The influence of environmental exposure to complex mixtures including PAHs and lead on genotoxic effects in children living in Upper Silesia, Poland

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Environmental exposure is a complex mixture of hazardous compounds with different mechanisms of toxicity. In case of concomitant exposure to carcinogenic substances—such as polycyclic aromatic hydrocarbons (PAHs)—and to heavy metals—such as lead (Pb)—the level of DNA damage may be enhanced. Children are considered more vulnerable than adults to chemical toxicants because they take in more toxicants as a proportion of body mass and because of inherent biological growth and developmental factors. The objective of the study was to measure cytogenetic effects in Silesian children and to investigate their relation with the environmental exposure to PAHs and Pb. The examined population included 74 children 5-14-year-old who lived in two cities located in the most polluted centre of the Silesia province. Individual exposure to lead was assessed for each child by measuring lead in blood (PbB), and to PAH by measuring 1-hydroxypyrene in urine (1-OHP), urinary mutagenicity and DNA adducts in circulating lymphocytes. Biomarkers of genetic effects were assessed by measuring micronuclei (MN) and sister chromatid exchanges (SCE) in children’s peripheral lymphocytes. The mean levels of biomarkers of exposure were as follows: PbB 7.69 µg/dl, DNA adducts 9.59 adducts per 10^9 nt, 1-OHP 0.54 µmol/mol creatinine, and urinary mutagenicity presented as the number of revertants per mmol of creatinine: 485 for TA 98 and 1318 for YG1024. Mean value of MN was 4.44 per 1000 binucleated cells and SCE frequency ranged between 6.24 and 10.06 with a mean value of 7.87. The results suggest the influence of exposure to environmental agents on the induction of cytogenetic effects in peripheral lymphocytes of children: namely Pb on MN and PAHs on SCE. The sources of that exposure may be outdoor and indoor. Emissions from coal-burning stoves are important contributors to the total exposure to PAHs and Pb in Silesian children.

Introduction

The Province of Silesia is located in the south of Poland that occupies 2% of the total area of Poland and includes 11% of the national population. The area is rich in natural resources, mainly coal, zinc, lead and other ore mines, that have stimulated the development of many industrial activities after World War II which has caused an intensive deterioration of the natural environment. The main sources of pollution are mining and smelting activities and the presence of heavy industry with coal-based power, steel and coke plants, as well as heavy automobile traffic and use of coal for domestic heating (1). Lead mining and processing have been operating almost exclusively in this area of Poland. In the early 1990s ~5 million tons of lead and zinc ores, corresponding to 4% of the world total production, were extracted in the region. In the late 1980s, annual lead fallout in the urban center of the Province, known as Katowice Agglomeration, was ~180 mg/m² (2). In 1991 average annual concentration of benzo(a)pyrene of airborne particulate matter from 24 sampling stations ranged from 15 to 125 ng/m³, always exceeding the permissible standard of 1 ng/m³ (average annual concentration) and 5 ng/m³ (average daily concentration) (3). The maximum daily concentration was as high as 950 ng/m³ (4). Although in the mid-1990s reduction of industrial activities reduced further degradation of the environment, new hazards emerged, including air pollutants from automobile exhaust and combustion of coal for domestic heating. Owing to the further reduction of industrial activities the concentration of some chemical air pollutants, including sulphur dioxide, nitrogen dioxide and particulate matter, has further decreased in recent years. By 2004 annual lead fallout had gradually decreased with only 13 measurement points out of 707 exceeding the permissible level of 100 mg/m². Conversely annual concentrations of benzo(a)pyrene were still exceeding the standard value of 1 ng/m³ in all sampling stations of the District Sanitary-Epidemiological Station in Katowice with values as high as 18.6 ng/m³ (5).

It has been hypothesized that children are more susceptible than adults to environmental toxins (6–8). Such susceptibility can be attributed to the fact that they have greater exposures to environmental toxicants than adults in relation to their body weight, they undergo rapid growth and development and their developmental processes are easily disrupted (9). Children have also a longer life span to develop chronic diseases that may be triggered by early exposures. Chronic diseases (e.g., cancer) that are caused by environmental toxicants are thought to arise through a series of stages that require years or even decades to evolve from their initiation to clinical manifestation. Carcinogenic and toxic exposures sustained early in life appear more likely to lead to disease than similar exposures encountered later (9).

Environmental exposure to lead in childhood was and still remains a relevant environmental health problem in Silesia Province (1,10). Given its genotoxic potential (11–21), research focused on the identification and specification of DNA damage in children exposed to lead is of particular importance. Children play in leaded dust and dirt and have...
more hand-to-mouth activity than adults and they have higher gastrointestinal absorption once they have ingested lead (1). Blood lead levels as low as 10 µg/dl can lower subsequent scores on IQ tests in children whereas the same exposure would not be expected to be of much consequence in adults (22).

The evidence of lead carcinogenicity remains unclear. Lead and lead compounds have been reviewed three times by the International Agency for Research on Cancer (IARC) (23-25). Currently, lead and inorganic lead compounds are classified by IARC in Group 2B, possibly carcinogenic to humans, on the basis of sufficient evidence for carcinogenicity in experimental animals. Exposure of cells in culture has shown that lead can induce chromosomal aberration, micronuclei (MN) and sister-chromatid exchange (SCE) (13,26) but the biological mechanism is still unknown (18). The evidence for chromosomal toxicity of lead has been reported inconsistently in exposed humans (10,11,14,17,19,26,27). A few studies on DNA damage were carried out in children environmentally exposed to lead (27–31) and their results remain inconclusive. Polycyclic aromatic hydrocarbons (PAHs) are the most prominent among the genotoxic and carcinogenic agents present in polluted urban air. They have been the focus of attention of most air pollution-related biomarker field studies including biomarkers of exposure like 1-hydroxypyrene (1-OHP) (32) or DNA adducts (33) and biomarkers of genotoxicity (34). Uranium mutagenicity reflecting global exposure to a mixture of known and unknown mutagens/carcinogens has also been used as an unspecific biomarker of exposure to PAHs (35,36). Both epidemiological and experimental evidence indicate that PAHs are carcinogenic to animals and possibly to human beings (25). The mechanism of genotoxicity of PAHs is well known and many studies on the genotoxic effect of airborne PAHs in humans have been carried out. They refer both to occupational (37–40) and environmental exposure including children and adolescents (30,41,42).

Environmental exposure is a complex mixture of hazardous compounds with different mechanisms of toxicity. Hazardous pollutants contained in ambient air are the main source of environmental pollution in Silesia region. PAHs and their nitro-derivatives are responsible for the high mutagenic concentrations were expressed in 229 nm excitation and 400 nm emission wavelengths.

The significance of linear dose–response relationship was examined by the least square method with the assumption of P-level ≤0.05. Mutagenic effect was expressed as a number of induced revertants per mmol of creatinine (50).

**Materials and methods**

**Study design**

The examined population included 74 children (47 boys and 27 girls): 5–14-year-old who lived in Katowice and Sosnowiec, two cities located in the most polluted centre of the Silesia province. The study was approved by the Ethics Committee of the Silesian Medical Academy in Katowice. According to the Polish law parents of all children participating in this study gave their written consent.

All parents completed a self-administered questionnaire form including items concerning children’s demographic variables (age, gender) health status (past and present diseases including chronic diseases), current and past intake of medicines, and dietary habits, including consumption of fruits and vegetables grown in Silesia Province. For each child included in the study parent’s education and smoking habit (including number of cigarettes smoked daily) were collected. Information concerning X-ray exposure (diagnostic, dentistry) and the presence of cancer within the child’s family and among parent’s first-order relatives was also collected. Other questionnaire items included the presence of industrial activities, the intensity of traffic at the dwelling site and the type of heating system (electric, oil, gas, coal stoves).

Individual exposure was assessed for each child by measuring lead in blood (PbB), 1-OHP in urine, PAH-DNA adducts in circulating lymphocytes and urinary mutagenicity. Biomarkers of genetic effects were assessed by measuring MN and SCE in children’s peripheral lymphocytes.

**Blood and urine sampling**

Blood sampling took place in the outpatient clinic of environmental health at the Institute of Occupational Medicine and Environmental Health in Sosnowiec during the cold season ranging between November (1998) and March (1999) when heating is required. Blood samples were collected into sodium–heparin tubes--Vacutte for setting cell cultures and Vacutainer for PbB determination. Twenty-four hour urine samples were collected into sterilized plastic containers.

**PbB determination**

Lead in whole blood was determined by electrothermal atomic-absorption spectrophotometry (EAAS) according to Stoeppler et al. (45). Vortex-mixed blood (200 µl) was added to 800 µl of 5% HNO3 in a pre-cleaned 2.2 ml Eppendorf tube. The mixture was vortexed and left for 24 h in refrigerator for better deproteinization. After centrifuging at 10 000 r.p.m. for 15 min the supernatant was transferred to the Perkin–Elmer polyethylene autosampler cups.

The solution (20 µl) was automatically injected into the pyro-coated graphite tube with L’vow platform. The Pb in the sample was vaporized at the optimized sequential dry-atomize furnace program (Transversely Heated Graphite Atomizer, THGA) developed in the laboratory. The atomic absorption signal of lead was measured in the absorbance-peak area mode using Zeeman Graphite furnace spectrometer (Perkin–Elmer 4100ZL).

The detection limit of PbB was 6 µg/l.

**Determination of 1-OHP in urine**

The determination of 1-OHP in urine was carried out using the high-pressure liquid chromatography (HPLC) method developed by Jongeneelen and Anzian (46). 1-OHP in the 20 ml urine samples was enzymatically deconjugated and then transferred to primed C18 Octadeyl cartridges (J.T.Baker), washed with 10 ml of water, and eluted with 9 ml of Methanol. The components of the eluate were separated by means of HPLC on the HP 1090 apparatus (Hewlett Packard) with the ODS C18, 200 × 2.1 mm column whereas 1-OHP was quantitatively determined using the fluorescence detector HP 1046 (Hewlett Packard) with 229 nm excitation and 400 nm emission wavelengths.

The detection limit was 0.14 nmol of 1-OHP/l of urine. 1-OHP concentrations were expressed in µmol/mmol creatinine to account for differences in urine dilution.

**Determination of creatinine in urine**

Urinary creatinine concentrations were analyzed using a standard colorimetric method following the picric acid reaction and absorption at 520 nm (47).

**Uranium mutagenicity**

The analysis included the preparation of urine extracts according to Tamasaki and Ames (48). The organic substances present in urine were condensed using adsorption and desorption methods on columns filled with organic resin XAD-2. The extracts were examined by the Ames incorporation plate test (49). Before the examination the urine extracts of urine were dissolved in DMSO (dimethyl sulfoxide).

Three doses, 20, 40 and 80 µl of DMSO solution (representing 6, 12 and 24 ml of urine), were examined twice with *Salmonella typhimurium* strains: TA98 and its derivative with elevated levels of O-acetyltransferase enzymes: YG1024, both with metabolic activation (+S9 mixture).

The significance of linear dose–response relationship was examined by the least square method with the assumption of P-level ≤0.05. Mutagenic effect was expressed as a number of induced revertants per mmol of creatinine (50).

**Determination of DNA adducts in white blood cells**

Peripheral blood (10 ml) was collected into heparinized tubes and stored in −20°C till total WBC DNA extraction but not longer than 1 month.

**DNA extraction**

DNA isolation was carried out using proteinase K digestion, phenol–chloroform extraction and ethanol precipitation as described in standard protocols. The concentration of DNA was determined spectrophotometrically by measuring the UV absorbance at 260 nm and the purity was ascertained by the ratio at 260/280 nm.
Aromatic DNA adducts analysis

DNA adducts were assessed using the 32P-postlabelling method mainly described by Randerath and Randerath (51). DNA (5 μg) was digested with micrococcal nuclease, phosphodiesterase from calf spleen and nuclease P1 from Penicillium citrinum (the last one is used to enrich the mixture in modified nucleotides). Then the digested was labeled with γ-32P-ATP catalyzed by T4 polynucleotide kinase. Redundant γ-32P-ATP were eliminated by apyrase. The material was then separated by thin layer chromatography on polyethyleneimine-cellulose plates and estimated by autoradiography. The diagonally radioactive zone was excised and quantified by scintillation counting. The parts of the plates without spots were used as background.

Determination of cytogenetic endpoints in lymphocytes

Cultures. Venous blood was taken from each subject using heparinized vacutainer tubes. Lymphocyte cultures were set up by adding 0.5 ml of heparinized blood to 4.5 ml of medium (RPMI 1640; Gibco) supplemented with 20% heat-inactivated fetal bovine serum (FBS; Gibco), antibiotics (penicillin and streptomycin) and 1-glutamine. Lymphocytes were stimulated by 1% phytohaemagglutinin (Gibco).

MN assay. The cultures were incubated at 37°C for 72 and 44 h after the initiation of cultures, cytochalasin-B (Sigma) at a concentration of 0.6 μg/ml was added to arrest cytokinesis. MN slides were stained with 10% Giemsa in phosphate buffer. A total of 1000 binucleated cells with well preserved cytoplasm was examined for each subject on coded slides and the total number of MN and the frequency of binucleated cells with MN (BNMN) were scored (52).

SCE assay. The cultures were incubated for 72 h at 37°C with 0.25 ml of 5-bromo-2'-deoxyuridine (BrdU; Sigma). Colcemid (Gibco) was added 2 h before harvesting. The cells were collected by centrifugation, resuspended in a pre-warmed hypotonic solution (0.075 M KCl) for 20 min and fixed in acetic acid/methanol (1:3, v/v). Chromosome preparations and stained slides were prepared following the Anthosina and Foriaikowa procedure (53). SCE were scored in 50 metaphases and presented as a number of SCE per cell. Data from <50 metaphases were discarded (54).

Statistical analysis

Questionnaire and analytical data were stored in a database and statistically analyzed using STATISTICA for Windows, Version 9.9, 1997. Biomarkers data were log transformed to meet normality assumptions if required by the test statistics (comparison of mean values). Test results were considered statistically significant for the \( P < 0.05 \).

The levels of SCE were normally distributed (Shapiro–Wilks test). The distribution of urinary 1-OHP concentration and the levels of DNA adducts and MN were skewed to the right. Therefore, they were transformed as log \( (X+1) \) to make their distribution normal (1-OHP and DNA adducts) or stabilize the variance (MN). The arithmetic mean and the standard deviation as well as the median and the range were used to describe the frequency distribution of biomarkers of exposure (PbB, 1-OHP and DNA adducts) and effects [MN and SCE in peripheral blood lymphocytes (PBL)]. Univariate statistics (Student’s t or Mann–Whitney tests) were used to compare subgroups dichotomized according to individual covariates including gender (boys and girls), age (<9 and ≥9 years old), exposure to ETS (yes = 1, no = 0), exposure to indoor emission (coal stoves inside flat = 1, other heating system = 0), and parents’ education (defined as the highest level of education achieved by the mother or the father: primary education = 1, secondary or higher education = 0), PbB levels (<10, ≥10 μg/dl). Correlations between biomarkers of exposure and effects were calculated by Spearman’s rank test.

Multiple linear regression analysis (stepwise procedure) was used to assess the influence of PbB, white blood cells DNA adducts and urinary 1-OHP used as continuous covariates—on MN and SCE levels separately. Gender, age, exposure to ETS and/or indoor emissions due to coal stoves and parents’ education were included in the regression model.

A total of 74 children, 5–14-year-old, participated in the study between November 1998 and March 1999 (Table I). Examined children included 47 boys (63.5%) and 27 girls (36.5%). Mean age of children was 8.4 years. Of the children 60.6% were exposed to ETS by co-habitation with smokers (mother, father or both), and 34.3% were exposed to indoor pollution generated by the system of heating based on coal-burning stoves. Primary education was achieved by the parents of 36 children and secondary or higher level by the parents of 29. For 9 children parent’s education was not available (Table I).

### Table I. Selected characteristics of the Silesian children population by gender

<table>
<thead>
<tr>
<th>Silesian children</th>
<th>N</th>
<th>Age in years [mean (SD)]</th>
<th>Exposure to ETS [N (%)]</th>
<th>Presence of coal stove in home [N (%)]</th>
<th>Parents’ education* [N (%)]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Primary</td>
</tr>
<tr>
<td><strong>Girls</strong></td>
<td>27</td>
<td>8.9 (2.3)</td>
<td>15 (60.0)</td>
<td>9 (34.6)</td>
<td>14 (58.3)</td>
</tr>
<tr>
<td><strong>Boys</strong></td>
<td>47</td>
<td>8.0 (1.7)</td>
<td>27 (60.9)</td>
<td>15 (34.1)</td>
<td>22 (53.7)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>74</td>
<td>8.4 (2.0)</td>
<td>42 (60.6)</td>
<td>24 (34.3)</td>
<td>36 (55.4)</td>
</tr>
</tbody>
</table>

*Not available for nine children.

### Table II. Levels of biomarkers of exposure and genetic damage in Silesian children

<table>
<thead>
<tr>
<th>Biomarker/assay (unit)</th>
<th>No.</th>
<th>Mean (SD)</th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Biomarkers of exposure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PbB (μg/dl)</td>
<td>74</td>
<td>7.69 (4.29)</td>
<td>6.77</td>
<td>2.7–23.0</td>
</tr>
<tr>
<td>DNA adducts (per 10⁶ nt)</td>
<td>72</td>
<td>9.59 (7.97)</td>
<td>7.17</td>
<td>0.64–44.92</td>
</tr>
<tr>
<td>1-OHP (μmol/mol creatinine)</td>
<td>66</td>
<td>0.54 (0.31)</td>
<td>0.46</td>
<td>0.04–1.95</td>
</tr>
<tr>
<td><strong>Urine mutagenicity (revertants/mmol creatinine)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TA98+S9</td>
<td>65</td>
<td>485 (415)</td>
<td>398</td>
<td>95–2934</td>
</tr>
<tr>
<td>YG 1024+S9</td>
<td>64</td>
<td>1318 (909)</td>
<td>1021</td>
<td>45–4987</td>
</tr>
<tr>
<td><strong>Biomarkers of effect</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MN (MN/1000)</td>
<td>71</td>
<td>4.44 (3.76)</td>
<td>3</td>
<td>0–17</td>
</tr>
<tr>
<td>SCEs (SCE/cell)</td>
<td>72</td>
<td>7.87 (0.87)</td>
<td>7.86</td>
<td>6.24–10.06</td>
</tr>
</tbody>
</table>

**Results**

A total of 74 children, 5–14-year-old, participated in the study between November 1998 and March 1999 (Table I). Examined children included 47 boys (63.5%) and 27 girls (36.5%). Mean age of children was 8.4 years. Of the children 60.6% were exposed to ETS by co-habitation with smokers (mother, father or both), and 34.3% were exposed to indoor pollution generated by the system of heating based on coal-burning stoves. Primary education was achieved by the parents of 36 children and secondary or higher level by the parents of 29. For 9 children parent’s education was not available (Table I).

Table II shows the average levels of biomarkers of exposure measured in children’s blood (PbB and DNA adducts), and in urine (1-OHP and urine mutagenicity). The mean PbB concentration was 7.69 μg/dl, with levels as high as 23.0 μg/dl. DNA adducts were detected in all children ranging between 0.64 and 44.92 adducts per 10⁷ nt and with mean level of 9.59 adducts per 10⁸ nt. 1-OHP mean value was 0.54 μmol/mol creatinine with highest values close to 2 μmol/mol creatinine. The mean level of revertants per mmol of creatinine corrected for urine dilution was 485 for TA98+S9 (range 95–2934) and 1318 (range 45–4987) for YG1024+S9.

MN and SCE measured in PBL are reported in Table II. MN mean value was 4.44 per 1000 binucleated cells, with individual values reaching 17 MN cells per 1000 binucleated cells. SCE frequency ranged between 6.24 and 10.06 with a mean value of 7.87.

Correlation between biomarkers of exposure and effects was examined by Spearman’s correlation. Positive significant correlation was found between the level of SCE and PAH–DNA adducts \( (r = 0.250, P < 0.05) \) (Figure 1) and between PbB and MN levels \( (r = 0.347, P < 0.05) \) (Figure 2). The mean level of MN in children with PbB concentration exceeding the threshold PbB level (10 μg/dl) was significantly higher than...
in children with PbB level equal or below the threshold value (6.4 versus 3.9, respectively, \( P < 0.05; \) Fig. 3). SCE mean levels were not found to be associated with the PbB threshold (data not shown). Significant correlation was also found between biomarkers of exposure: 1-OHP and DNA adducts \( (r = 0.471, P < 0.05) \) and 1-OHP versus urinary mutagenicity by YG1024 \( (r = 0.291, P < 0.05) \).

We examined the influence of gender, age, indoor exposure to ETS, emissions from indoor coal burning stoves, as well as parents' education on the level of biomarkers measured in children (Table III). Blood lead concentration was significantly affected by age, ETS, emission from coal stoves and parents' education. Increased PbB levels were found in children who were at least 9 years old, exposed to ETS and to indoor...
emission from coal operating stoves. Increased PbB concentrations were detected in children whose parents had a primary education compared to those with secondary or higher education. The influence of gender was not statistically significant although PbB level in boys tended to be higher than in girls (Table III). Indoor coal burning stoves and parents’ education affected the level of 1-OHP and urinary mutagenicity detected by both bacterial strains: TA98+S9 and YG1024+S9, resulting in increased concentration of these biomarkers in children exposed to emissions from coal stove and in children whose parents were low educated. The levels of DNA adducts and SCE were clearly and significantly associated with gender, with higher levels of these biomarkers in girls than in boys. Increased levels of DNA adducts were detected among children exposed to indoor emissions from coal stove although the difference was not statistically significant. ETS exposure was not associated with increased DNA levels. The highest level of DNA adducts was detected among girls exposed to indoor coal stove emissions (13.17 per 10^8 nt SD = 4.87, data not shown). The level of MN was significantly higher in boys than in girls and in older (≥9 years old) than in younger (<9 years) children. MN mean level was

![Fig. 3. Box and whiskers plot showing the number of MN cells/10^3 binucleated cells in children with PbB level ≤10 μg/dl and >10 μg/dl.](https://academic.oup.com/mutage/article-abstract/21/5/295/1135253)

**Table III. Influence of selected covariates on biomarkers of exposure and effects**

<table>
<thead>
<tr>
<th>Covariates (n)</th>
<th>Pb (μg/dl) Mean (SD)***</th>
<th>1-OHP (μmol/mol creat.) Mean (SD)***</th>
<th>TA98+S9 (rev./mmol creat.) Mean (SD)***</th>
<th>YG1024+S9 (rev./mmol creat.) Mean (SD)***</th>
<th>DNA adducts (per 10^8 nt) Mean (SD)***</th>
<th>MN (per 1000 cells) Mean (SD)***</th>
<th>SCE (per cell) Mean (SD)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
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<tr>
<td>Boys (47)</td>
<td>8.43 (4.85)</td>
<td>0.51 (0.29)</td>
<td>458 (237)</td>
<td>1286 (835)</td>
<td>8.54 (8.72)**</td>
<td>4.91 (3.44)</td>
<td>7.63 (0.83)**</td>
</tr>
<tr>
<td>Girls (27)</td>
<td>6.40 (2.67)</td>
<td>0.58 (0.35)</td>
<td>529 (600)</td>
<td>1369 (1030)</td>
<td>11.47 (6.17)</td>
<td>3.67 (4.16)</td>
<td>8.28 (0.78)</td>
</tr>
<tr>
<td>Age</td>
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<tr>
<td>&lt;9 (52)</td>
<td>6.43 (3.90)*</td>
<td>0.57 (0.34)</td>
<td>521 (453)</td>
<td>1262 (816)</td>
<td>9.40 (6.90)</td>
<td>3.46 (3.09)*</td>
<td>8.05 (0.92)</td>
</tr>
<tr>
<td>≥9 (20)</td>
<td>9.25 (4.20)</td>
<td>0.48 (0.20)</td>
<td>396 (317)</td>
<td>1410 (1166)</td>
<td>10.39 (9.50)</td>
<td>5.60 (4.22)</td>
<td>7.69 (0.70)</td>
</tr>
<tr>
<td>ETS*</td>
<td></td>
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<tr>
<td>None (26)</td>
<td>6.18 (3.19)*</td>
<td>0.51 (0.40)</td>
<td>552 (633)</td>
<td>1455 (1144)</td>
<td>8.27 (6.53)</td>
<td>4.64 (3.19)</td>
<td>7.95 (0.92)</td>
</tr>
<tr>
<td>Yes (40)</td>
<td>8.69 (4.80)</td>
<td>0.56 (0.25)</td>
<td>470 (238)</td>
<td>1292 (787)</td>
<td>9.40 (6.83)</td>
<td>4.36 (4.11)</td>
<td>7.78 (0.86)</td>
</tr>
<tr>
<td>Coal stove</td>
<td></td>
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<tr>
<td>No (46)</td>
<td>6.83 (3.95)*</td>
<td>0.43 (0.21)*</td>
<td>387 (241)*</td>
<td>1064 (750)*</td>
<td>8.15 (7.07)</td>
<td>4.02 (3.95)</td>
<td>7.77 (0.88)</td>
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<tr>
<td>Yes (24)</td>
<td>9.39 (4.70)</td>
<td>0.74 (0.36)</td>
<td>674 (589)</td>
<td>1804 (1002)</td>
<td>10.68 (6.30)</td>
<td>5.30 (3.31)</td>
<td>7.98 (0.87)</td>
</tr>
<tr>
<td>Parents’ education</td>
<td></td>
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<tr>
<td>Low (36)</td>
<td>8.89 (4.83)*</td>
<td>0.63 (0.35)*</td>
<td>582 (505)*</td>
<td>1529 (932)*</td>
<td>9.57 (6.03)</td>
<td>4.77 (3.70)</td>
<td>7.85 (0.97)</td>
</tr>
<tr>
<td>Median (29)</td>
<td>5.61 (2.82)</td>
<td>0.43 (0.20)</td>
<td>368 (266)</td>
<td>1045 (888)</td>
<td>8.18 (7.09)</td>
<td>4.33 (4.12)</td>
<td>7.85 (0.87)</td>
</tr>
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*None or one or both parents; **P < 0.050; ***Mann–Whitney U-test; **Student’s t-test (performed on log transformed data); #Student’s t-test (performed on original data).
increased in children exposed to indoor emissions from coal burning stoves although the difference was not statistically significant.

The relationship between the level of PbB, 1-OHP and DNA adducts on the number of MN and SCE in PBL was also tested using multiple linear regression analysis, taking into account all confounding factors (Table IV). As far as MN were considered the only clearly significant association was found for PbB level ($\beta = 0.335$, $P = 0.007$). Exposure to ETS was inversely associated with the number of MN ($\beta = -0.254$, $P = 0.046$). SCE were statistically affected by gender (higher in girls) ($\beta = 0.305$, $P = 0.016$). Despite the lack of statistical significance DNA adducts were found to be associated with SCE ($\beta = 0.234$, $P = 0.063$).

Discussion

Children are considered highly vulnerable to chemical toxicants because of potentially higher exposure caused by higher intake as a proportion of body mass and higher absorption compared with adults as well as inherent biological growth and developmental factors (8). It is therefore important to investigate whether exposure to common and ubiquitous environmental pollutants, such as Pb and PAHs may result in genotoxic effects in exposed children.

Initiatives aimed at the prevention of lead poisoning in Polish children began in the early 1980s. They were focused on children living close to industrial sources of lead. In 1993 the Institute of Occupational Medicine and Environmental Health (Sosnowiec, Poland), began a childhood lead poisoning prevention program oriented to children living in the urban center of Silesia, beyond the direct impact of industrial sources of lead (1). It was based on the determination of the most important biomarker of the exposure to lead i.e. lead in whole blood concentration. Kaszna-Kocot et al. (10) determined the level of PbB in 2–4-year-old children attending nursery school in three districts of the city. The geometric mean was 13.1, 13.2 and 11.9 $\mu$g/dl depending on the district, with the highest observed value of 30 $\mu$g/dl. Concentrations >20 $\mu$g/dl were found in 19.5, 11.8 and 15% of the children. In the period of 1993–1998, 11 877 Polish children aged 24–84 months living in six cities of Silesia were examined for PbB concentration. The geometric mean of PbB was 6.3 $\mu$g/dl (range 0.6–48 $\mu$g/dl), and >13% of them had PbB level $\geq$10 $\mu$g/dl. Poor housing, lack of recreational trips outside Silesia, and the time spent outdoors were associated with elevated PbB (1). Similar or even higher concentrations of PbB were detected in our research as well as in 222 children aged 11–15 years from Portoscuso, Sardinia, Italy (55) and in 131 schoolchildren 6–8 years old from Jakarta (56). The boys of Portoscuso had a mean blood lead level of 11.30 $\mu$g/dl and girls of 7.39 $\mu$g/dl. Mean PbB concentration of Jakarta children was 8.3 ± 2.8 and 6.9 ± 3.5 $\mu$g/dl, depending on the region and 26.7% of children had PbB level $\geq$10 $\mu$g/dl. Much lower PbB levels were found in 1994 among 1230 boys and 1211 girls, aged between 3 and 19 years (median age = 10 years) from the south of Sweden. The geometric mean was 2.7 $\mu$g/dl (range 1.2–12.2 $\mu$g/dl) in boys and 2.3 $\mu$g/dl (range 1.2–9.7 $\mu$g/dl) in girls (57). In our study geometric mean of PbB concentration was 6.7 $\mu$g/dl with the values ranging between 2.7 and 23 $\mu$g/dl. PbB level exceeded current threshold of concern of 10 $\mu$g/dl in 21.6% of Silesian children. PbB levels were higher in children aged ≥9 years, in children exposed to indoor emissions from coal-operated stoves and ETS, and whose parents were with low education. There was no significant effect of gender on the PbB level, however, PbB concentration tended to be higher in boys than in girls.

High concentrations of PAHs in ambient air are also substantial environmental problem in Silesia province. Biomarkers of PAH exposure that are available for use in molecular epidemiology studies include adducts with DNA urinary metabolites like urinary 1-OHP and urinary mutagenicity. The concentration of 1-OHP in children was measured in three Silesian regions in late 1990s. Median concentration of 1-OHP in 7–8-year-old children not exposed to ETS ($n = 126$) was 0.32 $\mu$mol/mol creatinine and children exposed to ETS and living in the town of Bytom, an old urbanized area with an out-dated industry ($n = 108$) was 0.49 $\mu$mol/mol creatinine (58). Median 1-OHP level in 30, 8-year-old children from the town of Sosnowiec, Silesia province, sampled in July on 6 consecutive days ranged from 0.28 to 0.59 $\mu$mol/mol creatinine (59) with the highest value reaching 3.62 $\mu$mol/mol creatinine. In our study median concentration of 1-OHP was 0.46 $\mu$mol/mol creatinine with highest values close to 2 $\mu$mol/mol creatinine and arithmetic mean was 0.54 ± 0.31 $\mu$mol/mol creatinine. Concentrations of 1-OHP in Silesian children are high as compared with two reports on urinary 1-OHP levels in children from Japan and The Netherlands. In the study involving 644 Dutch children the mean 1-OHP creatinine adjusted concentration was 0.34 $\mu$mol/mol creatinine (60). Japanese authors did not correct the values of 1-OHP for creatinine concentration. Mean levels of 1-OHP in urine samples collected in October from 10-year-old Japanese children attending elementary schools in three urban areas ranged between 0.46 and 0.73 mmol/l (61), whereas mean value found in Polish children was 2.72 mmol/l. The relatively high concentration of 1-OHP in Polish children reflects high environmental exposure to PAHs in Silesia province. The results of the study confirmed our previous findings that indoor pollution resulting from coal heating and/or cooking strongly affects the level of urinary 1-OHP (58).

Besides one paper (62), there are no published research papers on urinary mutagenicity in children. Moreover, different methods of preparation of urine extracts made inter-research comparisons very difficult. In order to make the comparison possible we recalculated our data from the number of revertants per mmol creatinine into mutagenic rate (MR/24 ml). The mutagenic rate (MR) is a ratio of a number of induced revertants to a number of revertants in the control sample. Median mutagenic rate of urinary mutagenicity in Silesian children determined with TA98+S9 is at the same level as in

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<tr>
<th>Table IV. Determinants of MN and SCE levels in children’s PBL (multiple linear regression analysis)</th>
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<tr>
<td>Dependent variable</td>
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<tr>
<td>MN/1000 cells PbB (µg/dl)</td>
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$\beta$, standardized regression coefficient; SE, standard error; adjusted for age, coal stove’s indoor emissions and parents’ education.
not occupationally exposed men from Zabrze (62) and Dąbrowa Górnicza (63): 1.46, 1.4 and 1.32, respectively, but higher than in non-smoking men: 1.2 (55) and 0.66 (63). We did not observe the differences in urinary mutagenicity (expressed as a number of revertants in 24 ml) detected by TA98+S9 and YG1024+S9 between population of children and non-smoking women from Silesia (environmental exposure) and Białystok (control group) (64). Median values for the strain TA98 were: 27 versus 24.1 versus 20.9 and for YG1024: 130 versus 101.6 versus 84.9, respectively. Urinary mutagenicity in no-smoking men living in two Silesian towns (35) was slightly lower than in Silesian children. Mutagenic rate (MR/24 ml) for TA98 was 0.9 versus 1.42 and for YG1024: 2.8 versus 3.06. The only research on urinary mutagenicity in children concerned boys and girls living in central Poland, close to the landfill. They had the same MR/24 ml of urine tested by TA98+S9 (1.4 versus 1.42) (65) as our children but significantly lower in case of the strain YG1024: 1.46 versus 3.07 (D. Mielżyńska, E. Siwińska, unpublished data). As in the case of PbB and urinary 1-OHP concentration, the level of urinary mutagenicity was influenced by coal stoves emissions as well as by parents’ education. The influence of the level of education may be connected with worse living conditions of low educated people who live in old buildings and use coal burning stoves placed in rooms and kitchens for heating and cooking purposes.

DNA adducts are commonly employed biomarkers in molecular epidemiology. The great majority of studies focused on DNA adducts is related to tobacco smoking and occupational exposures. There are very few studies investigating DNA adducts in children including newborns. DNA adducts in nasal respiratory epithelium were significantly increased in 86 children, 6–13-year-old, exposed to urban air pollutants in the Metropolitan Mexico City, compared with 12 unexposed children aged 11 years (66). A significant increase of PAH–DNA adducts was also detected in cord blood from newborns of unemployed mothers who lived in polluted areas of the city of Krakow, Poland (n = 17) compared with six newborns of mothers from low polluted areas (8.14 versus 1.7 adducts/10⁶ nt) (67,68). Aromatic DNA adduct level was studied in various subpopulations living in Silesia region (64). For example, a level of bulky DNA adducts equal to 5.0/10⁶ nt was established in leukocytes of people living in vicinity of coke oven plant (69). Our findings showed a 30% increased level of DNA adducts in children exposed to indoor emissions from coal stoves. Conversely, ETS was not associated with higher DNA adducts levels suggesting a major role of the exposure to coal stove emissions in adducts formation during the winter season. Although DNA adducts are considered to be capable of detecting children exposure to urban air pollutants and ETS (7), a recent paper (70) has shown that the chemical nature of the lung DNA adducts in smokers (measured by the nuclease P1-mediated 32P-post-labeling assay) that appear on TL chromatograms as diagonally radioactive zone (DRZ) is different from the typical adducts generated by PAH or aromatic amines. Indeed, DRZ adducts reflect exposure to aldehydes, butadiene, and cathecol, the levels of which are orders of magnitude higher in cigarette smoke than the carcinogenic PAHs. This raises the issue of the validity of the interpretation of the measured DRZ adducts as PAH-specific-smoking related DNA adducts. However, DNA adducts in circulating lymphocytes, as measured in this study and most of the epidemiologic researches, remain useful markers of exposure to environmental pollutants including those generated during the combustion process of organic material. Higher levels of DNA adducts were detected in girls than in boys, particularly in indoor emission exposed girls. We have no clear explanation of the role of gender although a higher susceptibility to PAHs contained in cigarette smoke has been reported for females (71–73).

Cytogenetic markers were used to assess exposure of children to airborne pollutants, soil and drinking water contaminants. There are few studies on DNA damage in children environmentally exposed to lead. Dalpra et al. (29) did not find the difference in SCE frequency between 3- and 14-year-old children living in a widely contaminated area and showing an increased absorption of lead (n = 19, SCE = 9.0) and 5–11-year-old referents (n = 12, SCE = 8.9). Bauchinger et al. (27) carried out a study in 30 children living in a town with a lead plant. Despite a significantly increased lead level in exposed children as compared with referent ones no increase of chromosomal aberrations could be detected. Yanex et al. (31) indicated increased SSB in children exposed to arsenic (n = 20, urinary arsenic = 136 μg/g creatinine) and lead (n = 20, PbB = 11.6 μg/dl) in the mining site as compared with control children (n = 35, urinary arsenic = 34 μg/g creatinine) and lead (n = 35, PbB = 8.3 μg/dl). The increased DNA damage detected in Mexican children exposed to metals could be explained by genotoxicity induced either by arsenic or by lead, with a more significant effect of the latter. Chromosome damage has been reported in adults occupation-ally exposed to lead (11,12,15,20,26,74). A more clear relation than in the case of environmental exposure to lead was found between the level of biomarkers of genetic damage and exposure to air pollutants with clearly increased MN frequency detected in exposed compared with unexposed children (7). The findings were confirmed by the analysis of MN frequency in buccal epithelium cells measured in 60 children aged 16–17 years from Katowice, the geographical area from which our study population comes from, 60 children from Częstochowa, and 62 children from Nowy Sącz, a rural area (75). The results of the study showed a relationship between the MN frequency and exposure to air pollutants, mainly PAHs, with mean MN level of 9.85; 4.2 and 2.9 in Katowice, Częstochowa, and Nowy Sącz, respectively. Moreover, in the late 80s three groups of 7–15-year-old Polish children from three areas with different level of air pollution were studied and SCE in PBL were measured (76). The highest mean value of SCE was observed in children from the area surrounding a metallurgical plant (10.7), compared with lower levels detected in children from central Krakow (7.4) and from a rural area (6.5).

The mean level of MN found in our study was 4.44 per 1000 binucleated cells, a mean value that is in line with that reported as a baseline value estimated by meta-analysis based on the results of 440 subjects (4.48) (77). However, although total MN level was similar to the expected baseline, it was significantly higher (6.4) in children with PbB level ≥10 μg/dl, the current CDC threshold of concern for Pb exposure (78). The contribution of lead exposure in MN induction is also suggested by the significant correlation between PbB concentration and MN level in PBL (Figure 1). The increased level of MN in boys may be also related to higher concentrations of PbB in boys than in girls. The observed increase of MN level with age is in accordance with the findings obtained from a pooled analysis showing a clear positive association of MN frequency with age (77). Although the association between
PbB and MN levels was revealed by multiple regression analysis, this finding needs to be confirmed by future studies given the non-chemical-specificity of this cytogenetic biomarker.

The mean number of SCE per cell detected in our children (7.87) was similar to that reported for children living in central Krakow (7.4) (67), and lower than in children exposed to ETS in the USA (10.07) (79). The positive significant correlation between DNA adducts and SCE suggests that increased level of SCE can be related to the exposure to environmental agents that are capable to react with DNA. The frequency of SCE was significantly associated with gender, with more SCE scored in girls than in boys. This is likely to be associated with a higher level of DNA adducts in girls. Although SCE in children have not been consistently reported to reflect specific environmental exposures (7), a high frequency of SCE was found in children living downwind of a chemical disposal site (80). A significantly increased level of SCE was detected in our study population among children who were probably exposed to ambient PAHs as we can conclude from the increased level of DNA adducts in these children. The association between DNA adducts and SCE was also detected in multiple regression analysis although it failed to reach statistical significance.

Our findings are suggestive of a potential role of exposure to environmental agents, namely Pb and PAHs, in the occurrence of cytogenetic effects as MN and SCE in peripheral lymphocytes of children. It is confirmed by the observed relationship between DNA adducts and SCE as well as between PbB and MN (all measured in blood). The study indicated that there are at least two main sources of PAHs and Pb contributing to environmental pollution: outdoor and indoor and that emissions from coal stoves are important contributors to the total exposure to PAHs and Pb in our region. Environmental tobacco smoke seems to be more important as the source of lead than of PAHs. However, because of the limited sample size of our study, the non-exposure specific nature of the cytogenetic markers and their high variability, and the fact that the measured biomarkers may reflect exposures that occurred shortly before blood sampling (e.g. PbB, 1-OHP and DNA adducts) or a persistent/cumulative genotoxic effect (e.g. MN or SCE), our findings need to be confirmed by other independently conducted research.

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