Mutagenicity/genotoxicity of PM 0.5 collected during winter 2014-2015 in five Italian cities: MAPEC (Monitoring Air Pollution Effects on Children for supporting public health policy) study

Salvatori T.[1], Ceretti E.[2], Bonetta Si.[3], Levorato S.[1], Viola G.C.V.[2], Schilirò T.[3], Vannini S.[1], Zerbini I.[2], Carraro E.[3], Carducci A.[4], Grassi T.[5], Fatigoni C.[1], Bonizzoni S.[6], Bonetti A.[7], Villarini M.[1], Gelatti U.[2], MAPEC_LIFE Study Group.

[1]Department of Pharmaceutical Sciences, University of Perugia, Italy; [2]Department of Medical and Surgical Specialties, Radiological Sciences and Public Health, University of Brescia, Italy; [3]Department of Public Health and Pediatrics, University of Torino, Italy; [4]Department of Biology, University of Pisa, Italy; [5] Department of Biological and Environmental Science and Technology, University of Salento, Italy; [6]Comune di Brescia, Italy; [7]Centro Servizi Multisettoriale e Tecnologico – CSMT Gestione Scarl, Brescia, Italy.

INTRODUCTION

Air pollutants, such as carbon monoxide (CO), sulfur dioxide (SO2), nitrogen oxides (NOx), volatile organic compounds (VOCs), ozone (O3), heavy metals, and respirable particulate matter (PM), have acute and chronic effects on human health, affecting a number of different systems and organs. The World Health Organization's (WHO) International Agency for Research on Cancer (IARC) asserts that exposure to outdoor air pollution causes lung cancer and have a positive association with an increased risk of bladder cancer. Furthermore, the IARC classified PM, a major component of outdoor air pollution, as "*Carcinogenic to humans"* (Group 1).

The MAPEC study aims to evaluate the associations between the concentrations of urban air pollutants and biomarkers of early biological effect in oral mucosa of 1,000 children recruited from first grade schools of 5 Italian towns (Torino, Brescia, Pisa, Perugia and Lecce), which are characterized by different PM levels.

STUDY AIM

The purpose of this study was to:

- Evaluate child exposure to urban air pollution by collecting ultra-fine PM (PM 0.5) samples in the school areas of the five Italian cities involved in the study, which varies significantly in the annual average level of some air pollution parameters (e.g. PM 10, PM 2.5, NOx, etc…);
- Measure the concentration of some toxic airborne pollutants (polycyclic aromatic hydrocarbon [PAH] and nitro-PAH) in the air samples;
- Evaluate the *in vitro* mutagenicity and genotoxicity of the particulate samples.

MATERIALS & METHODS

COLLECTION OF ENVIRONMENTAL SAMPLES

In order to assess the air quality of the cities involved in the research, a high-volume air sampler was located near to the schools where children were recruited. PM 0.5 fractions were collected on membranes made of fiberglass filter for 72 hours. Two sampling were carried out in two different seasons: winter 2014 and spring 2015. Data relative to samples collected in the winter season are discussed.

CHEMICAL ANALYSIS

The filters were weighed for gravimetric determination of PM 0.5 and then subjected to organic extraction using sonication.

The organic extracts were chemically analyzed using high pressure liquid chromatography to determine the concentrations of PAHs and nitro-PAHs.

***IN VITRO* ANALYSIS**

Different air volumes (10, 25, 50, 75 m^3) of the PM 0.5 organic extracts were tested with

- **Ames test** to evaluate *mutagenicity* on different *S. typhimurium* strains (TA100, TA98, TA98NR and YG1021);
- **Comet test** and **Micronucleus assay** to detect *genotoxicity* in a human lung adenocarcinoma cell line (A549).

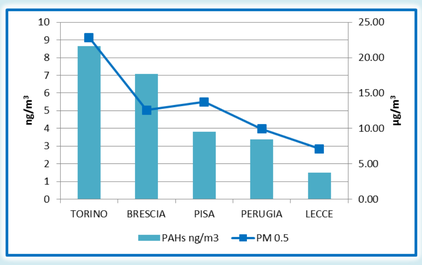
RESULTS

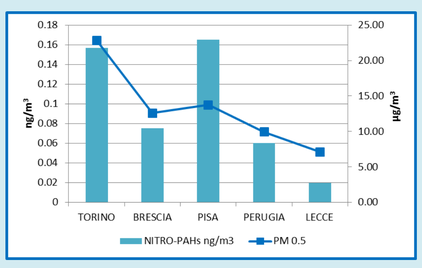
CHEMICAL ANALYSIS

As expected, PM 0.5 levels (µg/m^3) varied significantly among the five considered towns ($p<0.05$, ANOVA) with an increase of concentration from Southern to Northern Italy.

PM 0.5 and PAHs, but not NITRO-PHAs, were significantly correlated with the geographical distribution of towns (South-North) (Pearson's correlation analysis; PM 0.5: $r=0.677$, $p<0.01$; PAHs: $r=0.642$, $p<0.01$).

Figure 1. PAHs, NITRO-PAHs and PM 0.5 air levels





AMES TEST

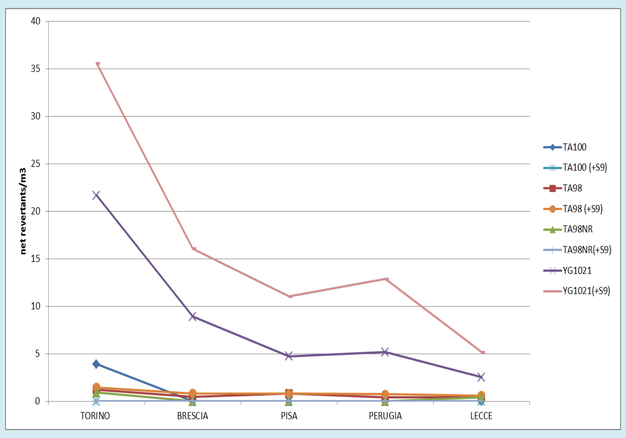
TA100: mutagenic effects in Torino (net revertants/m^3 range 3.0-4.8);

TA98: low mutagenic effects in all samples (net revertants/m^3 range 0.3-1.5); slight increase +S9, possible presence of PAHs;

TA98NR: low mutagenic effects in Torino (range 0.6-1.2);

YG1021: all samples showed mutagenic effect, possible presence of nitroaromatic compounds.

Figure 2. Net revertants/m^3 of different *S. typhimurium* strains exposed to PM 0.5 sampled

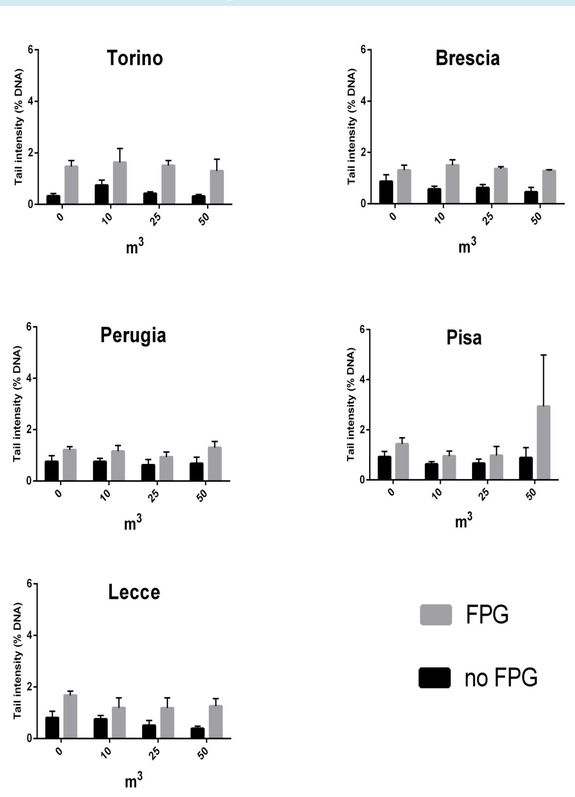


COMET ASSAY

No statistically significant increase of tail intensity was detected in treated cells compared to negative control (ANOVA).

No correlation with PM 0.5, PAHs or NITRO-PAHs levels was found.

Figure 3. Tail intensity of A549 cells exposed to different air volumes of the PM 0.5 organic extracts.

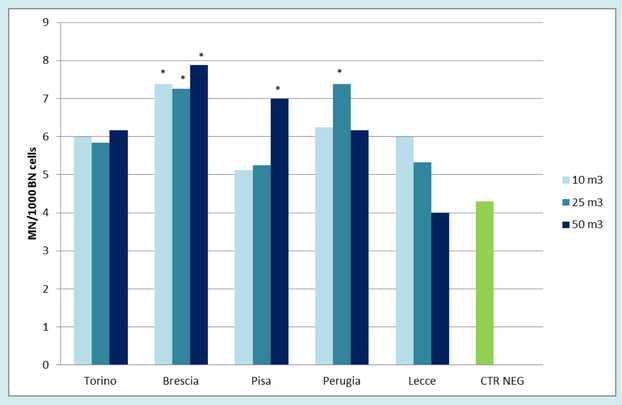


MICRONUCLEUS ASSAY

A statistically significant increase of micronuclei (MN) frequency was observed in cells exposed to Brescia (all concentrations), Pisa (50 m^3) and Perugia (25 m^3) air samples.

MN frequency was not correlated to PM 0.5, PAHs or NITRO-PAHs concentration.

Figure 4. Frequency of MN in A549 cells exposed to different air volumes of PM 0.5 organic extracts



DISCUSSION

- Chemical analysis confirmed that air pollution levels are greater in Northern Italian cities with respect to Centre and Southern ones.
- Data from *in vitro* analysis are controversial as we did not observe a significant correlation between the mutagenicity/genotoxicity of the PM samples and the concentration of toxic pollutants.
- These results could be explained by the low level of air pollution observed in winter 2014 compared to those of the previous years.
- Further comparison will be performed between the results of winter and summer seasons, among all towns and between those at higher (Brescia and Torino together) and lower (all the others) levels of urban air pollution.

Components of the MAPEC_LIFE Study Group:

Sara Compiani, Francesco Donato, Andrea Festa, Gaia Claudia Viviana Viola, Ilaria Zerbini, Department of Medical and Surgical Specialties, Radiological Science and Public Health, University of Brescia, Brescia, Italy;

Marcello Guido, Adele Idolo, Francesca Serio, Maria Rosaria Tumolo, Tiziano Verri, Department of Biological and Environmental Science and Technology, University of Salento, 73100 Lecce, Italy;

Cristina Fatigoni, Tania Salvatori , Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy;

Beatrice Bruni, Elisa Caponi,Gabriele Donzelli, Department of Biology, University of Pisa, Pisa, Italy;

Giorgio Gilli, Cristina Pignata, Department of Public Health and Pediatrics, University of Torino, Torino, Italy;

Silvia Bonizzoni, Camilla Furia, Comune di Brescia, Brescia, Italy;

Francesco Braga, Roberta Codenotti, Paolo Colombi, Daniela Lini, Evelyn Mario, Centro Servizi Multisettoriale e Tecnologico —CSMT Gestione S.c.a.r.l., Brescia, Italy.

Correspondence to: tania.salvatori@unipg.it