

# Effects of Air Pollution on Red Blood Cells in Children

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## Abstract

The aim of our study was to evaluate any effects in red blood cells in children exposed to air pollution. The subjects were 354 pupils, aged 11-14 years, living for more than ten years in the same home. The exposed group of children (n=215) were attending school in a city area with a high level of air pollution, while the children in the comparison group (n=139), designated the non-exposed group, were attending school in an area with a lower level of air pollution. The mean value of hemoglobin (g/mL) for exposed children was  $10.97 \pm 0.38$  and for non-exposed children  $11.09 \pm 0.78$ . The diagnosis of iron deficiency anemia was made using the pre-defined criteria. The air concentrations of black smoke, nitrogen dioxide, sulfur dioxide and lead in sediment matter were determined from 1990 to 2000. The red blood cell count and average of hemoglobin blood levels of exposed children differ significantly from those of the non-exposed ( $P < 0.001$ ). There was also a significant difference in the prevalence of anemia in children exposed to higher concentrations of air pollutants (RR = 3.76; 95% CI: 2.06-6.88). These findings suggest that air pollution could have negative effects on red blood cells in children.

**Keywords:** air pollution, children, iron deficiency anemia, red blood cells

## Introduction

Although air pollution has long been thought to exacerbate minor acute illnesses in children, there is increasingly strong evidence that air pollution is associated with nontrivial increases in infant mortality and chronic disease in children [1-3]. Overall, evidence for effects of air pollution on children has been growing, and effects are seen at concentrations that are common today. Although many of these associations seem likely to be causal, additional investigation is required.

Air pollution is the source of many substances that may enter the human bloodstream through the nose, mouth, skin, and the digestive tract. Most air pollutants reach the blood quickly without previous bio-transfor-

mation and have been shown to produce harmful effects on the blood, bone marrow, spleen, and lymph nodes. Some studies were undertaken to determine consequences on blood [4, 5]. Blood cells are constantly undergoing turnover, making the blood system highly sensitive to environmental poisoning. For example, lead interferes with normal red blood cell formation by inhibiting important enzymes. In addition, lead damages red blood cell membranes and interferes with cell metabolism, thus shortening the survival of each individual cell [6-8]. Some studies has suggested that red blood cell changes may occur in the winter months, when air pollution is higher [9]. Each of these harmful effects can result in clinical anemia.

The aim of the study was to evaluate any difference in the prevalence of anemia and any possible effects in red blood cells in two groups of children exposed to different levels of air pollution.

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## Subjects and Methods

### Subjects

The study comprised children aged 11 to 14 years (n=354), non-smokers, who attended two different schools. The exposed group of children (n=215), were attending school in a city area with a high level of air pollution, while the children in the comparison group (n=139), designated the non-exposed group, were attending school in an area with a lower level of air pollution. All children lived in the neighbourhood of their schools, so that exposure to local air pollution levels was homogenous, and not limited to children's presence in schools.

Exclusion criteria were any acute or chronic illnesses, to avoid bias in red blood cell results, and residence within the studied area for less than 10 years prior to the study, to guarantee a significant and homogenous exposure to the local air pollution area.

Parents were informed about the aims of the study, the performance and the expected results of the study took place in the two schools. The parents agreed with involvement of the children in the study and they were requested to fill in a questionnaire. Collected data included demographic characteristics, parents' smoking habits, parental education level, density of habitation and wood or coal heating.

### Laboratory Data

Venous blood was analyzed for hemoglobin concentration and total red blood cell counts in the laboratory of the Primary Health Care Center, Niš (Serbia). The presence of anemia was diagnosed according to the following criteria: hemoglobin < 120 g/l and erythrocyte count <  $4.3 \times 10^{12}/l$ .

### Ambient Air Monitoring

Outdoor air pollutants were monitored during the 10-year period. The concentrations of black smoke, nitrogen dioxide (NO<sub>2</sub>), sulfur dioxide (SO<sub>2</sub>) and lead (Pb) in sediment matter were determined in 24-hour samples of air collected from January 1990 to December 2000. Sampling equipment was placed at 1.5 m above floor level at two sampling sites near the school buildings. The sampling sites were selected to ensure diversity regarding the outdoor environment.

The ambient level of black smoke concentrations was measured by reflectance. The sampling was performed by means of a pump operating with flow rate of 1 L/min through Whatman N°1 paper filters. At the same time, the air concentration of sulfur dioxide was measured [10]. A measured volume of air was bubbled through a solution of potassium tetrachloromercurate. The sulfur dioxide present in the air stream reacted with the solution to form a stable monochlorosulfonatomercurate complex. During the sub-

sequent analysis, this complex was brought into reaction with acid-bleached pararosaniline dye and formaldehyde, yielding intensely colored pararosaniline methyl sulfonic acid. The optical density of this species was determined spectrophotometrically at 548 nm and was directly related to the amount of sulfur dioxide collected. The total volume of the air sample was determined from the flow rate and the sampling time. The concentration of sulfur dioxide in the ambient air was calculated and expressed in  $\mu\text{g}/\text{m}^3$ . The lowest limit of detection was  $1.7 \mu\text{g}/\text{m}^3$ .

Ambient nitrogen dioxide was collected with a pump containing triethanolamine in its tube with the exact amount of the reacted nitrogen dioxide was determined using the standard spectrophotometry [11]. The minimum detectable limit of the method had been determined to be  $2.0 \mu\text{g}/\text{m}^3$ .

Lead in sediment matter was collected with absorbed solution of sulfur acid and was detected by graphic furnace atomic absorption spectrometry (12). The lowest limit of detection was  $0.5 \mu\text{g}/\text{m}^3$ .

### Statistical Analysis

A statistical package SPSS was used for data analysis. The variables of hematological parameters were analyzed with T-test. Air pollution data were analyzed using the Mann-Whitney U test. Statistically significant differences in anemia incidence in children exposed to different and substantial concentrations of air pollutants were analyzed using a Pearson's chi-squares test.

A *P* value <0.05 was required for significance. Statistical analyses were performed using SAS version 8.2 software (SAS Institute, Inc., Cary, North Carolina).

## Results

Baseline characteristics of study population are reported in Table 1. There were no statistically significant differences in age between the two groups.

Table 1. Distribution of children by gender and age\*.

Characteristics of children	Total n=354	Exposed n=215	Non-exposed n=139
Male/Female <sup>b</sup> , n	174/180	101/114	73/66
Age, yr <sup>a</sup> (mean ±SD)	12.96±1.54	12.78±1.56	12.95±1.52
11 yr, n	109	60	49
12 yr, n	107	54	53
13 yr, n	79	55	24
14 yr, n	59	46	13

<sup>a</sup> t-test; <sup>b</sup> chi-square test; \*no statistically significant differences between the two groups

Table 2. Characteristics of examined schoolchildren.

Home environment	Exposed (n=215)	Non-exposed (n=139)	Significance
Parental education level <sup>b</sup>			
Elementary	15%	31%	n.s.
Above elementary	85%	69%	
Parental smoking habit <sup>b</sup>			
YES	43%	45%	n.s.
Density of habitation <sup>a</sup> (person/room) mean ± SD	0.82 ± 0.32	0.88 ± 0.29	n.s.
Wood or coal heating <sup>b</sup>	20.0%	15.8%	n.s.

<sup>a</sup> t-test; <sup>b</sup> chi-square test; n.s.-no statistically significant

Also, there were no statistically significant differences in parents' smoking habits, parental education level, density of habitation, wood or coal heating between the two groups (Table 2).

The results of air pollution measurements are summarized in Fig. 1. All concentrations of the air pollutants measured during the period 1990-2000 at the location in School 1 were higher when compared to the concentrations of the same pollutants measured at the location in School 2. This difference is statistically significant (Mann-Whitney U test: P<0.05).

The red blood cell count of exposed children (4.16±0.32) differ significantly from those of the non-exposed group – 4.48±0.29 (t=9.53; P<0.001). Also, there were statistically significant differences in red blood cell counts between girls and boys of the exposed and non-exposed groups (Fig. 2).

In both sexes, the mean value of hemoglobin blood levels was significantly higher in the non-exposed group than in exposed subjects (Fig. 3).

Out of a total number of children exposed to higher concentrations of air pollutants in school1, 29.8% were anemic. In school 2, the percentage of anemia was lower (7.9%).Chi-square test ( $\chi^2= 22.85, P<0.01$ ) confirmed that there was a significant difference in the frequency of anemia in children exposed to higher concentrations of air pollution, when compared to those who were exposed to

lower concentrations of air pollution. The values of relative risk were more than 1 (RR =3.75; 95%CI:2.06-6.88).

### Discussion of Results

It is well known that air pollution has many effects on the health of both adults and children. However, few stud-

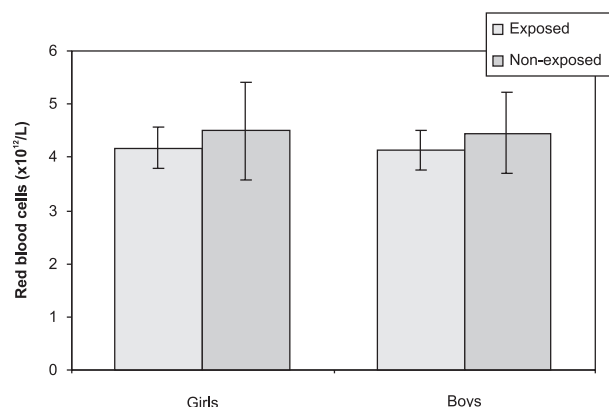


Fig. 2. Red blood cell counts in exposed and non-exposed groups.

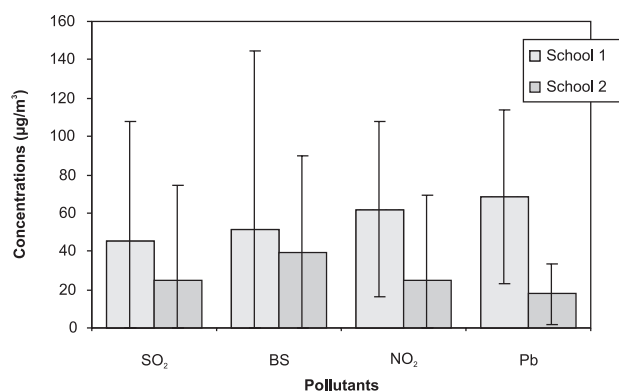


Fig. 1. Levels of air pollution studied during the period 1990-2000.

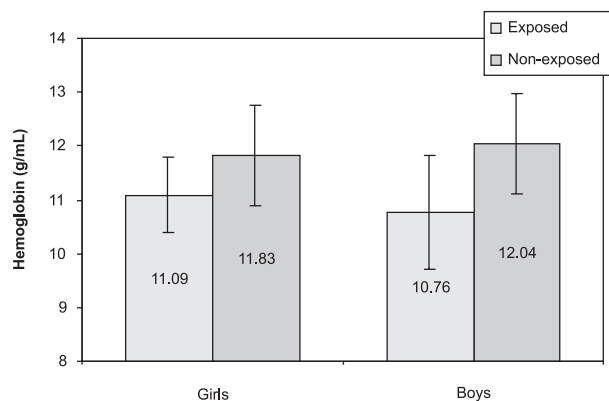


Fig. 3. Hemoglobin levels in exposed and non-exposed groups (P<0.001).

ies have investigated exposure to this environmental risk factor in association with children's anemia [13-15].

This investigation has shown that long-term exposure to air pollution positively corresponds to the occurrence of anemia among children. Analysis of our data revealed a significant decrease in red blood cell numbers and hemoglobin concentrations in association with higher air pollution exposure.

The decrease in hematocrite and hemoglobin in association with NO<sub>2</sub> exposure has been observed previously [16]. Small, but significant decreases in hemoglobin and hematocrite in adults were observed immediately after 2.5-h exposures to 1 or 2 ppm NO<sub>2</sub>. In addition, mice exposed to 5 ppm NO<sub>2</sub> after 1 h demonstrated reductions in hemoglobin and erythrocyte counts along with increases in bilirubin and methemoglobin concentrations, suggesting a mild hemolytic anemia [17]. It is likely that NO<sub>2</sub> exposure, even at low concentrations, leads to small reductions in circulating red blood cells. The duration of this effect is unknown. Mechanisms may involve red blood cell membrane changes, methemoglobin formation, or cellular redistribution within circulation [18]. Future studies of NO<sub>2</sub> exposure should consider assessment of red blood cell membranes, reticulocyte counts, and methemoglobin levels. Children might be more susceptible to nitrogen dioxide exposure effects on red blood cells.

Also, highly toxic lead can damage the erythrocyte membranes, resulting in anemia. Lead interferes with many biochemical systems, particularly the heme biosynthetic pathway. Sensitivity to lead is higher in children than adults [7]. Lead ions inhibit enzymes that catalyze the reactions for biosynthesis of hemoglobin thus lead poisoning that causes anemia. According to the data of another study [6], it is proposed that 25 µg Pb/100 ml blood be regarded as the maximum biologically allowable concentration of lead in blood of school-age children. But, in a nested case-control study among Ukrainian children no increased risks of overall morbidity or anemia were observed [19].

Although concentrations of sulfur dioxide and black smoke have been significantly decreasing worldwide in the last ten years [20], the present air concentrations in the city of Niš are still an important threat to children's health.

It is difficult to determine whether one of the measured pollutants, alone or in combination, was responsible for the observed health effects in children. It is also less clear which pollutants are most responsible for anemia of children. Little is known about the possible adverse effects of exposure to complex mixtures of chemicals. In an analytical cross-sectional study, neonates born to mothers exposed to carbon monoxide air pollution had increased circulating absolute nucleated red blood cell counts compared with those of the control group [21]. It was speculated that exposure to increased levels of ambient carbon monoxide during pregnancy may contribute to the occurrence of hypo-oxygenation. Differences in the results may be due to the combination effects of air pollution and also the level of these parameters.

Further study is needed to disentangle this interaction and the underlying mechanisms. Moreover, the balance of evidence maintains that outdoor air pollution has a modest effect on the occurrence of anemia in schoolchildren. Exposure to air pollution may increase the risk of anemia in children through several mechanisms. The biological mechanisms by which air pollutants may interfere with the process of red blood cell production are reflected in the synthesis of hem, the forming of red blood cells, and its life-expectancy. Toxic materials from the air lead to significant damage of red blood cells such as reduced hemoglobin concentrations, the number of erythrocytes and hematocrite, thus leading to anemia.

The results of our study are similar to the previously found effects of air pollution on red blood cells in children [22]. Naturally, other factors (genetic disposition, nutrition habits, etc.) contributed to the detected low concentrations of hemoglobin and the prevalence of anemia.

Air pollutants present in the environment largely damage cell immunity and change the intensity and course of iron metabolism in the body, resulting in iron-deficient anemia with very low values of hematocrite and hemoglobin.

Although there was a difference between design of the present study and that of Wichmann and Heinrich [23], both studies established positive association of children's anemia with outdoor air pollution. The strength of the association was comparable.

A recent study determined the relationship between exposure to particulate matter, measured as PM<sub>10</sub>, and changes in hemoglobin concentration, hematocrite (packed cell volume) and red blood cell count [24]. Also, the authors measured plasma albumin and concluded that the decrease in haemoglobin was caused by increased peripheral sequestration of red blood cells, rather than generalized haemodilution [25]. The study pointed to the particulate air pollution, or a very closely associated confounding factor, had the potential to affect cardiovascular events.

As we mentioned earlier, children's exposure to air pollution is a special concern because their immune system and lungs are not fully developed. When exposure begins, different responses are evident in children than those seen in adults [25-27].

## Conclusion

In conclusion, we found that children exposed to higher levels of air pollution had an elevated prevalence of anemia. The study provides support for the hypothesis that even exposure to low levels of environmental pollutants is hazardous to the children's health.

The occurrence of anemia in children may be initiated by numerous integrated risk factors. The air pollution is definitely one of the reasons for this state.

The associations between red blood cell reduction and chronic high levels of air pollution is appealing, but further confirmation studies are necessary.

This is the first study to describe air pollution levels and anemia-associated variables among Serbian children in the peer-reviewed literature. The results obtained are a baseline for further analytic epidemiological research.

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