Micronucleus cytome assay in exfoliated buccal cells of children for the evaluation of early biological effects of air pollution exposure. The MAPEC_LIFE project.

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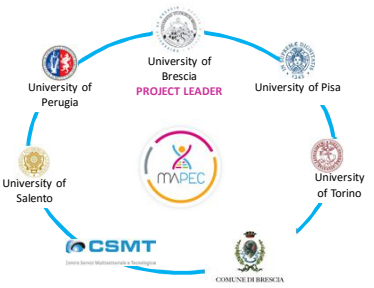
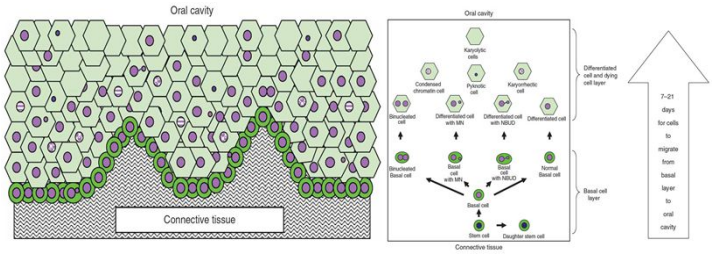
Introduction

Air pollution is a global problem. In 2013, air pollution and particulate matter were classified as carcinogenic to human by the IARC. Children are a high-risk group in terms of the health effects of air pollution, and early exposure during childhood can increase the risk of developing chronic diseases in adulthood.

The MAPEC_LIFE (Monitoring Air Pollution Effects on Children for supporting public health policy, www.mapec-life.eu) is a project founded by EU Life+ Programme (LIFE12 ENV/IT/000614) which intends to evaluate the associations between air pollution and early biological effects in children and to propose a model for estimating the global risk of early biological effects due to air pollutants and other factors in children (Feretti et al., 2014). This work aims to investigate micronuclei frequency in child cells in association with air pollutant levels and other factors, as lifestyle.

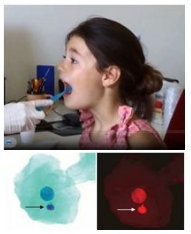




Representation of a cross section of normal buccal mucosa, **on the left**, and origins and spatio-temporal sequence of the various buccal mucosa cell types, **on the right** (from Thomas et al., 2009)

Methods

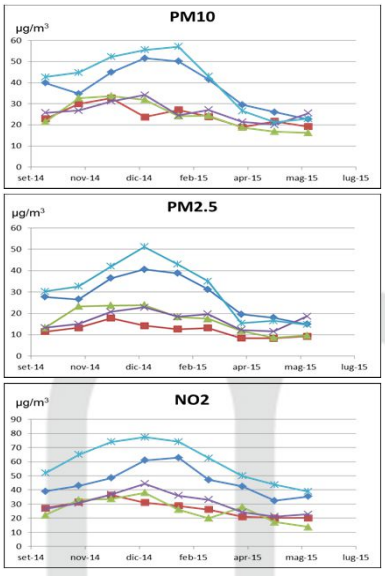
The micronucleus cytome assay was performed in exfoliated buccal cells of 6–8-year-old children from 5 Italian towns (Torino, Brescia, Pisa, Perugia and Lecce) with different air pollution levels. Data on air quality during the study period were obtained from Regional Agencies for Environmental Protection. Details of child diseases, socio-economic status, exposures to other pollutants and lifestyle were collected using a questionnaire (Zani et al., 2015) administered to children's parents.



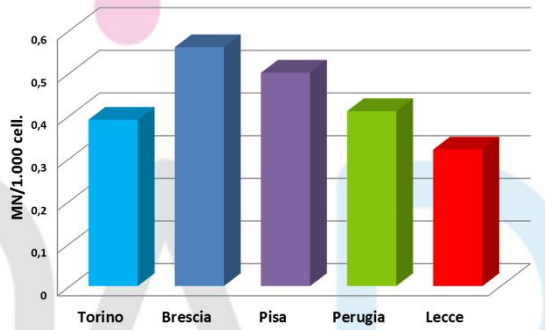
Sampling of buccal cells, and a differenziated buccal cell with micronucleus (double staining).

Results

During the winter campaign (Nov 2014 - Mar 2015), 1318 children were recruited and their buccal cells sampled. Micronucleus frequency was evaluated only for 1089 children, because some samples were not suitable for test performing. As about air quality, the levels of main pollutants were, as expected, higher in the North of Italy, with a PM10 mean values of 50 and 45 μg/m^3 in Torino and Brescia, respectively, than in the other towns (Pisa, Perugia, Lecce). In contrast, micronucleus frequency in buccal cells of children was higher in Brescia (0.54/1000 cells) than in any other towns (from 0.32 to 0.50/1000 cells). From a preliminary analisys, MN frequency in child cells did not show a clear relationship with air pollutant levels or socio-demographic and lifestyle factors.



Graphs on the left show the monthly concentration of PM10, PM2.5 and NO_2 in the five towns between September 2014 and May 2015.
The pictures and the table below describe MN and nuclear bud frequencies (the second one only in the table) in buccal cells of children of the five towns (a: p-value calculated with Kruskal-Wallis test).
The table on the right shows the distribution of some socio-demographic and lifestyle features of children, collected by questionnaire administered to children's parents (b: p-value calculated with chi square test; c: p-value calculated with ANOVA, Tukey *post hoc*).



Biomarkers	Torino	Brescia	Pisa	Perugia	Lecce	Total	p-value[a]
MN	0.39 ± 0.48	0.54 ± 0.59	0.50 ± 0.65	0.41 ± 0.59	0.32 ± 0.44	0,43 ± 0,56	0.001
NBUDs	0.25 ± 0.39	0.24 ± 0.46	0.60 ± 0.74	0.19 ± 0.39	0.15 ± 0.31	0,27 ± 0,49	0.000

Socio-demographic and lifestyle factors	Turin 19,8%	Brescia 23,2%	Pisa 16,1%	Perugia 21,1%	Lecce 19,7%	Total 100,0%	p-value
Sex							
M	52.8%	47.4%	44.0%	57.4%	50.2%	50.6%	0.068[b]
F	47.2%	52.6%	56.0%	42.6%	49.8%	49.4%	
Children's age	7,33 ± 0,73	7,41 ± 0,86	7,23 ± 0,96	7,35 ± 0,91	7,41 ± 0,88	7,35 ± 0,87	0.182[c]
Parents' education							
Secondary school or less	43.1%	50.8%	44.6%	32.6%	45.6%	43.4%	0.002[b]
College or university	56.9%	49.2%	55.4%	67.4%	54.4%	56.6%	
Smokers in home	9.3%	10.3%	9.8%	5.7%	7.0%	8.4%	0.328[b]
Heating systems							
District heating	29.4%	60.2%	1.7%	1.7%	0.5%	20.7%	0.000[b]
Wood/pellet	0.5%	2.8%	2.3%	4.8%	3.0%	2.7%	
Fossil fuels/diesel	10.0%	0.4%	1.1%	5.2%	2.0%	3.7%	
Gas	59.2%	33.1%	93.1%	85.6%	94.1%	70.9%	
Electric	0.9%	3.6%	1.7%	2.6%	0.5%	2.0%	
Physical activity							
Three times a week	38.4%	41.5%	38.9%	46.1%	59.1%	44.9%	0.000[b]
In outdoor environments	24,1%	32,8%	25,1%	31,3%	26,5%	28,3%	0.155[b]
Swimming pool	3.7%	0.2%	6.3%	4.3%	14.0%	6.2%	0.000[b]
BMI	16.3 ± 2.5	16.4 ± 2.5	16.7 ± 2.4	16.9 ± 2.9	16.7 ± 2.9	16.6 ± 2.7	0.106[c]
Underweight	2.8%	4.0%	2.3%	3.9%	3.7%	3.4%	0.525[b]
Normal weight	71.8%	68.8%	62.3%	65.2%	68.4%	67.5%	
Overweight	8.3%	13.0%	14.3%	11.7%	13.5%	12.1%	
Obese	17.1%	14.2%	21.1%	19.1%	14.4%	17.0%	
Adherence to Mediterranean diet	3.62 ± 1.74	3.53 ± 1.67	3.33 ± 1.67	3.83 ± 1.81	3.07 ± 1.66	3.49 ± 1.73	0.000[c]
Low adherence	47,7%	52,6%	58,3%	45,7%	63,7%	53,3%	0.006[b]
Medium adherence	38,4%	32,8%	31,4%	37,4%	27,4%	33,6%	
High adherence	13,9%	14,6%	10,3%	17,0%	8,8%	13,1%	

Conclusions

Even if children of the five towns showed significant differences in MN frequency, these differences seemed not to be associated with air pollutant concentrations or other investigated factors. A more complete analysis will be performed as data about second and third sampling campaign will be available.

The MAPEC_LIFE Study Group.
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