Environmental lead exposure increases micronuclei in children

Lucyna Kapka*, Adolf Baumgartner1,2, Ewa Siwińska, Lisbeth E. Knudsen3, Diana Anderson1 and Danuta Mielżyńska

Department of Genetic Toxicology, Institute of Occupational Medicine and Environmental Health, Sosnowiec, Poland, 1Department of Biomedical Sciences, University of Bradford, Bradford, UK, 2Obstetrics/Gynaecology and Reproductive Sciences, University of California, San Francisco, CA, 2: 94720, USA and 3Department of Occupational and Environmental Health, Institute of Public Health, University of Copenhagen, Panum, Denmark

The objective of this pilot study was to investigate the contribution of environmental exposures to lead in the development of cytogenetic damage detected as the frequency of micronuclei (MN) in children. The other aim was to apply the MN assay in combination with fluorescence in situ hybridization (FISH) using a pan-centromeric chromosome probe to elucidate the formation mechanism of induced MN. The examined population was composed of 9-year-old children (n = 92), living in the region where non-ferrous ores are extracted and processed. The non-exposed group consisted of 49 children of the same age from an unexposed recreational area. Exposure to lead was assessed by determination of lead concentrations in blood (PbB) by atomic absorption spectrophotometry, whereas the level of selenium (Se) in serum was detected by using graphite furnace atomic-absorption spectrometry. The frequency of MN was determined by the cytokinesis-block MN assay and fluorescence in situ hybridization performed using a specific pan-centromeric probe. Environmental exposure to lead resulted in significantly increased levels of PbB (5.29 ± 2.09 versus 3.45 ± 1.20 µg/dl in controls), although the average level was much below the value of the biological lead exposure limit = 10 µg/dl. A negative correlation between lead in blood and Se in serum concentrations (P = 0.006) was found for the pooled study population. The results showed a significant difference (P < 0.0001) in the level of MN between the exposed and control group (standard MN test: 2.96 ± 2.36 versus 1.16 ± 1.28; FISH technique: 3.57 ± 3.02 versus 1.43 ± 1.69, respectively). The frequencies of both centromere-positive (C+MN) and centromere-negative (C-MN) micronuclei were significantly increased in exposed children; however, the contribution of C+MN in the total number of MN in peripheral blood lymphocytes of exposed children was significantly higher than in the controls what may suggest a pro-aneugenic effect of the exposure to lead. The results of multiple regression analysis indicated that the exposure to lead was an important factor affecting the increase in MN frequency what was confirmed by significant correlation between the PbB and MN levels. In conclusion, our results suggest that the exposure to lead may be associated with an increased frequency of MN, especially of C+MN; however, the influence of other factors (e.g. vitamins and minerals in the diet) cannot be excluded.

Introduction

Environmental exposure to lead in childhood remains an obvious and important public health problem in Poland, especially in urban and industrial regions. Locations, where non-ferrous ores are extracted and processed, in particular the Silesia Province, represent specific areas of concern. Gradually decreasing levels of lead emissions and declining air lead concentrations are not followed by immediate elimination of potential health hazards related to lead exposure, such as, neurodevelopmental deficiencies, adverse effects on the haematopoietic system and genotoxic risk.

Determination of blood lead concentration is a widely accepted biological marker of exposure to lead. The currently accepted ‘safe’ level of blood lead is 10 µg/dl, although recent epidemiological studies suggest impairment of cognition at blood lead levels even below 10 µg/dl (1). Biological monitoring of environmental lead exposure, conducted by the Institute of Occupational Medicine and Environmental Health between 1993 and 1998, including >14 000 children, showed that 13–15% of the tested children had elevated PbB (>10 µg/dl). In the year 2000, testing of >1000 children revealed that the highest percentage of elevated PbB (~24%) was seen in children living in Katowice-Szopieniec, a city district where a non-ferrous metallurgical plant is located (HNM ‘Szopieniec’) (2).

Lead is known to be a toxin affecting both the nervous and haematopoietic systems. Its genotoxic potential has also been shown, although exact mechanisms are not explained (3). Lead and inorganic lead compounds are classified by International Agency for Research on Cancer in group 2B as possibly carcinogenic to humans. Organolead compounds are to be found within group 3, not classifiable as carcinogenic to humans (3), as only a single study on tetraethyl lead in mice was available for review (4).

With respect to the health hazards related to lead exposure in children, not only identification of clinically overt cases of lead poisoning (which currently occur very rarely) is important but also the enhanced understanding of the underlying mechanisms of lead toxicity. Of particular interest are those biomarkers, which may be indicative of the risk of diseases that can develop as delayed consequences of exposure to lead.

In the studies on the mechanisms of lead toxicity, a role of selenium (Se), one of the trace elements is also discussed. Selenoprotein glutathione peroxidase plays a crucial protective role in the defence against peroxidation of the cellular membranes and of lipids by reducing the levels of hydrogen peroxide and lipid hydroperoxides (5). Epidemiological and
The population studied consisted of 92 children living in Bukowno, a town in the south of Poland at the western part of the Małopolska Province. In total, 11,025 inhabitants of Bukowno are currently living in an area of 63.4 km². It is an industrial town with mining and metallurgy as the main industries. As early as in the 15th century, lead exploitation and smelting started in this area. Today, the main industrial plant is the mining and metallurgical plant 'Bolesław', which is one of the greatest producers of zinc and lead concentrates in Poland. The plant activities involve mining of zinc and lead ores, processing to concentrating and production of non-ferrous metals. In our study, the exposed group comprised 44 boys and 48 girls attending two primary schools in the vicinity of the plant.

To detect a mean level of MN in a general population (non-exposed environmentally to lead), we recruited 49 children (27 boys and 22 girls, control group) of the same age as the exposed group living in Ustron, a town in the Silesia Province, far from its industrial centre. It is in the piedmont of the Beskidy Mountains at 350–995 m above the sea level. The area of Ustron encompasses 58.9 km², with 15,514 inhabitants. The town is divided into two parts by the Wisła River. The main part of the town is situated on one side of the river (there is also the school where samples have been collected). On the other side, there is a health resort specializing in rheumatism, respiratory tract diseases and circulatory system diseases.

Children and their parents were informed of the study aims and the parents were asked to sign an informed consent form and to complete a self-administered questionnaire. The questionnaire provided information about lifestyle, e.g. exposure to environmental tobacco smoke (ETS) and diet. The study was approved by the local ethics committee which limited the amount of blood to be collected from each child to 10 ml.

**Blood sampling**

Blood samples were collected into sodium–heparin Vacutette tubes for setting up cell cultures and Vacutainers for determining the concentration of lead in the blood. Blood sampling was performed in the schools that children attend. Tubes with blood specimens were delivered to the laboratory within 2 h and processed immediately.

**Lead in blood (PbB) determination**

The levels of lead in whole blood were determined by electrothermal atomic absorption spectrophotometry according to Stoeppler and Brandt (33). 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 the refrigerator for better deproteinization. After centrifuging at 10,000 rpm for 15 min, the supernatant was transferred to the Perkin-Elmer polystyrene autosampler cups. Then, 20 µl of the solution was automatically injected into the pyro-coated graphite tube with a L’vov platform. Lead in the sample was vapoaurized at the optimized sequential dry-atomize transverse-heated graphite atomizer furnace programme developed in the laboratory. The atomic absorption signal of lead was measured in the absorbance-peak area mode using the Zeeman effect for background correction (Perkin-Elmer 4100ZL). The amount of lead in the blood samples was calculated by reference to matrix-matched calibration plots.

**Selenium (Se) in serum determination**

The level of Se was determined directly in serum using graphite furnace atomic absorption spectrophotometry (34,35). Blood, which was collected in glass tubes without additives, was centrifuged after clotting at 3,000 rpm for 15 min and the serum was transferred into 2 ml polyethylene tubes and frozen until analysis. Before analysis, the serum samples were diluted 1 : 1 with 0.2% Triton-X100 directly in autosampler cups. The solution of 10 µl was automatically injected into graphite tube together with 10 µl of the matrix modifier solution. Copper, magnesium and silver nitrate in 0.4% nitric acid were used as mixed-matrix modifiers. Se in the sample was vapourized at the optimized sequential dry-atomize furnace programme with oxygenashing during the pyrolysis step. The atomic absorption signal of Se was measured in the absorbance-height mode and the deuterium background correction system was used to correct for the background signal (Unicam 939 atomic absorption spectrometer fitted with a GF90 graphite furnace). The calibration was performed with a matrix-matched calibration curve.

**Cell cultures**

Cultures of peripheral blood lymphocytes (PBL) were set up by adding 0.5 ml of heparinized blood to 4.5 ml of chromosome medium (RPML 1640) with 1-glutamine, Gibco) supplemented with 20% heat-inactivated foetal bovine serum (Gibco) and antibiotics (penicillin and streptomycin). Lymphocytes were stimulated with 1% phytohaemagglutinin (Gibco) in the medium. The cultures were incubated at 37°C for 72 h.

**Cytokinesis-block MN assay**

Forty-four hours after the initiation of cultures, cytochalasin B (Sigma) was added at the concentration of 6 µg/ml to arrest cytokinesis for the rest of the
incubation time. At the end of incubation (72 h after culture start), the cultures were centrifuged, resuspended in 75 mM KCl, centrifuged again and treated with fixative (Carnoy’s solution, three parts of methanol and one part of glacial acetic acid) with the addition of three drops of formaldehyde (38%). The fixation was repeated twice (without formaldehyde). Then, a pellet suspension was dropped on clean slides and allowed to dry (36). For each subject, eight slides were prepared. Four slides for the determination of the MN frequency were stained with 10% Giemsa in phosphate buffer (pH 6.8). Slides for centromere analysis by FISH were stored at −20°C and subsequently processed as described below.

Fluorescence in situ hybridization

FISH was performed using a human pan-centromeric probe labeled with fluorescein isothiocyanate (Cambio, UK) labelled with fluorescein isothiocyanate. After thawing, the slides were dehydrated at room temperature in an ethanol series (70, 90, 100%; 2 min each) and air dried. DNA denaturation was performed in 70% formamide (Sigma) in 2 × saline–sodium citrate buffer (SSC) at 76°C for 3 min and immediately transferred into cold (−20°C) 70% ethanol, further dehydrated in 90 and 100% ethanol and then air dried. The hybridization mixture containing the probe (3 μl) and 25-μl hybridization buffer was denatured at 80°C for 10 min according to the protocol of Cambio (Protocol G1 for Pan-Centromeric Chromosome Paint, Human and Mouse). An aliquot of 27 μl per slide was applied to the slide, which were then covered with cover slips and sealed with rubber cement. Hybridization was performed for 48 h at 37°C in a moist box. After the incubation, the slides were put into 2 × SSC at 37°C for 5 min (to let cover slips fall off) and washed twice in 50% formamide for 5 min at 45°C. After the last wash (in PN buffer for 10 min at room temperature), the slides were stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI, Sigma) and finally cover slipped with Vectashield, a commercial anti-fading solution (Vector Laboratories, Inc.) and stored in a light protected place at −20°C.

Slide scoring

For the scoring of MN, stained slides were coded and scored by light microscopy at ×400 magnification. The scoring of bi-, tri- and tetra-nucleate cells and MN was carried out according to Fenech and Morley (15). For each individual, the MN frequency and the presence of nucleoplasmic bridges were assessed in 1000 BN lymphocytes. In addition, 500 lymphocytes were scored to determine the percentage of cells with one to four nuclei. Then, the cytokinesis-block proliferation index (CBPI) was calculated (37).

For FISH analysis, the slides were scored with a Leica DMLB epifluorescence microscope. The MN present in the BN lymphocytes with intact centromere were examined for the presence and the number of centromeric signals were classified as centromere-positive (C+MN) or centromere-negative (C−MN). A total of 1000 BN cells were analysed for each subject. For scoring, all slides were coded to exclude any bias (double blind study).

Statistical analysis

Questionnaire and analytical data were stored in a database and statistically analysed using STATISTICA for Windows, Version 9.9, 1997. Normal distribution was tested according to Shapiro–Wilks test. The level of Se in serum was normally distributed. The distribution of lead in blood and MN as well as in standard test as in FISH method were skewed to the left. Therefore, they were transformed as log(x + 1) to make their distribution normal (PbB) or stabilize the variance (MN, C+MN, C−MN). The differences between the groups were analysed using the Student’s t-test when the variance was equal or Levene’s t-test for unequal variance. Test results were considered statistically significant for the P-value <0.05. All data are presented as mean ± standard deviation.

Student’s t-test was also used to compare subgroups dichotomized according to individual covariates including gender (boys and girls), exposure to ETS (yes = 1, no = 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). Statistical comparison of C+MN/C−MN ratio among the various subgroups was made using chi-square test.

Table I. General characteristics of exposed and control children

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Age (years)</th>
<th>Gender</th>
<th>ETS (at least one of the parents was a smoker)</th>
<th>Parents’ education level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Boys</td>
<td>Exposed</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Girls</td>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Total</td>
<td>141</td>
<td>9</td>
<td>71 (50.4%)</td>
<td>63 (45.5%)</td>
<td>86 (61.0%)</td>
</tr>
<tr>
<td>Control</td>
<td>49</td>
<td>9</td>
<td>27 (55.1%)</td>
<td>22 (44.9%)</td>
<td>26 (53.1%)</td>
</tr>
<tr>
<td>Exposed</td>
<td>92</td>
<td>9</td>
<td>44 (47.8%)</td>
<td>48 (52.2%)</td>
<td>60 (65.2%)</td>
</tr>
</tbody>
</table>
Exposed children (1.64 versus 0.53, \( P = 0.0001 \) and 1.93 versus 0.90, \( P = 0.001 \), respectively). Significantly increased levels of C+MN in the exposed group were observed for all examined subgroups, while the differences between exposed and control children in C−MN were not significant for girls and children not exposed to ETS. No statistically significant differences in the level of C+MN were found both within the exposed and the control group. Referring to the frequency of C−MN, significant differences in the exposed group of children were connected with gender and parents’ education status (Table III).

In the control group, the frequency of C+MN was much lower than the frequency of C−MN (31 versus 69%). The contribution of C+MN in the total MN was increased in the exposed group (C+MN = 52%; C−MN = 48%, \( P = 0.003 \)). Significant differences in the ratio of C+MN to C−MN were also observed between girls and boys and children exposed and not exposed to ETS in the control group. The type of chromosomal damage did not depend on ETS exposure of children which were environmentally exposed to lead (Table III). The difference between C+MN and C−MN was significant only for the subgroup of children with different level of education of their parents (\( P = 0.016 \)).

The relationship between the concentration of lead in blood and the frequency of MN in PBL was assessed by Pearson’s correlation. The results of analysis are presented in Table IV. All studied relations were statistically significant. We also examined a degree of the accordance between the results of two methods of MN determination by Pearson’s correlation and we found it significant (\( P < 0.001 \)).

The relationship between MN frequency (standard and FISH technique; C+MN, C−MN) and the factors, such as exposure to lead, gender and parents’ education level, was assessed with multiple regression analysis. We have found a statistically significant relationship between the frequency of MN and PbB level for MN determined by standard test (\( \beta = 0.262, P = 0.002 \)), for MN determined by FISH method (\( \beta = 0.248, P = 0.004 \)), for C+MN (\( \beta = 0.238, P = 0.005 \)) and for C−MN (\( \beta = 0.167, P = 0.046 \)). The effect of other factors on MN level were statistically insignificant at \( P < 0.05 \).

**Discussion**

Children as developing organisms may be particularly susceptible to environmental pollution and toxins. They are more heavily exposed per unit of body weight to environmental toxins than adults. They drink more water, eat more food and breathe more air than adults in relation to their body weight. Some detoxifying enzymes are also less developed in children (39,40). That is why children are a population of great concern.

Silesia province is the most industrialized region in Poland where lead mining and processing operate almost exclusively. In young children, elevated levels of PbB can have adverse effects on their health and intellectual development. Because of these effects, lead screening programmes were introduced aimed at detecting children with increased PbB and protecting them from further exposure. In Poland, children were exposed to lead mainly from combustion of leaded gasoline and industrial processes. Leaded fuel used to be a major source of exposure to lead in the early 1990s up to 2005 (41). Concentrations above 20 µg/dl were found in 19.5, 11.8 and 15% of 2–4 years old children attending three nursery schools.
Exposed PbB and Se/C0
Control group for biomonitoring chromosome damage in human secondary smelter workers (46).

European countries (44,45). An interaction between occupational levels of Se in blood were much lower than reported from other studies of urban children from Silesia Province. Observed insufficiency in lead-exposed children was also shown in a study of Silesian children (41). Parental education (or socioeconomic status which is usually connected with the level of education) was a risk factor for elevated PbB in these studies.

Our research revealed increased levels of lead in blood concentration in the exposed group compared to the controls (5.29 versus 3.45 µg/dl). Children with low educated parents had significantly increased level of PbB compared with children whose parents were highly educated (6.10 versus 4.99 µg/dl) only in the exposed group.

Significantly decreased levels of Se in exposed children compared to the controls and a negative correlation between lead in blood and Se in serum was found in our study. Se insufficiency in lead-exposed children was also shown in a study of urban children from Silesia Province. Observed levels of Se in blood were much lower than reported from other European countries (44,45). An interaction between occupational lead exposure and Se status was also found in Swedish secondary smelter workers (46).

MN expression in PBL is well established as a standard method for biomonitoring chromosome damage in human populations. MN tests are gaining increasing attention among laboratories active in the field of environmental mutagenesis and the number of published studies based on this biomarker has increased recently (47).

Neri et al. (48) recently reviewed published studies, which referred to MN in children. A meta analysis of data from 13 studies revealed a clear age-dependent increase in MN while no effect of gender was seen after the reanalysis of 448 children selected in the HUman MicroNucleus data set (49). The same results referring to gender were obtained in our study. There is a lack of studies involving analyses of MN frequencies in PBL of children environmentally exposed to lead except for a research carried out in 11 children of 9 years of age from Silesia region (50).

The influence of air pollution on children resulting mainly from traffic was examined in an Italian study. Children showed a lower MN frequency than adults, regardless of sex, with the mean value of 2.20 (51). In a family pilot study conducted in the Czech Republic, significantly higher frequencies of MN were found in children living in Teplice mining region as compared with those living in the rural area of Prachatice (7.0 versus 4.9) (52).

Our results showed significantly higher MN frequency observed in standard Giemsa staining in PBL of children environmentally exposed to lead: 3.14 ± 2.66 (boys) and 2.79 ± 2.06 (girls) compared to the control children: 1.19 ± 1.49 (boys) and 1.14 ± 0.99 (girls).

In the study of Baier et al. (53) in 2- to 15-year old ETS-exposed children showed significantly higher MN frequencies (mean: 8.0/1000 BN cells; P = 0.001) than non-exposed children (mean: 6.2/1000 BN cells). We found the effect of passive smoking on the level of MN neither in the exposed (P = 0.943) nor in the control (P = 0.970) group of children (Table III).

### Table III. The frequency of MN determined by the FISH technique [with (C+MN) or without (C–MN) centromere signals] and standard MN test in 1000 BN lymphocytes of the exposed group and the controls by gender, ETS exposure and parents’ education status

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Number of MN/1000 BN (mean ± S.D.)</th>
<th>Standard test</th>
<th>FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Total MN</td>
<td>C+MN</td>
<td>C–MN</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>1.16 ± 1.28</td>
<td>1.43 ± 1.69</td>
<td>0.53 ± 1.02</td>
</tr>
<tr>
<td>Boys</td>
<td>27</td>
<td>1.19 ± 1.49</td>
<td>1.33 ± 1.61</td>
<td>0.33 ± 0.68</td>
</tr>
<tr>
<td>Girls</td>
<td>22</td>
<td>1.14 ± 0.99</td>
<td>1.54 ± 1.81</td>
<td>0.77 ± 0.97</td>
</tr>
<tr>
<td>Exposed to ETS</td>
<td>26</td>
<td>1.23 ± 1.48</td>
<td>1.46 ± 1.77</td>
<td>0.35 ± 0.80</td>
</tr>
<tr>
<td>High parents’ education</td>
<td>23</td>
<td>1.09 ± 1.04</td>
<td>1.39 ± 1.64</td>
<td>0.74 ± 1.21</td>
</tr>
<tr>
<td>Low parents’ education</td>
<td>28</td>
<td>1.07 ± 1.05</td>
<td>1.14 ± 1.56</td>
<td>0.36 ± 0.87</td>
</tr>
<tr>
<td>Exposed</td>
<td>21</td>
<td>1.28 ± 1.55</td>
<td>1.81 ± 1.83</td>
<td>0.76 ± 1.18</td>
</tr>
</tbody>
</table>

*aStatistically significant difference (P < 0.05) versus control girls; χ2 test.

*bStatistically significant difference (P < 0.0001) versus control exposed to ETS; χ2 test.

*cStatistically significant difference (P < 0.001) versus total control; Student’s t-test.

*dStatistically significant difference (P < 0.05) versus control; Student’s t-test.

*eStatistically significant difference (P < 0.05) versus exposed girls; Student’s t-test.

*fStatistically significant difference (P < 0.05) versus exposed children whose parents had low education level; Student’s t-test.

**Statistically significant difference (P < 0.05) versus exposed children whose parents had low education level; χ2 test.

### Table IV. The relationships between PbB and (i) Se in serum and (ii) the number of MN in PBL by Pearson’s simple linear correlation

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>r²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>PbB and Se</td>
<td>–0.230</td>
<td>0.053</td>
<td>0.006</td>
</tr>
<tr>
<td>MN total (standard method)</td>
<td>0.250</td>
<td>0.062</td>
<td>0.003</td>
</tr>
<tr>
<td>MN total (FISH method)</td>
<td>0.255</td>
<td>0.065</td>
<td>0.002</td>
</tr>
<tr>
<td>C+MN</td>
<td>0.223</td>
<td>0.050</td>
<td>0.008</td>
</tr>
<tr>
<td>C–MN</td>
<td>0.190</td>
<td>0.036</td>
<td>0.023</td>
</tr>
</tbody>
</table>

r, correlation coefficient; r², coefficient of determination and P, level of statistical significance.

In 1993 (42). Much later, the currently used threshold of concern of 10 µg/dl was detected in 21.6% (43) and 13% of Silesian children (41). Parental education (or sociodemographic status which is usually connected with the level of education) was a risk factor for elevated PbB in these studies.

Our research revealed increased levels of lead in blood concentration in the exposed group compared to the controls (5.29 versus 3.45 µg/dl). Children with low educated parents had significantly increased level of PbB compared with children whose parents were highly educated (6.10 versus 4.99 µg/dl) only in the exposed group.

Significantly decreased levels of Se in exposed children compared to the controls and a negative correlation between lead in blood and Se in serum was found in our study. Se insufficiency in lead-exposed children was also shown in a study of urban children from Silesia Province. Observed levels of Se in blood were much lower than reported from other European countries (44,45). An interaction between occupational lead exposure and Se status was also found in Swedish secondary smelter workers (46).

MN expression in PBL is well established as a standard method for biomonitoring chromosome damage in human populations.
The use of the cytokinesis-block MN assay in combination with FISH with centromeric probes allows for distinguishing MN induced by chromosome breakage and those formed by malsegregation of whole chromosomes. A centromeric signal observed in the MN suggests that it contains a whole chromosome and has been generated by mitotic spindle disturbance. The absence of fluorescent signal in a MN indicates that it originated from chromosome breakage, i.e., as a result of clastogenic effects. This method has been mainly applied in the assessment of occupational exposure to pesticides, nitrogen oxide and radiation (27–29,31,54). Limited research on the assessment of occupational exposure to pesticides, nitrogen oxide and radiation (27–29,31,54). Limited research on the assessment of occupational exposure to pesticides, nitrogen oxide and radiation (27–29,31,54).

In our study, the total number of MN observed with FISH technique was statistically increased in exposed children, similarly to the results obtained in the standard MN test. Also the frequency of C+MN and C−MN was significantly higher in the exposed group compared to the controls. Significantly increased levels of C+MN in the exposed group were observed for all examined subgroups (Table III).

Our results of multiple regression analysis indicated that the exposure to lead was the factor affecting the increase in MN frequency. It was confirmed by significant relationship between the level of PbB and the total number of MN (both standard and FISH) as well as C+MN and C−MN (Table IV). Our results suggest that exposure to lead is the most important factor affecting the increase in MN frequency. However, the influence of other factors as for example diet cannot be excluded. Unfortunately, the available biological material was insufficient for the determination of concentration of some vitamins like Vitamin B12 or plasma folate.

The overall frequency of C+MN in the exposed group was significantly increased compared to the controls (52 versus 31%, respectively). The contribution of C+MN in PBL in the total number of MN in exposed children is slightly higher than of C−MN (52 versus 48%), opposite to the findings from the study performed in children and their mothers (13.7 versus 8.6%—results for children) (50). The type of chromosomal damage did not depend on ETS of children which were environmentally exposed to lead (Table III). Significant increase of C+MN in exposed children may suggest pro-anneugenic effect of exposure to lead.

In conclusion, the results indicate that environmental exposure to lead may be associated with an increased frequency of MN. Neither in the exposed children nor in the control group was an influence of gender and ETS exposure at the level of MN. MN–FISH showed that both C+MN and C−MN were responsible for increased levels of MN in the exposed group. However, the contribution of C+MN in the total number of MN in PBL of exposed children was significantly higher than in the controls what may suggest a pro-anneugenic effect of exposure to lead.

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References

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