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BIOMONITORAMENTO DE POPULAÇÕES HUMANAS EM ÁREAS DE EXPOSIÇÃO A POLUENTES ATMOSFÉRICOS MUTAGÊNICOS

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Porto Alegre, Março de 2008

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RESUMO

Processos industriais utilizam e geram uma variedade de compostos que são liberados e dispersados no ambiente. Sobre a maioria dessas substâncias, muitas sendo recentemente sintetizadas, não se tem o devido conhecimento e controle, o que pode causar diversos prejuízos ao ambiente e à saúde humana, sendo as populações do entorno das fontes emissoras as primeiras a se exporem. Nesse contexto, uma avaliação que estime o potencial efeito de uma mistura de substâncias à determinada população requer um trabalho extenso e multidisciplinar. O presente estudo teve como objetivo avaliar, através de marcadores genéticos, a presença de atividade mutagênica no material particulado atmosférico como marcador de exposição e diagnóstico ambiental em áreas sob influência industrial petroquímica, associando o biomonitoramento humano em população urbana exposta à atividade industrial. Foram avaliadas amostras de material particulado atmosférico, através do ensaio *Salmonella*/microssoma, de áreas que recebem as emissões atmosféricas do complexo petroquímico do sul, Triunfo (RS), e de uma refinaria de petróleo em Esteio (RS) e, para comparação, uma área urbana em Porto Alegre (RS) também foi avaliada. Todos os locais estudados apresentaram respostas positivas para mutagenicidade e indicaram a presença de mutágenos diretos e indiretos, e de nitrocompostos, como nitroarenos, aminas aoromáticas e nitro-PAHs. Amostras de sangue e mucosa oral de homens residentes e/ou trabalhadores na área influenciada pela refinaria de petróleo foram avaliadas quanto ao dano no DNA através do ensaio cometa e micronúcleo, respectivamente. Este grupo de indivíduos foi comparado a um grupo de referência similar entre os habitantes do município de Santo Antônio da Patrulha (RS), caracterizado como fora das principais áreas industriais do Estado. Não houve diferença entre a freqüência de células micronucleadas entre os grupos. No entanto, o ensaio cometa se mostrou sensível na detecção de dano ao DNA

em indivíduos do grupo exposto. Nenhum dos fatores com possibilidade de interferência no parâmetro avaliado (fumo, idade e radiação) mostraram associação com aumento de dano detectado pelo ensaio cometa. Os ensaios biológicos utilizados no diagnóstico ambiental e no biomonitoramento da população foram ferramentas úteis na avaliação de áreas influenciadas por atividades humanas. Assim, o ensaio *Salmonella*/microssoma além de fornecer informações sobre os efeitos biológicos resultantes das misturas presentes no ambiente, também indicou as classes dos compostos e sua contribuição ao efeito observado. Já o ensaio do cometa foi indicativo importante no biomonitoramento da exposição humana à mistura de agentes genotóxicos ambientais. Esses resultados indicam que os atuais parâmetros de qualidade do ar não são suficientes para evitar efeitos adversos ao ambiente e à saúde humana.

Palavras chave: genotoxicidade ambiental, PM10, biomonitoramento humano, *Salmonella*/microssoma, ensaio cometa, teste do micronúcleo.

ABSTRACT

Industrial processes use and generate a variety of compounds that are liberated and dispersed in the environment. Most of those substances, many recently synthesized, still do not have the understanding and control they deserve, which can cause several damages to the environment and human health, and the populations surrounding the emission sources are the first to be exposed. In this way, any evaluation estimating the potential effect of a mixture of substances to a particular population require an extensive multi-disciplinary approach. The present study had as objective to evaluate, through genetic biomarkers, the presence of mutagenic activity in the airborne particulate matter as an exposure marker and environmental diagnosis in areas under influence of petrochemical industry, associating the human biomonitoring in urban population exposed to industrial activities. Airborne particulate matter samples were evaluated through *Salmonella*/microsome assay. Samples from areas receiving atmospheric emissions from a petrochemical complex (Triunfo, RS), an oil refinery (Esteio, RS) and an urban area (Porto Alegre, RS) were evaluated. All studied areas showed positive responses for mutagenicity, indicating that direct and indirect-acting mutagens were present in airborne particulate matter. Also, the mutagenic responses indicate the participation of nitrocompounds, like nitroarenes, hydroxylamines, nitro-PAHs and aromatic amines in the total mutagenicity. Samples of blood and buccal mucosa, from males residing and/or working downwind from an oil refinery, were evaluated in single-cell gel electrophoresis assay (comet assay) and micronucleus (MN) assay, respectively. This studied group was compared to males from another town (Santo Antônio da Patrulha, RS, Brazil) situated in an urban area with restricted traffic and industrial influence, constituting the reference group. No difference in micronucleated cells frequencies was observed between groups.

Comet assay was sensitive to detect DNA damage in subjects from exposed group. No association was found between possible confounding factors (tobacco smoking, age and radiation exposure) and increased DNA damage. Biological tests in monitoring and environmental diagnosis studies, in areas under influence of anthropogenic activities, were useful tools to screen which chemical genotoxic compound classes are present. It also indicates that the current air quality standards are not sufficient to avoid damage to the environment and human health.

Keywords: environmental genotoxicity, PM10, human biomonitoring, *Salmonella*/microsome, comet assay, micronucleus assay.

LISTA DE ABREVIAÇÕES

Português/Inglês

DCM	Diclorometano
DMSO	Dimetilsulfóxido
FEPAM	Fundação Estadual de Proteção Ambiental Henrique Luís Roessler
HPA /PAH	Hidrocarbonetos Policíclicos Aromáticos
MOE/EOM	Matéria Orgânica Extraída
PAC	Compostos Policíclicos Aromáticos
PM10	Material particulado inalável (<10μm diâmetro aerodinâmico)
PTS/TSP	Partículas Totais em Suspensão (<100μm diâmetro aerodinâmico)
S9	Fração de metabolização de mamífero *in vitro*
2AF	2 amnifluoreno
2NF	2 nitrofluoreno
4NQO	Óxido de nitroquinolina

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1. INTRODUÇÃO

Processos industriais utilizam e geram uma variedade de compostos que são liberados e dispersados no ambiente, sendo a maioria dessas substâncias recentemente sintetizadas, não dispondo do devido conhecimento e controle toxicológico sobre essas (Irigaray et al., 2007). Essas substâncias podem causar diversos prejuízos ao ambiente e à saúde humana, sendo as populações do entorno das fontes emissoras as primeiras a se exporem. Os riscos e efeitos da exposição a contaminantes estão sendo uma preocupação crescente nos últimos tempos. Um reflexo pode ser percebido na comunidade científica pelo aumento de estudos de biomonitoramento humano (Needham et al., 2007).

As substâncias apresentam características específicas que determinam sua mobilidade, interação e concentração entre os diferentes compartimentos ambientais, como ar, água solo e sedimento. Além disso, os efeitos da exposição dessas substâncias nos organismos dependem da via de contato e das susceptibilidades intrínsecas. Assim sendo, uma avaliação que estime o potencial efeito de uma única ou de uma mistura de substâncias a determinada população requer um trabalho extenso e multidisciplinar (Greim, 2001).

A utilização de biomarcadores genéticos sensíveis permite a identificação da presença de substâncias perigosas em misturas ambientais complexas de diferentes compartimentos ambientais, através de seus efeitos genotóxicos (Vargas, 2003; Claxton et al., 2004; Ohe et al., 2004). Estas metodologias possibilitam definir, de forma preventiva, fontes de contaminantes favorecendo ações preventivas no controle da qualidade ambiental. Métodos específicos de abordagem para o diagnóstico da presença de substâncias mutagênicas em amostras de água, sedimentos e material particulado de ar têm sido desenvolvidos na área de pesquisa da FEPAM (Vargas et al., 1988, 1993,

1995, 2001; Ducatti & Vargas, 2003; Vargas, 2003; Horn et al., 2004; Tagliari et al., 2004; Cardozo et al., 2006; Pereira et al., 2007; Coronas et al., 2008; Vargas et al., 2008).

Devido à dinâmica dos ecossistemas, aos ciclos biogeoquímicos e às propriedades das substâncias, os poluentes se dispersam e transitam entre os compartimentos ambientas. No entanto, o compartimento atmosférico destaca-se por sua complexa composição, reações e abrangência. Além disso, a fragilidade dos organismos terrestres diante desse compartimento ambiental faz com que o constante e regular monitoramento da qualidade do ar seja indispensável para a segurança e integridade do ambiente.

Atualmente, o Conselho Nacional do Meio Ambiente (CONAMA) estabelece padrões primários e secundários de qualidade do ar para diversos parâmetros, representando as concentrações dos poluentes que ultrapassadas podem causar prejuízos à saúde da população humana e concentrações de poluentes abaixo das quais se prevê o mínimo efeito adverso sobre o bem-estar da população, assim como o mínimo dano à fauna, à flora, aos materiais e ao meio ambiente em geral, respectivamente. Entre esses parâmetros estão as Partículas Totais em Suspensão (PTS) e Inaláveis (PM10), que corresponde a partículas menores que 10μm, para o controle da poluição do ar (Brasil, 1990). No entanto, esses parâmetros não abrangem a imensa variedade de compostos presentes na atmosfera, muitos ainda desconhecidos, nem o potencial prejuízo das interações entre eles. Nesse contexto, a avaliação da atividade mutagênica, através do ensaio de mutação reversa com *Salmonella typhimurium*, também chamado de ensaio *Salmonella*/microssoma ou Teste de Ames, torna-se, entre os biomarcadores, uma medida eficaz para estimar, qualitativa e quantitativamente, o dano à exposição.

O ensaio *Salmonella*/microssoma vem sendo extensivamente utilizado como marcador de evento mutagênico em diferentes tipos de amostras, desde substâncias químicas isoladas até amostras ambientais complexas (Maron & Ames, 1983; Mortelmans & Zeiger, 2000; Umbuzeiro & Vargas, 2003). Recentemente Claxton et al. (2004) revisaram a literatura reunindo os estudos que empregaram esse ensaio e suas variações em amostras de ar urbano. A presença de compostos mutagênicos em extratos orgânicos de material particulado atmosférico foi relatada pela primeira vez em 1975, e desde então pesquisas por todo o mundo têm buscado quantificar, caracterizar, apontar as fontes emissoras e fatores interferentes, além do risco de exposição diante dessse grupo de poluentes atmosféricos (Claxton et al., 2004; Claxton & Woodwall Jr., 2007)

No Brasil estudos com PTS nas regiões urbanas e industriais no Brasil relatam atividade mutagênica mesmo em amostras em que os valores de material particulado se apresentam dentro dos padrões estabelecidos em legislação (Sato et al., 1995; Vargas et al., 1998; Ducatti & Vargas, 2003; Vargas, 2003 Umbuzeiro et al., 2008). Quanto à fração inalável, as publicações de pesquisas feitas no Brasil ficam ainda mais restritas (De Martinis et al., 1999; Coronas et al., 2008). Estudos epidemiológicos relacionando parâmetros de poluição atmosférica com morte de neonatos e baixo peso ao nascer encontraram associações com a concentração de PM10, embora suas concentrações não tenham ultrapassado os valores previstos em legislação durante o período investigado (Lin, et al., 2004; Medeiros & Gouveia, 2005). Trabalhos investigando a presença de substâncias químicas na fração PM10 também não encontram valores acima dos 150µg/m^3 (padrão primário e secundário – Brasil, 1990), embora hidrocarbonetos policíclicos aromáticos (HPAs) cancerígenos tenham sido identificados (Braga et al., 2005; Dallarosa et al., 2005). Investigação da atividade mutagênica com o uso do ensaio *Salmonella*/microssoma em extratos orgânicos fracionados de PM10 foi realizada em

área residencial/comercial na zona oeste da cidade de São Paulo, relatando resultados positivos em diferentes frações e tanto em ensaios com ou sem fração de metabolização de mamífero *in vitro* (De Martinis et al., 1999).

A fração de partículas inaláveis do ar (PM10) é responsável por diversos prejuízos aos organismos, pois além da capacidade de penetrar e se depositar nas vias respiratórias, os compostos mutagênicos presentes no ar estão freqüentemente associados a esse material (De Martinis et al., 1999; Claxton et al., 2004). A associação entre o tamanho da partícula e a presença de nitropirenos foi relatada, sendo a quantidade desses compostos maior quanto menor for o tamanho das partículas analisadas (Hayakawa et al., 1995; Pagano et al., 1996).

Diversos trabalhos avaliaram conjuntamente a contribuição de nitrocompostos à atividade mutagênica do material particulado do ar, sendo essa influência encontrada entre diferentes períodos e locais (urbano e industrial) avaliados (DeMarini et al., 1994; Sato et al., 1995; Vargas et al., 1998; Ducatti & Vargas, 2003; Vargas, 2003; Coronas et al., 2008; Umbuzeiro et al., 2008). Esses compostos nitroderivados são freqüentemente encontrados no ar, pois são resultantes da combustão incompleta e das reações que ocorrem na atmosfera (Sato et al., 1995; Finlayson-Pitts & Pitts, 1997; Claxton et al., 2004).

São conhecidos os efeitos de médio e longo prazo para a saúde humana frente à exposição constante aos agentes mutagênicos. Essas substâncias podem agir em diferentes etapas dos processos de carcinogênese e reprodutivo (malformações congênitas e baixo peso ao nascimento) além de sua contribuição para várias doenças degenerativas (Oliveira et al., 2002; Pope et al., 2002; Vargas, 2003; Irigaray et al., 2007; Lewtas, 2007). Estudos relatam que a exposição a poluentes atmosféricos, particularmente ao material particulado fino, aumenta o risco de câncer de pulmão e

doenças cardiovasculares (Hemminki & Pershagen, 1994; Pope et al., 2002; Englert, 2004; Vineis & Husgafvel-Pursiainen, 2005; Boffetta, 2006).

Assim, o monitoramento utilizando metodologias genéticas em populações expostas a potenciais mutagênicos e/ou carcinogênicos é um eficiente sistema de advertência para doenças genéticas ou cânceres (Kassie et al., 2000). Entre os vários ensaios que têm sido utilizados o teste de células individualizadas em gel, mais conhecido como ensaio do cometa, além do de avaliação de micronúcleos, se destacam por serem técnicas rápidas e sensíveis para medir lesões genômicas precoces. No entanto, a aplicação desses marcadores em estudos de exposição a misturas complexas ambientais é mais restrita (Kassie et al., 2000; Faust et al, 2004; Möller, 2006; Angerer, et al., 2007).

O micronúcleo é um biomarcador citogenético e sua ampla utilização está fundamentada na relação com estágios iniciais de doenças crônicas, especialmente o câncer (Bonassi et al., 2005). O uso do ensaio do micronúcleo como biomarcador em estudos populacionais já é reconhecido na literatura (Majer, et al., 2001; Bonassi et al., 2005). No entanto, o seu emprego com populações humanas avaliando o efeito da poluição, em particular a atmosférica, é restrito. Majer et al. (2001) em extensa revisão de trabalhos de biomonitoramento utilizando o teste de micronúcleo em esfregaço de células epiteliais não citam nenhum estudo avaliando o efeito à exposição de misturas complexas do ambiente.

O ensaio cometa é capaz de detectar quebras na fita de DNA e suas diversas modificações acrescentam a possibilidade de observação de outros tipos de danos à molécula (Möller, 2006). A utilização do ensaio do cometa em estudos de monitoramento vem, progressivamente, aumentando (Möller, 2006). No entanto, sua

aplicação tem sido mais limitada a pesquisas de exposições ocupacionais ou de compostos específicos (Kassie et al., 2000; Möller, 2000; Collins, 2004).

A investigação da exposição humana à poluição atmosférica com o emprego do ensaio cometa foi realizada na cidade do México, afetada por altos índices de poluição atmosférica (Valverde, 1997; Calderón-Garcidueñas, 1999; Rojas, 2000). Altas concentrações de ozônio são encontradas ao sul da Cidade do México, enquanto o norte é afetado especialmente por altas taxas de partículas e hidrocarbonetos. Estudos utilizando o ensaio do cometa em diferentes tipos celulares (como células da mucosa nasal e oral, do canal lacrimal e linfócitos) em habitantes das duas diferentes partes da cidade encontraram o maior número de danos no DNA na população de adultos jovens residente no sul (Valverde, 1997; Rojas, 2000).

Investigações em Teplice (República Checa), cidade escolhida como modelo de monitoramento e pesquisas do efeito à saúde da poluição atmosférica, relatam resultados diferentes. Um dos estudos encontrou associação entre os danos medidos pelo cometa em linfócitos e a exposição pessoal a partículas inaláveis e HPAs (Binková et al., 1996). Já outra investigação na mesma cidade, utilizando células brancas totais do sangue, não relatou diferença significativa entre a população alvo e a controle (Sram et al., 1998). Neste caso os autores ressaltam que linfócitos isolados parecem ser mais sensíveis do que células brancas totais, principalmente frente a baixas dosagens de mutágenos (Sram et al., 1998).

Apesar de alguns estudos utilizarem o ensaio cometa em células epiteliais (Valverde et al., 1997; Rojas et al., 2000), com a vantagem de uma abordagem não invasiva, Pinhal et al. (2006) investigaram se células da mucosa oral seriam adequadas para esse tipo de estudo. Esses autores mostraram que poucas dessas células formam

cometas possíveis de serem analisados e outras ainda formam cometas atípicos, concluindo que esse ensaio pode não ser um bom marcador nesse tipo celular.

A combinação desses dois métodos, micronúcleo e cometa, permitem a detecção de uma grande variedade de danos genotóxicos, pois enquanto o primeiro traz informações de danos cumulativos, como danos em cromossomos e aneuploidias, o ensaio do cometa registra danos recentes ainda passíveis de reparo (Laffon et al., 2002). Além disso, como a metodologia do ensaio cometa é mais recente e também mais sensível, a utilização conjunta de outro biomarcador genético é de fundamental importância para confirmar os relatos da literatura de sua maior sensibilidade à exposição que cause dano à molécula de DNA (Möller, 2000).

A avaliação conjunta utilizando marcadores de danos genéticos na população e de presença de substâncias mutagênicas no ambiente amplia as interpretações sobre a exposição e efeito dos contaminantes ambientais. Enquanto os métodos mais diretamente relacionados à saúde humana levam a conclusões genéricas, outros métodos aplicados a amostras ambientais (como mutagenicidade em *Salmonella*/microssoma) fornecem mais informações sobre os mecanismos de ação das substâncias e são mais facilmente utilizados para estudos comparativos (Claxton et al., 2004; Claxton & Woodall Jr., 2007). Nesse contexto, a identificação da presença de substâncias genotóxicas e seus efeitos nas populações das áreas de potencial contaminação tornam-se indispensáveis para uma estimativa de risco e para adoção de medidas mitigadoras.

O presente estudo teve como objetivo avaliar, através de marcadores genéticos, a presença de atividade mutagênica no material particulado atmosférico como marcador de exposição e diagnóstico ambiental em áreas urbanas e industriais, associando o biomonitoramento humano em população urbana exposta à atividade industrial.

Considerando os objetivos específicos e locais avaliados, a presente dissertação apresenta três capítulos, correspondentes aos artigos científicos resultantes do projeto e experimentos desenvolvidos.

O primeiro artigo *"Mutagenic activity of airborne particulate matter in a petrochemical industrial area"* (publicado na *Mutation Research* vol. 650 n. 2 p. 196–201, 2008) apresenta um estudo de diagnóstico, monitoramento e avaliação comparativa da atividade mutagênica do material particulado atmosférico em local sob influência do Complexo Petroquímico do Sul. Neste primeiro artigo dados atuais de experimentos com material particulado PM10 foram comparados com dois outros períodos de estudos, realizados anteriormente no grupo de pesquisa, utilizando material particulado total (PTS). Assim, além da comparação de respostas de diferentes frações do material particulado atmosférico de um mesmo local em diferentes períodos, foram apresentadas e discutidas análises com linhagens que permitem o diagnóstico da presença nitrocompostos, foram apresentados e discutidos.

O segundo artigo "*Mutagenicity of PM10 in an area under the influence of an oil refinery*" (a ser submetido para *Journal of Environmental Monitoring*) apresenta os resultados da avaliação da atividade mutagênica da fração inalável do ar em área de influência de uma refinaria de petróleo. Esse trabalho também compara os dados da área industrial com área urbana e relaciona a atividade mutagênica com outros parâmetros químicos e físicos do compartimento atmosférico.

O terceiro artigo "*Genetic biomonitoring of a population living in area under the influence of an oil refinery*" (a ser submetido na revista *Mutation Research*) mostra os resultados da avaliação de marcadores genéticos em populações humanas urbanas que habitam em área sob a influência da refinaria de petróleo.

2. MUTAGENIC ACTIVITY OF AIRBORNE PARTICULATE MATTER IN A PETROCHEMICAL INDUSTRIAL AREA*

*Artigo publicado na *Mutation Research/Genetic Toxicology and Environmental Mutagenesis* vol. 650 n. 2 p. 196–201, 2008. Co-autores: Rubem Cesar Horn, Adriana Ducatti, Jocelita Vaz Rocha e Vera Maria Ferrão Vargas

Abstract

Exposure to airborne particulate matter has adverse effects on human health and ecosystem. Mutagenic activity of airborne particulate organic matter extracts in three time periods from total suspended particles (TSP) and particles less than 10μm (PM10) was evaluated in an area under the influence of a petrochemical industry located in the town of Triunfo, Brazil. The extracts were investigated using the *Salmonella*/microsome assay, with the microsuspension method. The extracts were obtained by sonication extracted using dichloromethane (DCM) solvents. The fractions were tested for mutagenicity with the *Salmonella typhimurium* strains TA98 (with and without metabolic activation), TA98NR and TA98/1,8DNP$_6$; or YG1021 and YG1024. A positive frameshift mutagenic response was observed for the environmental samples during the different periods. The responses according to percentage of extractable organic matter (EOM%), EOM/m^3, revertants/μg and revertants/m^3 were lower for TSP than for PM10 extracts. The highest rev/m^3 values were observed in PM10 extract samples collected in winter, July 2005, in the presence (13.79 rev/m^3) or absence (6.87 rev/m^3) of S9 fraction. Similarly in the first (1995) or second period (2000) the highest values for TSP were observed in winter, but with lower activity (3.00 and 0.89 rev/m^3 respectively). The responses observed for the nitrosensitive strains suggest the contribution of nitro, amino and/or hydroxylamino derivatives of PAHs to the total mutagenicity of matter extracted from airborne particles. The *Salmonella*/microsome assay was a sensitive method to define areas contaminated by genotoxic compounds, even in samples with TSP or PM10 values that are acceptable according to legal environmental quality standards, favoring environmental control measures with an effective response seen in the population's improved quality of life.

Keywords: *Salmonella/*microsome; microsuspension method; TSP; PM10; mutagenicity; nitrocompounds.

1. Introduction

Airborne particulate matter is an air pollutant comprised of a complex mixture of compounds [1], and the main sources in industrial countries are transportation and industrial activities [2]. Exposure to this pollutant has adverse effects on human health [3] and the ecosystem [4]. The inhalable fraction of particulate matter (PM10 - particles less than 10μm) presents a significant risk to health because of their ability to penetrate and deposit in the respiratory tract [5]. Several studies assessed the mutagenic activity of atmospheric particulate matter concluding that the mutagenic compounds are almost exclusively located on particles less than 2.0–3.3 μm in diameter [6,7].

Polycyclic aromatic hydrocarbons (PAHs) are formed during incomplete combustion or pyrolysis of organic material and in connection with the worldwide use of oil, gas, coal and wood in energy production. These emissions are complex mixtures of hundreds of chemicals, including PAHs and PAH-derivatives, such as nitro-PAHs and oxygenated products, and also polycyclic aromatic compounds (PAC) [3]. Studies from several countries reported the presence of carcinogenic PACs and nitroarenes compounds adsorbed to particulate matter in air samples from urban and industrial areas [8-11].

In Brazil, although primary and secondary standards for total suspended particles (TSP) and inhalable (PM10) are regulated by the National Council of the Environment, studies report mutagenic activity even in samples in which the particulate matter values are within the established standards [9, 12, 13]. According to some of the studies, emissions from vehicles, industrial activities and waste incineration are the main

sources associated with mutagenic activity in airborne particulate matter samples [9, 10, 14, 15].

Considering the complexity of the composition and chemical reactions involved, an approach to evaluate the risks of exposure to the compounds present in the environment is essential for environmental diagnosis or monitoring. In this context, the *Salmonella*/microsome assay is a convenient, practical assay to compare genotoxic activity [10].

The present study analyses an area under the influence of a petrochemical industry during three periods. It investigates the mutagenic activity and the effect of nitrocompounds through specific strains. This study also compares the observed results for the organic extracts in 1995 and 2000 obtained from airborne particulates of TSP and in 2005 from PM10.

2. Material and Methods

2.1 Site and sampling procedures

2.1.1 Site

Airborne particulate matter was collected at a sampling station (29°49'35"S, 51°24'56"W) located under influence of petrochemical emissions inside the industrial area situated in the town of Triunfo, Rio Grande do Sul state, in southern Brazil (Fig. 1). This sampling station is 6.1 km away from the main smokestack of the of the central raw material (benzene, toluene, xylene, ethene, propane and butadiene) production area of this petrochemical district, located in the main atmospheric dispersion quadrant of this complex and 1.4 km from a federal highway. This petrochemical complex (14,600 ha) is located 30 km upstream from Porto Alegre, the capital of Rio Grande do Sul, in a mixed rural, urban and industrial area. Particulate matter samples (TSP or PM10) were

collected using 24h high volume sampling in these three different periods (Table 1). The samplings were performed monthly during winter and spring of 1995, 2000 and weekly in 2005 (Table 2). The filters were weighed and stabilized before and after sampling (45% humidity) to calculate the TSP or PM10 expressed in units of $\mu g/m^3$ of sampled air [16].

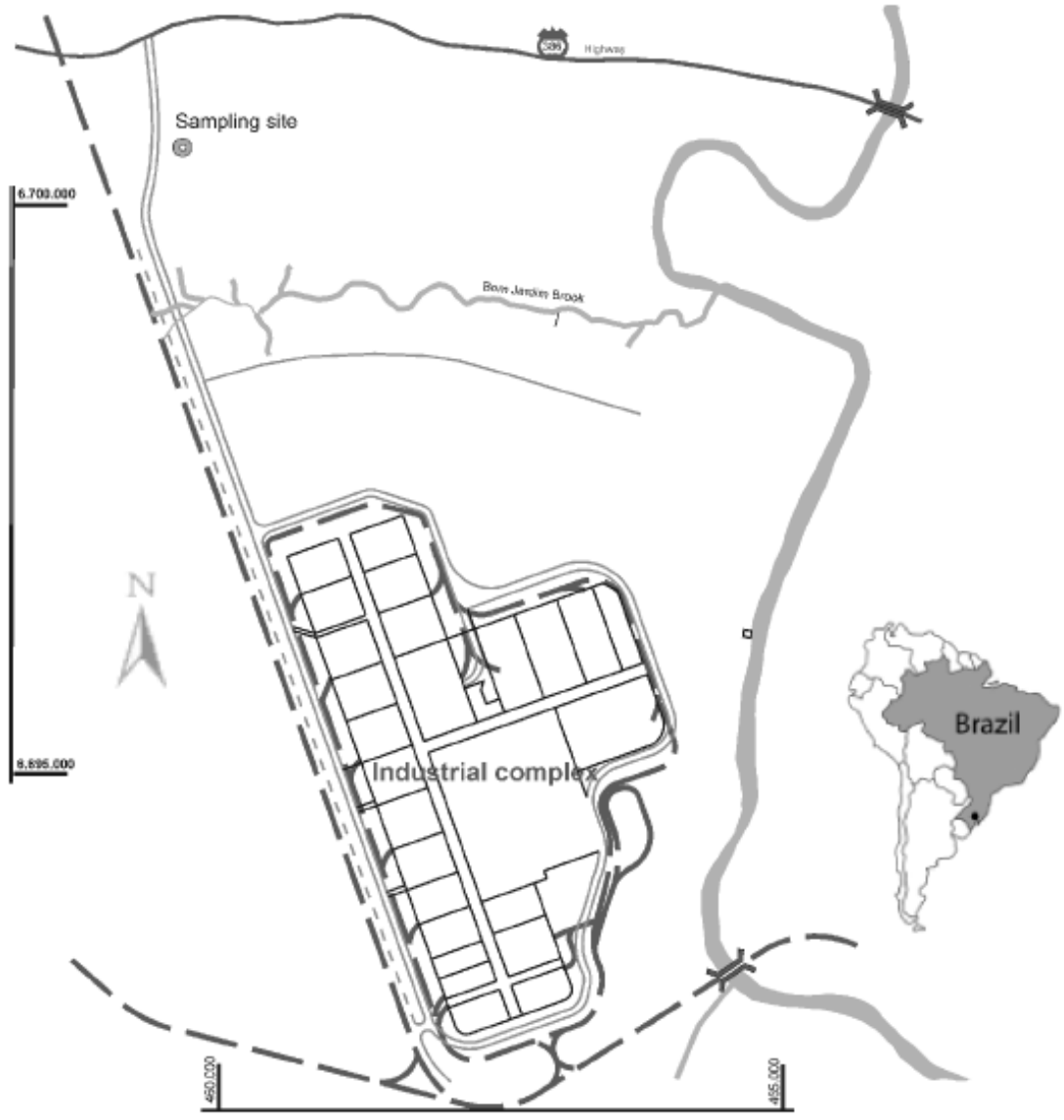

Fig. 1. Location of sampling site.

Table 1. Data on sampling: period, particle size, filter, sampler and time.

First period (1995)			
Particulate matter	*Filter*	*Sampler*	*Samples*
TSP (particles <100μm)	Fiber glass (AP40-810, 20cm×25 cm Millipore)	High-volume (General Metal Works Inc.)	1-Winter, 2- Spring
Second period (2000)			
Particulate matter	*Filter*	*Sampler*	*Samples*
TSP (particles <100μm)	Fiber glass (AP40-810, 20cm×25 cm Millipore)	High-volume (General Metal Works Inc.)	3- Fall/Winter 4- Spring
Third period (2005)			
Particulate matter	*Filter*	*Sampler*	*Samples*
PM10 (particles <10μm)	Teflon (TX40HI20WW, 254X203mm)	High-volume (AVG MP10, 1200/CCV)	5, 6, 7 -Winter 8- Spring

The references used were values already described for an area located in the Botanical Gardens neighborhood at Porto Alegre in spring (negative response) [13] and winter (unpublished data) 1998 (1.67 ± 0.41 and 2.62 ± 0.39 for TA98-S9 and TA98+S9 respectively).

2.1.2 Extraction of organic compounds

Half of each filter was used for the extraction of the organic compounds. The filters were pooled to obtain monthly samples. Each sample was submitted to extraction by sonication with dichloromethane (DCM, CASRN. 75-09-2) [15]. Dichloromethane extracts the moderately polar compounds and is the most representative fraction of mutagenic activity [5] extracting a wide range of compounds from airborne particulate [17]. DCM is preferred when the investigator did not want to extract non-organic components that would be toxic to the bacteria used in the assay [10, 17]. The percentage of extractable organic matter (EOM%) was calculated, and the mass obtained was compared to half the volume of air sampled (EOM in $\mu g/m^3$), since half the filters were used. Prior to bioassay performance, the organic extract was dried with gaseous nitrogen and resuspended in dimethyl sulfoxide (DMSO, CASRN. 67-68-5).

2.2 Salmonella/microsome assay

Mutagenicity of the organic extracts was assessed using the *Salmonella*/microsome assay [18], through the microsuspension method [19]. *Salmonella typhimurium* strains used were: frameshift strain TA98, with and without metabolic activation (S9 mix fraction); the nitroreductase deficient (TA98NR.) or overexpressing (YG1021) strains and the *O*-acetyltransferase -deficient (TA98/1,8-DNP_6) or overexpressing (YG1024) [20-22]. During the first and second periods the deficient strains were used and in the last period the overexpressing strains started to be used in our institution (Table 2). The different sets of these strains allow similar conclusions about the presence of nitrocompounds. Six doses of each sample (1.25, 2.50, 5.0, 10.0, 20.0 and 40 µg/plate) were tested in duplicate. All assays were carried

out in the presence of negative (5µl DMSO solvent 5µl/plate) and positive (4-nitroquinoline oxide—4NQO, 0.5µg/plate, CASRN. 56-57-5; 2-nitrofluorene—2NF, 0.15µg/plate, CASRN. 607-57-8; and 2-aminofluorene—2AF, 1µg/plate, CASRN. 153-78-6 from Sigma Chemical Company, St.Louis, MO) controls for the different strains.

2.3 Data Analysis

The assay response was considered a significant effect when the number of *his*$^+$ revertants per plate observed was double that of the spontaneous yields observed in the negative control, a significant ANOVA ($p \leq 0.05$) accompanied by a significant dose-response curve ($p \leq 0.05$). The response was considered indicative when one of the criteria was not fulfilled. The significance of linear regressions from the dose–response curves was evaluated by the Salmonel program [23] choosing the linear or Bernstein model, as described in Vargas et al. [15]. The positive or indicative responses were expressed in revertants/µg of extract (rev/µg), number of revertants per unit mass of particles and revertants/m^3 (rev/m^3), number of revertants per unit volume of air (rev/µg × EOM in µg/m^3).

3. Results

The air volume drawn through each filter ranged from 1349 to 2593 m^3 for TPS and from 1349 to 1469 m^3 for PM10 samples; the mass ranged from 20.4 to 147.4 mg/filter for TSP and 13 to 163.4 for PM10; the concentration of total suspended particles ranged from 8 to 92 µg/m^3 for TSP and 9 to 111 µg/m^3 for PM10 (Table 2).

Table 2. Particle concentration and strains used for nitrocompounds detection.

	Data collection period	Particle concentration ($\mu g/m^3$)	Nitrocompounds sensitive strains
First period 1995	**Sample 1**		TA98 NR; TA98/1,8DNP$_6$
	July	52	
	August	41	
	August	86	
	Sample 2		
	November	56	
	November	92	
Second period 2000	**Sample 3**		TA98 NR; TA98/1,8DNP6
	May	17	
	May	13	
	June	14	
	July	57	
	Sample 4		
	September	8	
	October	38	
	November	31	
	December	38	
Third period 2005	**Sample 5**		YG1021; YG1024
	June	46	
		44	
		10	
		9	
		37	
	Sample 6		
	July	27	
		26	

Sample 7		
		28
		20
	August	41
		10
		23
Sample 8		
		35
		111
	November	88
		71
		58

Standard concentrations for TSP / PM10 (μg/m^3) for exposure of 24-hours: Primary Standards 240/150 μg/m^3; Secondary Standards 150/150 μg/m^3 [24].

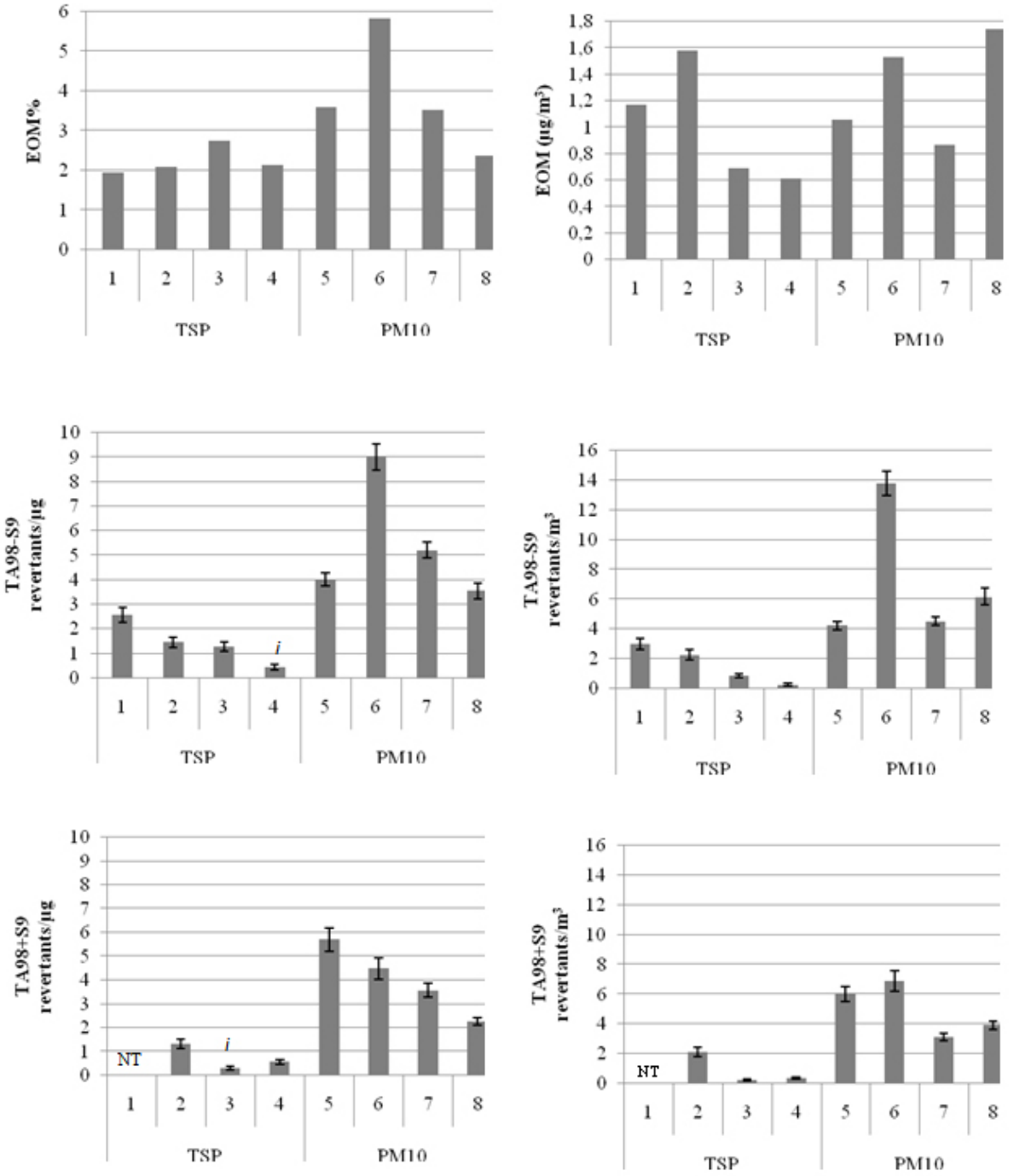

Fig. 2. Variation in extractable organic matter in percent (EOM%) per unit of air sampled (EOM µg/m^3) and mutagenic activity for TA98 strain (revertants/µg or m^3). -S9,+S9 absence, presence of S9 mix; NT not tested; Negative control (DMSO): 47±17.11 (TA98-S9), 55±38.57 (TA98+S9); Positive control: 4NQO (TA98-S9) 483±14.85; 2AF (TA98+S9) 365±204.29.

Fig. 2 presents the results observed for TSP or PM10 during the different periods (1995, 2000, 2005) and months, in pool values of EOM%, EOM µg/m^3, rev/µg or rev/m^3 for TA98 and TA98 with and without S9 mix. There appears to be a relationship between EOM% and rev/µg for direct mutagenesis in the highest value, corresponding to the samples of PM10. The highest values were observed in the samples of PM10, with sample 6 being outstanding (5.83% and 9.01 rev/µg). In the presence of metabolic activation, mutagenic activity diminishes except in samples 4 and 5.

The amount of extractable organic matter (EOM) varied during the periods sampled, and the lowest values were obtained in the samples of the second sampling period (2000), where the lowest values of revertants per amount of extracted matter (µg) and volume of air (m^3) were also found. The volume of air needed to observe a mutagenic effect, calculated from double the spontaneous mutation and the number of rev/m^3 also varied throughout the period, values varying from 12.54 to 253.85 m^3 for direct mutation and 13.23 to 293.94 m^3 for promutagens (Fig. 3).

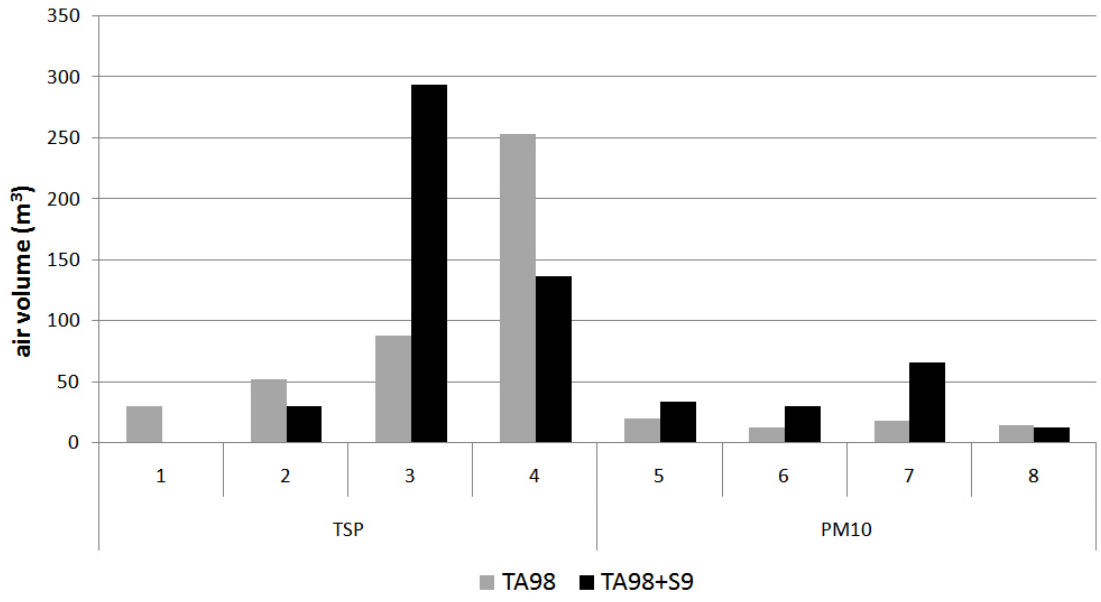

Fig. 3 Variation of air volume required to obtain a mutagenic effect.

The nitro compounds contributions to direct mutagenic activity were investigated through specific strains (Table 3). The results for the first and second periods showed a major decrease in the mutagenic responses observed for deficient strains when compared with the TA98 (48% to total absence), highlighting that mononitro e dinitroarenes contribute to the observed mutagenicity. For the third period the increase of mutagenic activity in overexpressing strains (1.27 to 10.38 times the spontaneous index) indicates the nitroarenes and/or aromatic amines presence.

Table 3. Mutagenicity of airborne particulate matter organic extracts in revertants/µg

	Sample	TA98	TA98NR	TA98/1,8DNP$_6$
First period	1	2.57 ± 0.32	NT	NT
	2	1.45 ± 0.21	0.28 ± 0.26	absence
Second period	3	1.28 ± 0.19	0.66 ± 0.07	0.69± 0.16
	4	*0.43 ± 0.122*	absence	0.53 ± 0.13
		TA98	YG1021	YG1024
Third period	5	4.02 ± 0.27	35.9 ± 2.48	41.74 ± 1.91
	6	9.01 ± 0.53	17.17 ± 2.24	11.45 ± 1.24
	7	5.21 ± 0.32	15.23 ± 2.82	18.21 ± 1.45
	8	3.55 ± 0.33	13.97 ± 0.85	9.4 ± 0.76

Negative control: 47±17.11 (TA98), 38.67±1.53 (TA98NR), 31±7,12 (TA98/1,8DNP$_6$), 53±16.15 (YG1021), 42±10.31 (YG1024); Positive control: 4NQO (TA98) 483±14.85; 2NF 236±52.33, TA98NR; 233±10.61, TA98/1,8DNP$_6$; 3519±1055.73, YG1021; 3313±1670.5, YG1024; *Italic*: indicative response. The values are mean ± standard error. NT: not tested

4. Discussion

The present study focuses on a comparative approach to the mutagenic potency of EOM from airborne particulate matter (TSP and PM10) in an industrial petrochemical area. The determinations of TSP or PM10 (μg/m^3) with a 24-hour sampling time are quality evaluation measures in our state [24]. In the samples investigated, the concentration of particles present (Table 2) agrees both with the primary (240, TSP or 150, PM10 μg/m^3) and the secondary (150 μg/m^3, for TSP or PM10) Brazilian legal standards [24].

The results obtained do not present a clear variation during the seasons studied. The diversity of responses during the three periods investigated was observed for possible correlations between TSP or PM10, EOM and mutagenic response. During the first and second periods (TSP), samples 1 (winter) and 2 (spring) presented the highest mean concentrations of TSP, the highest values of mutagenic potential in rev/μg and the lowest percentage of EOM. However, during the third period, sample 6 (winter) presented lower mean PM10 values, a higher percentage of EOM and positive mutagenicity in rev/μg or m^3. On the other hand, sample 8 (spring), which presented a higher mean PM10, had a lower percentage of EOM and positive mutagenicity in rev/μg.

The amount of air volume necessary to double the frequency of reversion was lower in the samples of the third period analyzed, mainly in direct mutagenesis. This result may represent the magnitude of the mutagenic effect of organic compounds adsorbed to the particulate matter in the environment and help estimate the risk of exposure. Independent of these aspects, all extracts examined from the airborne particulate matter TSP or PM10 were mutagenic for the different strains used (Fig. 2 and Table 3).

The results were higher in PM10 than TSP organic extracts, and the highest mutagenic activity was detected for PM10 sample 6 (July, winter). In a review of literature, Claxton et al. [10] emphasize that mutagenicity increased with decreasing particle size as well as their capacity to penetrate and deposit more deeply in the respiratory tract. Also related to particle size, Hayakawa et al. [25] showed that the relative amounts of 1- nitropyrene and dinitropyrenes increased as particle size decreased. This finding increases health concerns regarding finer particles. It should also be pointed out that the highest values observed in 2005, might be related to the elevation of mutagenic compounds caused by anthropic sources. Vargas [9] reports a pilot study in the same petrochemical district area using the Kado assay to investigate mutagenicity of airborne particulate matter in four sampler points during the period from January to April 2000.The results show that due to preferential wind directions, samples from a site inside the industrial Complex, in an area where most particulate deposition occurs, present the highest levels of mutagenic activity measured in rev/μg and rev/m^3. Two locations in an area outside plant area 7 and 9 km from the petrochemical complex, considering the same wind directions, presented 83 and 89% decrease in rev/m^3, respectively. The ratio was similar in assays with S9 fraction but showed lower absolute values. Comparing responses for TSP values in rev/m^3, no relationship can be seen between the largest TSP magnitudes and the levels of rev/m^3. These results agree with others that indicate that mutagenic activity is more highly concentrated in the fine particulate matter [6].

All samples showed frameshift mutagens and the observed responses for nitrosensitive strains indicate a significant contribution of nitro and amino derivatives of PAHs to the total mutagenicity of suspended particulates. In fifty percent of the cases, samples 4, 6 and 8, the nitroreductase strains (TA98NR or YG 1021) were more

sensitive and sample 4 gave better responses. These strains are more sensitive to the nitro derivatives of PAHs due to increased nitroreductase activity. But for samples 2, 5 and 7 the *O*-acetyltransferase strains (TA98/1,8-DNP_6 or YG 1024) were more sensitive to nitro and amino derivatives of PAHs present in the organic extracts. For sample 3, a mixture of responses were observed. The results indicate the participation of nitroarenes or hydroxylamines and aromatic amines in the total mutagenicity of the extracts studied. The hydroxylamine and aromatic amine content was higher in PM10 sample 5 (winter, June). These responses were associated with increased mutagenicity in S9 fraction metabolic activation tests due to the presence of promutagens in the suspended particulates.

Ducatti and Vargas [13] investigated mutagenic activity in the urban area of Porto Alegre, Brazil, comparing the studies performed in the industrial area during the summer and spring periods (1998). These authors observed that mutagenic frameshift responses in organic airborne particulate matter varied in different seasons of the year and the highest rev/m³ values were observed at a site with heavy traffic in spring for DCM-fractions, and in summer for CX-fractions. As to reference site, a negative response was observed in spring and a positive one in summertime. The mutagenic responses of most of the samples showed the presence of a mixture of mononitroarenes and dinitroarenes in different proportions, or even other groups of chemicals, suggesting different pollution sources. In a different way from the industrial area measured, the data in this previous study suggest an association between the increased TSP and the mutagenicity of EOM in rev/m^3.

The results of the Rio Grande do Sul studies confirm the usefulness of this biological approach as an air quality parameter. This also enables measuring genotoxic properties of samples and the variability of mutagenic properties of the airborne mixture

over time. It determines the presence of polycyclic aromatic hydrocarbons (PAHs) and nitro-derivative compounds and favors environmental control measures with an effective response in the population's improved quality of life.

Acknowledgments

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3. MUTAGENICITY OF PM10 IN AN URBAN AREA UNDER THE INFLUENCE OF AN OIL REFINERY*

*Artigo a ser submetido para *Journal of Environmental Monitoring*. Co-autores: Andréia Torres de Lemos, Jocelita Aparecida Vaz Rocha e Vera Maria Ferrão Vargas

Abstract

Industrial and urban areas are extensively studied for the presence of genotoxic compounds and their effects on human health and ecosystems. These areas show mutagenic activity in airborne particulate worldwide. Organic extracts of PM10 airborne particulate matter (particles less than 10μm) in the area under the influence of an oil refinery plant (site 1) and an urban area (site 2) were investigated for mutagenicity in *Salmonella*/microsome assay with TA98 (with and without S9 mix fraction), YG1021 and YG1024 strains. All samples showed mutagenic positive responses. For most of the samples, mutagenic effect decreased in the presence of S9 mix indicating that the predominant compounds present in the organic particulate matter were direct-acting mutagens. The responses of YGs strains indicated that aromatic amines and nitroarenes were present in PM10 extracts from site 1, under the influence of an oil refinery plant, while at site 2, urban area, nitroarenes predominated. In site 1 there are two automatic stations belonging to the Rio Grande do Sul Environmental Protection State Foundation (FEPAM) that monitor some air quality parameters. During the present study the parameters exceeded the concentrations established by Brazilian air quality standards in only three episodes. The data of these parameters showed that these air pollutants reach a substantial concentration. These air pollutants have important role in atmospheric reactions that may originate mutagenic products. This study reinforces the importance of biological tests in environmental monitoring and characterizes areas under the influence of anthropogenic activities. It also indicates that the current air quality standards are not sufficient to avoid damage to the environment and human health.

Keywords: *Salmonella/*microsome; microsuspension method; PM10; nitrocompounds.

1. Introduction

Ambient air is reported to be mutagenic in many urban and industrial areas worldwide.[1-4] Air pollution adverse effects increase concern about the regulatory policies and stimulate the development of new efficient air standards. Due to the complexity of composition and reactions comprised in the atmospheric environment, biological assays present as an alternative to show the effects of exposure.

Initially, most of the mutagenicity of airborne combustion particles was attributed primarily to polycyclic aromatic hydrocarbons (PAHs), but early researches demonstrated that these compounds are not the predominant class of mutagens found in airborne particles, although they contribute significantly to mutagenicity.[2,5] A wide range of aromatic compounds (PAC), 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_2 and NO_3 radicals.[5-7]

In Brazil, total suspended particles (TSP) and inhalable (PM10) are regulated by the National Council of the Environment.[8] However, because of the complex system that forms the airborne particulate matter, no safe threshold level or no main component of these air pollutants or adverse effects are established.[9] Studies associating emissions sources, mutagenic activity diagnosis and chemical characterization of airborne particulate matter help understand the effects of pollutants and establish more restrictive and safer concentrations of the main biologically active components.

Most of the studies investigating mutagenic and carcinogenic properties in urban air reported positive responses.[2,3] Areas affected by industrial activities have also shown mutagenic activity.[4,10-12]

The objective of the present study was to evaluate the mutagenic activity of inhalable airborne particulate matter in an area under influence of an oil refinery plant. At the same time an urban area was also investigated to compare both areas. Furthermore, the mutagenic activity investigated for the presence of different classes of nitrocompounds, were assessed through specific strains.

2. Material and Methods

2.1 Site and sampling procedures

2.1.1 Sampling site and airborne particulate matter collection

Airborne particulate matter (PM10) samples were collected on Teflon® filters (TX40HI20WW, 254mm × 203mm) using a high-volume collector (AVG MP10, 1200/CCV) for 24 hours. The samples were collected weekly from October to December 2006. The collector was placed in the town of Esteio (87,000 inhabitants), Rio Grande do Sul state, in southern Brazil in an urban/residential area (29°51'29"S; 51°09'25"W) under the influence of an oil refinery plant (Fig. 1). The refinery is located in the town of Canoas (330,000 inhabitants), close to the region that borders with the neighboring municipality, Esteio (site 1). The dominant winds there are from the Southeast, and therefore Esteio is most influenced by the plant, and the PM10 collector was installed at a site predisposed to particle deposition. In this area there are two automatic stations from the Rio Grande do Sul Environmental Protection State Foundation (FEPAM) monitoring network which regularly measure PM10, SO_2, NO_X, O_3, CO and meteorological parameters (Fig. 1). Among these stations, one is located in the municipality of Canoas (29°52'58"S; 51°08'39"W) southeast from the oil refinery, and the other in the municipality of Esteio (29°51'31"S; 51°10'43"W), to the northeast. In order to compare it with an urban area free of petrochemical contamination samples

were collected in the city of Porto Alegre (1.4 million inhabitants), capital of the state of Rio Grande do Sul, far from large industries and near the Botanical Gardens at 8th District station (site 2).

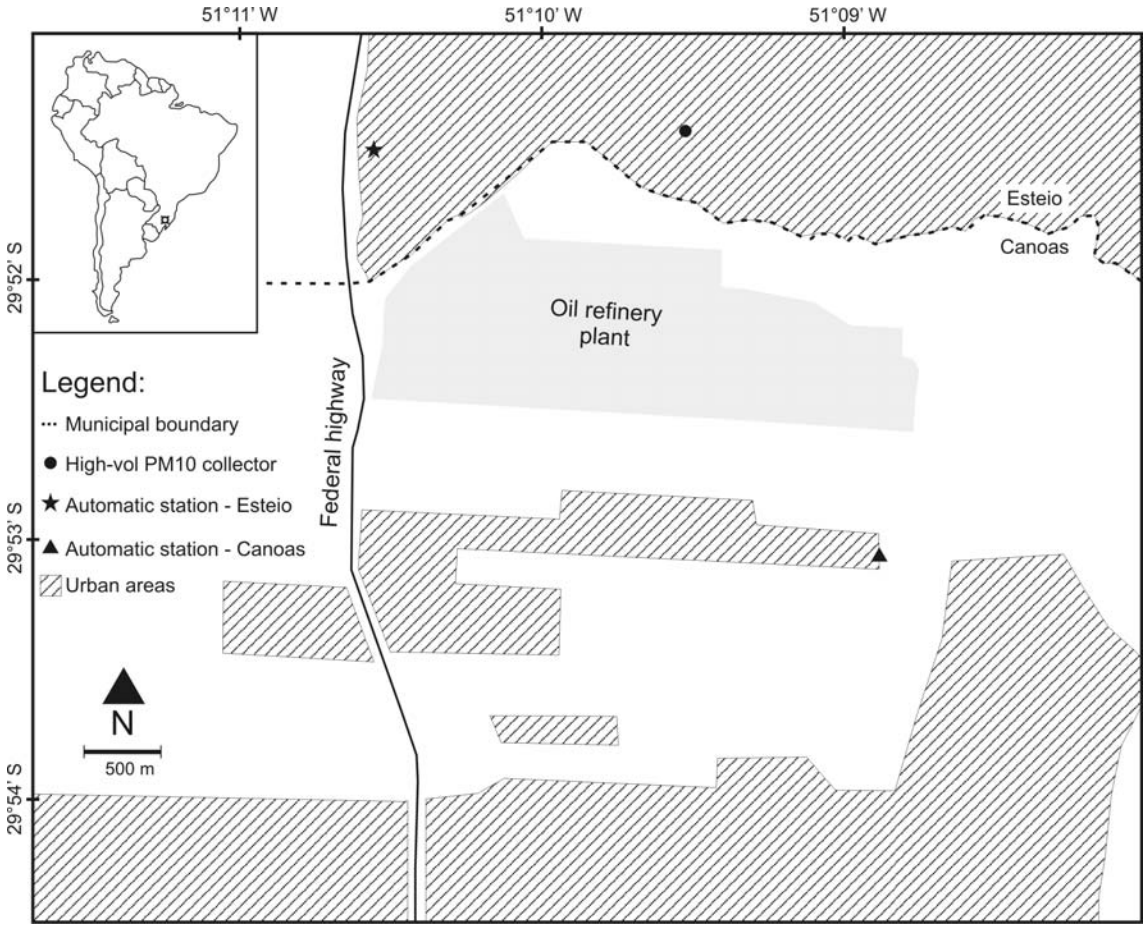

Fig.1 Location of sampling site

2.1.2 Extraction of organic compounds

Half of each filter was used for the extraction of the organic compounds. The filters were pooled two by two to obtain biweekly samples. Each sample was submitted to extraction by sonication with dichloromethane (DCM, CASRN. 75-09-2).[13] Dichloromethane extracts the moderately polar compounds and is the most representative fraction of mutagenic activity,[14] extracting a wide range of compounds

from airborne particulate matter.[15] DCM is preferred when the investigator did not want to extract non-organic components that would be toxic to the bacteria used in the assay.[2,15] The percentage of extractable organic matter (EOM%) was calculated, and the mass obtained was compared to half the volume of air sampled (EOM in μg/m^3), since half the filters were used. Prior to bioassay performance, the organic extract was dried with gaseous nitrogen and resuspended in dimethyl sulfoxide (DMSO, CASRN. 67-68-5).

2.2 *Salmonella*/microsome assay

The organic extracts were assayed for mutagenicity through the *Salmonella*/microsome assay,[16] in the microsuspension method.[17] For each sample *Salmonella typhimurium* TA98 (frameshift strain) was used, with and without metabolic activation (S9 mix fraction), and also was used the derivatives strains YG1021 (nitroreductase -overproducing) and YG1024 (*O*-acetyltransferase -overproduction).[18,19] Six doses of each sample (1.25, 2.50, 5.0, 10.0, 20.0 and 40 μg/plate) were tested in duplicate. All assays were carried out under yellow light and in the presence of negative (DMSO solvent, 5μl/plate) and positive (4-nitroquinoline oxide—4NQO, 0.5μg/plate, CASRN. 56-57-5; 2-nitrofluorene—2NF, 0.15μg/plate, CASRN. 607-57-8; and 2-aminofluorene—2AF, 1μg/plate, CASRN. 153-78-6 from Sigma Chemical Company, St.Louis, MO) controls for the different strains.

2.3 Data Analysis

The assay response was considered a significant effect when the number of *his*$^+$ revertants per plate observed was double that of the spontaneous yields observed in the negative control, a significant ANOVA ($p \leq 0.05$) accompanied by a significant dose-response curve ($p \leq 0.05$). The response was considered indicative when one of the criteria was not fulfilled.[13] The significance of linear regressions from the dose–

response curves was evaluated by SALANAL software (*Salmonella* Assay Analysis, version 1.0, Integrated Laboratory Systems of Research Triangle Institute, RTP, North Caroline, USA) choosing the linear or Bernstein model.[20] The positive or indicative responses were expressed as the number of revertants in function of the mass of PM10 extracts (rev/μg) and volume of air sampled (rev/ m^3) *i. e.*, rev/μg multiplied by EOM in $\mu g/m^3$.

3. Results

The air volume drawn through each filter ranged from 1417 to 2030 m^3 for site 1 and from 1699 to 1944 m^3 for site 2 samples; the concentration of PM10 particles ranged from 9 to 62 $\mu g/m^3$ and 15 to 76 $\mu g/m^3$ for site 1 and 2, respectively (Table 1). The extractable organic matter (EOM) in $\mu g/m^3$ and in relation to total particulates collected (%) are shown in Table 1. The samples from site 1 (refinery influenced) showed the highest values of EOM ($\mu g/m^3$). All samples showed positive responses (except sample 7 for the TA98 strain which had an indicative response) for mutagenesis for all strains (Table 2 and Fig. 2). The highest value observed was for sample 9 (site 2), 18.89 rev/μg without S9 mix and 10.90 rev/μg in the presence of S9 mix. For most of the samples, the mutagenic activity decreased in the presence of S9 mix, except for sample 5 where there was a slight increase. This response indicating that the predominant compounds present in the organic particulate matter were direct-acting mutagens.

Table 1 Sample sites, dates, volume, PM10 concentration and extractable organic matter (EOM) from the two sites analyzed in Rio Grande do Sul, Brazil in 2006.

Sample	Date	Air volume (m^3)	PM10 ($\mu g/m^3$)	EOM ($\mu g/m^3$)	EOM%
Site 1 *(industrial)*					
1	October, 14th	1785	9	0.48	2.92
	October, 18th	1958	23		
2	October, 24th	1770	62	2.03	4.36
	October, 30th	1770	27		
3	November, 5th	1958	19	0.77	2.51
	November,13th	1785	46		
4	November, 17th	1944	14	0.91	5.5
	November, 25th	2030	20		
5	November, 29th	1417	15	1.12	4.36
	December, 5th	1828	37		
6	December. 11th	1829	47	1.65	3.79
	December, 18th	1880	40		
Site 2 *(urban)*					
7	October, 14th	1913	15	0.57	7.14
	October, 20th	1944	P		
8	November, 12th	1944	24	0.79	2.58
	November, 18th	1846	37		
9	December, 13th	1872	76	0.95	2.05
	December, 19th	1699	14		

P prejudiced filter

Table 2 Mutagenic activity of extractable organic PM10 in *Salmonella*/microsome assay in TA98 strain

Samples	TA98-S9		TA98+S9	
	rev/µg	rev/m^3	rev/µg	rev/m^3
Site 1 (industrial)				
1	6.78 ± 0.43	3.26 ± 0.21	2.60 ± 0.24	1.25 ± 0.12
2	6.32 ± 0.65	12.85 ± 1.32	4.53 ± 0.20	9.21 ± 0.41
3	5.03 ± 0.36	4.03 ± 0.29	3.02 ± 0.45	2.42 ± 0.36
4	11.69 ± 0.53	10.64 ± 0.48	9.14 ± 0.29	8.32 ± 0.26
5	2.19 ± 0.29	2.46 ± 0.33	2.50 ± 0.35	2.81 ± 0.39
6	4.39 ± 0.30	7.26 ± 0.50	2.78 ± 0.30	4.60 ± 0.50
Site 2 (urban)				
7	*2.35 ± 0.53*	1.33 ± 0.30	1.60 ± 0.25	0.91 ± 0.14
8	2.67 ± 0.56	2.11 ± 0.44	1.90 ± 0.22	1.50 ± 0.17
9	18.89 ± 1.44	17.95 ± 1.37	10.90 ± 0.55	10.36 ± 0.52

-S9,+S9 absence, presence of S9 mix fraction; Negative control (DMSO): 50.7 ± 27.4 rev/plate (TA98-S9), 45 ± 18.9 rev/plate (TA98+S9); Positive controls: 4NQO (TA98-S9) 456 ± 340 rev/plate; 2AF (TA98+S9) 453± 224 rev/plate. The values are mean ± standard deviation. *Italic:* indicative response

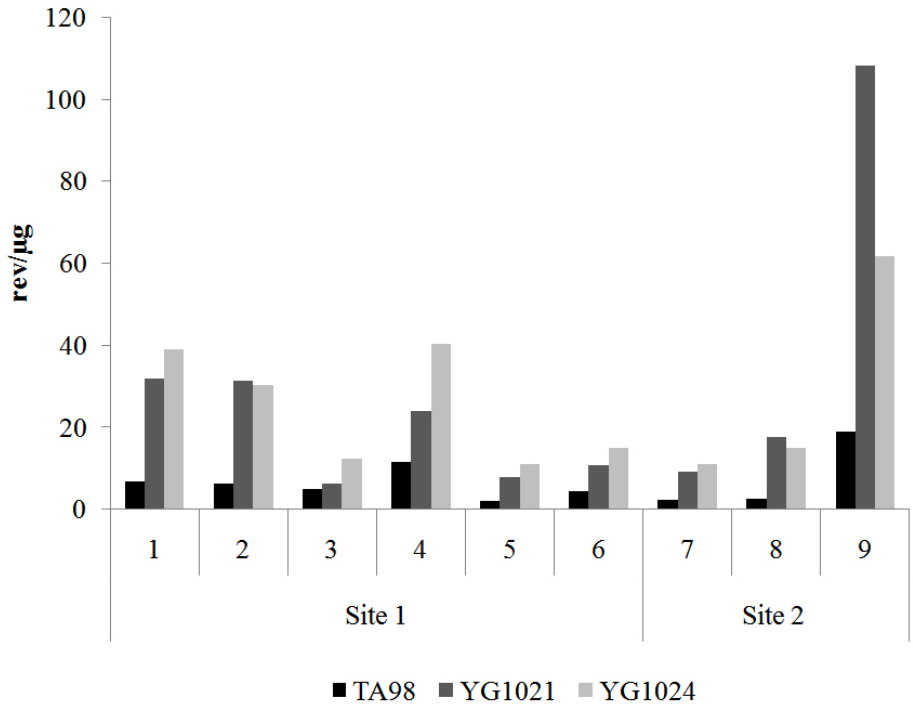

Fig. 2 Mutagenic activity of PM10 organic extracts for TA98, YG1021 and YG1024 strains.

Negative control (DMSO): 50.7 ± 27.4 (TA98), 75.13 ± 29.7 (YG1021), 44.8 ± 16.2 (YG1024); Positive controls: 4NQO (TA98) 456 ± 340; 2NF (YG1021) 4146 ± 3743, (YG1024) 5416 ± 2293. The values are mean ± standard deviation.

All samples showed increase response for mutagenic activity for the YGs strains, indicating the presence of nitrocompounds in the evaluated sites. At site 1, under the influence of oil refinery, strain YG1024 produced higher values of revertants/µg of EOM in five of the six samples, while at site 2, urban area, strain YG1021 showed higher values in most of the samples.

Air pollutants measured by automatic stations are shown in Table 3, the concentrations are monthly means and standard deviation; maximum concentrations measured during the period are also shown. Exceedance data are based on Brazilian Brazilian air quality parameters.[8] During this period the parameters were below the values established in Brazilian air quality standards except in three episodes, one for O_3 at Canoas station and two for NO_2 at Esteio station. These episodes did not coincide with the sampling days.

Table 3 Parameter measured at FEPAM automatic stations during sampling period.

Station	Period	PM10 (μg/m^3)		NO (μg/m^3)		NO_2 (μg/m^3)		NO_x (μg/m^3)		O_3 (μg/m^3)		CO (ppm)		SO_2 (μg/m^3)	
		Mean ± S.D.	Max	Mean ± S.D.	Max	Mean ± S.D.	Max	Mean ± S.D.	Max	Mean ± S.D.	Max	Mean ± S.D.	Max	Mean ± S.D.	Max
Canoas	October	36.17 ± 27.28	260.9	6.46 ± 11.80	138.2	36.44 ± 22.65	159.4	43.37 ± 30.56	294.8	40.89 ± 25.45	168	0.35 ± 0.13	1.1	2.46 ± 9.80	119.7
	November	32.69 ± 23.85	172.9	7.22 ± 14.96	131.4	32.97 ± 23.60	176.8	39.64 ± 35.45	275.3	39.81 ± 21.38	124.5	0.29 ± 0.14	1	4.49 ± 23.12	449.5
	December	44.49 ± 27.49	270	6.77 ± 10.77	126.8	30.32 ± 17.59	142.1	37.53 ± 25.16	236.4	39.74 ± 26.87	151.2	0.29 ± 0.13	0.9	4.68 ± 16.79	218.1
	Exceedances*	0		0		0		0		1		0		0	
Esteio	October	30.26 ± 38.06	429.7	6.80 ± 11.81	183.3	18.43 ± 12.49	105.8	25.72 ± 21.44	262	32.58 ± 20.10	107.5	0.55 ± 0.23	1.7	24.76 ± 37.20	325.2
	November	19.29 ± 12.75	127	10.33 ± 20.67	241.7	23.58 ± 28.92	272.6	34.41 ± 44.20	466.9	29.02 ± 16.21	90	0.54 ± 0.25	1.6	23.77 ± 35.98	269.4
	December	27.90 ± 15.44	91.5	17.97 ± 25.27	273.5	48.71 ± 34.64	390.5	67.16 ± 54.39	634	30.60 ± 20.10	110.7	0.57 ± 0.22	1.5	16.71 ± 30.96	255.6
	Exceedances*	0		0		2		0				0		0	

* Exceedances are how many days the concentrations went over Brazilian air quality standards.[8] Canoas O_3 exceeeds on October, 10th, Esteio NO_2 exceeds on December, 05th and December, 15th. The values are monthly means ± standard deviation (S.D.); Max values are maximum concentration measured.

4. Discussion

The major objective of the present study was to investigate the mutagenicity of organic particulate matter present in PM10 extracts in an area under influence an oil refinery, relating the mutagenic activity detected to nitrocompounds and compare it to an urban area. All samples showed positive responses for mutagenicity in *Salmonella*/microsome assay for all strains tested. These results increase health concerns regarding this air pollutant.

Although mutagenic activity is not directly related to particle concentration, in the present study the samples that had the highest and lowest PM10 concentration were those that presented the highest [sample 9, 18.89 ± 1.44 (-S9); 10.90 ± 0.55 (+S9) in revertants/μg EOM] and lowest direct and indirect mutagenic activity [sample 7, 2.35 ± 0.53 (-S9); 1.6 ± 0.25 (+S9) in revertants/μg EOM]; both samples were from the urban area (site 2). The extractable organic matter (EOM) did not present a clear relationship with the particle concentration and mutagenic activity, but generally, the highest EOM concentrations resulted in a higher mutagenic potential (Table 1 and Table 2). Mutagenic activity is more closely related to the types of compound and reaction products than to the particle mass. Although vehicular emissions contribute a relatively small amount of the organic mass, they contribute a relatively substantial amount of the genotoxicity potential.[2]

Much of the S9 independent mutagenic activity is due to nitroarenes.[21, 22] The main sources of these compounds are vehicular emissions and photochemical atmospheric reactions.[6,21] Nitroarenes such as 1NP that elute in the neutral/base (DCM) fraction account for at least 50% of the direct-acting mutagenicity and induce only the hotspot frameshift mutation in strain TA98.[22] This occurrence, with

higher activity for direct-acting mutagens (-S9), as observed in the present study (Table 2), has already been found in other studies evaluating urban air samples.[10,13]

Most of the S9 dependent mutagenic activity is due PAHs.[2] PAHs and others compounds extracted in the base/neutral fraction (DCM) are responsible for mutagenicity in organic samples.[21] These compounds in urban areas originate manly by traffic emissions.[23]

Site 2 (urban) used to be a reference site for the urban area in Porto Alegre (RS, Brazil) presenting in previous studies absence or low mutagenic activity.[13,24] These studies evaluated total suspended particulate matter (TSP), which might account for the low indexes of mutagenesis, since the lowest particle fractions concentrate organic and mutagenic compounds.[25,26] However, increased vehicle traffic in the area indicates that this is an important factor for the high values found in the present study. A more recent study detected the 16 most important PAHs in PM10 at this site.[27] According to the 8th District meteorological station records, the dominant winds at the site are from the southeast, and it receives the influence of the place in the city with the lowest occupation and least industries, coming from the direction of the municipality of Viamão (250,000 inhabitants), RS. In the present study lower mutagenic responses were observed in this site than industrial area (site 1). However, on the days when the December sampling was performed (pool 9) there was a record of wind from the north, bringing the influence of the area with the highest traffic and most industries in Porto Alegre (1,420,000 inhabitants) which may indicate the elevated mutagenic activity observed in this sample.

The samples at site 1, under the influence of the refinery, showed elevated values for YG1024, indicating the presence of aromatic amines and nitroarenes in PM10 extracts while at site 2 (urban area) nitroarenes predominated (YG1021). Brits *et al.*[11]

evalueted PM10 in urban, rural and industrial areas in Belgium found higher particle and PAHs concentration in the urban area, where the strongest mutagenic activity was also found, followed by the industrial area. Zwozdziak *et al.*[28] evaluated PM10 organic matter extracted with DCM in an urban area in Poland and reported higher sensitivity of YG1021 (nitroarenes) than YG1024. Likewise, Vargas *et al.*[13] and Ducatti & Vargas[24] observed for Porto Alegre that, in DCM fraction, TA98NR (nitroreductase deficient) strain was more sensitive. These results are in agreement with those observed in the present study in which the YG1021 strain was more sensitive in urban area, in two of the three samples. However, in petrochemical industrial area more mutagenic activity has been observed for YG1024 strain, in accordance with early studies performed by our research group.[1,4,29] These results indicate that at site 1, under influence of an oil refinery, the aromatic amines and nitroarenes were present in PM10 extracts while nitroarenes predominated at site 2 (urban area).

Approximately half the ambient mutagenicity can be ascribed to atmospheric reaction products of two-to-four-ring PAH. Not all of the mutagenicity can be ascribed to particular compounds, but much of it is due to nitro-PAH lactones and to simpler nitro-PAH.[5] This supports the importance of co-pollutants in the mutagenic activity of airborne particles. Reactive gases (NO_X, O_3) under photoactive conditions can produce mutagenic compounds from non-mutagenic organic compounds commonly present in ambient air.[2] In the present study, site 1 is continually monitored by parameters foreseen in law, but these parameters sometimes go over the limits. However, the range of concentration of these factors (Table 3) indicates that they may reach high concentrations, which favors the occurrence of atmospheric reactions that may give rise to mutagenic products.

The present study emphasizes the importance of biological tests in monitoring and characterizes areas under the influence of anthropogenic activities, and they could be useful tools to screen which chemical compound classes are present. It also indicates that the current air quality standards are not sufficient to avoid damage to the environment and human health.

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4. GENETIC BIOMONITORING OF A POPULATION LIVING IN URBAN AREA UNDER THE INFLUENCE OF AN OIL REFINERY*

*Artigo a ser submetido para *Mutation Research/Genetic Toxicology and Environmental Mutagenesis*. Co-autores: Tatiana da Silva Pereira, Jandyra Maria Guimarães Fachel, Daisy Maria Favero Salvadori e Vera Maria Ferrão Vargas

Abstract

Biomonitoring studies have increased as a consequence of risks and effects to human health on exposure to environmental contaminants, mainly air pollutants. Genetic biomarkers are useful tools for the early assessment of exposure to occupational and environmental pollution. The objective of the present study was to investigate genotoxic effects on people residing and/or working downwind from an oil refinery in southern Brazil. This exposed group (*n*=37) was compared to a reference group (*n*=37) of subjects living in an urban area with limited traffic and industrial influence, located far from the main industrial areas. Samples of peripheral blood and buccal mucosa cells were evaluated using the single-cell gel electrophoresis assay (comet assay) and the micronucleus (MN) assay, respectively. The group in the area under the influence of the oil refinery (exposed group) showed significantly higher DNA damage in lymphocytes than the reference group. The MN frequencies in buccal mucosa were very low for both groups and no difference between groups was observed. No association was found between age and tobacco smoking habit and level of DNA damages measured by the comet assay. The results indicate that the comet assay was a sensitive tool to detect DNA damage in subjects under the influence of an oil refinery, with marked genotoxic activity in the atmospheric environment.

Keywords: G*enotoxicity, Biomonitoring, Air pollution, Micronucleus assay, Comet assay*

1. Introduction

Biomonitoring studies have increased at a faster rate since the mid-1980's [1], because risks of the exposures to contaminants, due to intense anthropic activities. Considering these activities, air pollution is an important and constant source of exposure. The mutagenic activity of the airborne particulate matter has been widely investigated and the presence of genotoxic compounds in urban and industrial areas has been recognized [2-4]. The main source of airborne pollutants in these areas is combustion emissions, mainly from fossil fuel [5,6].

In this context, genetic biomarkers are a useful tool for the early assessment of exposure to occupational and environmental pollution. Genotoxic effect, measured by DNA adduct, was reported in individuals occupationally exposed and nearby residents of a refinery and petrochemical complex [7]. Increased frequencies of chromosome aberrations, micronuclei and primary DNA damage were detected in peripheral blood leukocytes from workers at an oil refinery [8]. Environmental air pollution had also induced increase DNA damage in humans when evaluated facing different biomarkers and target cells [9-12]. Increased primary DNA damage as measured by the comet assay was found in subjects exposed to high ozone concentration in Mexico City [10]. Similarly, a positive correlation between genetic biomarkers and personal exposure to polycyclic aromatic hydrocarbons and respirable particles (PM2.5) was reported in northern Bohemia [9].

The single-cell gel electrophoresis assay (comet assay) is a sensitive and reliable method for detecting DNA double- and single-strand breaks and alkali-labile sites in individual cells [13-15]. The use of the comet assay in biomonitoring studies has progressively increased [16,17], however, its application has been restricted to

occupational exposure or to specific compounds [15,16,18]. Some reports had assessed the effects of environmental air pollution in exposed subjects through the comet assay [9-11,19], although these studies are limited in number compared to the possibilities of this approach.

Micronucleus has been used as indicator of chromosome damage, induced by substances that cause chromosomes breakage (clastogens) as well as by agents which affect the spindle apparatus (aneugens) [20,21]. The MN assay in exfoliated cells is a fast and simple method for *in situ* biomonitoring of human populations exposed to environmental genotoxicans [21]. The advantages of the use of the MN assay in human exfoliated buccal cells are the noninvasive collection of sampling material and because the oral epithelium is in contact with inhaled and ingested genotoxic agents. Therefore, the use of MN and cometa assays extends the sort of damages induced by exposure to genotoxins.

In the present study, we investigated DNA damage in people residing and/or working downwind from an oil refinery. Samples of peripheral blood and buccal mucosa were evaluated using the comet assay and micronucleus assay, respectively.

2. Material and Methods

2.1. Site

The target population inhabits an urban/residential area in the town of Esteio (87,000 inhabitants) under the influence of atmospheric emissions from an oil refinery (Fig. 1) located in Canoas (330,000 inhabitants), Rio Grande do Sul state, in southern Brazil. In this area there are two automatic air quality stations belonging to the Rio Grande do Sul Environmental Protection State Foundation (FEPAM) (Fig. 1). One of these stations is located in the municipality of Canoas (29°52'58"S; 51°08'39"W),

upwind (southeast) from the oil refinery and the other one in the municipality of Esteio - Esteio1 (29°51'31"S; 51°10'43"W), downwind (northwest) near a federal highway. At a third site close to the target population, airborne particulate matter PM10 (particles less than 10μm) were sampled in a high-volume collector located north of the Esteio 2 refinery (29°51'29'S; 51°09'25"W), in an area assessed as having the greatest propensity for the deposition of particulate matter, since the dominant winds there come from the Southeast (Fig.1). This area is situated 20 Km North from Porto Alegre, capital of Rio Grande do Sul state. These samples were tested for the presence of organic compounds with mutagenic activity, through *Salmonella*/microsome assay, and the data showed positive responses [22].

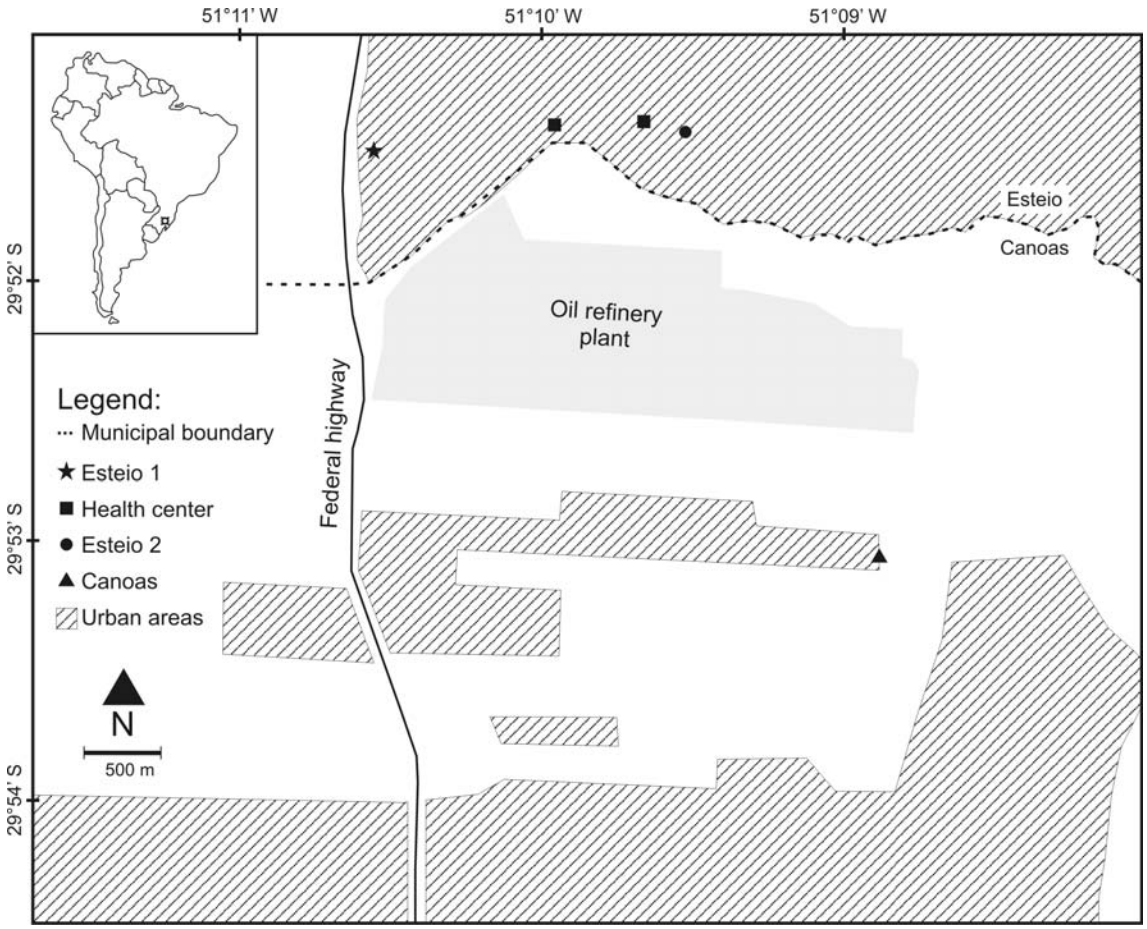

Fig.1 Location of sample site from exposed group under influence of a refinery.

2.2.Subjects and sampling

Healthy men, 18-40 years old, living and/or working at the target site of the present study, most non-smokers, non-excessive alcohol consumers and do not working with agricultural inputs or in chemical industries, were recruited. All volunteers signed an Informed Consent Form and answered a detailed questionnaire about their lifestyle (smoking habits, exposure to radiation, dietary habits, alcohol consumption) and health status (use of prescription medicines). The main characteristics of the study group are presented in Table 1. The reference group had 37 males from Santo Antônio da Patrulha, RS, Brazil (41,000 inhabitants), situated in an urban area with restricted traffic and industrial influence, located far from the main Rio Grande do Sul industrial areas, 73 Km Northeast from Porto Alegre, constituting the reference group. This area was also investigated for mutagenicity of total suspended particulate matter (TSP) from September 2004 to May 2005 (data still unpublished).

Table 1 Main characteristic of studied groups.

Subjects	Reference group *n*	Exposed group *n*
Number	37	37
Smokers	1	8
Exposed to X-ray[1]	9	6
Age (in years) [mean ± SD]	26.0 ± 6.90	29.2 ± 6.82

[1] X-ray for diagnosis (dental or medical), ≤ 2 months before sampling

Samples of peripheral blood (2ml) were taken by venipuncture, stored in ice and protected from light until to be processed. Before buccal mucosa cells sampling, volunteers were asked to rinse their mouth thoroughly with physiological saline solution. Buccal mucosa cells were obtained by scraping the inner cheek with a polyethylene brush. The collected cells were rinsed into ice-cold physiological saline solution (0.9%) using individual coded centrifuge tubes, and were kept on ice until to be processed. The Ethics Committee of Federal University of Rio Grande do Sul, UFRGS, Porto Alegre, Brazil, approved the study protocol (CEP/UFRGS nº 2004256). The biological samples were at municipal health centers (from October to December 2006) at Esteio city.

2.3.Micronucleus assay

Buccal mucosa cells in methanol:acetic acid (3:1) were centrifuged three times at 2000g, for 5 min, and then the pellet was dropped in duplicate slides. Slides were stained with Feulgen/fast green method and cells were scored under 1000× magnification [23]. Two slides from each volunteer were blindly scored by two readers (1000 from each of the duplicate slides and for each reader).

2.4.Comet assay

Lymphocytes from the peripheral blood samples were isolated in Ficoll® gradient. The alkaline version of the comet assay was performed according to Singh et al. [24] and Tice [25]. Isolated lymphocytes (10 µl) were added to 120 µl of 0.5% low melting point agarose (LMA), at 37 °C and layered onto precoated slides with 1.5% regular agarose, covered with a coverslip, and left, for 10min, at 4 °C to solidify the

agarose. The coverslips were carefully removed and the slides immersed in a lysis solution (2.5M NaCl, 100mM EDTA, 10mM Tris [pH 10], 1% N-lauroyl sarcosine sodium, 1% Triton X-100 and 10% DMSO) overnight. After lysis, the slides were washed in PBS for 5 min, immersed in a freshly prepared alkaline buffer (10N NaOH; 200mM EDTA; pH>13) and randomly distributed in a horizontal electrophoresis chamber. After a 20min DNA unwinding period, electrophoresis was conducted at 25V and 300mA for 20min. After electrophoresis the slides were rinsed three times with neutralization buffer (0.4M Tris; pH 7.5), fixed in absolute ethanol, and stored at 4 °C. Negative and positive (lymphocyte treated with 200µM H_2O_2) controls were also included. Before analysis, the slides were stained with 50 µl of 20 µg/ml ethidium bromide and scored using a fluorescent microscope at 400× magnification. Images of 50 randomly chosen "nucleoids" were analyzed using the Comet Assay II Image System (Perceptive Instruments, Haverhill, Sufolk, UK). Parameters used to estimate DNA damage were tail intensity (TI- percentage of pixels DNA tail), and tail moment (TM- product of tail DNA/total DNA).

2.5.Data analysis

Two main analyses were carried out: 1) comparisons between exposed (Esteio) and reference (Santo Antônio da Patrulha) groups; 2) interference of tobacco smoking, age and X-ray (confounding factors) on DNA damage. Differences in tail intensity and tail moment values between groups were evaluated by analysis of variance using hierarchical models, where the various factors are nested in a specific order ("nucleoids" are nested inside the subject and the subject inside the town). The mean values of DNA damage were compared using Student's t test for confounding factors. Statistical

evaluations were conducted using the SPSS for Windows statistical package, version 13 and Proc Mixed -Statistical Analysis System (SAS) version 9.1

3. Results

Frequencies of micronucleated buccal mucosa cells and level of primary DNA lesions (tail moment and tail intensity) are present in table 2. Data show statistically significant ($p<0.05$) increase of damage in individuals from Esteio city (exposed group). No difference was detected regarding MN in buccal mucosa cells.

Table 2 Micronucleated cells in buccal mucosa (MN‰), and primary DNA damage (tail moment and tail intensity) in peripheral lymphocytes of individuals from Santo Antônio da Patrulha and Esteio

Groups	DNA damage		
	MN(‰)	Tail intensity Mean ±S.D. (range)	Tail moment Mean ±S.D. (range)
Reference Santo Antônio da Patrulha	0,055	7.09 ± 3.85 (2.43 - 19.10)	0.82 ± 0.68 (0.20 – 4.11)
Exposed Esteio	0,055	10.04 ± 7.13* (2.31 - 26.62)	2.53 ± 2.28* (0.31 – 7.53)

* $p<0.05$; ANOVA - Hierarchical Models. Negative and positive controls, respectively: tail moment 0.33 ± 0.68 and 1.20 ± 1.66; tail intensity 3.70 ± 5.25 and 9.12 ± 9.99, in mean ± standard deviation.

Tables 3, 4, 5 and 6 presented the effect of age, tobacco smoking habit and X-ray exposure on primary DNA damage as detected by the comet assay. Data show no significant interference of the confounding factors, except for X-ray, but only in the reference group (Table 6).

Table 3 Effect of age, tobacco smoking and radiation exposure on primary DNA damage (tail intensity and tail moment) in the studied subjects

	DNA damage	
	Tail intensity	Tail moment
Age (years)		
< 30 (*n*=44)	8.25 ± 5.87	1.51 ± 1.82
>30 (*n*=30)	9.03 ± 5.97	1.92 ± 1.96
Tobacco smoking habit		
Non smokers (*n*=65)	8.66 ± 5.97	1.65 ± 1.89
Smokers (*n*=9)	7.93 ± 5.47	1.79 ± 1.90
X-ray exposure [1]		
Non exposed (*n*=59)	8.39 ± 5.96	1.70 ± 1.93
Exposed (*n*=15)	9.28 ± 5.68	1.58 ± 1.74

[1] X-ray for diagnosis (dental or medical), ≤ 2 months before sampling

Table 4 Effect of age on primary DNA damage (tail intensity and tail moment) as detected in lymphocytes of reference and exposed groups.

	Age (years)	DNA damage	
		Tail intensity	Tail moment
Reference group	< 30 (*n*=26)	7.49 ± 4.27	0.88 ± 0.77
Santo Antônio da Patrulha	>30 (*n*=11)	6.18 ± 2.55	0.67 ± 0.39
Exposed group	< 30 (*n*=18)	9.36 ± 7.61	2.41 ± 2.47
Esteio	>30 (*n*=19)	10.68 ± 6.78	2.64 ± 2.15

Table 5 Effect of tobacco smoking on primary DNA damage (tail intensity and tail moment) as detected in lymphocytes of reference and exposed groups.

	Tobacco smoking habit	DNA damage	
		Tail intensity	Tail moment
Reference group	Non smokers (*n*=36)	7.14 ± 3.90	0.83 ± 0.68
Santo Antônio da Patrulha	Smokers (*n*=1)	5.47	0.45
Exposed group	Non smokers (*n*=29)	10.54 ± 7.47	2.69 ± 2.37
Esteio	Smokers (*n*=8)	8.24 ± 5.76	1.96 ± 0.69

Table 6 Effect of X-ray exposure on primary DNA damage (tail intensity and tail moment) as detected in lymphocytes of reference and exposed groups.

	X-ray exposure[1]	DNA damage	
		Tail intensity	Tail moment
Reference group	Non exposed (n=28)	6.12 ± 2.99a	0.64 ± 0.36
Santo Antônio da Patrulha	Exposed (n=9)	10.13 ± 4.79b	1.36 ± 1.10
Exposed group	Non exposed (n=31)	10.43 ± 7.18	2.65 ± 2.25
Esteio	Exposed (n=6)	8.00 ± 7.09	1.92 ± 2.52

[1] X-ray for diagnosis (dental or medical), ≤ 2 months before sampling

(a, b) significantly different groups ($p<0.05$), according to Student's t test

The air quality parameters of the air monitoring stations belonging to FEPAM, Canoas and Esteio1, during the study period were within the values determined by law, except for three episodes in which the parameters O_3 (October, 10^{th}) in Canoas and NO_2 (December, 05^{th} and December, 15^{th}) in Esteio were inadequate, surpassing the Brazilian air quality standards (data not shown). The mean of two days of measurements, for two consecutive weeks, of airborne particulate matter (PM10) in the three monitoring stations near the refinery is shown in Fig. 2.

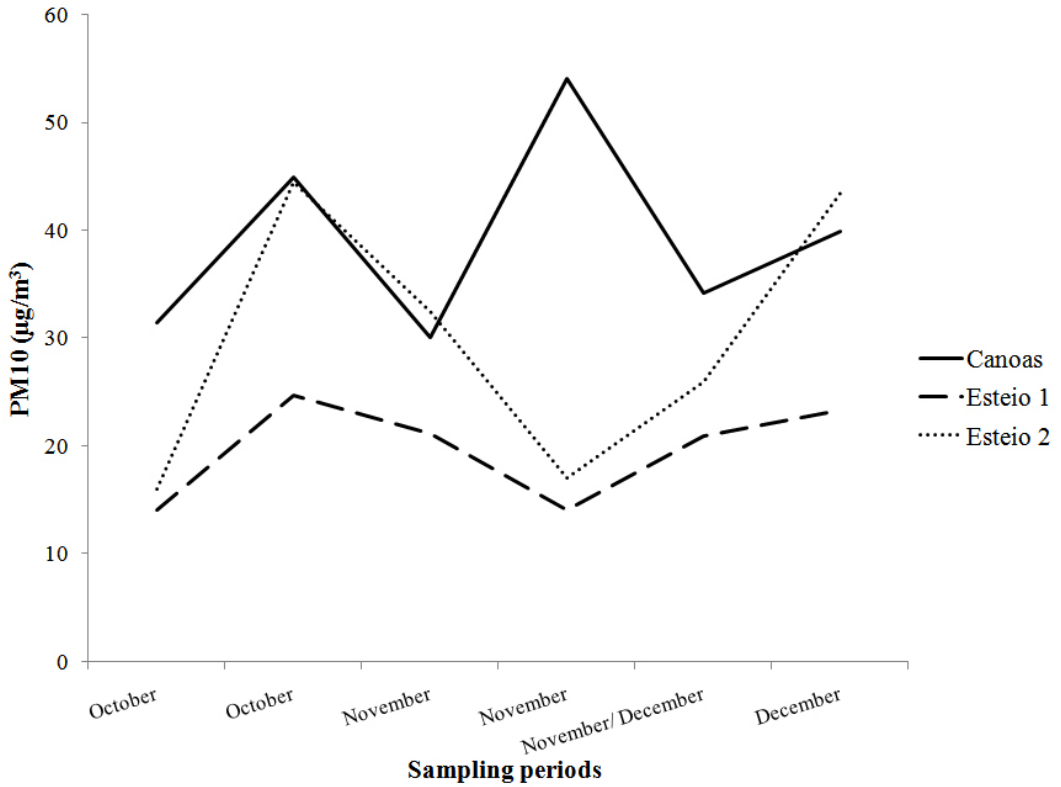

Fig. 2 Average airborne particulate matter PM10 concentrations at three stations during the study period in the exposed area.

4. Discussion

In the present study we investigated primary DNA damage and cytogenetic alterations in individuals exposed to environmental air pollution in an area under influence of the oil refinery. Increased levels of DNA damage detected in the comet assay were found in subjects under the influence of an oil refinery comparing to people living in a reference area with restricted traffic and industries. However no mutagenic effect was detected in buccal mucosa cells using the MN assay.

The mutagenic responses for organic extracts of PM10 from Esteio 2 station were positive for mutagenicity. The mutagenic potencies in this area ranged from 2.46 to 12.85 revertants/m^3 of sampled air in TA98-S9 and from 1.25 to 9.21 revertants/m^3 of

sampled air in TA98+S9. This data showed that direct (TA98 without S9 mix fraction) and indirect-acting mutagens (TA98 in the presence of S9 mix fraction) were present in the samples [22]. The reference area (Santo Antônio da Patrulha) was measured during the sampling period for mutagenicity in organic extracts of TSP. The results ranged from negative to 1.5 revertants/m^3 of sampling air for TA98 strain in assays without S9 mix, and from indicative responses of 0.2 to positives of 2.3 revertants/m^3 in the presence of S9 mix (unpublished data). These values are below those recorded in the capital of Rio Grande do Sul State, Brazil, where most of the responses are positive and reach 17.13 revertants/m^3 in area with heavy traffic [26].

In all studies mentioned above that evaluated organic extracts of airborne particulate matter the concentrations of PM10 and TSP were inside the limits of Brazilian legal standards for air quality [27]. The higher values of PM10 at Canoas station than the other stations reflect mainly the anthropic activities upwind from the oil refinery. However, Esteio 2 Station, the site selected because it represents the area with the greatest deposition of particulates, taking the refinery as a source, presents higher PM10 values compared to Esteio 1. The highest mutagenic activity in atmospheric particulate matter in the industrial areas is not related to the concentration of particulates, but to the reactivity of chemical substances present in the sample both for analyses with TSP or PM10 [2,22,28]. Tests performed on particulates from Esteio 2 station agree with these results [22].

The other parameters measured at the Canoas and Esteio 1 automatic stations were within the limits allowed during most of the period, except in three episodes, twice NO_2 at Esteio 1 and once O_3 at Canoas, where the values surpassed those determined by Brazilian law [27]. Although there have been few episodes in which parameters surpass the legal maximums, during about half the period observed (October to December), air

quality was considered fair, according to the air quality index adopted by FEPAM (http://www.fepam.rs.gov.br/qualidade/monitor_ar.asp) at the two stations.

Exposure to ambient air pollution has adverse effects on human health ranging from modest transient changes in the respiratory tract to mortality [29]. Short and long-term exposure to NO_2, O_3, SO_2 and particulate matter are related to respiratory symptoms, reduced lung function, increased hospital admissions and mortality [29,30]. Besides the effects of these air pollutants on health, they react in the atmosphere with others compounds present in ambient air and can produce mutagenic compounds [3]. In the present study, the area under the influence the oil refinery is continually monitored by legally determined parameters, but these parameters sometimes go above the limits.

Tovalin et al [19] evaluating indoors and outdoors workers reported that high levels of DNA damage were associated with concentration levels of particulate matter, benzene and ozone. Roma-Torres et al. [8] found a positive genotoxic effect in three genetic biomarkers (chromosome aberrations, micronuclei and primary DNA damage) measured in peripheral blood leukocytes in men working at a petroleum refinery aromatics plant who were occupationally exposed to benzene, toluene and xylene.

Some studies had reported associations between air pollution parameters and primary DNA damage measured in comet assay [9, 10, 12]. A positive significant correlation between respirable particles and tail intensity in lymphocytes were found in women who worked outdoors in Teplice city [9]. Also a study investigating DNA damage in lymphocytes in outdoor policemen from Prague showed increased damages compared to the control group (indoor policemen) and a strong association between the levels of oxidative DNA damage and personal exposure to cPAH [31].

Since some studies have discussed the interference of confounding factors on DNA damage [15, 16, 21, 32-35], we analyzed the effect of age, tobacco smoking habit and X-Ray exposure on the data obtained by the comet assay. Due to the low frequencies of micronucleated buccal mucosa cells in both groups no considerations can be made about the interference of confounding factors.

Because of the high sensitive of the comet assay, some confounding factors may interfere in the results. Age and gender are some of the factors which are being discussed, although data are controversial [15, 36]. Faust et al. [36] reviewing the use of comet assay in lymphocytes in human biomonitoring found that these factors showed a significant correlation with the level of DNA damage only in a minority of studies.

Kassie et al. [16] reviewing the use of the comet assay in biomonitoring studies, reported that tobacco smoking did not interfere in the results of individuals exposed to atmospheric pollution, different from other studies that evaluated occupational exposure, such as pesticides and anesthetic agents. Similarly, Möller et al. [15] have also described that the habit of smoking presents in a contradictory influence on DNA damage as detected by the comet assay. Valverde et al.[10], evaluating the effect of atmospheric pollution in Mexico City, in 42 young adults, did not find a significant difference between the smokers (less than ten cigarettes per day) and non-smokers.

In the present study no associations were found between age and tobacco smoking habit and increased DNA damage in the comet assay. Exposure to X-ray was associated with increased DNA damage only in the reference group. This sort of exposure has been previously evaluated in subjects occupationally exposed to low levels of X-radiation and the results showed increased primary DNA damage and micronucleus in peripheral blood [37]. It should be stressed that the smoking habit was

a restriction factor and age was defined between 18 and 40 years old, thus this non-association between these factors and DNA damage, in the comet assay, can be an effect of the limited number of subjects and restricted age range

In conclusion, our data showed an increased primary DNA damage as detected by the comet assay in the individuals living and/or working under the influence of an oil refinery. However, the genetic lesions were not associated with the tobacco smoking habit, age and recent exposure to X-ray for diagnosis. The current results indicate that the values established for air quality in Brazilian law are not safe to ensure the integrity of the environment and human health.

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5. CONSIDERAÇÕES FINAIS

Metodologias capazes de monitorar e identificar de forma rápida e precoce os efeitos de compostos constantemente liberados no ambiente são necessárias para aliar o desenvolvimento social e econômico à manutenção da integridade do meio ambiente e da saúde das populações humanas. Entre os compartimentos ambientais, o atmosférico permite uma dispersão de contaminantes a longas distâncias e, dependendo da fonte, dos regimes de ventos e das condições atmosféricas, pode representar uma fonte de exposição constante a contaminantes. Nesse contexto, o atual estudo buscou avaliar, através de marcadores genéticos, a presença de atividade mutagênica no material particulado atmosférico como marcador de exposição e diagnóstico ambiental em áreas industriais e urbanas, associando o biomonitoramento humano em população urbana exposta à influência petroquímica.

Os padrões vigentes para a qualidade do ar no Brasil são aqueles estabelecidos na Resolução CONAMA nº 03 de 1990 (Brasil, 1990). Esta resolução estabelece padrões primários e secundários para Partículas Totais em Suspensão (PTS), Partículas Inaláveis (PM10), Monóxido de Carbono (CO), Dióxido de Nitrogênio (NO_2), Dióxido de Enxofre (SO_2), Ozônio (O_3) e Fumaça. A atividade mutagênica no material particulado atmosférico (PTS e PM10) investigada através do ensaio *Salmonella*/microssoma em áreas sob a influência de indústrias petroquímicas e em área urbana encontrou respostas positivas mesmo as concentrações de partículas estando abaixo dos limites previstos na legislação.

O ensaio *Salmonella*/microssoma se mostrou uma eficiente ferramenta na avaliação de misturas complexas influenciadas por fontes diversas e difusas, como o compartimento atmosférico. As respostas associadas de diferentes linhagens e presença de fração de metabolização de mamífero *in vitro* (fração S9) obtidas nesse ensaio

indicam as classes de compostos presentes nas amostras. Assim sendo, a atividade mutagênica dependente de S9 diante da linhagem TA98 (TA98+S9) está, por exemplo, associada à presença de hidrocarbonetos policíclicos aromáticos (HPAs) enquanto que a independente (TA98-S9) é devida a nitroarenos (DeMarini et al., 1996). As linhagens nitro-sensíveis (TA98NR, TA98/1,8DNP$_6$, YG1021 e YG1024) também permitem diferenciar a contribuição na atividade mutagênica de diferentes classes de nitrocompostos e indicam, através dos padrões de respostas, as diferenças em áreas urbanas e industriais.

Além de uma avaliação qualitativa de classes de compostos mutagênicos, o ensaio *Salmonella*/microssoma permite abordagem quantitativa o que possibilita fazer uma estimativa do risco de exposição. Um exemplo desse tipo de abordagem foi apresentado na figura 3 do primeiro artigo que mostra o volume de ar necessário para dobrar a freqüência de reversão, representando a quantidade de ar para que um evento mutagênico ocorra. Por expressar de forma quantitativa, este ensaio também permite comparações com estudos de diferentes locais e períodos.

O presente estudo investigou áreas anteriormente avaliadas para mutagenicidade (complexo petroquímico-capítulo 1 e 8º Distrito de Meteorologia-capítulo 2) e comparou as respostas entre os diferentes períodos. Além disso, permitiu avançar nos estudos que investigam a atividade mutagênica na fração inalável (PM10), que ainda são escassos no Brasil (De Maritinis et al., 1999) e inéditos para o Estado do Rio Grande do Sul. A fração PM10 está mais relacionada com a saúde humana por penetrar mais profundamente no trato respiratório, bem como as concentrações de compostos orgânicos e mutagênicos serem maiores nas menores frações de partículas (Hayakawa et al., 1995; Pagano et al., 1996). As pesquisas devem avançar na caracterização de frações ainda menores de material particulado atmosférico (PM2,5).

Muito da atividade mutagênica de misturas complexas é devido a uma ou poucas classes de compostos (DeMarini et al., 1994; DeMarini et al., 1996) e, embora, HPAs contribuam significativamente para a atividade mutagênica não são a classe dominante de mutágenos presentes em amostras de ar (Claxton et al., 2004). Assim, somente a análise de HPAs é insuficiente para predizer o risco da exposição (Brits et al., 2004).

Estima-se que aproximadamente metade da mutagenicidade avaliada em amostras de ar é devido às reações atmosféricas originando produtos de HPAs de dois a quatro anéis (Lewtas, 2007). Portanto, a presença de co-poluentes como gases reativos (O_3, NO_X) sob condições fotoativas podem produzir compostos mutagênicos a partir de compostos orgânicos não mutagênicos comumente presentes no ambiente (Claxton et al., 2004). O monitoramento associado desses compostos com a resposta mutagênica permite uma compreensão mais ampla dos processos envolvidos e dos efeitos desses poluentes atmosféricos.

A poluição atmosférica pode causar efeitos adversos ao ambiente (Grantz, et al., 2003; Vargas, 2003) e à saúde humana (WHO, 2004). Os efeitos à saúde vão desde breves mudanças no trato respiratório à mortalidade (WHO, 2004). Uma abordagem que permita um diagnóstico precoce é de grande utilidade para avaliar populações potencialmente expostas.

Os biomarcadores genéticos podem sinalizar de maneira precoce o dano à exposição e indicar os mecanismos de ação. No presente estudo o ensaio cometa se mostrou sensível na detecção de dano ao DNA em indivíduos que moram e/ou trabalham em área sob a influência de uma refinaria de petróleo. Além disso, apesar de restringir na amostra as possíveis variáveis que pudessem aumentar o dano ao DNA, alguns indivíduos estiveram expostos a possíveis fatores, como o fumo, por exemplo. Dentro dos fatores avaliados nenhum se mostrou associado ao aumento de dano

detectado pelo ensaio cometa, exceto a exposição à radiação no grupo de referência. Esta investigação contribui para o atual conhecimento do ensaio cometa e sua aplicação como uma ferramenta de biomonitoramento humano.

A avaliação conjunta de ensaios *in vitro* e *in vivo* permite ampliar a compreensão e caracterização do tipo e do efeito da exposição. Enquanto ensaios *in vitro*, como o *Salmonella*/microssoma, apresentam algumas limitações para a extrapolação dos efeitos nos indivíduos, eles controlam outros fatores que interferem nas respostas, como por exemplo, as susceptibilidades individuais e aqueles relacionados ao estilo de vida (como fumo, dieta e consumo de bebidas alcoólicas), presentes nos ensaios *in vivo*, como, no caso do presente estudo, o ensaio do cometa e micronúcleo.

Ensaios biológicos para monitoramento e diagnóstico ambiental se apresentam como uma ferramenta útil principalmente na avaliação de áreas influenciadas e impactadas por atividades humanas. Além disso, esses ensaios não se limitam aos efeitos biológicos, mas também podem indicar as classes dos compostos presentes e sua contribuição no efeito observado. A utilização do ensaio *Salmonella*/microssoma, em suas diferentes abordagens, também permitiu constatar que os atuais parâmetros de qualidade do ar e os limites estabelecidos não asseguram a integridade do ambiente e da saúde humana. Assim sendo, a revisão dos limites vigentes, bem como a complementação com novos parâmetros de análise, como ensaios biológicos, devem ser considerados na implementação dos padrões de qualidade do ar para assegurar a proteção necessária.

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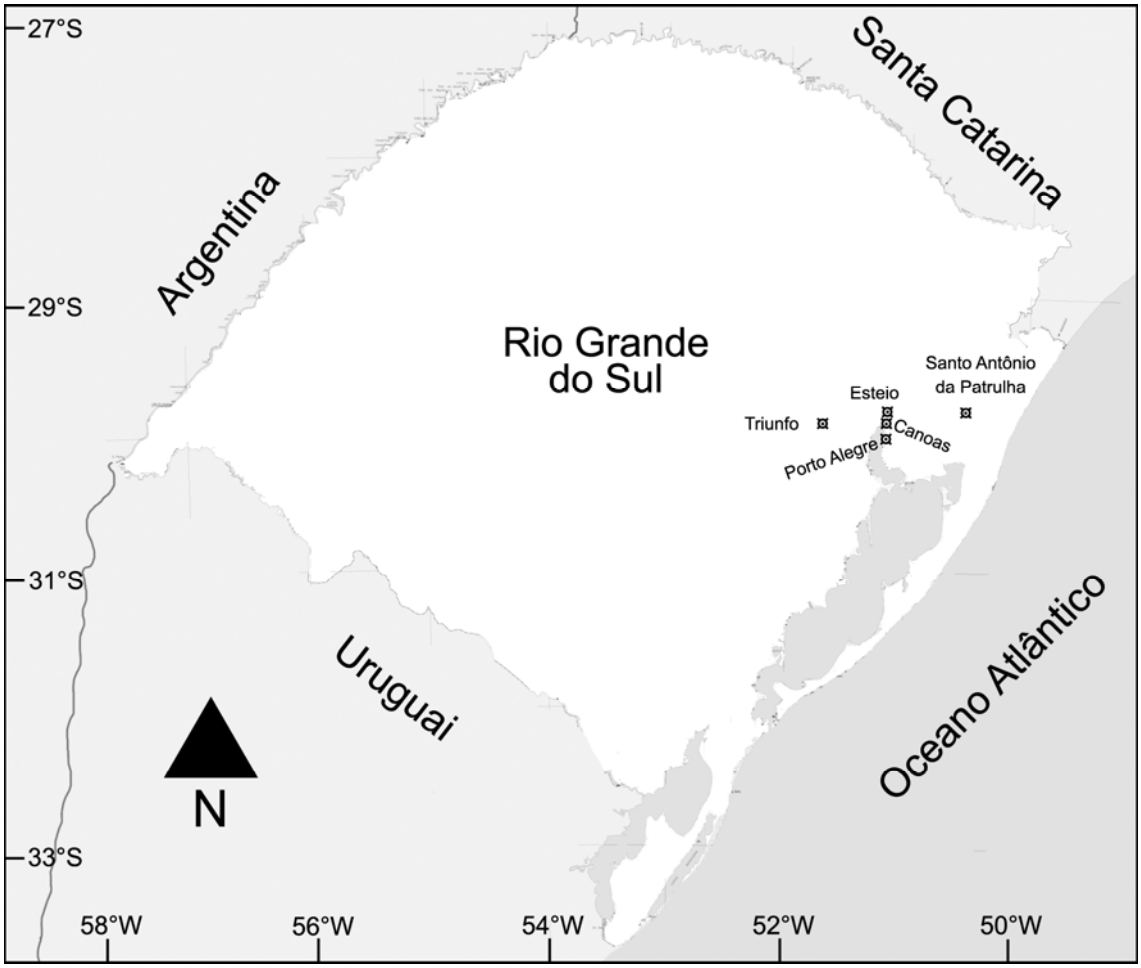
APÊNDICES

Áreas de estudo



Localização das cidades investigadas no presente estudo.

Termo de Consentimento entregue aos voluntários

TERMO DE CONSENTIMENTO INFORMADO

A pesquisa “Biomonitoramento de populações humanas através de genotoxicidade em área sujeita a risco ecotoxicológico", tem como objetivo avaliar sinais iniciais para detectar efeitos da poluição ambiental na saúde humana. Estes sinais podem ser observados através de testes (que avaliam danos nas células) e entrevista individual (informações sobre alimentação, uso de medicamentos, profissão, moradia).

As análises serão realizadas no Laboratório de Biologia da Fundação Estadual de Proteção Ambiental, FEPAM, em trabalho conjunto com o Curso de Pós-graduação em Ecologia da Universidade Federal do Rio Grande do Sul, UFRGS. Todos os resultados ficarão sob a total responsabilidade dos pesquisadores deste laboratório. A identidade de cada voluntário será mantida em sigilo e você poderá a qualquer momento desistir da sua participação, sem que isto leve a qualquer prejuízo.

O desconforto que você passará será mínimo, estando basicamente relacionados à coleta de sangue (4 mL), que implica em uma sensação dolorosa temporária na região da coleta, havendo possibilidade de formação de um pequeno hematoma na região. A coleta de sangue é feita com material limpo, esterilizado e descartável (usado para cada pessoa), sem risco de transmitir AIDS ou outra doença qualquer. Será realizada, também, coleta de células bucais de forma suave, sem dor. Não há risco na coleta destes exames.

O resultado destes exames não apresenta finalidade individual, mas servirá para avaliar a sensibilidade da população de seu município para os poluentes ambientais. O sangue coletado será utilizado para avaliar se há danos no seu material genético (DNA) e para extração do DNA, a fim de verificar sua sensibilidade à exposição a alguns agentes tóxicos e para estudos que esclareçam o papel genético no aumento de risco a danos no DNA.

Os pesquisadores envolvidos no Projeto garantem a você o direito a qualquer pergunta e/ou esclarecimentos mais específicos dos procedimentos realizados.

Esta pesquisa, na qual você será voluntário, poderá trazer grande benefício para a população humana, possibilitando selecionar testes que identifiquem os efeitos iniciais de substâncias perigosas aos organismos antes que ocorram problemas graves de saúde.

Eu, _____ portador da CI_____, residente em _____- RS, fui informado dos objetivos específicos e da justificativa desta pesquisa, de forma clara e detalhada. Recebi informações sobre cada procedimento no qual estarei envolvido e do desconforto, tanto quanto dos benefícios esperados. Todas as minhas dúvidas foram respondidas com clareza e sei que poderei solicitar novos esclarecimentos a qualquer momento. Além disso, sei que as informações obtidas durante o estudo serão fornecidas e que terei liberdade de retirar meu consentimento de participação na pesquisa.Os pesquisadores garantiram que as informações geradas terão caráter confidencial.

Caso tiver novas perguntas sobre este estudo, posso chamar os pesquisadores integrantes da equipe de pesquisa do Laboratório de Biologia da FEPAM pelo telefone (51) 3334-6765.

Declaro ainda que recebi cópia do presente Termo de Consentimento.

Data ___/___/_____

_____ _____

Assinatura do voluntário Vera Maria Ferrão Vargas

Pesquisadora Responsável

Questionário feito aos voluntários

QUESTIONÁRIO

Data ___/___/_____

I – Identificação
N° de registro: _____

Nome: _____

Identidade: _____

Profissão: _____

Data de nascimento: ___ / ___ / _____

Idade: _____

Endereço: _____

CEP: _____-_____ Telefone para contato: _____

Fatores para inclusão da amostra: residir e permanecer maior parte do dia no local de estudo; não trabalhar com insumos agrícolas; preferentemente não fumar.

N° de registro do voluntário no estudo: _____

II – História Social

- Escolaridade:

( ) não alfabetizado ( ) ensino médio incompleto ( ) ensino superior incompleto

( ) ensino fundamental incompleto ( ) ensino médio completo ( ) ensino superior completo ( ) ensino fundamental completo

- Tempo de residência na área do município: _____
- Trabalha: ( ) Sim ( ) Não Trabalho atual:_____ ___

Local de trabalho: _____

Tempo de serviço: _____ Tipo:_____

Data das últimas férias: _____

Ausentou-se do município nos últimos 12 meses por um periódico de 1 semana no mínimo? ( ) sim ( ) não

Trabalho anterior: _____ Tempo de serviço: _____ Tipo: _____

- Renda familiar média: Em Reais: R$_____

( ) menos de 1 salário mínimo ( ) de 1 a 3 salários ( ) de 3 a 5 salários

( ) de 5 a 10 salários ( ) mais de 10 salários ( ) não sabe ( ) não informou

- Tipo de casa: ( ) alvenaria ( ) madeira ( )pau-a-pique ( ) outros

Número de cômodos na casa: _____

- De onde vem a água que bebem (abastecimento de água da residência)?

( ) encanada (CORSAN) ( ) poço artesiano ( ) bombeada de arroio
( ) poço comum ( ) abastecido por carro-pipa ( ) cacimbas e/ou nascentes
( ) bica pública ( ) não sabe ( ) não informou ( ) outros

- Qual o destino do esgoto:

( ) esgoto público encanado (cloacal) ( ) valo direto e/ou arroio ( ) fossa e/ou sumidouro ( ) não sabe ( ) não informou ( ) outros

III – Hábitos

- Fuma? ( ) Sim ( ) Não Há quanto tempo (meses) _____

Quantos cigarros por dia _____

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

- Já fumou? ( ) Sim ( ) Não Há quanto deixou de fumar (meses)? _____

Quantos cigarros por dia _____

Durante quanto tempo fumou (meses)?_____

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

- Convive diariamente com fumante (s) – fumante passivo? ( ) Sim ( ) Não

- Bebe chimarrão? ( ) Sim ( ) Não Quantas cuias por dia? _____
- Quantos dias da semana bebe? ( )1 a 2 dias ( )3 a 5 dias ( ) todos os dias Consome bebida alcoólica? ( ) Sim ( ) Não ( ) eventualmente Há quanto tempo (meses)? _____

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

Quantidade por dia (número de copos por dia)? _____

- Já bebeu? ( ) Sim ( ) Não Há quanto deixou (meses)? _____

Quantidade (número de copos por dia): _____

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

- Já usou drogas? ( ) Sim ( ) Não Tipo:_____ Quantidade: _____

Por quanto tempo (meses)? _____ Consome ainda? ( ) sim ( ) não

- 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 ( ) Vitamina ( ) Antiinflamatório ( ) Xarope ( )Outros: _____

Freqüência por dia: _____

- Usou algum tipo de medicamento nos últimos 12 meses? ( ) Sim ( ) Não

Quais: ( ) Antibiótico ( ) Vitamina ( ) Antiinflamatório ( ) Xarope ( ) Outros: _____

Freqüência por dia: _____ Há quanto tempo deixou? _____

- Fez alguma cirurgia no último ano? ( ) Sim ( ) Não

Data: _____ Tipo: _____

- Alimenta-se de:

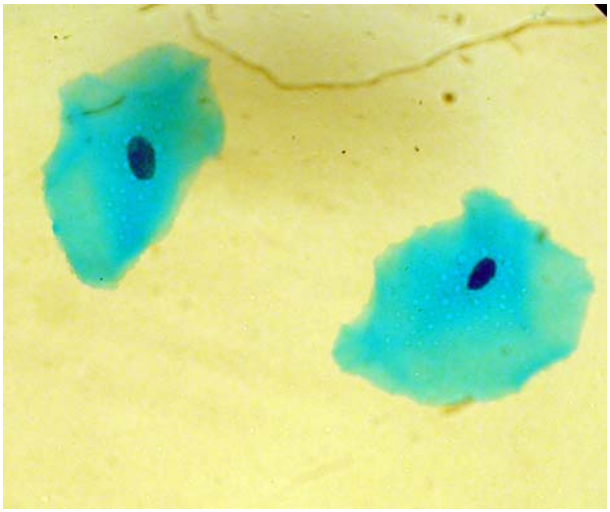
Alimentos construtores: ( ) carne ( ) peixe ( ) frango ( ) ovos ( ) leite e derivados (queijo e iogurte) ( ) feijão ou lentilha ( ) grão-de-bico ou soja.

Alimentos reguladores: ( ) verduras ( ) frutas ( ) legumes

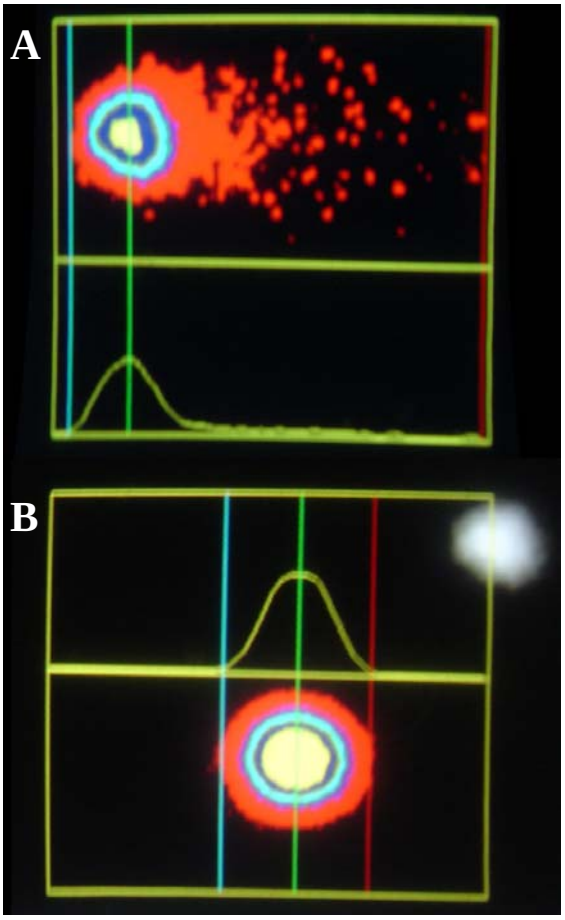
Carboidratos: ( ) pão ( ) macarrão ( ) cereais-arroz, milho ( ) doces ( ) batata ( ) mandioca

OBSERVAÇÃO: Termo de Consentimento e Questionário aprovados pelo Comitê de Ética da UFRGS, dentro do projeto cadastrado no CEP/UFRGS com o nº 2004256, e pelo CONEP de Brasília, Proc. Nº 23078.200270/04-17

Imagens



Células de esfregaço bucal coradas pelo método Feulgen/f*ast green* para análise de micronúcleos



Análise de imagem de nucleóides de linfócitos sob microscopia de fluorescência através do programa computacional *Comet Assay 2.2, Perceptive Instruments* (Suffolk, UK), em A, apresentando fragmentação do DNA em B, sem danos.

Dados da estação automática de monitoramento da qualidade do ar da Fepam – Canoas Parque Universitário - nos dias de amostragem de partículas inaláveis.

	PM10 (μg/m^3)		NO (μg/m^3)		NO_2 (μg/m^3)		NO_X (μg/m^3)		O_3 (μg/m^3)		CO (ppm)		SO_2 (μg/m^3)	
	Média ± D.P.	Máx.	Média ± D.P.	Máx.	Média ± D.P.	Máx.	Média ± D.P..	Máx.	Média ± D.P.	Máx.	Média ± D.P.	Máx.	Média ± D.P..	Máx.
14/10/2006	30,80 ± 16,23	61,90	6,52 ± 18,78	94,00	23,90 ± 7,52	38,70	30,89 ± 21,69	125,90	43,41 ± 16,14	42,70	0,53 ± 0,12	0,80	0,39 ± 0,40	1,40
18/10/2006	32,21 ± 12,20	53,00	6,90 ± 9,44	37,10	28,41 ± 10,65	63,40	35,75 ± 18,89	90,80	38,82 ± 13,44	56,90	0,46 ± 0,07	0,60	0,76 ± 0,97	3,50
24/10/2006	58,69 ± 26,04	129,70	9,02 ± 10,83	38,40	45,73 ± 22,17	99,10	55,25 ± 27,85	117,00	46,89 ± 37,83	106,00	0,36 ± 0,10	0,60	0,86 ± 0,92	2,90
30/10/2006	31,29 ± 12,97	62,60	6,48 ± 7,31	25,20	47,36 ± 36,35	153,50	54,29 ± 41,52	173,00	50,00 ± 34,48	148,70	0,42 ± 0,08	0,60	14,90 ± 27,48	91,10
05/11/2006	30,12 ± 22,06	79,20	1,32 ± 0,83	3,70	16,21 ± 6,88	32,90	17,99 ± 6,93	34,40	38,58 ± 8,18	50,20	0,39 ± 0,05	0,50	0,05 ± 0,08	0,20
11/11/2006	--	--	5,82 ± 8,16	35,00	24,91 ± 11,27	61,20	31,17 ± 18,43	96,40	39,79 ± 12,74	53,60	0,43 ± 0,06	0,60	0,15 ± 0,19	0,70
17/11/2006	--	--	3,67 ± 4,46	21,60	41,35 ± 21,29	92,20	45,50 ± 22,75	95,70	44,64 ± 13,80	66,20	0,29 ± 0,07	0,40	0,70 ± 0,71	2,80
23/11/2006	54,16 ± 14,16	76,90	12,37 ± 23,29	98,30	35,13 ± 25,45	123,00	47,97 ± 42,43	167,20	37,46 ± 22,05	69,50	0,23 ± 0,06	0,40	0,34 ± ,75	3,00
29/11/2006	13,29 ± 7,80	28,00	3,53 ± 3,31	12,70	19,86 ± 7,41	39,60	23,90 ± 10,24	53,20	32,20 ± 5,66	42,90	0,30 ± 0,04	0,40	0,34 ± 0,32	0,90
05/12/2006	55,15 ± 27,39	116,40	3,03 ± 2,47	7,90	20,38 ± 9,09	52,30	23,88 ± 10,86	60,50	45,18 ± 7,86	59,90	0,30 ± 0,04	0,40	0,42 ± 0,71	3,00
11/12/2006	46,07 ± 19,49	77,00	6,25 ± 10,73	48,20	18,44 ± 8,04	38,00	24,73 ± 16,15	77,10	33,70 ± 14,96	58,40	0,30 ± 0,06	0,40	0,34 ± 0,24	0,90
18/12/2006	33,79 ± 21,21	92,90	4,13 ± 3,06	11,60	27,65 ± 10,59	54,30	32,14 ± 12,21	62,70	41,12 ± 16,18	65,00	0,35 ± 0,07	0,50	0,19 ± 0,34	1,20

Valores são médias diárias e desvio padrão. Máx. representa o valor máximo registrado no dia, -- sem registros

Dados da estação automática de monitoramento da qualidade do ar da Fepam – Esteio Vila Ezequiel - nos dias de amostragem de partículas inaláveis.

	PM10 (μg/m^3)		NO (μg/m^3)		NO_2 (μg/m^3)		NO_X (μg/m^3)		O_3 (μg/m^3)		CO (ppm)		SO_2 (μg/m^3)	
	Média ± D.P.	Máx.	Média ± D.P.	Máx.	Média ± D.P.	Máx.	Média ± D.P..	Máx.	Média ± D.P.	Máx.	Média ± D.P.	Máx.	Média ± D.P..	Máx.
14/10/2006	14,26 ± 10,72	42,40	2,55 ± 1,76	7,40	10,66 ± 6,08	27,40	13,69 ± 7,74	35,20	37,83 ± 15,10	65,50	0,53 ± 0,18	0,80	12,98 ± 15,79	49,20
18/10/2006	14,00 ± 8,34	37,50	4,03 ± 3,38	13,90	14,02 ± 6,55	35,40	18,57 ± 9,73	50,10	31,34 ± 8,55	46,20	0,75 ± 0,12	0,90	37,08 ± 36,32	121,90
24/10/2006	32,9 ± 17,26	58,50	5,33 ± 7,55	35,30	19,03 ± 7,70	36,90	24,90 ± 14,01	69,80	38,81 ± 33,03	107,50	0,41 ± 0,19	0,80	19,64 ± 28,45	132,40
30/10/2006	21,05 ± 10,51	45,50	6,95 ± 7,92	36,70	18,37 ± 7,00	37,90	25,86 ± 12,54	58,50	34,97 ± 27,44	96,70	0,35 ± 0,08	0,60	28,48 ± 35,45	127,00
05/11/2006	13,96 ± 10,64	36,90	1,59 ± 0,60	2,90	5,22 ± 1,52	9,00	7,35 ± 1,42	10,90	33,18 ± 7,61	43,70	0,26 ± 0,12	0,50	1,94 ± 4,67	21,20
11/11/2006	28,43 ± 6,92	41,40	1,73 ± 1,63	7,60	6,45 ± 2,80	11,80	8,58 ± 4,00	19,90	33,27 ± 9,71	44,80	0,70 ± 0,14	0,90	22,51 ± 22,30	77,40
17/11/2006	14,17 ± 8,15	32,80	7,05 ± 10,86	49,70	19,89 ± 13,65	54,70	27,45 ± 23,56	99,80	27,22 ± 12,86	52,50	0,63 ± 0,13	1,00	11,43 ± 15,53	66,90
23/11/2006	--	--	16,17 ± 28,65	107,80	32,82 ± 19,23	85,20	49,48 ± 45,79	184,90	29,18 ± 15,20	53,60	0,54 ± 0,12	0,80	54,04 ± 57,63	204,50
29/11/2006	12,16 ± 7,00	29,40	10,28 ± 7,93	33,90	36,85 ± 19,93	86,00	47,69 ± 26,36	110,40	23,48 ± 4,30	30,80	0,49 ± 0,09	0,70	51,99 ± 37,68	154,10
05/12/2006	29,73 ± 9,65	45,90	20,88 ± 54,18	273,50	53,37 ± 70,79	360,20	74,72 ± 123,52	634,00	35,26 ± 7,71	47,70	0,31 ± 0,08	0,60	39,67 21,51	89,70
11/12/2006	24,02 ± 8,99	44,40	7,70 ± 4,18	18,90	25,20 ± 11,59	49,10	33,38 ± 15,30	68,70	28,35 ± 13,48	54,00	0,44 ± 0,13	0,60	32,99 ± 30,49	90,70
18/12/2006	22,74 ± 17,50	73,30	13,57 ± 14,03	60,20	56,88 ± 30,05	123,30	70,92 ± 42,81	184,20	31,35 ± 14,33	57,20	0,57 ± 0,10	0,80	0,27 ± 0,73	3,30

Valores são médias diárias e desvio padrão. Máx. representa o valor máximo registrado no dia, -- sem registros

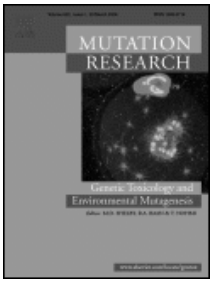
ANEXOS

PADRÕES NACIONAIS DE QUALIDADE DO AR
Resolução CONAMA nº. 03 de 28/06/1990

Poluente	Tempo de Amostragem	Padrão Primário µg/m³	Padrão Secundário µg/m³	Método de Medição****
Partículas Totais em Suspensão (PTS)	24 horas*	240	150	Amostrador de Grandes Volumes
	MGA**	80	60	
Partículas Inaláveis (PM10)	24 horas*	150	150	Separação Inercial/Filtração
	MAA***	50	50	
Fumaça	24 horas*	150	100	Refletância
	MAA***	60	40	
Dióxido de Enxofre (SO_2)	24 horas*	365	100	Pararosanilina
	MAA***	80	40	
Dióxido de Nitrogênio (NO_2)	1 hora*	320	190	Quimiluminescência
	MAA***	100	100	
Monóxido de Carbono (CO)	1 horas*	40.000	40.000	Infravermelho não Dispersivo
		35 ppm	35 ppm	
	8 horas*	10.000	10.000	
		9 ppm	9 ppm	
Ozônio (O_3)	1 horas*	160	160	Quimiluminescência

*** Não deve ser excedido mais que uma vez ao ano.**
**** Média geométrica anual.**
***** Média aritmética anual.**
****** A resolução permite a utilização de método equivalente.**

Normas : **Mutation Research** - Genetic Toxicology and Environmental Mutagenesis



Guide for Authors

Types of Articles

Mutation Research - Genetic Toxicology and Environmental Mutagenesis publishes the following types of article: (I) **Research papers**- papers reporting results of original, fundamental research. (II) **Short communications** of up to 5 printed pages. (III)**Rapids** - are accelerated publications - research papers identified by the Editor as being of significant quality and thereby qualifying for rapid reviewing, and publication within 8-10 weeks of acceptance. (IV) **Current issues** are generally short, 1-2 page comments on a topical theme, and are published within 10 weeks of acceptance. (V) Volunteered and invited **Mini-reviews** of less than 10 printed pages, using references generally no later than 2 years old.

Please note that Full-length reviews comprehensively covering and critically analysing a topic are published in *Mutation Research Reviews*. Also published in the *Reviews* section are invited papers in the series **Reflections in Mutation Research**, in which research and techniques that have played an important part in the development of the field of mutation research are revisited and their significance discussed. **Special issues**, comprising multiple original and/or review articles written from a particular viewpoint, on a central theme, are published on a regular basis in the appropriate section of *Mutation Research* by topic or article type.

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Tables should be typed with double line spacing and should have a heading.

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Examples:
[1] B.N. Ames, J. McCann, E. Yamasaki. Methods for detecting carcinogens and mutagens with the Salmonella/mammalian-microsome mutagenicity test, Mutation Res. 31 (1975) 347-363.[2] L. Ehrenberg, C.A. Wachtmeister. Safety precautions in work with mutagenic and carcinogenic chemicals, in: B.J. Kilbey, M.S. Legator, W. Nichols and C. Ramel (Eds.), Handbook of Mutagenicity Test Procedures, Elsevier, Amsterdam, 1977, pp. 401-410.
Nomenclature. Authors are requested to adopt the nomenclature system for human gene mutations recommended by the HUGO MDI Nomenclature Working Group. Guidelines for this system can be found at http://journals.wiley.com/1059-7794/nomenclature.html.

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Normas : **Journal of Environmental Monitoring**

1.0 Organization of material

Every latitude, consistent with brevity, in the form and style of papers is permitted, and no rigid pattern for either is prescribed. **The suggestions outlined here are for guidance only**.

1.1 Full articles

1.1.1 Title. A paper should have a short, straightforward title directed at the general reader. Lengthy systematic names and complicated and numerous chemical formulae should therefore be avoided where possible. The use of non-standard abbreviations and symbols in a title is not encouraged. Brevity in a title, though desirable, should be balanced against its accuracy and usefulness. The use of Series titles and Part numbers in titles of papers is discouraged. Instead the Series title and Part number can be included as a footnote to the first page together with a reference (reference 1) to the preceding Part. When the preceding part has been submitted to the Society but is not yet published, the paper reference number should be given.

1.1.2 Author names. Full names for all the authors of an article should be given; initials should not be used.

1.1.3 Graphical contents entry. Graphics are included in the contents list. The format incorporates, a small graphic (maximum size 8 cm wide x 4 cm high) alongside one sentence of text, which should be presented in such a way as to encourage further perusal of the article, by highlighting the novelty and main feature(s) of interest; excessive lists of results and, in particular, cumbersome formulae should therefore be avoided. In view of the space available graphics should be as clear as possible. Simple schematic diagrams or reaction schemes are preferred to ORTEP-style crystal structure depictions and complicated graphs, for example. The graphic used in the Contents entry need not necessarily appear in the article itself. Authors should bear in mind the final size of any lettering on the graphic. For examples of graphical contents entries check the online version of the appropriate journal.

1.1.4 Summary. Every paper must be accompanied by a summary (50-250 words) setting out briefly and clearly the main objects and results of the work; it should give the reader a clear idea of what has been achieved. The summary should be essentially independent of the main text; however, names, partial names or linear formulae of compounds may be accompanied by the numbers referring to the corresponding displayed formulae in the body of the text.

1.1.5 Introduction. This should give clearly and briefly, with relevant references, both the nature of the problem under investigation and its background.

1.1.6 Results and discussion. It is usual for the results to be presented first, followed by a discussion of their significance. Only strictly relevant results should be presented and figures, tables, and equations should be used for purposes of clarity and brevity. The use of flow diagrams and reaction schemes is encouraged. Data must not be reproduced in more than one form, *e.g.* in both figures and tables, without good reason.

1.1.7 Experimental. Descriptions of experiments should be given in detail sufficient to enable experienced experimental workers to repeat them; the degree of purity of materials should be given, as should the relative quantities used. Descriptions of established procedures are unnecessary. Standard techniques and methods used throughout the work should be stated at the beginning of the section. Apparatus should be described only if it is non-standard; commercially available instruments are referred to by their stock numbers (*e.g.* Perkin-Elmer 457 or Varian HA-100 spectrometers). The accuracy of primary measurements should be stated. Unexpected hazards encountered during the experimental work should be noted. In general there is no need to report unsuccessful experiments.

1.1.8 Conclusion. This is for interpretation and to highlight the novelty and significance of the work. The conclusions should *not* summarise information already present in the text or abstract.

1.1.9 Acknowledgements. Contributors other than co-authors may be acknowledged in a separate paragraph at the end of the paper; acknowledgements should be as brief as possible.

1.1.10 Dedications. Personal dedications of an appropriate nature may be included as a footnote to the title of the paper. Dedications for significant birthdays (from 60 years onwards) and *in memoriam* dedications would be considered appropriate. Other forms of dedication may require approval of the relevant journals Editorial Board.

1.1.11 Bibliographic references and notes. These should be listed at the end of the manuscript in numerical order.

1.2 Communications

Individual articles should be as brief as possible; depending on the journal in question, a page limit my apply. Formatting should be as for Full Articles, except for the following topics.

1.2.1 Summary. This is restricted to one sentence of text.

1.2.2 Article. No section headings are used in Communications. Brief details of key experiments are permitted and should include the amounts of reagents used in chemical reactions. Extensive spectroscopic and other supporting data are not required, but authors are encouraged to supply such data as Electronic Supplementary Information to aid the referees in their assessment of the work. Description for routine procedures should *not* be included.

1.2.3 Notes and bibliographic references. These should not be extensive and inclusion of 5-10 references is recommended.

1.2.4 Figures. These should be kept to a minimum bearing in mind the restrictions to the length of most Communications.

2.0 Style and presentation

2.1 Brevity

For reasons of economy, brevity in the presentation of papers is essential. Authors should note that the following practices are likely grounds for rejection of a manuscript, or acceptance only after substantial revision.

- Unnecessary division of work into separate parts of a series of papers.
- Submission of fragmentary work which can be included in a larger article.
- Undue elaboration of hypotheses.
- Over-detailed and verbose exposition of ideas.
- Excessive use of diagrams; for example, a straight-line plot can be adequately expressed as an equation together with, if necessary, a table of deviations.
- Duplication of data in text, tables and figures, *etc.*
- Descriptions of slight variations of essentially the same technique.

2.2 Linguistic and typographical conventions

2.2.1 Grammar and spelling. Standard English or American spelling is used but consistency should be maintained within a paper.

2.2.2 Abbreviations. The use of common or standard abbreviations is encouraged.

2.2.3 Use of italics. Foreign words and phrases and Latin abbreviations are given in italics: *e.g.*, *in toto*, *in vivo*, *ca.*, *cf.*, *i.e.*

In the names of chemical compounds or radicals italics are used for prefixes (other than numerals or symbols) when they define the positions of named substituents, or when they define stereoisomers: other prefixes are printed in roman. (*Note:* Initial capital letters are not to be used with italic prefixes or single-letter prefixes: full stops are not to be associated with letter prefixes.) For example, *o-, m-* and *p*-nitrotoluenes, but *ortho-*, *meta-* and *para-* compounds (*o-*, *m-* and *p-* are used only with specific names; *ortho-*, *meta-* and *para-* are used with classes), *N*,*N*-dimethylaniline, *trans-* and *cis*-bis(glycinato)platinum(II), *gem-* and *vic*-diols, benzil *anti*-oxime.

The names of periodicals or their abbreviations are set in italics.

2.2.4 Headings.

(a) Main sections (Experimental, Results and discussion, *etc.*): side-heading, bold, first initial capital letter only, no final fullstop.
(b) Main side-heading: bold, first initial capital letter only, no final fullstop.
(c) Subsidiary side-heading: bold, first initial capital letter only, final fullstop.
(d) Further subdivision: italic, first initial capital letter only, final fullstop.

3.0 Presentation of experimental data

3.1 Physical characteristics of compounds

Data associated with particular compounds should be listed after the name of the compound concerned, following the description of its preparation. **The following is suggested as the order in which the most commonly encountered data for a new compound should be cited**: yield, melting point, optical rotation, refractive index, elemental analysis, UV absorptions, IR absorptions, NMR spectrum, mass spectrum. Appropriate formats for the citation of each are as follows.

3.1.1 Yield. In parentheses after the compound name (or its equivalent). Weight and percentage are separated by a comma, *e.g*. the lactone (7.1 g, 56%).

3.1.2 Melting point. In the form mp 75 °C (from EtOH), *i.e*. the crystallization solvent in parentheses. If an identical mixed melting point is to be recorded, the form mp and mixed mp 75 °C is appropriate.

3.1.3 Optical rotation. The *units* should be stated in the preamble to the Experimental section, *e.g*. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}. Shown in the form $[\alpha]_D^{22}$ -22.5 (*c* 0.95 in EtOH), *i.e*. concentration and solvent in parentheses.

3.1.4 Refractive index. Given in the form n_D^{22} 1.653.

3.1.5 Elemental analysis. In the presentation of elemental analyses, both forms (Found: C, 63.1; H, 5.4. $C_{13}H_{13}NO_4$ requires C, 63.2; H, 5.3%) and (Found: C, 62.95; H, 5.4. Calc. for $C_{13}H_{13}NO_4$: C, 63.2; H, 5.3%) are acceptable. Analyses are normally quoted to the nearest 0.1%, but a 5 in the second place of decimals is retained. For identification purposes for new compounds, an accuracy to within ±0.3% is expected, and in exceptional cases, to within ±0.5% is required. If a molecular weight is to be included, the appropriate form is: [Found: C, 63.1; H, 5.4%; M (mass spectrum), 352 (or simply M^+, 352). $C_{13}H_{13}NO_4$ requires C, 63.2; H, 5.3%; M, 352].

3.1.6 UV absorptions. These are given in the form λ_{max}(EtOH)/nm 228 (ε/dm^3 mol^{-1} cm^{-1} 40 900), 262 (19 200) and 302 (11 500). Inflections and shoulders are specified as 228infl or 262sh. Alternatively the following form may be used: λ_{max} (EtOH)/nm 228, 262 and 302 (ε/dm^3 mol^{-1} cm^{-1} 40 900, 19 200 and 11 500). log ε may be quoted instead of ε.

3.1.7 IR absorptions. As follows: ν_{max}/cm^{-1} 3460 and 3330 (NH), 2200 (conj. CN), 1650 (CO) and 1620 (CN). The type of signal (s, w, vs, br) can be indicated by appended letters (*e.g*. 1760vs).

3.1.8 NMR data. For all spectra δ values should be used, with the nucleus indicated by subscript if necessary (*e.g*. δ_H, δ_C). A statement specifying the units of the coupling constants should be given in the preamble to the Experimental section, *e.g*. *J* values are given in Hz. Instrument frequency, solvent, and standard should be specified. For example: δ_H(100 MHz; $CDCl_3$; Me_4Si) 2.3 (3 H, s, Me), 2.5 (3 H, s, COMe), 3.16 (3 H, s, NMe) and 7.3-7.6 (5 H, m, Ph). A broad signal may be denoted by br, *e.g*. 2.43 (1 H, br s, NH). Order of citation in parentheses: (i) number of equivalent nuclei (by integration), (ii) multiplicity (s, d, t, q), (iii) coupling constant, *e.g*. $J_{1,2}$ 2, J_{AB} 4, (iv) assignment; italicisation can be used to specify the nuclei concerned (*e.g*. CH_3CH_2). The proton attached to C-6 may be designated C(6)H or 6-H; the methyl attached to C-6, 6-Me or C(6)Me. Mutually coupled protons in ^{1}H NMR spectra must be quoted with precisely matching *J* values, in order to assist thorough interpretation. In instances of any ambiguities when taking readings from computer print-outs, mean *J* values should be quoted, rounded to the nearest decimal point.

3.1.9 Mass spectrometry data. Given in the form: *m*/*z* 183 (M^+, 41%), 168 (38), 154 (9), 138 (31) *etc*. The molecular ion may be specified as shown if desired. Relative intensities in parentheses (% only included once). Other assignments may be included in the form *m*/*z* 152 (33, M - CH_3CONH_2). Metastable peaks may be listed as: M* 160 (189⟶174), 147 (176⟶161), *etc*. The type of spectrum (field desorption, electron impact, *etc*.) should be indicated. Exact masses quoted for identification purposes should be accurate to within 5 ppm (EI and CI) or 10 ppm (FAB or LSIMS).

3.1.10 Literature citations. If comparison is to be made with literature values, these should be quoted in parentheses, *e.g*. mp 157 °C (from chloroform) (lit.,[19] 156 °C), or ν_{max}/cm^{-1} 2020 and 1592 (lit.,[24] 2015 and 1600).

3.1.11 Experiments involving microorganisms. For work involving microorganisms, sufficient detail should be provided to identify the species being used.

4.0 Bibliographic references, notes and footnotes

Footnotes or Notes may be used to present material which, if included in the body of the text, would disrupt the flow of the argument but which is, nevertheless, of importance in qualifying or amplifying the textual material. Footnotes are referred to with the following symbols: †, ‡, §, ¶, ‖ *etc.* Alternatively the information may be included as Notes (end-notes) to appear in the Notes/references section of the manuscript. Notes should be numbered using the same numbering system as the bibliographic references.

Bibliographic reference to the source of statements in the text is made by use of *superior numerals* at the appropriate place. The reference numbers should be cited in the correct sequence through the text (including those in tables and figure captions, numbered according to where the table or figure is designated to appear). The references themselves are given at the end of the final printed text along with any Notes.

Authors are encouraged to check the RSC Reviews web site to ensure that they have cited relevant recent reviews.

4.1 Journals

The style of journal abbreviations to be used in the Society's publications is that defined in Chemical Abstracts Service Source Index (CASSI). The abbreviations listed in CASSI are based upon internationally recognised systems. A list of CASSI-style abbreviations covering the most commonly cited journals is available from our web site. It is not, of course, a full list; CASSI plus its quarterly supplements run to more than 2000 pages.

If you cannot locate an authoritative abbreviation for a journal, and if it is not obvious how the title should be abbreviated, please cite the full title.

Bibliographic details should be cited in the order: **year**, **volume**, **page**. Where possible, page number ranges are preferred over single values, but either format is acceptable.

Where page numbers are not yet known, articles may be cited by DOI (Digital Object Identifier). *e.g.* A. R. Jones, *Dalton Trans.*, 2005, DOI: 10.1039/B503459J.

Please note that journal citations in articles submitted to the journal *Photochemical & Photobiological Sciences* should include the article titles.

4.2 Books

For example:

J. Barker, in *Catalyst Deactivation*, ed. B. Delmon and C. Froment, Elsevier, Amsterdam, 2nd edn., 1987, vol. 1, ch. 4, pp. 253-255.

4.3 Patents

Patents should be indicated in the following form:

Br. Pat., 357 450, 1986. *US Pat.*, 1 171 230, 1990.

4.4 Reports and bulletins, *etc.*

For example:

R. A. Allen, D. B. Smith and J. E. Hiscott, *Radioisotope Data*, UKAEA Research Group Report AERE-R 2938, H.M.S.O., London, 1961.

4.5 Material presented at meetings

For example:

H. C. Freeman, Proceedings of the 21st International Conference on Coordination Chemistry, Toulouse, 1980.

4.6 Theses

For example:

A. D. Mount, Ph.D. Thesis, University of London, 1977.

4.7 Reference to unpublished material

For material presented at a meeting, congress or before a Society, *etc*., but not published, the following form is used:

A. R. Jones, presented in part at the 28th Congress of the International Union of Pure and Applied Chemistry, Vancouver, August, 1981.

For material accepted for publication, but not yet published, the following form

A. R. Jones, *Dalton Trans.*, 2003, DOI: 10.1039/paperno.

is used for RSC journals, and

A. R. Jones, *Angew. Chem.*, in press.

is used for non-RSC journals. If DOI numbers are known these should be cited in the form recommended by the publisher

For material submitted for publication but not yet accepted the following form is used:

A. R. Jones, *Angew. Chem.*, submitted.

For personal communications the following is used:

G. B. Ball, personal communication.

If material is to be published but has yet to be submitted the following form is used:

G. B. Ball, unpublished work.

Reference to unpublished work should not be made without the permission of those by whom the work was performed.

4.8 Names

The names and initials of all authors are always given in the reference; they must not be replaced by the phrase *et al*. This does not prevent some, or all, of the names being mentioned at their first citation in the cursive text: initials are not necessary in the text.

4.9 Composite references

Whenever possible, composite references should be used rather than a series of individual references. The style for composite references is as follows:

A. B. Jones, *J. Am. Chem. Soc.*, 1956, **78**, 1234-1246; A. B. Jones and C. D. Brown, *J. Am. Chem. Soc.*, 1957, **79**, 567-569; A. B. Jones and E. F. Green, *J. Am. Chem. Soc*., 1957, **79**, 999-1048.

Idem, *loc. cit.*, and *op. cit* are not used in references.