from internal combustion engines, especially diesel [14]. Owing to their utility as tracers, it is essential to document their atmospheric abundance to identify the sources in different environments [24].

The highest PAH concentrations detected at site 3 are caused by vehicular emissions and restricted ventilation. These PAHs are classified according to their carcinogenicity by the International Agency for Research on Cancer [25]: benzo[a]pyrene is in Group 1 (carcinogenic to humans), and benzo[a]anthracene and chrysene are in Group 2B (possibly carcinogenic to humans).

Although site 2 has heavy traffic, it is in an area that has many dispersion factors, such as wind and rain, which could explain why its PAH levels are lower than at site 3. When comparing the two periods, we observed similar PAH values at sites 1 and 2 [10]. However, in November (during Period II), only benzo[a]anthracene and chrysene were detected at site 1, and only benzo[a]pyrene was detected at site 2. Periods with high temperatures and photochemical decomposition are propitious for PAHs dispersal. Several studies

that monitor levels of PAHs have detected a reduction in these pollutants during the summer [26–28].

Site 3 had the highest values for benzo[a]anthracene, chrysene and benzo[a]pyrene during period II (November and December). The increase in these PAHs may be related to gasoline and diesel vehicular emissions [29, 30].

Nitro-PAHs present in the atmosphere originate from primary sources, such as vehicle emissions, especially from diesel-fueled vehicles [31–33]. In addition, nitro-PAHs are also formed in the atmosphere via a reaction of their parent PAHs initiated by hydroxyl (OH) radicals during the day and by nitrate ( $\text{NO}_3^-$ ) radicals (in the presence of  $\text{NO}_x$ ) during the night [33–35] and/or the heterogeneous gas–particle interaction of the parent PAHs adsorbed onto particles with nitrating agents [36]. Nitro-PAHs have  $2.10^5$  times the mutagenic and ten times the carcinogenic potential of PAHs [33, 37].

In period I no nitro-PAH analysis was performed, which prevents a comparison between the two periods. Site 3 showed the highest concentrations of nitro-PAHs. This result may be related to vehicular emissions and inadequate ventilation. The nitro-PAH levels detected at site 3 were up to 21 times higher than in other tunnels: Allegheny Mountain ( $0.12 \text{ ng/m}^3$  1-nitropyrene) [38]; Baltimore Harbour ( $0.34 \text{ ng/m}^3$  1-nitropyrene and  $0.29 \text{ ng/m}^3$  9-nitroanthracene) [39] and Queensway ( $0.56 \text{ ng/m}^3$  1-nitropyrene and  $0.36 \text{ ng/m}^3$  9-nitroanthracene) [40].

Meanwhile, site 1 was found to have the lowest nitro-PAH values. This may be related to the lower automotive emissions than at the other two sites under study. The highest nitro-PAH values were at site 2, especially during the hottest months of the year. Similar nitro-PAH values were detected during the summer in the metropolitan area of Porto Alegre [33]. These results may be related to the interaction between PAHs and nitro compounds in photochemical smog.

All strains detected higher  $\text{rev/m}^3$  in period I [9, 10] than in period II at all three sites. There was less rainfall in period I than in period II. This seasonal characteristic could lead to a concentration of pollutants and thereby increased values of  $\text{rev/m}^3$ . Increased mutagenic responses during the winter have been detected in several studies that evaluate the mutagenicity of PM seasonally [2, 4, 26]. Decreased levels of  $\text{rev/m}^3$  were detected mainly in the rainy months (December 2010 to February 2011 – period II). Rain cleanses some particles from the atmosphere, but this does not mean that there is no further risk of exposure to contaminants associated with the particles that remain in the environment [2, 41, 42].

For period I, mutagenic frameshift responses in the presence of metabolic activation were detected at all three sites [9, 10]. However for period II mutagenic frameshift responses in the presence of metabolic activation were detected only at site 3. These results can be attributed to the presence of promutagens such as PAH, resulting from intense vehicular emissions and inadequate ventilation in this tunnel.

Mutagenic frameshift responses in the absence of metabolic activation were detected at all the sites during periods I [9] and II. These results could indicate the predominance of direct-acting frameshift activity in the airborne particulate

material. Several authors have recognized the contribution of nitrocompounds to direct frameshift mutagenicity in urban atmospheric samples, associating this capacity with the presence of PAH derivatives, such as mono- or dinitro PAHs [3, 4, 12]. The contribution of nitrocompounds to direct mutagenic activity was investigated through the *Salmonella/microsome* assay with specific strains YG1021 (pYG216) and YG1024 (pYG219), which over express highly active enzymes with a high sensitivity to nitrocompounds such as nitroarenes or dinitroarenes, hydroxylamines and aromatic amines [18]. In period I, nitroarenes and dinitroarenes were detected at all three sites [9, 10]. During period II, the presence of nitroarenes was also detected at all the sites, but dinitroarenes were only detected at sites 2 and 3. Nitroarenes and dinitroarenes result from direct emissions of diesel combustion and can be produced by atmospheric reactions of PAH with gaseous copollutants found in photochemical smog [43, 44]. Studies performed in the urban region of Porto Alegre detected a similar response during the hot season, when there is marked mutagenic activity due to the presence of mono and dinitroarene compounds in different sizes of atmospheric particles such as PTS, PM10 and PM2.5 [4, 45].

## 5 Conclusion

In conclusion, air quality in Rio de Janeiro is worsening as traffic becomes heavier. The data generated in this study show that in certain periods of the year, different levels of PAHs and nitroderivatives probably contribute to the airborne mutagenicity detected at different sites of Rio de Janeiro. However, less variation in the levels of PAHs and nitroderivatives was found in Rio de Janeiro than has been reported in other places where winter temperatures are lower. This may be attributed to the fact that the city of Rio de Janeiro does not have wide temperature fluctuations during the year.

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## References

- [1] Oh, S.M., Kim, H.R., Park, Y.J., Lee, S.Y. & Chung, K.H., Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells). *Mutation Research*, 723(2), pp. 142–151, 2011.



- [2] Pereira, T.S., Beltrami, L.S., Rocha, J.A.V., Broto, F.P., Comellas, L.R., Salvadori, D.M.F. & Vargas, V.M.F., Toxicogenetic monitoring in urban cities exposed to different airborne contaminants. *Ecotoxicology and Environmental Safety*, 90, pp. 174–182, 2013.
- [3] Claxton, L.D., Matthews, P. & Warren, S., The genotoxicity of ambient outdoor air, a review: *Salmonella* mutagenicity. *Mutation Research*, 567(2-3), pp. 347–399, 2004.
- [4] De Brito, K.C.T., de Lemos, C.T., Rocha, J.A.V., Mielli, A.C., Matzenbacher, C. & Vargas, V.M.F., Comparative genotoxicity of airborne particulate matter (PM2.5) using *Salmonella*, plants and mammalian cells. *Ecotoxicology and Environmental Safety*, 94, pp. 14–20, 2013.
- [5] De Martinis, B.S., Kado, N.Y., Carvalho, L.R.F., Okamoto, R.A. & Gundel, L.A., Genotoxicity of fractionated organic material in air borne particles from São Paulo, Brazil. *Mutation Research*, 446(1), pp. 83–94, 1999.
- [6] Lewtas, J., Air pollution combustion emissions: characterization of causative agents and mechanisms associated with cancer, reproductive, and cardiovascular effects. *Mutation Research*, 636(1-3), pp. 95–133, 2007.
- [7] Alerta Rio. Relatórios Anuais de Chuva. [www0.rio.rj.gov.br/alertario/?page\\_id=1110](http://www0.rio.rj.gov.br/alertario/?page_id=1110).
- [8] García-Suástegui, W.A., A., Huerta-Chagoya, K.L., Carrasco-Colín, M.M., Pratt, John, K., Petrosyan, P., Rubio, J., Poirier, M.C. & Gonsebatt, M.E., Seasonal variations in the levels of PAH-DNA adducts in young adults living in Mexico City. *Mutagenesis*, 26(3), pp. 385–391, 2011.
- [9] Rainho, C.R., Velho, A.M.A., Corrêa, S.M., Mazzei, J.L., Aiub, C.A.F. & Felzenszwab, I., Prediction of health risk due to polycyclic aromatic hydrocarbons present in urban air in Rio de Janeiro, Brazil. *Genetics and Molecular Research*, 12(3), pp. 3992–4002, 2013a.
- [10] Rainho, C.R., Corrêa, S.M., Mazzei, J.L., Aiub, C.A.F. & Felzenszwab, I., Genotoxicity of Polycyclic Aromatic Hydrocarbons and Nitro-Derived in Respirable Airborne Particulate Matter Collected from Urban Areas of Rio de Janeiro (Brazil). *BioMed Research International*, 2013, pp. 1–9, 2013b.
- [11] WHO (World Health Organization), Health Risks of Particulate Matter From Long Range Transboundary Air Pollution, Copenhagen, 99 pages, 2006.
- [12] Vargas, V. M. F., Horn, R.C., Guidobono, R.R., Mittelstaedt, A.B. & de Azevedo, I.G., Mutagenic activity of airborne particulate matter from the urban area of Porto Alegre, Brazil. *Genetics and Molecular Biology*, 21(2), pp. 247–253, 1998.
- [13] Coronas, M.V., Horn, R.C., Ducatti, A., Rocha, J.A.V. & Vargas, V.M.F., Mutagenic activity of airborne particulate matter in a petrochemical industrial area. *Mutation Research*, 650(2), 196–201, 2008.

- [14] Pereira, T.S., Gotor, G.N., Beltrami L.S., et al., *Salmonella* mutagenicity assessment of airborne particulate matter collected from urban areas of Rio Grande do Sul State, Brazil, differing in anthropogenic influences and polycyclic aromatic hydrocarbon levels. *Mutation Research*, 702(1), pp. 78–85, 2010.
- [15] Corrêa, S.M. & Arbillia, G., A two-year monitoring program of aromatic hydrocarbons in Rio de Janeiro downtown area. *Journal of the Brazilian Chemical Society* 18(3), pp. 539–543, 2007.
- [16] Kado, N.Y., Guirguis, G.N., Flessel, C.P., Chan, R.C., Chang, K. & Wesolowski, J.J., Mutagenicity of fine (<2.5  $\mu\text{m}$ ) airborne particles: diurnal variation in community air determined by a *Salmonella* micro preincubation (microsuspension) procedure. *Environmental Mutagenesis*, 8(1), pp. 53–66, 1986.
- [17] Maron, D.M. & Ames, B.N., Revised methods for the *Salmonella* mutagenicity test. *Mutation Research*, 113(3-4), 173–215, 1983.
- [18] Watanabe, M., Ishidate, M. & Nohmi, T., A sensitive method for the detection of mutagenic nitroarenes: construction of nitroreductase-overproducing derivatives of *Salmonellatyphimurium* strains TA98 and TA100. *Mutation Research*, 216(4), pp. 211–220, 1989.
- [19] Bernstein L., Kaldor, J., McCann, J. & Pike, M.C., An empirical approach to the statistical analysis of mutagenesis data from the *Salmonella* test. *Mutation Research*, 97(4), pp. 267–281, 1982.
- [20] Weijers, E.P., Khlystov, A.Y., Kos, G.P.A. & Erisman, J.W., Variability of particulate matter concentrations along roads and motorways determined by a moving measurement unit. *Atmospheric Environment*, 38(19), pp. 2993–3002, 2004.
- [21] Gertler, A.W., Diesel vs. gasoline emissions: does PM from diesel or gasoline vehicles dominate in the US? *Atmospheric Environment*, 39(13), pp. 2349–2355, 2005.
- [22] Onat, B. & Stakeeva, B., Personal exposure of commuters in public transport to PM2.5 and fine particle counts. *Atmospheric Pollution Research*, 4, pp. 329–335, 2013.
- [23] Reisen, F. & Arey, J., 2005. Atmospheric reactions influence seasonal PAH and nitro-PAH concentrations in the Los Angeles Basin. *Environmental Science Technology*, 39(1), pp. 64–73, 2005.
- [24] Rajput, P., Sarin, M. & Kundu, S.S., Atmospheric particulate matter (PM2.5), EC, OC, WSOC and PAHs from NE-Himalaya: abundances and chemical characteristics. *Atmospheric Pollution Research*, 4(2), pp. 214–221, 2013.
- [25] IARC, International Agency for Research on Cancer, Monographs on the evaluation of carcinogenic risks to humans. Some non-heterocyclic aromatic hydrocarbons and some related exposures, vol. 92, 1–18, Lyon, 2005.
- [26] Piekarska, K. & Karpińska-Smulikowska, J., Mutagenic Activity of Environmental Air Samples from the Area of Wrocław, Poland. *Polish Journal of Environmental Studies*, 16(5), pp. 745–752, 2007.

- [27] Dallarosa, J., Teixeira, E.C., Meira, L. & Wiegand, F., Study of the chemical elements and polycyclic aromatic hydrocarbons in atmospheric particles of PM10 and PM2.5 in the urban and rural areas of South Brazil. *Atmospheric Research*, 89(1-2), pp. 76–92, 2008.
- [28] Zaciera, M., Kurek, J., Dzwonek, L., Feist, B. & Jedrzejczak, A., Seasonal variability of PAHs and NITRO-PAHs concentrations in total suspended particulate matter in ambient air of cities of Silesian Voivodeship. *Environment Protection Engineering* 38(1), pp. 45–50, 2012.
- [29] Cadle, S.H., Mulawa, P.A., Hunsanger, E.C. et al., Composition of light-duty motor vehicle exhaust particulate matter in the Denver, Colorado area. *Environmental Science and Technology*, 33(14), pp. 2328–2339, 1999.
- [30] He, L.Y., Hu, M., Huang, X.F., Zhang, Y.H., Yu, B.D. & Liu, D.Q., Chemical characterization of fine particles from on-road vehicles in the Wutong tunnel in Shenzhen China. *Chemosphere*, 62(10), pp. 1565–1573, 2006.
- [31] Nielsen, T., Reactivity of polycyclic aromatic hydrocarbons towards nitrating species. *Environmental Science & Technology*, 18(3), pp. 157–163, 1984.
- [32] Bamford, H.A. & Baker, J.E., Nitro-polycyclic aromatic hydrocarbon concentrations and sources in urban and suburban atmospheres of the Mid-Atlantic region. *Atmospheric Environment* 37(15), pp. 2077–2091, 2003.
- [33] Teixeira, E.C., Garcia, K.O., Meincke, L. & Leal, K.A., Study of nitro-polycyclic aromatic hydrocarbons in fine and coarse atmospheric particles. *Atmospheric Research*, 101(3), pp. 631–639, 2011.
- [34] Atkinson, R., Arey, J., Atmospheric chemistry of gas phase polycyclic aromatic hydrocarbons: formation of atmospheric mutagens. *Environmental Health Perspectives*, 102(4), pp. 117–126, 1994.
- [35] Söderström, H., Hajlova, J., Kocourek, V., Siegmund, B., Kocan, A., Obiedzinski, M.W., Tysklind, M. & Bergqvist, P., PAHs and nitrated PAHs in air of Five European countries determined using SPMDS as passive samplers. *Atmospheric Environment*, 39(9), pp. 1627–1640, 2005.
- [36] Feilberg, A.B., Poulsen, M.W., Nielsen, T. & Skov, H., Occurrence and sources of particulate nitro-polycyclic aromatic hydrocarbons in ambient air in Denmark. *Atmospheric Environment*, 35(2), pp. 353–366, 2001.
- [37] Durant, J.L., Busby, W.F., Lafleur, A.L., Penman, B.W. & Crespi, C.L., Human cell mutagenicity of oxygenated, nitrated and unsubstituted polycyclic aromatic hydrocarbons associated with urban aerosols. *Mutation Research*, 371(3-4), pp. 123–157, 1996.
- [38] Gorse Jr., R.A., Riley, T.L., Ferris, F.C., Pero, A.M. & Skewes, L.M., 1-nitropyrene concentration and bacterial mutagenicity in on-road vehicle particulate emissions. *Environmental Science and Technology*, 17(4), pp. 198–202, 1983.
- [39] Benner, Jr. B.A., Mobile sources of polycyclic aromatic hydrocarbons (PAH) and nitro-PAH: a roadway tunnel study, USA, University of Maryland, 1988.

- [40] Dimashki, M., Harrad, S. & Harrison, R.M., Measurements of nitro-PAH in the atmospheres of two cities. *Atmospheric Environment*, 34(15), pp. 2459–2469, 2000.
- [41] Amador-Munoz, O., Delgado-Rodriguez, A., Villalobos-Pietrini, R., Ortiz-Martello, R., Diaz-Gonzalez, G., Bravo-Cabrera, J.L. & Gomez-Arroyo, S., Partículas suspendidas hidrocarburos aromáticos policíclicos y mutagenicidad en el suroeste de la Ciudad de México. *Revista Internacional Contaminación Ambiental*, 17(4), pp. 193–204, 2001.
- [42] Rinaldi, M.C.S., Domingos, M., Dias, A.P.L., Esposito, J.B.N. & Pagliuso, J.D., Leaves of *Lolium multiflorum* “Lema” and tropical tree species as biomonitoring of polycyclic aromatic hydrocarbons. *Ecotoxicology and Environmental Safety*, 79(2), pp. 139–147, 2012.
- [43] Pitts Jr., J.N., On the trail of atmospheric mutagens and carcinogens: a combined chemical/microbiological approach. *American Zoologist*, 25(2), pp. 415–431, 1985.
- [44] Sato, M. I. Z., Umbuzeiro, G.A., Coimbrão, C.A. et al., Mutagenicity of airborne particulate organic material from urban and industrial areas of São Paulo, Brazil. *Mutation Research*, 335(3), pp. 317–330, 1995.
- [45] Vargas, V.M.F., Brito, K.C.T. & Coronas, M.V., *Genetic biomarkers applied to environmental air quality: ecological and human health aspects*. In: Mazzeo, N. A. (Ed.), Air Quality Monitoring, [www.intechopen.com/books/air-quality-monitoring-assessment-and-management](http://www.intechopen.com/books/air-quality-monitoring-assessment-and-management), 2011.



# *Biomonitoring of tunnel workers exposed to heavy air pollution in Rio de Janeiro, Brazil*

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## APENDICE F - Biomonitoring of tunnel workers exposed to heavy air pollution in Rio de Janeiro, Brazil

# Biomonitoring of tunnel workers exposed to heavy air pollution in Rio de Janeiro, Brazil

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**Abstract** Epidemiological studies found an increased risk of cancers in occupants exposed to traffic air pollution. Fine particulate matter is toxic, can enter into the respiratory tract and circulatory system, and can adsorb various substances, such as polycyclic aromatic hydrocarbons and its amino, alkyl, and nitro derivates. The study was carried out with Rebouças tunnel workers (exposed group) and 11 healthy men (control group). This tunnel is very representative of Brazilian cities and the biggest tunnel of the city of Rio de Janeiro. Samples of buccal mucosa cells and peripheral blood were evaluated using micronucleus assay. Urine samples were used to estimate the concentration of 1-hydroxyprene (1-HOP) and 2-naphthol (2-NAP). A significantly higher frequency of micronucleus in buccal cells and binucleated lymphocytes was observed for the exposed workers than for the control group. Higher concentrations of 1-HOP and 2-NAP were detected in the exposure group. In conclusion, damage to the genetic material and the high concentrations of metabolites of polycyclic aromatic hydrocarbons detected in the biological samples taken from control group can be related to daily exposure to pollutants.

**Keywords** PAHs · Atmosphere · Genotoxic · Blood · Urine

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## Introduction

Urban air pollution is a complex mixture of particles and gases derived from a variety of sources (primary pollutants) and can produce secondary pollutants by photochemical and thermal reactions (DeMarini 2013). Traffic is a major source of this air pollution, emitting carbon dioxide, carbon monoxide, various hydrocarbons, nitrogen oxides, particulate matter (PM), volatile organic compounds, toxic metals, and secondary reaction products such as ozone, nitrates, and organic acids (DeMarini 2013; Health Effects Institute 2010). The exhaust from gasoline and diesel vehicles is frequently a major source of PM in urban air (DeMarini 2013; Han and Naeher 2006), especially PM<sub>2.5</sub> (fine particulates with a median aerodynamic diameter less than 2.5 μm), which enters the respiratory tract and potentially the circulatory system (DeMarini 2013; Valavanidis et al. 2008).

The prevalence of chronic nonspecific respiratory disease in road tunnel employees and the effect of automobile exhaust on respiratory symptoms and pulmonary function has been investigated (Speizer and Ferris 1963; Tollerud 1983). The toxic effects of PM are mainly attributed to PM<sub>2.5</sub>. Because of their large specific surface, these particulates can adsorb various organic substances, such as polycyclic aromatic hydrocarbons (PAHs), nitro-PAHs, and oxygenated PAHs (oxy-PAHs) (Oh et al. 2011; Rainho et al. 2013a). Previous studies have demonstrated that increased concentrations of PAHs in the workplace environment could induce DNA damage in workers involved in road paving and bitumen (Singaravelu and Sellappa 2013). In addition, an increased risk of oxidative damage has been found in PAH-exposed coke oven workers. According to Ulvestad et al. (2000), tunnel workers showed loss forced expiratory volume and increased chronic obstructive pulmonary disease when exposed to pollutants. Another study showed a higher frequency of symptoms of chronic

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bronchitis in PAH-exposed foundry workers (Singaravelu and Sellappa 2013), and an epidemiological study also found an increased risk of cancers in foundry workers (Singaravelu and Sellappa 2013; Bosetti et al. 2007) and in occupations with high exposure to traffic-related air pollution (Hayes et al. 1989; Guberan et al. 1992; Burgaz et al. 2002). The exposure of local residents and certain occupational groups to heavy traffic, such as bus drivers, street policemen, and street vendors, has been studied for its potential to assess the contribution of urban air pollution to DNA damage in urban residents (Burgaz et al. 2002; Anwar and Kamal 1988; Autrup et al. 1999; Knudsen et al. 1999).

Humans are exposed to genotoxic agents in the environment, at work, in medical treatments, and through lifestyle choices. Biomarkers can be employed as endpoints for assessing human genotoxicant interactions from exposure to effects and individual host susceptibility (Singaravelu and Sellappa 2013; Albertini et al. 1993). Two widely used biomarkers of carcinogen exposure are urinary metabolites that indicate the internal exposure dose and genetic biomarkers like micronucleus (MN) assays, which reflect the biologically effective dose (Singaravelu and Sellappa 2013).

In 2010, we conducted a PM<sub>2.5</sub> monitoring study at three sites of the city of Rio de Janeiro: the campus of the Rio de Janeiro State University (moderate traffic 119,000 vehicles/day), Brazil Avenue expressway (heavy traffic, ~250,000 vehicles/day and is the city's biggest highway), and the Rebouças tunnel (heavy traffic, ~190,000 vehicles/day) (Rainho et al. 2013a, b, 2014). In these studies, we detected PM<sub>2.5</sub> values that exceeded the levels established by the World Health Organization (2006), mainly in Rebouças tunnel. Furthermore, this site showed highest concentrations of PAHs and nitro-PAHs, and the highest mutagenicity values for *Salmonella typhimurium* strain TA98 and its derivatives, YG1021 and YG1024, both sensitive to nitro-compounds (Rainho et al. 2013a, b, 2014). The aim of the present study was to investigate the genotoxic effects on Rebouças tunnel workers exposed to PM<sub>2.5</sub>, originated from a unique fuel matrix of the Brazilian fleet (gasoline with 25 % of ethanol, 45.9 %; hydrated ethanol, 2.70 %; diesel with 7 % of biodiesel, 5.98 %; flex fuel, 45.5 %; and some of them use compressed natural gas).

## Experimental

### Subjects and sampling

The study was carried out on Rebouças tunnel workers (exposed group) and 11 healthy men working on the campus of the Rio de Janeiro State University (control group), without signs of any occupational exposure to potential genotoxic substances. The working shift of tunnel

workers is 24 for 72 h, but the exact exposure time of each worker can range according to the day, as for example when accidents occur. The participants were informed about the study and asked to sign an informed consent form and to complete a standard questionnaire to obtain necessary data on their lifestyle and personal factors (age, working hours, health, food consumption, medication, smoker, and X-ray exposure). All procedures were submitted to and approved by the National Research Ethics Committee, CONEP (CAAE N°. 27402014.6.0000.5259). The mean characteristics of the study group are presented in Table 1.

Buccal mucosa cells were obtained by scraping the inner cheek with a swab. The cells were rinsed in ice-cold physiological saline solution (0.9 %) using individual coded centrifuge tubes. Samples of peripheral blood (2 mL) were collected in heparinized vacuum tubes by vanipuncture, and urine samples (30 mL) were collected by the workers. All the samples were stored on ice and protected from light until processed.

### Micronucleus assay

Buccal mucosa cells were centrifuged three times in methanol: acetic acid (3:1) solution at 2000 g for 5 min, and then, the pellet was dropped on duplicate slides. Slides were stained with Feulgen/fast green and cells were scored under  $\times 1000$  magnification (Tolbert et al. 1992). Two slides from each volunteer were blindly scored by two readers (1000 cells from each of the duplicate slides and for each reader).

### Lymphocyte cultures, staining, and binucleated cells with micronuclei scoring

Lymphocyte cultures were set up by adding 0.5 mL whole blood to 5 mL RPMI 1640 medium supplemented with 500  $\mu$ L fetal bovine serum plus 100  $\mu$ L phytohemagglutinin

**Table 1** The characteristics of the study group

	Control group	Exposed group
N	11	15
Age	44 $\pm$ 12	41 $\pm$ 10
Smoked	0	0
Exposure to X-ray	2	6
Use of medications	4	4
Alcohol intake	6	5
Consumption of smoked foods	7	10
Consumption of fried foods	9	15
Consumption of fruits	10	12
Consumption of vegetables	10	14

Exposure to X-ray at 12 months; eventual alcohol intake; regular consumption of smoked, fried foods, fruits, and vegetables

A, and incubated in CO<sub>2</sub> 5 % for 44 h at 37 °C. Two cultures per subject were established. A final concentration of 6 µg mL<sup>-1</sup> cytochalasin B (Cyt B) was added to the cultures 28 h later to arrest cytokinesis. At 72 h incubation, the cultures were harvested by centrifugation at 800 rpm for 8 min and treated with a hypotonic solution (0.075 mol L<sup>-1</sup> KCl at 4 °C). The cells were centrifuged and a methanol:acetic acid (3:1) solution was gradually added. This fixation step was repeated twice and the resulting cells were resuspended in a small volume of fixative and dropped onto clean slides. The slides of all the samples were stained with 5 % Giemsa for 7 min (Salvadori et al. 2003). Following the criteria proposed by Fenech (1993) to determine the frequency of binucleated cells with micronuclei (BNMN) and the total number of MN in lymphocytes, a total of 1000 binucleated cells with well preserved cytoplasm (500 per replicate) were scored per subject on coded slides (Fenech 1993; Pastor et al. 2003).

### Urinary concentrations of 2-naphthol and 1-hydroxyprene

Urine samples (1.50 mL) were placed in a 2-mL vial and added to 100-mL buffer solution of sodium acetate to 0.2 mol L<sup>-1</sup> to adjust the pH to 5.0. Then 10 µL β-glucuronidase/arylsulfatase (Merck) was added to promote enzymatic hydrolysis at 37 °C for 18 h with shaking and 200 rpm. A SiO<sub>2</sub>-C18 cartridge (Supelco Supelclean ship-18 SPE 100 mg) was prepared by passing 5 mL HPLC-grade methanol and 5 mL bidistilled water. The prepared sample was transferred slowly to the cartridge using a glass syringe to retain the organic molecules. Five milliliter double-distilled water was added to the cartridge to remove the soluble compounds in water. Then, 1.5 mL HPLC-grade acetonitrile was passed through the cartridge into a 2-mL vial. Chemical analyses were performed by high-performance liquid chromatography with fluorescence detector (HPLC-FLU Perkin Elmer series 200). An injection volume of 30 µL was used, with a mobile phase of 50 % acetonitrile and 50 % double-distilled water at a fixed flow rate of 1.5 mL min<sup>-1</sup>. The chromatograph column was a Supelcosil LC-18 (column length 250 mm, internal diameter 4.6 mm, particle size 5.0 µm), operating at 40 °C. The fluorescence detector operated with an excitation wavelength of 240 nm and emission of 370 nm. Calibration curves were prepared with standard 2-naphthol (2-NAP) (Sigma) between 20 and 100 ng mL<sup>-1</sup> and 1-hydroxyprene (1-HOP) (Sigma) between 50 and 400 ng mL<sup>-1</sup>. The determination coefficients were 0.99 for both compounds. Calibration standards were prepared on a urine control and the same procedures were carried out as for the samples and calibration was done by injection of seven concentrations in triplicate. The limit of detection for 2-NAP and 1-HOP were estimated in 0.4 and 1.6 ng mL<sup>-1</sup>, respectively.

### Statistical method

Student's *t* test was used to assess the statistical significance of the results obtained in the different assays. Comparisons between the results of the micronucleus test and the data from the questionnaire were performed using the Pearson correlation test with a significance level of 0.05.

### Results

The MN frequencies observed in the buccal mucosa cells and binucleated lymphocytes and the 1-HOP and 2-NAP concentrations are given in Figs 1 and 2.

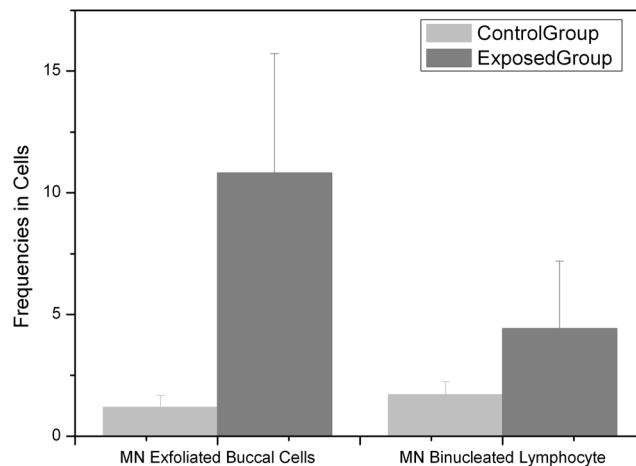
A significantly higher frequency of MN in binucleated lymphocytes was observed for the exposed workers than for the control group (Fig. 1). No correlation was observed between lifestyle factors and frequencies of MN in binucleated lymphocytes.

Significantly higher concentrations of 1-HOP and 2-NAP were detected in the exposure group (Fig. 2).

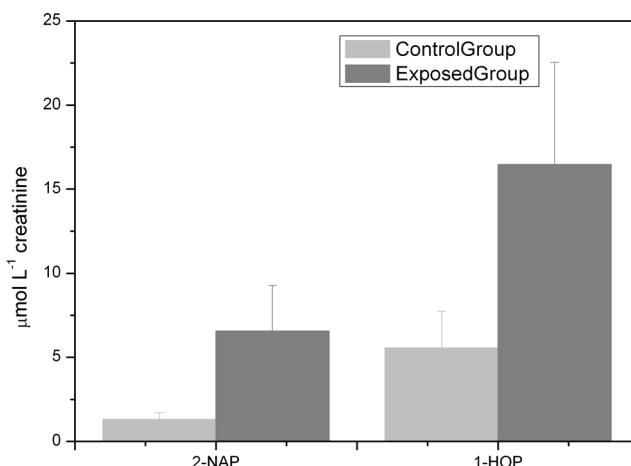
### Discussion

The assessment of MN frequencies in exfoliated buccal cells revealed a significant difference between exposed workers and control subjects (Fig. 1). The correlation test revealed a positive correlation (0.79) between alcohol intake and buccal MN frequency in the control group. None of the factors mentioned in the questionnaire (Table 1) were found to correlate with the MN frequencies detected for the exposure group.

Human biomonitoring is becoming increasingly important in occupational and environmental health studies (Hansen et al. 2008). The use of biomarkers as integrated measures of exposure and/or effects is increasing as a result of difficulties



**Fig. 1** The micronuclei frequencies in cells of the buccal mucosa and in binucleated lymphocytes of control and exposed groups



**Fig. 2** 1-HOP and 2-NAP concentrations of control and exposed groups

in identifying exposure sources and demands for more integrated data for human exposure risk assessments (Hansen et al. 2008). The exposure of the general population to PAHs and substituted PAHs has gained great importance in environmental health (Wilhelm et al. 2008). The significance of this class of substances from an environmental medicine viewpoint is determined by their ubiquitous occurrence in the environment and their carcinogenic nature (Wilhelm et al. 2008). Heavy traffic is a major source of exposure to PAHs in urban areas. After metabolic activation, many PAHs have been shown to induce lung and skin tumors in animals by mechanisms that also operate in exposed humans (Wilhelm et al. 2008). In this study, we analyzed the effect of exposure to environmental pollutants in Rebouças tunnel workers using different exposure biomarkers. The Rebouças tunnel has heavy traffic and has high ambient air concentrations of  $\text{PM}_{2.5}$  ( $141 \mu\text{g m}^{-3}$ ), PAHs ( $5.41 \text{ ng m}^{-3}$ ), and nitro-PAHs ( $13.02 \text{ ng m}^{-3}$ ) (Rainho et al. 2013a, b, 2014). Fifteen of the 50 Rebouças tunnel workers agreed to participate in this study, although  $N$  is small, it represents 30 % of the population. Other similar studies also show small samples, as these locations have low number of workers due to the risks associated of working inside a tunnel (Burgaz et al. 2002; Coronas et al. 2009; Brucker et al. 2015). The biological samples of these workers, including buccal mucosa cells and peripheral blood, were analyzed for MN frequency, and urine samples were used to estimate the concentration of hydroxyl metabolites of pyrene (1-HOP) and naphthalene (2-NAP).

The buccal mucosa cell samples taken from the Rebouças tunnel workers showed a significantly higher frequency of MN than the control group. Furthermore, this result showed no correlation with any of the data provided in the questionnaire. These results may be related to exposure to pollutants present in this tunnel. Other studies investigating human exposure to pollutants have found an increase in the frequency of MN in cells of the buccal mucosa in workers exposed to PAHs (Singaravelu and Sellapa 2013), heavy metals (Letaj et al.

2012), and ozone (Chen et al. 2006). Other workers also exposed to pollutants and found to have an increased frequency of MN in buccal mucosa cells are listed below: traffic police in China,  $5.72 \pm 2.57$  (Zhao et al. 1998), in Turkey,  $0.10 \pm 0.0$  (Karalahil et al. 1999) and in the Philippines,  $17.07$  (Hallare et al. 2009); gas station attendants in the Philippines,  $18.90$  (Hallare et al. 2009) and in India,  $12.76$  (Sellapa et al. 2010); and taxi drivers in Turkey,  $0.12 \pm 0.05$  (Karalahil et al. 1999). The correlation test revealed a positive correlation between alcohol intake and MN frequency in buccal mucosa cells for the control group. Several studies have indicated a relationship between the ingestion of alcohol and increased frequency of micronuclei (Singaravelu and Sellapa 2013; Dittberner et al. 1997). The same correlation was not observed in the exposure group, which reinforces the likelihood of the occurrence of MN being related to occupational exposure.

The binucleated lymphocyte samples from the Rebouças tunnel workers also showed a significantly higher frequency of MN than the control group. A similar increase was also observed in studies using a micronucleus assay in lymphocytes from tunnel workers ( $6.31 \pm 0.61$ ) in the Umbrian Apennine Mountains, Italy, compared with outdoor workers away from traffic ( $4.71 \pm 0.28$ ) (Villarini et al. 2008). An elevated frequency of MN in human lymphocytes compared with control groups has also been observed in individuals who have other occupations that expose them to pollutants in different parts of the world: garage mechanics in Hungary,  $23.5 \pm 5.7$  (Schoket et al. 1999); traffic police in Italy,  $3.75 \pm 1.65$  (Merlo et al. 1997) and in China,  $4.27 \pm 0.68$  (Bai et al. 2005); and diesel train attendants in China,  $0.166$  (IARC 2002).

PAHs are a major group of carcinogenic compounds in ambient urban air, and most recent biomarker studies have focused on assessing PAH exposure. To assess internal PAH exposure, the determination of 1-HOP and 2-NAP in urine has been successfully used in many studies in environmental and occupational medicine (Hansen et al. 2008; Wilhelm et al. 2008). Urinary excretions mainly contain metabolites of PAHs with a low molecular weight, such as naphthalene and pyrene. Assessments of humans exposed to naphthalene have attracted increasing interest in environmental health, since this most volatile PAH has been classified as a possible human carcinogen by international agencies (Wilhelm et al. 2008; IARC 2002; U.S.EPA 2002), and the general population's exposure to external naphthalene in the environment is reported to be higher than it is to other PAH compounds (Wilhelm et al. 2008). Our results showed a significant increase in the concentration of metabolites 1-HOP and 2-NAP in the urine of the exposure group. Other studies assessing occupational exposure to pollutants have also found higher concentrations of 1-HOP: coke oven and graphite electrode-producing plant workers (Buchet et al. 1992; Ferreira et al. 1994), coal liquefaction workers (Quinlan et al. 1995), road pavers (Levin et al. 1995), aluminum plant workers (Schoket et al. 1999), and

carbon black workers (Tsai et al. 2002). The same observation has been made in studies assessing 2-NAP concentrations in the urine of workers exposed to different classes of pollutants: charcoal workers (Kato et al. 2004 Kim et al. 2004) and emission inspectors (Kim et al. 2004). In general, exposure assessments and biomarkers have found differences between the control and exposed populations, suggesting a likely linkage between the class of agent measured by the exposure assessment and the damage detected by biomarker (DeMarini 2013). PAHs or its metabolites were the main class of chemical measured in the air and urine, respectively, and this class of compound is recognized as an important component of diesel and automobile exhaust and air pollution in general (DeMarini 2013).

## Conclusions

Damage to the genetic material and high concentrations of PAH metabolites were detected in biological samples taken from Rebouças tunnel workers (exposure group) and can be related to daily exposure to pollutants in the tunnel.

Despite the small sample number, the results are consistent with others studies presented. The importance of this type of monitoring is to provide information to reduce the exposure period of time of these workers or to identify procedures to be applied, as the use of masks or autonomous respiratory system, to avoid PAH exposure.

Other importance of this study relies on the increase use of tunnels in cities with irregular topography as the city of Rio de Janeiro. New tunnels were constructed in the last years and others are being constructed to face the increased number of vehicles and consequently trying to solve mobility problems.

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**Ethics approval** All procedures were submitted to and approved by the National Research Ethics Committee, CONEP.

**Informed consent** The participants were informed about the study and asked to sign an informed consent form and to complete a standard questionnaire to obtain necessary data on their lifestyle and personal factors.

## References

- Albertini RJ, Nicklas JA, Fuscoe JC, Skopek TR, Branda RF, O'Neill JP (1993) *In vivo* mutations in human blood cells: biomarkers for molecular epidemiology. Environ Health Perspec 99:135–141
- Anwar WA, Kamal AAM (1988) Cytogenetic effects in a group of traffic policemen in Cairo. Mutat Res 208:225–231
- Autrup H, Daneshvar B, Dragsted LO, Gamborg M, Hansen AM, Loft S, Okkels H, Nielsen F, Nielsen PS, Raffn E, Wallin H, Knudsen LE (1999) Biomarkers for exposure to ambient air pollution comparison of carcinogen-DNA adduct levels with other exposure markers and markers for oxidative stress. Environ Health Perspectives 107:233–238
- Bai YP, Li J, Fan XY, Yao SQ, Jiang SF, Jin YL (2005) Effects of traffic air pollution on the rate of micronucleus and sister chromatid exchange of traffic police in a city. Carcinog Teratog Mutagen 14:250–254
- Bosetti C, Boffetta P, La Vecchia C (2007) Occupational exposures to polycyclic aromatic hydrocarbons, and respiratory and urinary tract cancers: a quantitative review to 2005. Ann Oncol 18:431–446
- Brucker N, Moro A, Charão M, Bubols G, Thiesen FV, Garcia SC (2015) Biological monitoring in Brazilian workers occupationally exposed to different xenobiotics. Appl Res Toxicol 1:25–31
- Buchet JP, Gennart JP, Mercado-Calderon F, Delavignette JP, Cupers L, Lauwerys R (1992) Evaluation of exposure to polycyclic aromatic hydrocarbons in a coke production and a graphite electrode manufacturing plant: assessment of urinary excretion of 1-hydroxypyrene as a biological indicator of exposure. Br J Ind Med 49:761–768
- Burgaz S, Demircigil GC, Karahalil B, Karakaya AE (2002) Chromosomal damage in peripheral blood lymphocytes of traffic policemen and taxi drivers exposed to urban air pollution. Chemosphere 47:57–64
- Chen C, Arjomandi M, Qin H, Balmes J, Tager I, Holland N (2006) Cytogenetic damage in buccal epithelia and peripheral lymphocytes of young healthy individuals exposed to ozone. Mutagenesis 21: 131–137
- Coronas MV, Pereira TS, Rocha JAV, Lemos AT, Fachel JMG, Salvadori DMF, Vargas VMF (2009) Genetic biomonitoring of an urban population exposed to mutagenic airborne pollutants. Environ Int 35: 1023–1029
- DeMarini DM (2013) Genotoxicity biomarkers associated with exposure to traffic and near-road atmospheres: a review. Mutagenesis 28:485–505
- Dittberner U, Schmetzter B, Golzer P, Eisenbrand G, Zankl H (1997) Genotoxic effects of 2-trans-hexenal in human buccal mucosa cells in vivo. Mutat Res 390:161–165
- Fenech M (1993) The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. Mutat Res 285:35–44
- Ferreira M, Buchet JP, Burron JB, Moro J, Cupers L, Delavignette JP, Jacques J, Lauwerys R (1994) Determinants of urinary thioethers, D-glutaric acid and mutagenicity after exposure to polycyclic aromatic hydrocarbons assessed by air monitoring and measurement of 1-hydroxypyrene in urine: a cross-sectional study in workers of coke and graphite-electrode-producing plants. Int Arch Occupat Environ Health 65:329–338
- Guérard E, Usel M, Raymond L, Bolay J, Fioretta G, Puissant J (1992) Increased risk for lung cancer and cancer of the gastrointestinal tract among Geneva professional drivers. Br J Ind Med 49:337–344
- Hallare AV, Gervasio MK, Gervasio PL, Acacio-Claro PJ (2009) Monitoring genotoxicity among gasoline station attendants and traffic enforcers in the city of Manila using the micronucleus assay with exfoliated epithelial cells. Environ Monit and Assess 156:331–341
- Han X, Naeher LP (2006) A review of traffic-related air pollution exposure assessment studies in the developing world. Environ Int 32: 106–120
- Hansen AM, Mathiesen L, Pedersen M, Knudsen LE (2008) Urinary 1-hydroxypyrene (1-HP) in environmental and occupational studies—a review. Int J Hyg Environ Health 211:471–503
- Hayes RB, Thomas T, Silverman DT, Vineis P, Blot WJ, Mason TJ, Picle LW, Correa P, Fontham ETH, Schoenberg JB (1989) Lung cancer in motor exhaust-related occupations. Am J Ind Med 16:685–695

- Health Effects Institute (2010) Traffic-Related Air Pollution: A Critical Review of the Literature on Emissions, Exposure, and Health Effects, Special Report 17. Health Effects Institute, Boston, MA, USA
- IARC (2002) Monographs on the evaluation of carcinogenic risks to humans: some traditional herbal medicines, some mycotoxins, naphthalene and styrene, vol 82. IARC, Lyon
- Karahalil B, Karakaya AE, Burgaz S (1999) The micronucleus assay in exfoliated buccal cells: application to occupational exposure to polycyclic aromatic hydrocarbons. *Mutat Res* 442:29–35
- Kato M, Loomis D, Brooks LM, Gattas GFJ, Gomes L, Carvalho AB, Rego MAV, DeMarini DM (2004) Urinary biomarkers in charcoal workers exposed to wood smoke in Bahia State. *Brazil Cancer Epidemiol Biomarkers Prev* 213:1005–1012
- Kim MK, Oh S, Lee JH, Im H, Ryu YM, Oh E, Lee J, Lee E, Sul D (2004) Evaluation of biological monitoring markers using genomic and proteomic analysis for automobile emission inspectors and waste incinerating workers exposed to polycyclic aromatic hydrocarbons or 2,3,7,8-tetrachlorodibenz-p-dioxins. *Exp Mol Med* 36:396–410
- Knudsen LE, Norppa H, Gamborg MO, Nielsen PS, Okkels H, Søll-Johanning H, Raffn E, Jarventaus H, Autrup H (1999) Chromosomal aberrations induced by urban air pollution in humans: influence of DNA repair and polymorphisms of glutathione S-transferase M1 and N-acetyltransferase 2. *Cancer Epidemiol Biomarkers Prev* 8:303–310
- Letaj K, Elezaj I, Selimi Q, Kurteshi K (2012) The effects of environmental pollution with heavy metals in frequency of micronuclei in epithelial buccal cells of human population in mitrovica. *J Chem Health Risks* 2:1–4
- Levin JO, Rhén M, Sikström E (1995) Occupational PAH exposure: urinary 1-hydroxypyrene levels of coke oven workers, aluminium smelter pot-room workers, road pavers, and occupational non-exposed persons in Sweden. *Sci Total Environ* 163:169–177
- Merlo F, Bolognesi C, Peluso M, Valerio F, Abbondandolo A, Puntoni R (1997) Airborne levels of polycyclic aromatic hydrocarbons: 32P-postlabeling DNA adducts and micronuclei in white blood cells from traffic police workers and urban residents. *J Environ Pathol Toxicol Oncol* 16:157–162
- Oh SM, Kim HR, Park YJ, Lee SY, Chung KH (2011) Organic extracts of urban air pollution particulate matter (PM2.5)-induced genotoxicity and oxidative stress in human lung bronchial epithelial cells (BEAS-2B cells). *Mutation Res* 723:142–151
- Pastor S, Creus A, Parrón T, Cebulská-Wasilewska A, Siffel C, Piperakis S, Marcos R (2003) Biomonitoring of four European populations occupationally exposed to pesticides: use of micronuclei as biomarkers. *Mutagenesis* 18:249–258
- Quinlan R, Kowalczyk G, Gardiner K, Calvert IA, Hale KA, Walton ST (1995) Polycyclic aromatic hydrocarbon exposure in coal liquefaction workers: the value of urinary 1-hydroxypyrene excretion in the development of occupational hygiene control strategies. *Ann Occupat Hyg* 39:329–346
- Rainho CR, Corrêa SM, Mazzei JL, Aiub CAF, Felzenszwab I (2013a) Genotoxicity of polycyclic aromatic hydrocarbons and nitro-derived in respirable airborne particulate matter collected from urban areas of Rio de Janeiro (Brazil). *BioMed Res Int* 2013:1–9
- Rainho CR, Velho AMA, Corrêa SM, Mazzei JL, Aiub CAF, Felzenszwab I (2013b) Prediction of health risk due to polycyclic aromatic hydrocarbons present in urban air in Rio de Janeiro. *Brazil Genet Mol Res* 12:3992–4002
- Rainho CR, Corrêa SM, Mazzei JL, Aiub CAF, Felzenszwab I (2014) Seasonal variations in the level of mutagenicity: an assessment of respirable particulate matter in Rio de Janeiro, Brazil, In: CA Brebbia; JWS Longhors. (Org.). *Air Pollution XXII*. 1<sup>a</sup> ed. Boston: WITpress, 1:87
- Salvadori DM, Ribeiro LR, Fenech M (2003) Micronucleus test in vitro humans cells In: Environmental Mutagenesis (in portuguese). Canoas: Editora ULBRA.
- Schoket B, Poirier MC, Mayer G, Török G, Kolozsi-Ringelhann A, Bognár G, Bigbee WL, Vincze I (1999) Biomonitoring of human genotoxicity induced by complex occupational exposures. *Mutat Res* 445:193–203
- Sellappa S, Sadhanandhan B, Francis A, Vasudevan SG (2010) Evaluation of genotoxicity in petrol station workers in South India using micronucleus assay. *Ind Health* 48:852–856
- Singaravelu SR, Sellappa S (2013) Assessment of genotoxicity in exfoliated buccal epithelial cells of foundry workers occupationally exposed to polycyclic aromatic hydrocarbons. *Asian J Pharm Clin Res* 6:339–342
- Speizer FE, Ferris BG Jr (1963) The prevalence of chronic nonspecific respiratory disease in road tunnel employees. *Am Rev Resp Dis* 88: 205–212
- Tolbert P, Shy C, Allen J (1992) Micronuclei and other nuclear anomalies in buccal smears: methods development. *Mutat Res* 271:69–77
- Tollerud DJ, Weiss ST, Elting E, Speizer FE, Ferris B (1983) The health effects of automobile exhaust. VI. Relationship of respiratory symptoms and pulmonary function in tunnel and turnpike workers. *Arch Environ Health* 38(6):334–340
- Tsai PJ, Shieh HY, Lee WJ, Chen HL, Shih TS (2002) Urinary 1-hydroxypyrene as a biomarker of internal dose of polycyclic aromatic hydrocarbons in carbon black workers. *Ann Occupat Hyg* 46: 229–235
- U.S.EPA (2002) Health effects support document for naphthalene, external review draft. EPA 822-R-02-031; Washington DC
- Ulvestad B, Bakke B, Melbostad E, Fuglerud P, Kongerud J, Lund MB (2000) Increased risk of obstructive pulmonary disease in tunnel workers. *Thorax* 55:277–282
- Valavanidis A, Fiotakis K, Vlachogianni TJ (2008) Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. *Environ Sci Health, Part C: Environ Carcinog Ecotoxicol Rev* 26:339–362
- Villarini M, Moretti M, Fatigoni C, Agea E, Dominici L, Mattioli A, Volpi R, Pasquini R (2008) Evaluation of primary DNA damage, cytogenetic biomarkers and genetic polymorphisms for CYP1A1 and GSTM1 in road tunnel construction workers. *J Toxicol Environ Health, Part A* 71:1430–1439
- WHO (2006) World Health Organization. *Health Risks of Particulate Matter From Long - Range Transboundary Air Pollution*, Copenhagen, 99
- Wilhelm M, Hardt J, Schulz C, Angerer J, On behalf of the Human Biomonitoring Commission of the German Federal Environment Agency (2008) New reference value and the background exposure for the PAH metabolites 1-hydroxypyrene and 1- and 2-naphthol in urine of the general population in Germany: Basis for validation of human biomonitoring data in environmental medicine. *Int J Hyg Environ Health* 211:447–453
- Zhao X, Niu J, Wang Y, Yan C, Wang X, Wang J (1998) Genotoxicity and chronic health effects of automobile exhaust: a study on the traffic policemen in the city of Lanzhou. *Mutat Res* 415:185–190