Micronuclei in families exposed to air pollution
A pilot study in the Czech Republic
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Introduction
Biomarkers of effect may relate health outcomes to individual personal exposures and take the various individual factors like uptake, metabolism and excretion into consideration. Differences in genetic background and age are considered. Also, biomarkers often reflect aggregated exposures and confounders. In family biomonitoring studies, including more than one child and parents, useful information may be provided about how children differ from each other, and from the parents in response to genotoxic exposure.

Micronuclei (MN) is a biomarker of genotoxic exposure and early biological effect. A high frequency of MN indicates an early step in carcinogenesis, as DNA damage at the chromosome level is an important event in carcinogenesis [1] (Figure 1).

Children differ from adults in exposure to the environment due to differences in behaviour, e.g. inability to remove themselves from a noisy environment, hand-to-mouth behaviour and playing on the floor. Compared with adults, children have a higher daily intake of food, water and air per kg body weight, and therefore they may have a proportionally higher intake of toxic agents than adults.

The susceptibility of children is different from adults. Increased susceptibility is related to altered rates of distribution of toxins, detoxification, DNA repair processes, and cell proliferation. Children have immature organs, immune system, metabolic and excretory pathways, and alterations in target tissues, organs and CNS susceptibility, dependent on age. Moreover children have a longer life span in which to express illness [3]. Hence biomonitoring studies in children are necessary for risk assessment of children.

Epidemiological studies have been reviewed and the association between exposure to ambient particulate matter (PM) and mortality and morbidity of urban populations has been stated [4]. Especially ultrafine particles (UFP) and fine particles (PM) are of concern. Clinical studies indicate that UFP are retained in the alveolar regions of the lungs and penetrate to the bloodstream. UFP might carry PAHs, VOCs, and they may contain trace metals, e.g., Pb, Cu, Ni, Cu, Zn. Concentrations can reach 100.000 UFP/ml in urban air.

Exposure to air pollution in the Teplice district in the northeast of the Czech Republic (Figure 2) reveals a serious adverse respiratory health problems for children and affecting generation in adults. Elevated levels of air pollutants, even during short winter inversions, resulted in measurable uptake, metabolism and excretion of genotoxic organic compounds, as well as increased blood concentrations of toxic metals and DNA damage [5].

Objectives
The objective of the family pilot project was to assess the relationship between genotoxic exposure from ambient air and a range of biomarkers measured in children and mothers [6].

The exposure assessment included air sampling of ultrafine and fine particles at the front doors of 24 families living in the Czech Republic. Measurements and analyses of other particle fractions and gaseous air pollutants from stationary monitoring stations along with meteorological conditions on the sampling days were obtained. Relevant information on exposure to indoor sources, including ETS, was compiled from questionnaires and biomarker results.

The biological material (blood and urine) was collected in 48 children (two siblings) and their mothers. Families selected for the study were drawn from the Teplice area, a former mining region, and compared with families from the rural area of Frachotice (Figure 2).

Methods
The cytokinesis-block micronucleus (CBMN) test was used for measuring peripheral blood lymphocytes (Figure 3).

The principle of the CBMN test is to stimulate a culture of nondividing (G0) cells to divide with the mitogenic compound PHA (phyto-
haemagglutinin). Cytochalasin-B (CB-B), a cytokinesis-blocking agent, is added at the last part of the culture period, and the frequency of MN is counted only in binucleated cells (BN) to ensure that a single nuclear division that is essential for MN development [8].

The CBMN test is used in biomonitoring of human populations, screening of chemicals and other specific purposes [9].

Scoring criteria:
Only viable cells with an intact cytoplasmic membrane are scored.

The two main nuclei of the BN cell should be equal in size, staining pattern and staining intensity. They may touch but not overlap. The two nuclei within a BN cell may be attached by a fine nucleoplasmic bridge.

MN be scored morphologically identical to, but smaller than the main nucleus

The BN cells should not contain more than 6 MN

1000 BN lymphocytes are scored for MN

Cells and MN are not scored if uncertain and unclear [10]

Results
The air sampling at the front doors resulted in lower levels of UFP and PM in both areas. The difference between Teplice and Prachatice appeared to be small during this particular time of sampling. Air monitoring data and the results of the chemical analyses of the MN indicated a significant exposure difference between the areas, especially during winter.

Children and mothers living in the Teplice area have higher frequencies of MN cells as compared with children and mothers from the Prachatice area (Table 1 and Figure 4).

Table 1. Characteristics of the study population and MN frequency

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean</th>
<th>Median</th>
<th>Range</th>
<th>SD</th>
<th>CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Teplice (n=24)</td>
<td>7</td>
<td>1.21</td>
<td>0.65</td>
<td>0.00-6.75</td>
<td>1.04</td>
<td>0.87</td>
</tr>
<tr>
<td>Prachatice (n=24)</td>
<td>7</td>
<td>19.92</td>
<td>5.44</td>
<td>0.00-100</td>
<td>9.02</td>
<td>4.56</td>
</tr>
</tbody>
</table>

In order to analyze the effect of several variables bimodal mixed regression models were applied. As shown in table 2 children and mothers from the Teplice area had significantly increased MN frequencies of 50 % compared with the study population from Prachatice. A significant effect of age on the MN frequency was revealed in children. An age difference of one year increases the formation of MN by 9%. Moreover increased MN frequencies of 21 % was found in girls as compared to boys. A low inter family variation was found as expressed by the S.D.

MN frequencies were analyzed in order to test whether the effect of area could be explained by exposure to aromatics from traffic or indoor sources. Higher MN frequencies were found in the two families living in proximity to high level of traffic. Exposure to indoor sources showed an adverse effect, however these findings are not conclusive.

Conclusions
The family pilot study showed that MN is a valuable and sensitive biomarker for early effect, which can be used to reveal MN frequency differences in the blood exposed to different levels of pollution.

References

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