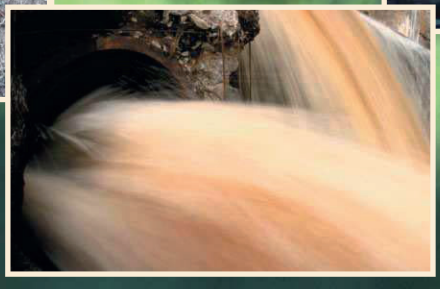
Environmental Health in Central and Eastern Europe

Edited by
K.C. Donnelly and Leslie H. Cizmas



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K.C. Donnelly

Texas A&M University System Health Science Center
College Station, Texas, USA

and

Leslie H. Cizmas

Texas A&M University System Health Science Center
College Station, Texas, USA

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Contributing Authors

Tomas Adamus, *Czech Republic*
Anahit Aleksandryan, *Republic of Armenia*
Gulbaram Arzygulova, *Kyrgyz Republic*
Miklós Bálint, *Romania*
Pavlina Bartová, *Slovakia*
Andrea Batariova, *Czech Republic*
Vladimír Bencko, *Czech Republic*
Ivan Beneš, *Czech Republic*
Milan Beno, *Slovakia*
Martin van den Berg, *Holland*
Frantisek Beska, *Czech Republic*
Olena Beskid, *Czech Republic*
Zhaukharia Bezverkhnyaya, *Kyrgyz Republic*
Blanka Binková, *Czech Republic*
William Brattin, *USA*
Mihály Braun, *Hungary*
Marek Buzga, *Czech Republic*
David Carpenter, *USA*
Milena Černá, *Czech Republic*
Silvia Cerná, *Slovakia*
Katerina Cimova, *Czech Republic*
Irena Chvátalová, *Czech Republic*
Jan Dejmek, *Czech Republic*

Lubomir Dobiáš, *Czech Republic*
Kirby C. Donnelly, *USA*
Ondřej Drábek, *Czech Republic*
Zbigniew Drąg, *Poland*
Marek Drímal, *Slovakia*
Zdik Dušek, *Czech Republic*
Eleonora Fabiánová, *Slovakia*
Peter Farmer, *Bulgaria*
Petr Franěk, *Czech Republic*
Aleksandra Fučić, *Croatia*
Alena Gábelová, *Slovakia*
Petra Gergelová, *Slovakia*
Miloslav Götzl, *Slovak Republic*
Molly M. Gribb, *USA*
Susan Griffin, *USA*
Hongxia Guo, *USA*
Eugen Gurzau, *Romania*
Bernhard Hennig, *USA*
Marta Hurbánková, *Slovakia*
Ana Marija Jazbec, *Croatia*
Zdenek Jirak, *Czech Republic*
Ivan Kalina, *Slovakia*
Iosif Kasler, *Romania*
Omor Kasymov, *Kyrgyz Republic*
Balvinder Kaur, *United Kingdom*
Artak Khachatryan, *Republic of Armenia*
Andrei Sárkány-*Kiss*, *Romania*
Martin Kočárek, *Czech Republic*
Radka Kodešová, *Czech Republic*
Kvetoslava Koppová, *Slovakia*
Frantisek Kotesovec, *Czech Republic*
Zuzana Kováciková, *Slovakia*
Josef Kozák, *Czech Republic*
Jacek Krzyzak, *Poland*
Ruzena Kubínová, *Czech Republic*
Jaromira Kůsová, *Czech Republic*
Soterios Kyrtopoulos, *Greece*
Tyler Lane, *USA*
Hana Lehocká, *Czech Republic*
Zdena Lnenickova, *Czech Republic*
Katarina Mahutova, *USA*
Michel Maignan, *Switzerland*

Zuzana Majkova, *USA*
Elvira Majkova, *Kyrgyz Republic*
Milena Menzlova, *Czech Republic*
Alena Milcova, *Czech Republic*
Iulia Neamtiu, *Romania*
Karel Němeček, *Czech Republic*
Jiří Novák, *Czech Republic*
Elizabeth Oesterling, *USA*
Jan Pavlovic, *Czech Republic*
Vít Penížek, *Czech Republic*
Joseph P. Pinto, *USA*
Ivan Pleško, *Slovak Republic*
Todor Popov, *Bulgaria*
Jadwiga Rachtan, *Poland*
Jiří Rameš, *Czech Republic*
Gudrun Reiterer, *USA*
Marcela Rohošková, *Czech Republic*
Pavel Rössner, *Czech Republic*
Zofia Rudek, *Poland*
Paula Schmittdiel, *USA*
Miljenko Sedlar, *Croatia*
Oksana Sevastyanova, *Czech Republic*
Ainash Sharshenova, *Kyrgyz Republic*
Raj Singh, *United Kingdom*
Jiri Skorkovsky, *Czech Republic*
Jiri Smid, *Czech Republic*
Katarina Slotová, *Slovakia*
Ivo Solansky, *Czech Republic*
Radim J. Šrám, *Czech Republic*
Margareta Šulcová, *Slovakia*
Almaz Sultashev, *Kyrgyz Republic*
Simona Surdu, *Romania*
Karla D. Thrall, *USA*
Michal Toborek, *USA*
Hana Tomášková, *Czech Republic*
Jan Topinka, *Czech Republic*
Tomáš Trnovec, *Slovak Republic*
Oldřich Vacek, *Czech Republic*
Zuzana Valovičová, *Slovakia*
Antonina Cebulska-Wasilewska, *Poland*
Sona Wimmerová, *Slovakia*
Ivona Závacká, *Czech Republic*

Vladislava Zavadilova, *Czech Republic*
Ariana Znaor, *Croatia*
Anne-Laure Zufferey, *Switzerland*

Dedication

These Proceedings are dedicated to Professor Joseph Hartman (1942-2004) of Boise State University, one of the founding sponsors of the Central and Eastern European Environmental Health Conference series, a dedicated teacher and student advocate, and a talented researcher who encouraged greater collaboration between the environmental science and environmental health communities.

Preface

The first Central and Eastern European Environmental Health Conference was convened in Prague, Czech Republic, on October 24, 2004. This conference had three primary objectives:

1. To gather scientists and students from the USA and Central and Eastern Europe (CEE) to discuss the magnitude of the problems in specific regions of Central and Eastern Europe;
2. To discuss improved methods for assessing exposure including biomarkers of exposure and integrated methods for predicting dose; and,
3. To discuss specific health effects associated with exposure to chemicals at these sites with a focus on developmental and reproductive health.

While the objectives of this conference were quite broad, the intent was to gather scientists from a range of disciplines to identify the major problems facing CEE countries where U.S. National Institute of Environmental Health Sciences and U.S. Environmental Protection Agency research would make a positive difference. Thus, the initial sessions sought to define the magnitude of the problems given the current knowledge. The subsequent sessions discussed methods of assessing both the extent of exposure and potential adverse effects associated with the release of hazardous chemicals in these countries.

Acknowledgments

The organizers wish to thank the financial sponsors of this conference, whose generosity made it possible to convene this international meeting. They not only supported the conference activities, but also provided travel awards for many students and other conference participants, thereby greatly enhancing the breadth of the scientific discussions and the potential for future scientific collaborations. The financial sponsors included the Agency for Toxic Substances and Disease Registry (USA); BIOGENIX (Czech Republic); Boise State University (USA); SlovAm (Slovak Republic); the Superfund Basic Research Program of the National Institute of Environmental Health Sciences, National Institutes of Health, Department of Health and Human Services (USA); TerraChem, Inc. (USA); Texas A&M University System (USA); the U.S. Environmental Protection Agency (USA); Wellington Laboratories (Canada); Wellington Science USA, Inc. (USA); and the World Health Organization.

A number of other institutions also provided valuable support to the conference. These included the Cancer Research Institute, Slovak Academy of Sciences (Slovak Republic); Charles University (Czech Republic); Comenius University, Faculty of Natural Sciences, Environmental Section (Slovak Republic); Czech Technical University (Czech Republic); Masaryk University, Faculty of Informatics (Czech Republic); Slovak Medical University, Faculty of Public Health (Slovak Republic); Slovak University of Technology (Slovak Republic); EU Center of Excellence, Institute of Experimental Medicine (Czech Republic); Ministry of the Environment

(Czech Republic); National Institute of Public Health (Slovak Republic); Slovak Environmental Agency (Slovak Republic); and the State Institute of Public Health (Czech Republic).

Many thanks also go to the conference organizing committee, an inter-institutional, international group that included B. Anderson, R. Autenrieth, M. Avakian, J. Barich, J. Blaha, K. Blaha, E. Bruce, D. Carpenter, L. Cizmas, K. Daniel, P. Dimitriou-Christidis, K.C. Donnelly, W. Farland, A. Gillespie, J. Hartman, J. Hrebicek, W. Kovalick, R. Kreizenbeck, J. Lewtas, K. Mahutova, S. McDonald, T. McDonald, B. Mournighan, T. Phillips, P. Preuss, L. Reed, I. Rovny, L. Safe, S. Safe, J. Silvan, R. Sram, W. Suk, E. Tesarova, J. Volf, T. Voltaggio, N. White and M. Wiles. We wish to extend particular thanks to the many local scientists who made this conference successful, including R. Sram and J. Volf.

Introduction

The first Central and Eastern European Environmental Health Conference (CEEHC) was held in Prague, Czech Republic, from October 24 – 27, 2004. The conference included more than 150 participants from 16 countries. During the three days of this conference, it became apparent that the scientists of this region have produced a remarkable body of information to characterize the frequency and severity of environmental health problems. In addition, data were presented from a number of ongoing studies designed to evaluate methods for reducing exposures or improving site remediation.

In many of the countries of the former Soviet Union, large blocks of land exist that were the sites of large industrialized zones. Often, these industrialized zones included a number of major industrial complexes involved in the production or manufacture of various products. For example, up to 80% of the petrochemicals of the former Soviet Union were produced in Sumgayit, Azerbaijan. The industrialized zone of Sumgayit housed a chlordane plant, a chloralkalai plant and a synthetic rubber plant, as well as other production facilities. It is not uncommon in many of these industrialized zones to find areas where environmental media contain elevated concentrations of a number of organic and/or inorganic contaminants. Workers employed in the industries that were located in these zones often lived nearby and in substandard housing. The combination of a severely impacted environment and poor housing conditions has resulted in significant numbers of individuals receiving elevated environmental exposures. Minimal information is available from which to accurately

characterize the human health impacts of many of these exposures. Even less information is available to understand the combined influence of genetics and nutrition on susceptibility to adverse health effects from these contaminants. Extensive research has been conducted by investigators in Central and Eastern Europe to evaluate these problems. This includes research to measure contaminant levels in the environment, studies to monitor biomarkers in exposed populations, and studies to investigate the incidence of disease. In addition, novel approaches are being investigated to identify appropriate procedures to contain and remediate these contaminated environments. The results from these and other environmental health studies in this region will be of value to health professionals in the region. In addition, it should be emphasized that these results may also be used by health professionals from around the world to implement methods to prevent exposures and thereby reduce disease.

Many of these research projects are described in these proceedings, which represent approximately one-quarter of the presentations from the conference. For most of the authors, English was not their first language. The manuscripts were edited as needed to improve the clarity, and all changes were reviewed by the authors prior to publication. These proceedings provide a useful venue for publicizing the current state of environmental health science research in Central and Eastern Europe.

Many of the senior scientists from the United States and Europe who attended the meeting noted that the quality of the students who were present, including those from the Central and Eastern European countries, was outstanding. We hope that these conference proceedings will help these students develop their careers, and will serve as a catalyst to expand collaboration between scientists within this region as well as with scientists in other parts of the world.

CHAPTER 1

MORTALITY IN NORTHWESTERN BOHEMIA IN PERIODS OF HIGH AND LOW AIR POLLUTION

Jiri Skorkovsky and Frantisek Kotesovec
Health Institute Usti nad Labem, Regional site Teplice, Wolkerova 3, Czech Republic

Abstract: Air pollution was higher in the industrial area of Northwestern Bohemia during the years 1982 – 1990 than from 1991 to 2000. The aim of the study was to determine whether daily total, cardiovascular (CVD) or respiratory mortality in this area was significantly different during the years 1982 – 1990 compared to the period 1991 – 2000. Poisson regression analyses were conducted with standardized daily mortality as the resulting variable, the time period as the variable of interest, and standardized daily mortality in the rest of the Czech Republic, day of the week, temperature, relative humidity and influenza as confounders. During the period 1982 – 1990, the average concentrations for SO_2 and total suspended particles (TSP) were 103 and 102 $\mu g/m^3$, respectively, and from 1991 – 2000 they were 45 and 62 $\mu g/m^3$, respectively. This reduction is thought to be a consequence of the reduction in combustion of brown coal. The yearly age-standardized total mortality was substantially higher in the period with higher air pollution (15.1 deaths/1000 in men and 13.8/1000 in women) than during the period with lower air pollution (13.5/1000 and 12.6/1000 in men and women, respectively). CVD mortality also dropped following the reduction in air pollution, from 7.6/1000 to 6.6/1000 in men and from 8.3/1000 to 7.4/1000 in women. The influence of air pollution period was assessed. For all ages, the relative risk (RR) and 95% confidence interval were: for total mortality in men, 1.055 (1.037, 1.074), and in women, 1.025 (1.007, 1.043); for CVD mortality in men, 1.087 (1.061, 1.114), and in women, 1.054 (1.031, 1.078); and for respiratory mortality in men, 1.331 (1.24, 1.43), and in women, 1.049 (0.964, 1.143). The RR was between 2.5%-33% depending on gender, cause of death and age group. The influence of the time period (1982 – 1990 or 1991 – 2000) was stronger for younger age groups, for CVD, and for men.

K. C. Donnelly and Leslie H. Cizmas (eds.), Environmental Health in Central and Eastern Europe, 1-11.

Key words: Northwestern Bohemia, coal burning, power plant, heating, mortality, standard, standardization, gender, age, cause of death, air pollution, Poisson regression, temperature, humidity, influenza.

1. INTRODUCTION

There is widespread concern about the potential adverse health effects of ambient air pollution. Studies of extreme episodes of air pollution in the Meuse Valley, Donora and London have provided compelling documentation of the serious adverse effects of this pollution on human health.

A remarkably consistent association between short term changes in daily mortality and particulate air pollution has been found in several US cities, (Dockery et al., 1992, 1993, Pope III et al., 1992, Schwartz, 1993, 1994, 1994, Schwartz and Dockery, 1992) Brazil and China (Saldiva et al., 1995, Xu, et al., 1994). Similar results have been obtained in 15 European cities from different countries within the Air Pollution and Health – A European Approach (APHEA) project (Katsouyani et al., 1996, 1997, Zmirou et al., 1996, Dab et al., 1996, Spix and Wichmann, 1996, Touloumi et al., 1996, Vigotti et al., 1996, Sunyer et al., 1996 Bacharova et al., 1996), as well as in studies concerning short term effects of air pollution on daily mortality in Northwestern Bohemia (Kotesovec et al., 2000) and Northern Bavaria (Peters et al., 2000).

Recent studies suggest that respirable particles are the causal factor responsible for the increase in daily mortality associated with increasing air pollution, whereas SO_2 is considered to be only an indicator of air pollution. Additional studies suggest that SO_2 or other air pollutants may act as independent factors (Xu et al., 1994, Zmirou et al., 1996, Spix and Wichmann, 1996, Bacharova et al., 1996).

Studies that present evidence of diminished daily mortality or increased life-expectancy following reduction in air pollution are rare. After the Irish government banned the marketing, sale and distribution of bituminous coal within the city of Dublin, a study in Ireland (Clancy et al., 2002) found a significant decrease in age-standardized total, cardiovascular and respiratory mortality following adjustment for temperature, humidity, day of week, respiratory epidemics and death rates in the rest of Ireland. This intervention was followed by an immediate and permanent reduction in particulate air pollution.

Until 1990, brown coal was used in Northern Bohemia by power plants, all industrial plants, and households, to produce energy and heat. Emission concentrations, particularly of SO_2 and particulate matter, reached levels

which were the highest in the Czech Republic and among the highest in Europe. After interventions in 1989 and 1990 (dust elimination and desulphurisation of power plants and changes in local heating from coal to gas), the level of air pollution decreased rapidly and remained low.

The aim of this study was to determine whether there was an association between air pollution and cause-specific mortality, and to evaluate whether age or gender influenced this association.

2. STUDY LOCATION

The study area is the gray area shown in Figure 1. As can be seen in this map, power plants and other major sources of pollution are concentrated in and around this region.

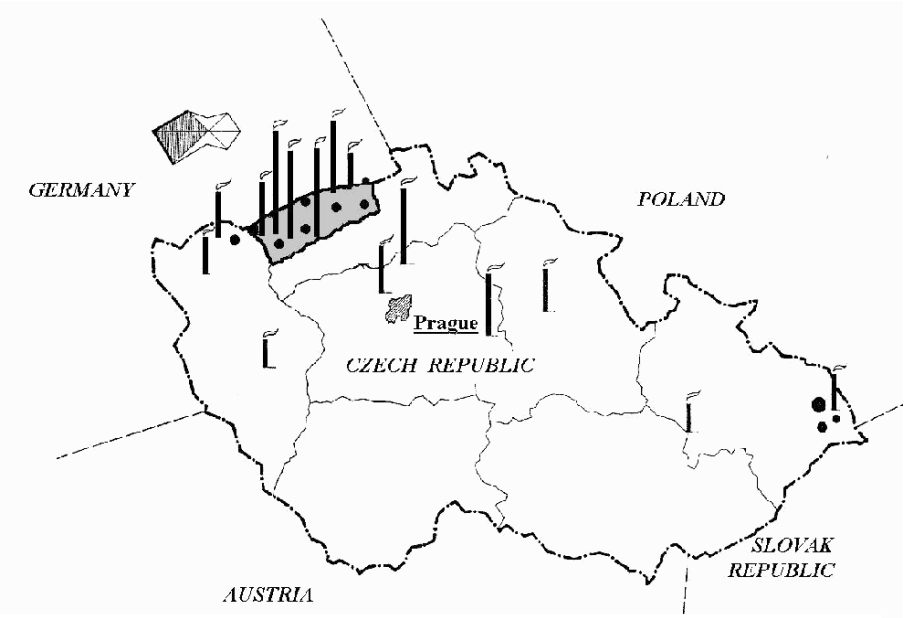

Figure 1. The study area (gray area). The chimneys represent power plants and other major sources of air pollution.

3. METHODS

The goal of this study was to determine if the period of high air pollution (1982 – 1990) was associated with changes in age-standardized mortality when compared with the period of relatively lower air pollution (1991–

2000). Air pollution was measured daily in the study area during the period 1982 – 2000. The goal of the study was to establish if the indicator of the period of high air pollution in the industrial area in Northwestern Bohemia in the years 1982 – 1990, compared with the period of relatively lower air pollution in the years 1991 – 2000, was associated with changes in age-standardized daily mortality. A similar study was conducted in Ireland (Clancy et al., 2002), where the periods before and after banning the sale of coal in Dublin were considered.

Data were available on daily mortality in the study area and in the Czech Republic as a whole, which served as the reference area. The data were stratified according to gender and cause of death, and population counts in five-year age groups were also available. For the present study, mortality data were indirectly age-standardized. In this method, the number of deaths in each age group (15-year intervals for 0-74 years and 5-year intervals for age 75 and older) in the reference area was multiplied by the ratio of the population in the study area to the population in the reference area for this age group. Thus, for each age group, the expected number of cases in the study area was calculated and the sum of the expected numbers was divided by the actual number of deaths in the study area. This was done separately for each gender, all causes of death, and all ages, and for the age groups <60, 60-74 and over 75. Multiplying this coefficient, called the SMR (standardized mortality ratio), by cause-specific mortality in the reference area per 100,000 inhabitants, yielded the standardized mortality in the study area. This method was used because the daily count of deaths in each age-group, particularly for cause-specific mortality in the study area, was often equal to zero while in the reference area the numbers were generally much higher. The method of indirect standardization uses only the total count in the study area and counts within age groups of the reference area for both genders and all considered causes of death.

The S-plus software package was used for evaluating the influence of the indicator of the period of high air pollution. The Procedure GAM (Generalized Additive Models), Poisson regression using the LOESS function to control for mortality in the rest of the Czech Republic, and meteorological variables were applied. The LOESS function is a method of piecewise regression where the size of the regressed part is defined by the parameter span. The right size should be chosen to fit the trends but should not be influenced by random fluctuation. For standardized mortality in the reference area and relative humidity we used span 0.8, for temperature 0.5.

Standardized daily mortality was considered to be a resulting dependent variable, and the indicator of the period was considered a variable of interest. The confounders were as follows:

1. standardized daily mortality in the rest of the Czech Republic

The standardized daily mortality was stratified and obtained in the same way as the mortality in the study area (described previously). The daily mortality in the rest of the Czech Republic was used to fit long-term trends caused by changes in life style (nutrition, smoking) or in medical care, and seasonal patterns. This variable in the model was highly significant in all cases except with women <60.

2. day of week was included for compatibility with other studies but did not prove to be significant.

3. temperature proved to be highly significant except with people <60. The lowest mortality was at about 15 °C.

4. relative humidity was again included for compatibility with other studies but did not prove to be significant.

5. influenza: weekly counts of influenza were available for all districts, and were used to calculate daily numbers using a seven-day moving average. This factor was highly significant except with people <60.

4. RESULTS

Average yearly concentrations for SO_2 and total suspended particles (TSP) were 103 and 102 μg/m^3, respectively, during 1982–1990, and 45 and 62 μg/m^3, respectively, during 1991–2000, following dust separation and desulphurization of power plants and the shift from changes from coal to gas in home heating.

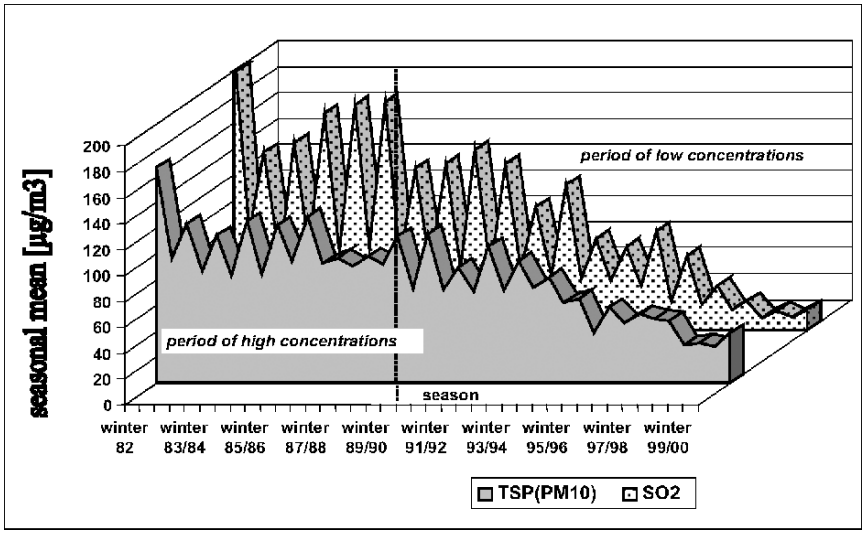

Figure 2. Seasonal concentrations of air pollutants. (Note: TSP = total suspended particles; particulate matter <10 μ m)

During the entire period of the study, the mean TSP and SO_2 concentrations in the study area were generally higher in winter seasons. During the period 1991 – 2000, the reduction in TSP and SO_2 was evident in all months, but the largest decline was noted in the winter (Figure 2).

Table 1. Yearly mean standardized mortality/1000 in periods of high and low air pollution.

Mortality	total		CVD		respiratory	
Gender	male	female	male	Female	male	female
high air poll.period	15.07	13.80	7.64	8.35	0.92	0.54
low air poll.period	13.49	12.59	6.65	7.42	0.60	0.45
Change	–1.58	–1.21	–0.99	–0.92	–0.32	–0.09
change[%]	10.50	–8.75	–12.94	–11.06	–34.42	–16.37
stat.significance	signif.	signif.	signif.	signif.	signif.	signif.

An average of 7,265 total deaths per year were recorded during the period of high air pollution, compared to an average of 6,867 total deaths per year during the period of lower air pollution (398 fewer deaths per year on average). Cardiovascular diseases (CVD) were responsible for approximately 51% of deaths in men and 61% in women. Respiratory diseases accounted for 5% of male and 4% of female deaths.

Mean age-standardized total non-trauma death rates as well as CVD and respiratory death rates also differed by season, with the highest mortality rates in the winter (Figure 3). The age-standardized total, CVD and respiratory mortality significantly decreased in the period with lower air pollution (Table 1).

Standardized mortality in the rest of the Czech Republic, in relation to daily temperature and the incidence of influenza were statistically significant for all groups except people younger than 60. On the contrary, relative humidity and day of the week did not appear to affect the mortality rate.

Table 2 presents the percent change in age-standardized mortality in Northwestern Bohemia during the periods of high and low air pollution. After adjusting the Poisson regression for temperature, humidity, day of the week, respiratory epidemics and age-standardized death rates in the rest of the Czech Republic, there was a significant change in total and CVD mortality for both genders and for respiratory mortality in men when these parameters were compared for the periods of high and low air pollution. The effects were mostly greater for men except age group <60. The effects were

larger for CVD than for total mortality. The greatest effect was observed for respiratory mortality in men, but due to the low number of cases in this category, this result should be interpreted with caution.

For men as well as for women, greater effects were observed for the age group <60 but again it should be noted that there were low numbers of cases in these categories, particularly for women (Figure 4).

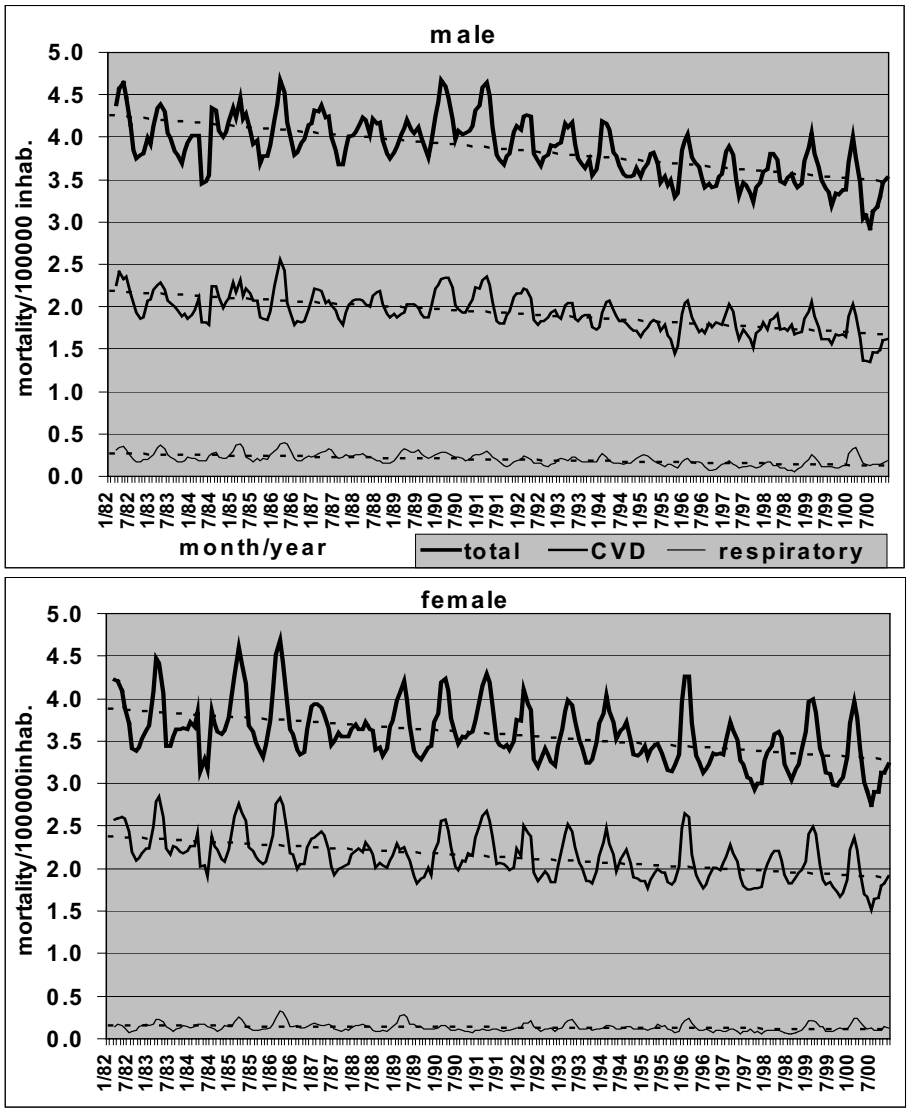

Figure 3. Mean age-standardized total non-trauma mortality rates in the study area for men and women for the period 1982 – 2000. (Note: inhab. = inhabitants).

5. DISCUSSION AND CONCLUSION

The substantial decrease in air pollution was followed by a decrease of 5.5% (men) and 2.5% (women) in age-standardized total mortality adjusted for temperature, humidity, incidence of respiratory diseases, day of week and death rates in the rest of the Czech Republic. The decrease in the cardiovascular death rate was 8.7% in men and 5.4% in women. The largest decrease was seen in estimated respiratory mortality in men (33.1%), whereas the decrease in respiratory mortality among women was not significant (4.9%). When the mortality was evaluated with regard to age, then the decrease in total and CVD mortality was generally highest in men as well as in women younger than 60 years.

Table 2. Adjusted relationship between indicator of air pollution period and mortality via Poisson regression.

Cause of death	Gender	Age	Stat. signif.	% Change (95% CI)
all	male	all	* * *	5.5 (3.7, 7.4)
all	female	all	* *	2.5 (0.7, 4.3)
CVD	male	all	* * *	8.7 (6.1, 11.4)
CVD	female	all	* * *	5.4 (3.1, 7.8)
all	male	<60	* * *	9.9 (6.8, 13)
all	female	<60	* * *	11.3 (6.6, 16.1)
CVD	male	<60	* * *	16.3 (10.5, 22.4)
CVD	female	<60	* * *	25.2 (15, 36.2)
all	male	60 – 74	* * *	6.7 (3.9, 9.6)
all	female	60 – 74	* *	4.2 (1.1, 7.4)
CVD	male	60 – 74	* * *	10.4 (6.4, 14.5)
CVD	female	60 – 74	* * *	7.8 (3.5, 12.3)
all	male	≥ 75	* * *	8.3 (5.1, 11.6)
all	female	≥ 75	* *	3.3 (1, 5.7)
CVD	male	≥ 75	* * *	10 (6, 14.1)
CVD	female	≥ 75	* * *	6.4 (3. 6, 9.3)
respir.	male	all	* * *	33.1 (24, 42.8)
respir.	female	all	n.s.	4.9 (-3.6, 14.3)

*Statistical significance: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$, n.s. $p > 0.05$*

Clancy and colleagues found a rapid reduction in air pollution to be associated with significant decreases in total mortality (5.7% decrease), CVD mortality (10.3% decrease) and respiratory mortality (15.5% decrease).

(Clancy et al., 2002) They found these decreases to be slightly higher if only the population younger than 60 years was considered. These results are consistent with the results of the present study in Northern Bohemia.

The results of this study show that the decrease in mortality associated with a reduction in air pollution is smaller in women than in men. A previous study (Peters et al., 2000) about daily mortality and air pollution found that the increase in daily mortality associated with increasing air pollution was smaller in women than in men, and was generally not significant in women. This suggests that women may be less susceptible than men to changes in air pollution.

The decrease noted in respiratory mortality could be explained by the direct influence of air pollution on the respiratory system. Nevertheless, in our conditions, only about 5% of total mortality is due to respiratory-related causes. As such, it is important to note that the results may be influenced by the low numbers of deaths, particularly in the younger age groups.

In conclusion, a substantial reduction in air pollution was followed by a significant decrease in age-standardized total, cardiovascular, and respiratory mortality adjusted for temperature, humidity, respiratory morbidity, day of the week and death rates in the rest of the Czech Republic. Thus, climatic factors, respiratory morbidity, and the other factors mentioned above do not appear to explain this decrease. The reduction in mortality in Northwestern Bohemia suggests that control of air pollution could substantially diminish daily mortality.

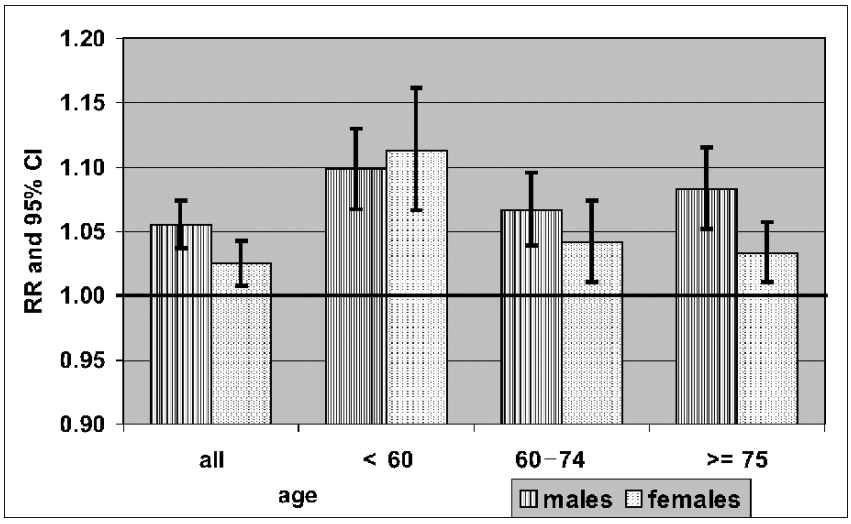

Figure 4. Adjusted relative risk (RR) of total mortality during the period of high air pollution. (Note: CI = Confidence Interval).

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CHAPTER 2

AIR POLLUTION IN TEPLICE AND PRACHATICE IN 1995 AND 2003

A Comparison After 8 Years

Ivan Beneš[1], Jiří Novák[2] and Joseph P. Pinto[3]

[1]Health Institute Ústí nad Labem, Regional Site 415 01 Teplice, Wolkerova 3, Czech Republic; [2]Czech Hydrometeorological Institute, Na Sabatce 17 , 143 06 Prague, Czech Republic; [3] National Center for Environmental Assessment, U.S. Environmental Protection Agency, 27711 Research Triangle Park, North Carolina, 27709 USA.

Abstract: Air pollution in Teplice and Prachatice, two cities in the Czech Republic, has been monitored from 1992 until the present. Since 1995, the same sampling and analytical methods, instruments for both sampling and analyses, and QA/QC procedures have been used in both cities. For these two locations, this study compared the annual concentrations of SO_2, NO, NO_2, CO, O_3, ambient particulate matter <2.5 μm and <10 μm ($PM_{2.5}$ and PM_{10}, respectively), polycyclic aromatic hydrocarbons (PAHs), the sum of carcinogenic PAHs, and benzo[*a*]pyrene (B*a*P). Time and site differences were also evaluated. Teplice and Prachatice were chosen for long term study because of the contrast in air quality and sources of pollution in the two cities. The area surrounding Teplice was highly industrialized and contaminated, while Prachatice was located in a rural area with better air quality. During the period from 1995 to 2003, all major air pollution sources were either closed or had contaminant control devices installed, and most residences switched to cleaner heating sources. On the other hand, automobile traffic increased during this period throughout the Czech Republic. In Teplice, concentrations of SO_2 and total PAHs were lower in 2003 than in 1995, whereas CO and ozone concentrations increased over this period. The data indicate that in the rural area of Prachatice, there was a decrease in SO_2, total PAHs, carcinogenic PAHs and BaP. Data from the rural community also reflect an increase in CO and particulate matter (both $PM_{2.5}$ and PM_{10}). Overall, the results from air quality monitoring in an industrialized and a rural area of the Czech Republic in 1995 and in 2003 appear to reflect changes in factory emissions and traffic density.

Key words: Czech Republic, Teplice, Prachatice, Air pollution, sulfur dioxide, nitric oxide, nitrogen dioxide, carbon monoxide, ozone, particulate matter 2.5, particulate matter 10, polycyclic aromatic hydrocarbon, benzo[a]pyrene.

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1. INTRODUCTION

The quality of air, food and water has a significant impact on the health status of any population. The World Health Organization website (http:// www.wpro.who.int/health_topics/air/) estimates that polluted air causes more than 500,000 deaths each year. Epidemiologic studies (reviewed in Nel, 2005) have observed a consistent increase in cardiac and respiratory morbidity and mortality from exposure to particulate matter. Namdeo and Bell (2005) found that respiratory hospital admissions were correlated with seasonal increases in PM_{10} in the United Kingdom. Ponka (1991) found that ozone and NO_X levels were significantly correlated with asthma admissions to hospitals for children in Helsinki. The potential for global transport of air pollution was demonstrated by Li et al. (2005), who detected organochlorine pesticides in air at an elevation of 4400m above sea level on Mt. Everest. The purpose of this manuscript is to describe changes in air pollution during the last ten years in two cities in the Czech Republic.

2. MATERIAL AND METHODS

Sulfur dioxide (SO_2), nitric oxide (NO), nitrogen dioxide (NO_2), carbon monoxide (CO) and ozone (O_3) were measured with continuous analyzers (all meeting U.S. Environmental Protection Agency [U.S. EPA] standards). Sulfur dioxide was measured by fluorescence, NO and NO_2 by chemi-luminescence, CO by infrared absorption and O_3 by UV absorption. Ambient particulate matter <2.5 μm and <10 μm ($PM_{2.5}$ and PM_{10}, respectively) samples were obtained with the VAPS sampler (University Research Glassware, Carrboro, NC) and their concentrations were determined by gravimetric analysis in a controlled environment. Air volume and filter weight were determined using verified instruments. In the $PM_{2.5}$ size fraction, polycyclic aromatic hydrocarbons (PAHs) were determined from the $PM_{2.5}$ filter using high performance liquid chromatography (HPLC) with fluorescence detection. The analytical method used was US EPA TO-13.

3. RESULTS

Air samples collected from the industrial area of Teplice and the rural city of Prachatice were analyzed for a range of parameters to investigate changes in pollution levels between 1995 and 2003. Sulfur dioxide concentrations in Teplice were reduced from 45 $\mu g/m^3$ in 1995 to 15 $\mu g/m^3$ in 2003. In Prachatice, air concentrations of SO_2 were also reduced, with a reduction of approximately 25% observed between 1995 and 2003 (Figure 1).

Airborne levels of nitric oxide and nitrogen dioxide were appreciably higher in Teplice when compared to airborne levels in Prachatice (Figures 2 and 3). However, NO_x (NO_x = NO + NO_2) levels in both cities were relatively unchanged from 1995 to 2003. Higher levels of both carbon monoxide and ozone were measured in air in the rural community than were observed in Teplice (Figures 4 and 5). In addition, concentrations of both chemicals in air increased between 1995 and 2003. Airborne concentrations of CO increased by approximately 20% in both cities between 1995 and 2003 (Figure 4). Ozone levels in Teplice increased from 21 µg/m^3 in 1995 to 43 µg/m^3 in 2005, while in Prachatice, 58 µg/m^3 ozone was measured in 1995 and 62 µg/m^3 ozone was measured in 2003 (Figure 5).

Air concentrations of particulate matter (PM_{10} or $PM_{2.5}$) and polycyclic aromatic hydrocarbons (PAHs) generally result from the combustion of fossil fuels. Concentrations of $PM_{2.5}$ in air from Teplice were approximately 30% higher than $PM_{2.5}$ levels in air from Prachatice in 1995; whereas $PM_{2.5}$ levels in both areas were approximately equal in 2003 (Figure 6). Similarly, PM_{10} concentrations in Teplice were much higher than Prachatice in 1995, while in 2003 the PM_{10} levels were approximately equal in the two locations (Figure 7).

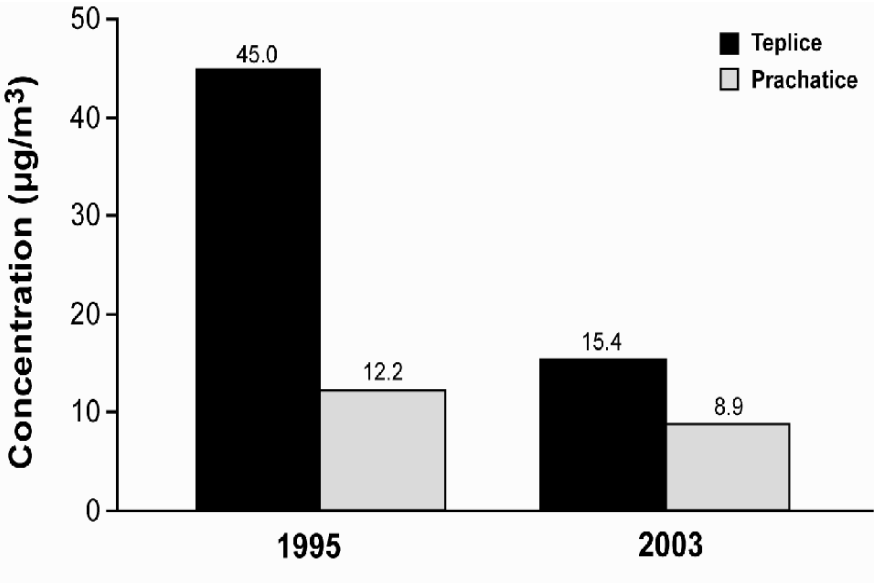

Figure 1. Annual average sulfur dioxide concentrations in Teplice and Prachatice.

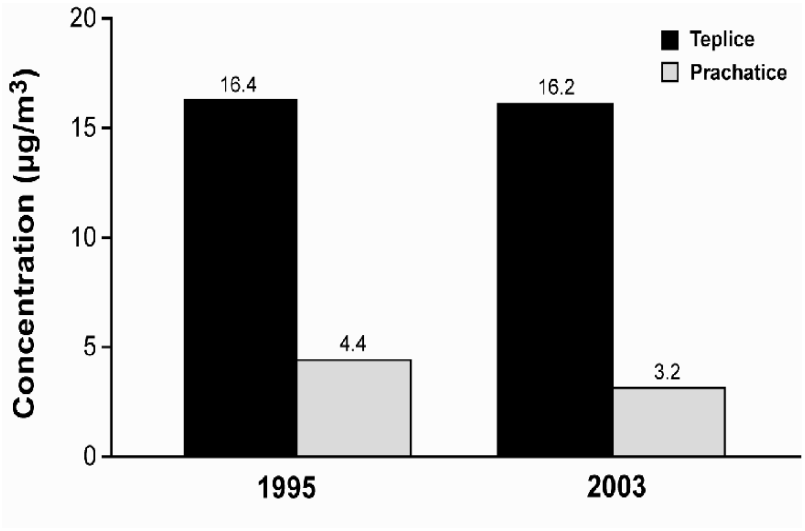

Figure 2. Annual average nitric oxide concentrations in Teplice and Prachatice.

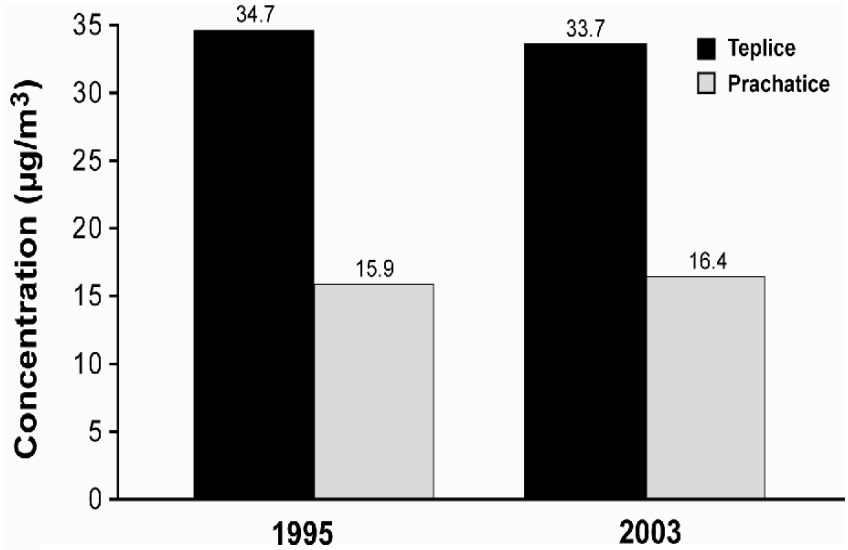

Figure 3. Annual average nitrogen dioxide concentrations in Teplice and Prachatice.

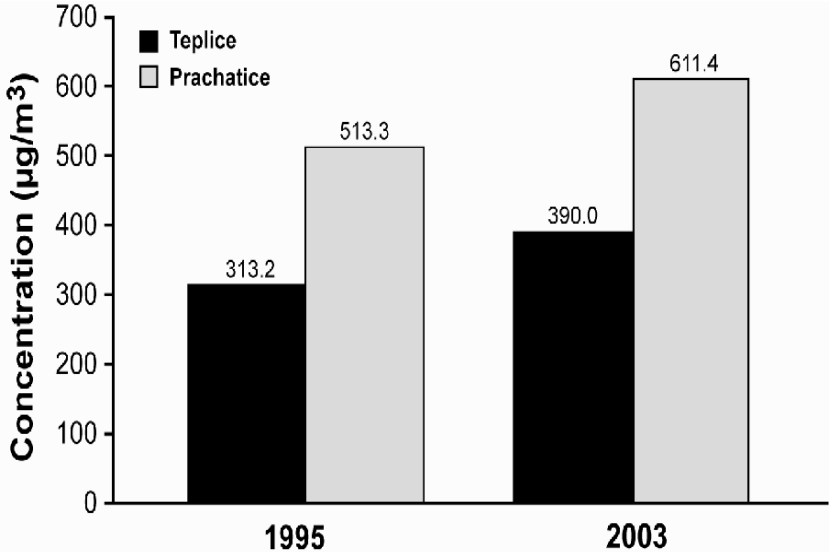

Figure 4. Annual average carbon monoxide concentrations in Teplice and Prachatice.

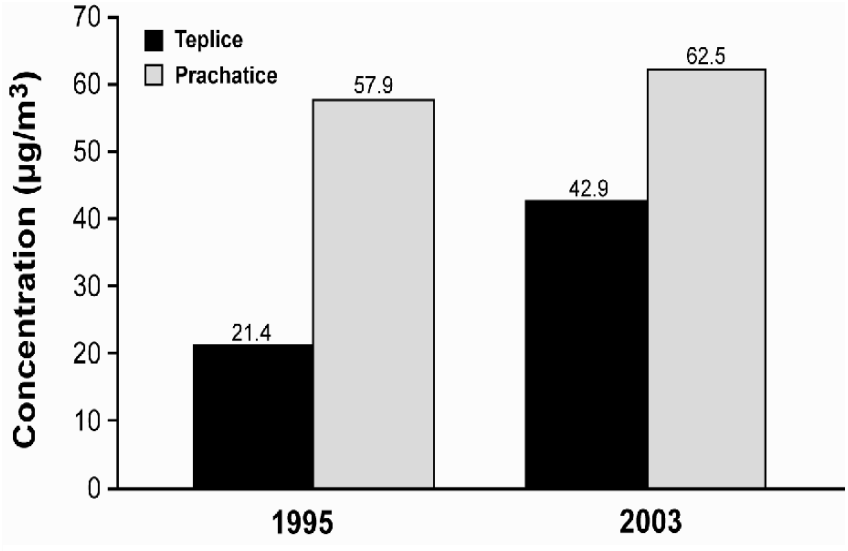

Figure 5. Annual average ozone concentrations in Teplice and Prachatice.

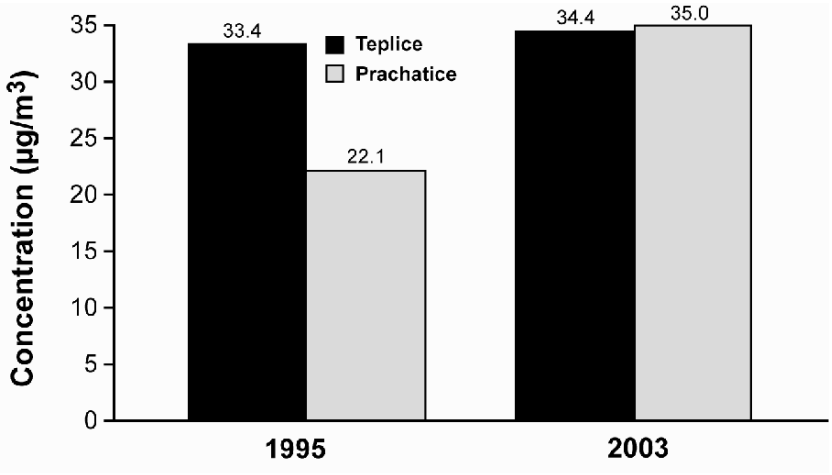

Figure 6. Annual average particulate matter 2.5 concentrations in Teplice and Prachatice.

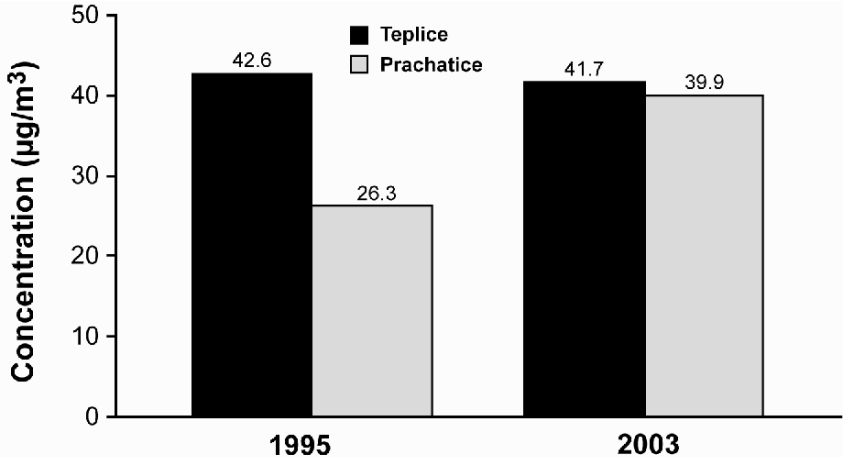

Figure 7. Annual average particulate matter 10 concentrations in Teplice and Prachatice.

Air levels of PAHs did not follow the same trend as PM. Total PAH levels in the rural community were slightly higher than were observed in Teplice in 1995, while total PAH concentrations in air were reduced in both communities in 2003 (Figure 8). Air levels of total PAHs in Prachatice fell from 72 ng/m^3 to 15 ng/m^3. Carcinogenic PAHs including BaP exhibited similar

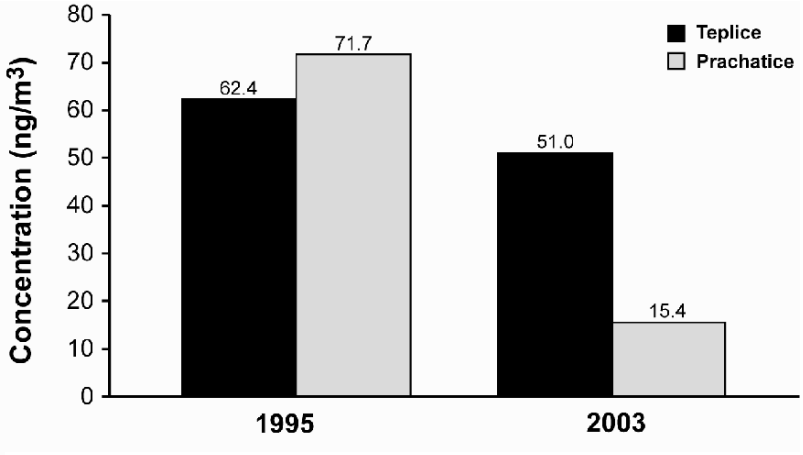

Figure 8. Annual average concentrations of total polycyclic aromatic hydrocarbons (PAHs) in Teplice and Prachatice.

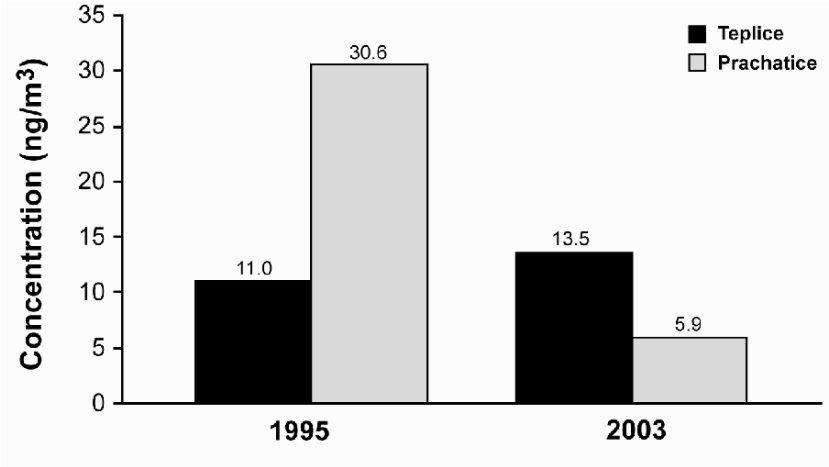

Figure 9. Annual average concentrations carcinogenic PAHs in Teplice and Prachatice.

trends (Figures 9 and 10). PAHs classified as carcinogens in the U.S. EPA's IRIS data base are benz[a]anthracene chrysene, benzo[b]fluoroanthene, benzo[k]fluoroanthene, benzo[a]pyrene, dibenz[a, h]anthracene, and indeno [1, 2, 3-cd]pyrene. The carcinogenic PAH concentration measured in air in 1995 was almost three times higher in the rural community than in Teplice.

However, in 2003, carcinogenic PAH levels in Prachatice were reduced by almost 80% from 1995 and were less than half the concentration measured in Teplice. BaP concentrations in air in Teplice were essentially unchanged from 1995 to 2003, while the BaP concentration in air from Prachatice was reduced by approximately 50% during the same time period.

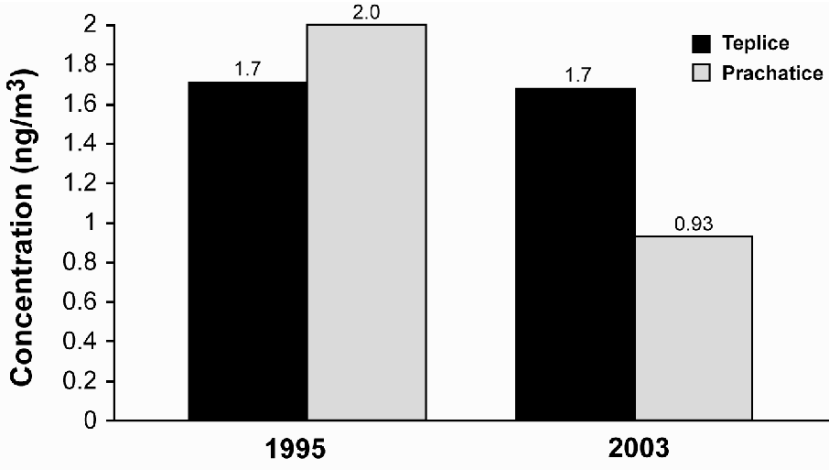

Figure 10. Annual average concentrations of Benzo(a)pyrene in Teplice and Prachatice.

4. DISCUSSION

The changes in annual average pollutant concentrations were quite different in both cities. The concentration of sulfur dioxide dropped appreciably in both cities following desulfurization of coal used by power plants and the change from coal to natural gas and electricity for residential heating in the 1990s.

The concentrations of both NO and NO_2 did not change appreciably in either city. This may be the result of a trade off between decreases in emissions from stationary sources (power plants, local sources) and increased emissions from mobile sources (traffic).

Carbon monoxide and ozone concentrations were higher in 2003 than in 1995 in both cities. Motor vehicle emissions influence atmospheric concentrations of CO, organic compounds, NO and NO_2. In the presence of UV radiation, these emissions are responsible for higher ozone concentrations.

The concentrations of particulate matter in both the PM_{10} and $PM_{2.5}$ size fractions did not change significantly in Teplice, but they increased in Prachatice. Because these particles contain many toxic components (e.g., metals, PAHs), there is some cause for concern regarding possible health effects.

Total PAH concentrations decreased in both cities, and this decrease was more dramatic in Prachatice. These reductions may be due to the closure of the largest source of emission of PAHs.

Carcinogenic PAHs and BaP were mainly found in the $PM_{2.5}$ fraction. Figures 9 and 10 show a decrease between 1995 and 2003 in the airborne concentrations of these contaminants in Prachatice. It will be particularly interesting to compare these findings with data from 2004. Since May 2004, when the Czech Republic was admitted to the EU, heavy truck traffic has increased by 30% on a highway near Teplice. The trucks on this road are powered by diesel engines, which are known to be a significant source of PAHs. It is speculated that such a dramatic increase in traffic will have caused a change in exposure patterns. Any changes in the outcome of health will be worth examining.

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CHAPTER 3

GENOTOXIC ACTIVITY OF AMBIENT AIR POLLUTION IN THREE EUROPEAN CITIES: PRAGUE, KOŠICE AND SOFIA

An 'in vitro' study

Alena Gábelová[1], Zuzana Valovičová[1], Blanka Binková[2], Radim J. Šrám[2], Raj Singh[3], Balvinder Kaur[3], Ivan Kalina[4], Todor A. Popov[5], Peter B. Farmer[3]

[1]Laboratory of Mutagenesis and Carcinogenesis, Cancer Research Institute, Vlárska 7, 833 91 Bratislava, Slovakia; [2]Laboratory of Genetic Ecotoxicology, Institute of Experimental Medicine, Vídeňská 1083, 140 20 Prague, Czech Republic; [3]Cancer Biomarkers and Prevention Group, Biocentre, University of Leicester, University Road, Leicester, United Kingdom; [4] Medical Faculty, University of P.J. Šafárik, Košice, Slovakia; [5]Medical Ecology and Nutrition, National Center of Hygiene, Sofia, Bulgaria

Abstract: Exposure of human hepatoma Hep G2 cells for two hours to extractable organic matter (EOM) adsorbed on respirable airborne particles <10 μm (PM_{10}) resulted in a linear dose-dependent increase in DNA damage ($p < 0.001$). There were clear location- and season-related differences in ambient air genotoxicity based on the amount of EOM associated with PM_{10} per unit volume of air (EOM μg/m^3). These data were correlated with the concentrations of benzo[a]pyrene, carcinogenic polycyclic aromatic hydrocarbons (PAHs) and total PAHs per cubic meter of air. No dose-dependent increase in the oxidative DNA damage (8-oxodG or M_1dG adducts) was detected in EOM-exposed cells.

Key words: air pollution, organic complex mixture, PM_{10}, genotoxicity, Hep G2 cells, single cell gel electrophoresis, LC-MS/MS, immunoslot blot assay, oxidative DNA damage.

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1. INTRODUCTION

It is well documented that increased exposure to respirable airborne particles <10 μm (PM_{10}) is associated with adverse respiratory and cardiovascular health effects as well as malignant lung diseases (Pope et al., 2002; Kelly, 2003). Ambient PM_{10} represents a complex mixture of anthropogenic and naturally occurring airborne particles. Anthropogenic emissions, including fossil fuel combustion and pyrolysis of organic materials and automobile exhausts, are believed to play a pivotal role in adverse health effects. Approximately three thousand chemicals including more than one hundred polycyclic aromatic hydrocarbons (PAHs) have been identified in ambient air (Lewtas and Gallagher, 1990). Many of these substances are known or suspected human mutagens and carcinogens (IARC, http://www.iarc.fr).

The mutagenicity and genotoxicity of organic complex mixtures of urban airborne particles have been demonstrated with different short-term tests using prokaryotic and eukaryotic organisms (Černá et al., 1999). However, only a limited number of studies have been conducted to assess the genotoxic potential of extractable organic matter (EOM) using human cells cultured *in vitro*. The mechanisms which are responsible for the adverse biological effects of air pollution are still unknown. It was recently suggested that the free radical-generating activity of airborne particles might underlie many of the adverse effects of PM_{10} (Donaldson et al., 2003). Enhanced production of reactive oxygen species (ROS) results in the induction of oxidative damage in cellular macromolecules such as lipids, proteins and DNA. The most prevalent and stable marker of base damage is the formation of 8-oxo-2′-deoxyguanosine (8-oxodG), which is believed to play a major role in mutagenesis and carcinogenesis. In addition, oxygen radical-induced lipid peroxidation generates a wide spectrum of reactive electrophiles that are capable of forming mutagenic DNA adducts (Niedernhofer et al., 2003). One of the most abundant adducts is the cyclic pyrimidopurinone adduct, M_1dG (Marnett, 2002).

Environmental air pollution is currently a matter of great interest because millions of people are chronically exposed to low doses of noxious chemicals, e.g., PAHs, that are common airborne contaminants. A European Community-funded study (Farmer et al., 2003) was initiated to assess the impact of air pollution on human health and to investigate the mechanisms underlying air pollution-induced adverse effects on biological systems. Part of this study included '*in vitro*' experiments.

The aim of the present study was: 1) to assess the genotoxic potential of organic complex mixtures extracted from PM_{10}, 2) to elucidate season- or location-related variability in the biological effects of individual EOMs due to variation in the crude organic mixture compositions and air pollutant concentration per cubic meter of air, 3) to estimate the capacity of the organic

complex mixtures to induce oxidative DNA damage, and 4) to determine the kinetics of DNA strand break rejoining after treatment.

2. MATERIAL AND METHODS

2.1 Cell line

The human hepatoma cell line Hep G2 was generously provided by Prof. Andrew R. Collins (University of Oslo, Norway). Hep G2 cells were maintained in William's modified medium supplemented with 10% fetal calf serum and antibiotics (penicillin 200 U/ml; streptomycin and kanamycin 100 μg/ml) in a humidified 5% CO_2 atmosphere.

2.2 Treatment of cells

Hep G2 cells (1.5×10^5) were grown for 48 h to semi-confluency, prior to exposure to mixtures or the positive control. The stock solutions of individual EOMs (50 mg/ml) were diluted in dimethyl sulfoxide (DMSO) immediately before use and added to serum-free medium to reach final concentrations ranging from 5 – 150 μg/ml. The concentration of DMSO never exceeded 0.5%. Control cells were exposed to 0.5% DMSO. After the cells were exposed to medium containing the treatment for two hours, the medium was removed and the cells were washed twice with PBS buffer. The cells were then either harvested and used for single cell gel electrophoresis (SCGE) or cultivated in fresh medium. The cells that were cultivated in fresh medium were harvested and processed at 2, 4, 16 or 24 h post-cultivation to measure the residual DNA strand breaks. Benzo[*a*]pyrene (BaP, 7.5 μM) was used as a positive control (internal standard) in each electrophoretic run.

2.3 Single cell gel electrophoresis (SCGE)

The single cell gel electrophoretic procedure of Singh et al. (1988), as modified by Collins et al. (1993) and published by Slameňová et al. (1997) was utilized in this research. The data from all independent experiments (at least 4) were pooled together and evaluated statistically by using the Student's *t*-test.

2.4 High performance liquid chromatography and tandem mass spectrometry (LC-MS/MS)

The purification of 8-oxodG from unmodified deoxynucleosides followed by high performance liquid chromatography and tandem mass spectrometry (LC-MS/MS) determination was conducted as described by Singh et al. (2003).

2.5 Immunoslot blot assay

The immunoslot blot assay was carried out as described previously (Singh et al., 2001). The level of the M_1dG in the DNA samples was determined from the calibration line generated by the dilution (with control DNA) of standard calf thymus DNA containing known amounts of M_1dG adduct (ranging from 0-10 fmol M_1dG per μg DNA) pipetted onto the same filter. The ChemiGenius2 Image Acquisition System (Syngene, Cambridge, UK) was used to capture a chemiluminescent image of the filter. The adduct level in each sample was corrected for the amount of DNA bound to the filter as determined by propidium iodide staining.

3. RESULTS

3.1 Particulate matter (PM_{10}) and EOM

The average seasonal concentrations of PM_{10}, EOM, BaP, carcinogenic PAHs (c-PAHs) and total PAHs (as analyzed by high performance liquid chromatography with fluorescence detection in crude extracts collected in individual localities during 3-month winter and summer sampling periods) have been already published (Farmer et al., 2003). Extractable organic matter from PM_{10} was at least 2-fold higher in winter than in summer, and c-PAHs were over 10-fold higher in winter air than in summer air. Location- and season-related variations in PAH concentrations in the crude extracts have already been published (Gábelová et al., 2004).

3.2 Genotoxicity of EOM

In general, a 1.5- to 4-fold increase in DNA strand breaks over the background level was determined in Hep G2 cells exposed to EOM for 2 hours using the SCGE. Nearly all EOM samples induced a statistically significant dose-dependent increase in DNA migration ($p < 0.05 – 0.001$;

r > 0.9). A slightly higher level of DNA damage was estimated for EOM samples from winter air in comparison with summer air; however, these differences were not statistically significant.

3.3 Ambient air genotoxicity

Substantial location- and season-related differences in air pollution genotoxicity, based on the amount of EOM associated with particulate matter per unit volume of air (EOM μg/m^3), were found (Fig. 1A, 1B). In general, the genotoxic potential of ambient air was higher during the winter than during the summer in all four monitoring sites. The samples with the highest genotoxicity were from Sofia, Bulgaria, during the winter and Prague (Smíchov district; PRG-SM), Czech Republic, during the summer sampling period.

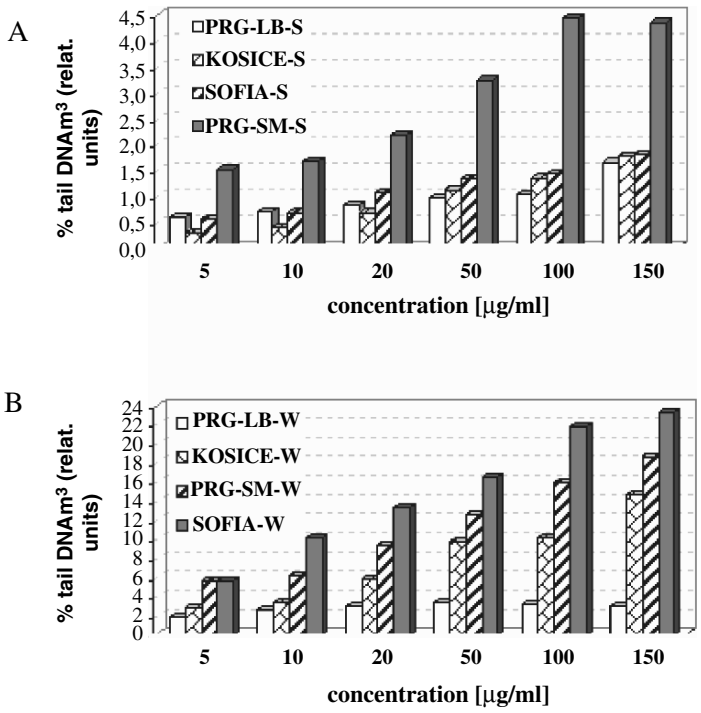

Figure 1. Ambient air pollution genotoxicity (% tail DNA/m^3) expressed in terms of EOM quantity per cubic meter air (EOM μg/m^3) in individual monitoring sites. **A:** summer (S); **B:** winter (W); localities: PRG-LB, Prague – Libuš area; PRG-SM, Prague – Smíchov district.

3.4 Oxidative DNA damage

Incubation of EOM-treated cells with the repair-specific DNA endonuclease, formamidopyrimidine DNA glycosylase (Fpg), did not result in any additional dose-dependent increase in DNA migration due to conversion of the oxidized purines to strand breaks (as determined by SCGE). A slight elevation of DNA strand breaks was found at some concentrations (approximately one dose per individual EOM sample).
The background, steady-state level of 8-oxodG that was estimated in control cells using LC-MS/MS was 59.6 per 10^6 nucleotides, while in EOM-treated cells the 8-oxodG levels ranged from 13.4 to 120.7 per 10^6 nucleotides. In most cases the number of 8-oxodG lesions determined in EOM-exposed cells was below the steady-state level.

In the DMSO control cells and in the BaP- and PRG-SM-treated cells, the M_1dG adduct level was below the limit of detection. In contrast, the M_1dG DNA-adduct number determined in Hep G2 cells treated with EOM samples from Prague – Libuš area (PRG-LB), Košice and Sofia collected during the winter and summer ranged from 14 to 92 per 10^8 nucleotides.

3.5 Kinetics of DNA strand rejoining

Reduction of DNA damage was either very slight or non-existent in Hep G2 cells 2 h after treatment in fresh medium. Although a significant decrease in DNA migration was determined 4 h after the treatment, approximately 60–80% of DNA strand breaks (sb) persisted in the treated cells. Sixteen hours post-cultivation in fresh medium, there was an 80 to 90% reduction in DNA damage in treated cells. Within 24 h after the treatment, the level of DNA sb had returned to near the background level.

4. CONCLUSIONS

This study confirmed the genotoxic properties of organic complex mixtures associated with respirable airborne particles collected in three European cities. No substantial season- or location-related variations in EOM genotoxicity (EOM μg/ml) were identified. However, when EOM concentration per cubic meter of air (EOM μg/m^3) was taken into account, there were appreciable differences in ambient air genotoxicity depending on location and season. These data were consistent with expected values based on the concentrations of BaP, carcinogenic PAHs, and total PAHs per cubic meter of air. The failure of *in vitro* systems to detect any substantial variation in EOM genotoxicity due to differences in the crude mixture

composition might be explained by the saturation of metabolic pathways needed to activate promutagenic agents.

No dose-dependent increase was detected in the number of 8-oxodG or M_1dG adducts in EOM-exposed cells. This suggests that these organic complex mixtures may play only a marginal role in oxidative stress and induction of oxidative damage in DNA. However, the organic compounds in these EOM samples may interact either additively or synergistically with other PM components, and in this way may enhance the adverse health effects of air pollution.

The DNA repair kinetics study showed that the removal of strand breaks induced by EOMs was relatively slow, taking around 24 h. However, it remains to be determined whether DNA damage induced by EOM was removed by the error-prone or error-free DNA repair mechanisms.

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CHAPTER 4

HUMAN EXPOSURE TO POLYHALOGENATED HYDROCARBONS AND INCIDENCE OF SELECTED MALIGNANCIES

Vladimír Bencko[1], Jiří Rameš[1], Martin van den Berg[2], Ivan Pleško[3], Tomáš Trnovec[4]

[1]*Charles University in Prague, First Faculty of Medicine & General University Hospital, Institute of Hygiene & Epidemiology, Prague, Czech Republic*
[2]*Institute for Risk Assessment Sciences, Utrecht University, Holland*
[3]*Slovak National Cancer Registry, Bratislava, Slovak Republic*
[4]*National Reference Centre for Dioxins and Related Compounds, Research Base of the Slovak Medical University, Institute of Preventive and Clinical Medicine, Slovak Medical University, Slovak Republic*

Abstract: Findings regarding the carcinogenicity of polyhalogenated hydrocarbons are inconclusive and even contradictory (Bencko 2003; Pavúk et al., 2004). This manuscript describes a preliminary study to analyze the incidence of selected malignancies in a population exposed to elevated concentrations of polychlorinated biphenyls (PCBs) by comparing data available in the Slovak National Cancer Registry database for Slovakia (~5 million inhabitants) to data for the population of Michalovce District, Slovakia (~55,000 inhabitants). The Michalovce District is recognized as one of the most heavily PCB-contaminated areas in the world. Data were analyzed for the 10-year period 1987–1996. The age adjusted world standard ratio (WSR) incidence of thyroid, pancreatic, breast, ovarian, bladder, and brain tumors in females and thyroid, pancreatic, breast, bladder, brain, prostate and testicular tumors in males were compared. Neither PCBs nor polychlorinated dibenzofurans (PCDFs) appear to contribute to the observed lower incidence of breast and prostate cancer in the Michalovce District. However, anti-estrogenic and anti-androgenic properties have been described for hydroxylated and methylsulfonyl PCB metabolites. These properties could contribute to a mechanism through which these metabolites influence the development of breast and prostate cancer. Further studies on the occurrence of these metabolites in exposed populations may clarify whether these PCB metabolites can modulate the incidence of breast and prostate cancer.

Key words: polyhalogenated hydrocarbons, anti-estrogenicity, anti-androgenicity, environmental cancer epidemiology, breast cancer, prostate cancer

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1. INTRODUCTION

A population-based cross sectional study (PCB Risk 5th FP) confirmed that the population of the Michalovce District in Eastern Slovakia was exposed to elevated polychlorinated biphenyl (PCB) levels due to the production of 21,500 metric tons of PCBs in a local chemical plant from 1959–1983 (Kočan et al., 2001; Trnovec et al., 2004). As such, the Michalovce District is recognized as one of the most heavily PCB-contaminated areas of the world. Due to the inconclusive and at times contradictory findings concerning the carcinogenicity of polyhalogenated hydrocarbons (Bencko 2003; Pavúk et al., 2004), this study was conducted to analyze the incidence of selected malignancies in the exposed population by comparing data from the Slovak National Cancer Registry database (established in 1975) for Slovakia (~5 million inhabitants) and the Michalovce District (~55,000 inhabitants). Data were analyzed for the ten-year period 1987–1996.

2. METHOD

The age-adjusted world standard ratio (WSR) incidence of malignant thyroid, pancreatic, breast, ovarian, bladder, and brain tumors in females and malignant thyroid, pancreatic, breast, bladder, brain, prostate and testicular tumors in males were compared for Slovakia and the Michalovce District. For the period 1987–1996, the study base for Slovakia was 50 M person-years and for Michalovce District was approximately 0.55 M person-years. Statistical significance was evaluated using two sample paired *t*-tests of the means in the STATISTICA 6.0 software.

3. RESULTS AND DISCUSSION

The WSR incidence at most cancer sties was not significantly different between the Michalovce District and all of Slovakia. For men, the incidence of malignant prostate tumors was significantly lower in the Michalovce District than in the rest of Slovakia during the period studied ($p = 0.03$; Table 1 and Figure 2). During this period, the WSR age-adjusted prostate cancer incidence per 100,000 individuals was 22.42 in Slovakia, but only 17.97 in the PCB-contaminated area of Michalovce. For women, the incidence of malignant breast tumors was also significantly lower in Michalovce than in Slovakia during the period studied (Table 2; Figure 1). The WSR age-adjusted breast cancer incidence values were 39.61/100,000 and 33.49/100,000 in Slovakia and Michalovce, respectively. This is noteworthy because previous studies have observed that the population in

Table 1. World standard ratio (WSR) age-adjusted incidence of selected malignancies in men in the exposed district (Michalovce) and Slovakia (per 100,000 individuals), during the period 1987 – 1996. (Adopted from reference Bencko et al., 2005)

	MICHALOVCE		SLOVAKIA				
CANCER SITE	**WSR**	**95% CI**	**WSR**	**95% CI**	**RR**	**t-test**	**p value**
PANCREAS	**8.78**	**± 4.38**	**10.15**	**± 0.40**	**0.87**	**–0.72**	**0.49**
BREAST	**0.38**	**± 0.58**	**0.46**	**± 0.09**	**0.83**	**–0.32**	**0.76**
PROSTATE	**17.97**	**± 3.76**	**22.42**	**± 1.13**	**0.80**	**–2.65**	**0.03**
TESTES	**6.08**	**± 2.85**	**5.44**	**± 0.58**	**1.12**	**0.48**	**0.65**
BLADDER	**12.34**	**± 3.55**	**14.44**	**± 0.43**	**0.85**	**–1.41**	**0.19**
BRAIN	**4.63**	**± 1.63**	**5.21**	**± 0.42**	**0.89**	**–0.78**	**0.45**
THYROID	**0.93**	**± 1.10**	**1.08**	**± 0.16**	**0.86**	**–0.31**	**0.77**

CI, confidence interval; RR, relative risk of WSR average values for this 10-year period; t-test, t-test value of WSR average values; p-value, p-value in t-test calculation of WSR average values.

Table 2. World standard ratio (WSR) age-adjusted incidence of selected malignancies per 100,000 individuals in women in the exposed district (Michalovce) and Slovakia, during the period 1987 – 1996. (Adopted from reference Bencko et al., 2005)

	MICHALOVCE		SLOVAKIA				
CANCER SITE	**WSR**	**95% CI**	**WSR**	**95% CI**	**RR**	**t-test**	**p value**
PANCREAS	**5.92**	**± 1.42**	**5.31**	**± 0.20**	**1.11**	**1.01**	**0.34**
BREAST	**33.49**	**± 5.53**	**39.61**	**± 2.22**	**0.85**	**–3.11**	**0.01**
OVARIUM	**9.73**	**± 3.04**	**10.81**	**± 0.27**	**0.90**	**–0.82**	**0.44**
BLADDER	**3.29**	**± 1.14**	**2.92**	**± 0.13**	**1.13**	**0.70**	**0.50**
BRAIN	**4.58**	**± 1.49**	**4.06**	**± 0.31**	**1.13**	**0.79**	**0.45**
THYROID	**4.95**	**± 2.83**	**3.12**	**± 0.38**	**1.59**	**1.52**	**0.16**

CI, confidence interval; RR, relative risk of WSR average values for this 10-year period; t-test, t-test value of WSR average values; p-value, p-value in t-test calculation of WSR average values

the Michalovce District was exposed to higher levels of PCBs than the rest of Slovakia (Kocan et al., 2001). The increasing trend in breast cancer in both populations was strikingly similar in this period.

During recent decades, the possible influence of PCBs on the development of breast tumors has been studied extensively. A few studies reported an association between PCB body burden and a higher incidence of breast cancer (Aronson et al., 2000; Guttes et al., 1998; Lucena et al., 2001), but the majority of the epidemiological studies did not find an association between PCB exposure and an increased incidence of breast cancer (Helzlsouer et al., 1999; Laden et al., 2002; Wolf et al., 2000; Zheng et al., 2000).

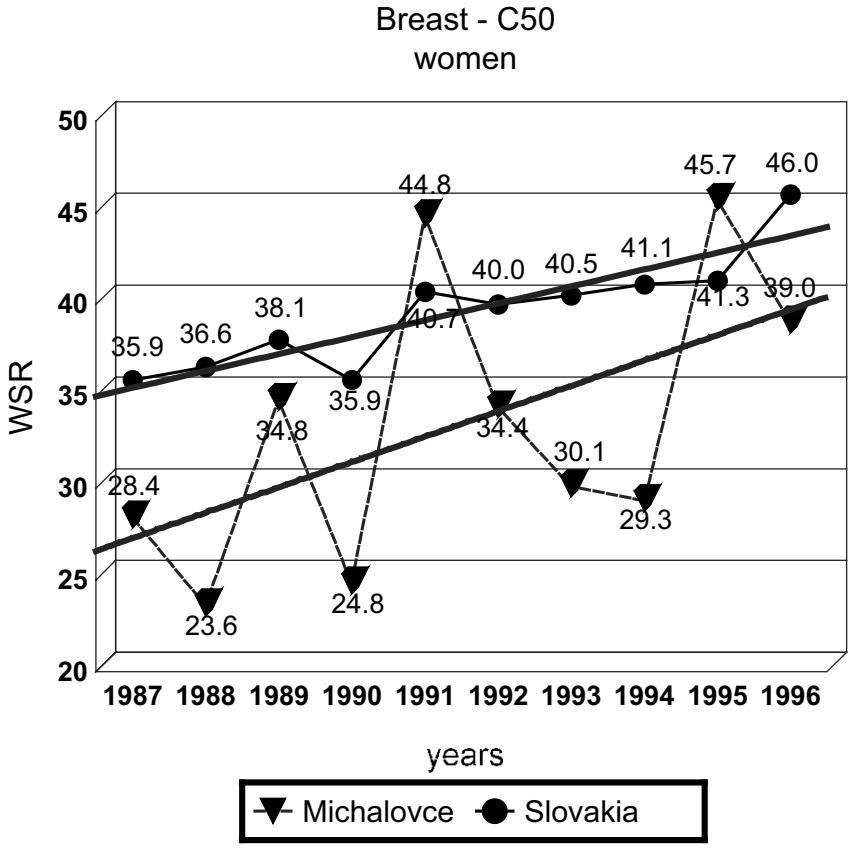

Figure 1. Linear regression of WSR values for breast tumors in women in Slovakia (solid line) and Michalovce District (dotted line).

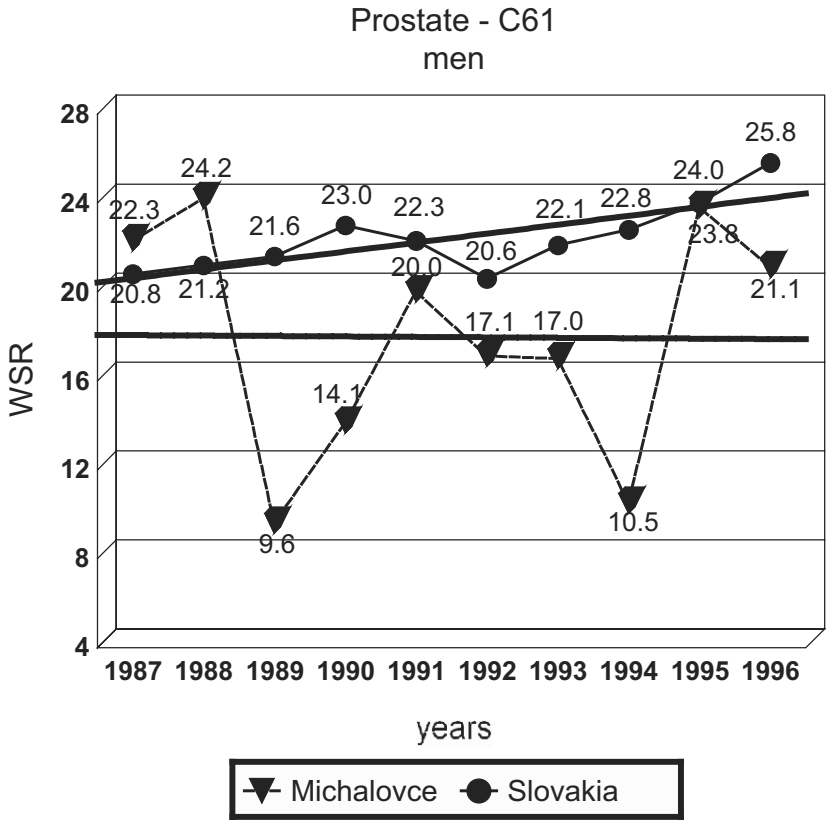

Figure 2. Linear regression of WSR values for prostate tumors in men in Slovakia (solid line) and Michalovce District (dotted line).

4. CONCLUSIONS

Laboratory studies have demonstrated that PCBs may induce a broad range of estrogenic and anti-estrogenic or androgenic effects (reviewed in Safe, 1994). A variety of factors including the dose and duration of exposure, and more importantly, the degree of chlorination will influence the nature and severity of effect. Mono-, di-, and tricholrinated biphenyls have very different affinities for biological receptors in comparison to PCBs with six or more chlorines. It is unclear if environmental agents have contributed to the lower incidence of breast or prostate cancer observed in the Michalovce district. The anti-estrogenic properties of hydroxylated and methylsulfonyl PCB metabolites may help explain the lower incidence of breast tumors in the Michalovce District. Further studies on the concentration and distribution

of these metabolites in exposed populations would provide valuable information regarding the potential for these PCB metabolites to be anti-estrogenic and/or anti-androgenic in humans.

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CORRESPONDING AUTHOR
Prof. Vladimír Bencko, M.D., Ph.D.,
Institute of Hygiene & Epidemiology, 1st Faculty of Medicine,
Charles University in Prague,
Studničkova 7, CZ 128 00 Praha 2, Czech Republic.

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CHAPTER 5

NON-MELANOMA SKIN CANCER INCIDENCE IN RELATION TO ARSENIC EXPOSURE

20 years of observation

Vladimír Bencko[1,2], Eleonora Fabiánová[3], Petr Franěk[1], Miloslav Götzl[4], Jiří Rameš[1,2]

[1]Charles University in Prague, First Faculty of Medicine & General University Hospital, Institute of Hygiene & Epidemiology, Prague, Czech Republic; [2]European Centre for Medical Informatics, Statistics and Epidemiology- Cardio, Prague, Czech Republic; [3]Institute of Health, Banská Bystrica, Slovak Republic; [4]Department of Oncology, District Hospital of Bojnice, Slovak Republic

Abstract: The subject of this analysis was a database of 1,503 non-melanoma skin cancer cases (756 in men and 747 in women) collected from 1977 to 1996 in a region in central Slovakia contaminated by emissions from a power plant that burned coal with a high arsenic content (ranging between 900 to 1,500 g per metric ton of dry coal). Exposure assessment of the non-occupationally exposed population was accomplished by analyzing hair and urine samples from groups of 10 year old boys living at different locations up to 30 km from the power plant. Basic epidemiological data of the cancer cases were obtained in a questionnaire about personal information and family, residential and occupational history. Our study base represented 1,335 thousands men/year and 1,337 thousands women/year for the 20 year study of a population of approximate size of 125,000 inhabitants. The age-standardized incidence of non-melanoma skin cancer (each confirmed by histological examination) ranged from 43.7 to 92.0 in men and from 34.6 to 79.1 in women calculated for four 5-year periods during the study). Analysis of our database demonstrates a positive correlation between human cumulative arsenic exposure and non-melanoma skin cancer incidence.

Key words: cancer epidemiology, biological monitoring, arsenic toxicity, non-melanoma skin cancer incidence, lung cancer incidence

1. INTRODUCTION

The trace element content of coal shows marked geographic variations (Thornton and Farago, 1997). A previous study by this research group

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(Bencko, 1997; Bencko et al., 2001) examined the adverse ecological and human health effects of environmental contamination due to emissions from a power plant in the central Slovakian district of Prievidza. This power plant had been burning coal with a high arsenic content (900-1,500 g/metric ton dry weight) since the mid-1950s. At the time of their recruitment, all human subjects included in that study had been living and/or working in the area surrounding the power plant. These subjects were followed longitudinally over a 20-year period to examine the trend in the incidence of non-melanoma skin cancer (NMSC), a type of cancer frequently associated with exposure to arsenic (ATSDR, 2000; WHO, 2000; Pleško et al., 2000). Our database of 1,503 NMSC cases (756 in men and 747 in women) was collected from 1977 through 1996. This publication summarizes the data that were gathered during this 20-year follow-up period.

2. MATERIALS & METHODS

2.1 Study base

During the study period, a population-based survey was conducted in the central Slovakian district of Prievidza. The aim of the survey was to study the trend in the incidence of all types of malignant diseases. A local cancer registry was also created for the entire administrative region with a population of about 125,000. During the study period, the actual size of the population in the Prievidza district remained more or less stable as was evident by several censuses.

As part of the study, any patient diagnosed or suspected to have a malignant lesion was referred to the district oncologist for final diagnosis and treatment. In all subjects, the diagnosis of cancer was confirmed by histological examination of tissue samples obtained by biopsy or at autopsy. Structured questionnaires were used for collecting data pertaining to the subjects' personal information, and residential, family and occupational histories. The data thus obtained, were stored in a central database.

Our study base represented 1,335 thousands men/year and 1,337 thousands women/year for the 20-year study of a population of approximate size of 125,000 inhabitants.

2.2 Statistics

The size of the population suspected to be at risk for residential exposure to arsenic was estimated (Breslow and Day, 1987) using census data, and the estimated figures are similar to those released by the Slovak Statistical Bureau.

To estimate the demographic data, the known sizes of the *i*-th age group of population *A* (obtained from census data) were denoted A_i^{1970}, A_i^{1980} and A_i^{1991}. Partially linear estimations of person-years during the time periods of interest were calculated.

The age-adjusted incidence rates (AIR) in different groups were evaluated using direct-standardisation methodology. The world standard population was used as a standard population. The study subjects were stratified into 10-year age groups (0-9, 60-69, 70 years old and older).

The ratio of rates (RR) was evaluated for two particular groups and the 95% confidence interval was calculated according to the methods of (Breslow and Day (1987) and Boyle and Parkin (1991). Both direct and indirect standardisations were used to cross-verify the results (Kahn, 1989). The statistical interpretations remained the same. The Microsoft Access 2000 and Microsoft Excel 2000 software were used for all statistical analyses.

2.3 Exposure assessment

Exposure assessment of the local non-occupationally exposed general population of the district was based on biological monitoring of arsenic in hair and urine samples obtained from groups of between 20 and 25 ten-year old boys from different localities situated up to 30 km from the power plant. The district of exposure was divided into two areas marked off by a 7.5 km circle around the power plant. The criteria for higher exposure included a mean arsenic concentration of $> 3\mu g/g$ of hair. Close to 20% of the study subjects lived within a 7.5 km radius of the power plant (i.e., exposed area). The individuals who lived between 7.5 and 30 km from the plant served as a "control" population.

Hair is a readily obtainable biological specimen for determining arsenic exposure. Our data lend further credence to the idea of using hair arsenic concentrations for monitoring population exposures to arsenic. As the levels of arsenic in various biological specimens show marked individual variations, a group-wise comparison of arsenic levels proved to be more meaningful (Bencko, 1995). The levels of arsenic in urine may be used to provide a more recent exposure by measurement of the amount of arsenic that an individual has inhaled or ingested recently.

Although not universally accepted, an arsenic level of $> 3\mu$ g/g of hair should be considered abnormally high, while values up to 0.2μ g/g may be considered acceptable levels of exposure to arsenic (Bencko, 1995; WHO, 2000).

3. RESULTS

The primary objective of the present study was to examine whether environmental pollution due to arsenic had any effect on the incidence of NMSC. The age-adjusted incidence of histologically-confirmed NMSC in non-occupational settings ranged between 43.7 to 92.0 per 100,000 males and 34.6 and 79.1 per 100,000 females. The data presented herein showed that over the first ten-year period, there was a dramatic increase in the incidence of NMSC in the most contaminated region of Prievidza district (Tables 1-2). This upward trend was gradually reversed, and the incidence of NMSC declined during the next five-year period. In our opinion, this downward trend in the incidence of NMSC is most likely attributed to the measures taken to reduce the levels of arsenic emissions from the plant. We strongly feel that the downward trend in the incidence of non-melanoma skin cancer following reduction in the arsenic emissions from the power plant may suggest a dose-effect relationship between the degree of environmental pollution due to arsenic and NMSC incidence. The biological plausibility of such a notion is understandable, considering the fact that arsenic is a known inducer of p53 mutations in basal cells (Seidl et al., 2001).

Table 1. Non-melanoma skin cancer incidence in population living in the vicinity of the power-plant burning the coal with high arsenic content and in the rest of the district (Male). (Bencko et al., 2005)

	1977-1981		1982-1986		1987-1991		1992-1996	
	EXP Cases (p-years)	REF Cases (p-years)	EXP Cases (p-years)	REF Cases (p-years)	EXP Cases (p-years)	REF Cases (p-years)	EXP Cases (p-years)	REF Cases (p-years)
Absolute number	44	125	32	134	30	142	27	222
Expected number	23.8		20.8		18.6		24.7	
Non-standardized rate	98.4	45.8	77.6	46.5	81.9	46.9	78.2	70.9
Age standardized rate	93.9	45.9	66.9	45.9	65.2	46.0	57.8	66.5
Person-years	(44 730)	(273 205)	(41 249)	(288 368)	(36 649)	(303 029)	(34 507)	(313 087)

Statistical parameters (Confidence interval (p = 0.05))

		Limit values		Limit values		Limit values		Limit values
RR	2.05	1.45 - 2.9	1.46	0.99 - 2.2	1.42	0.95 - 2.1	0.87	0.58 - 1.30
Mantel-Haensz. estimate	2.02	1.43 - 2.8	1.46	0.99 - 2.2	1.39	0.94 - 2.1	0.87	0.58 - 1.30
Chi - square	16.62		3.70		2.67		0.46	
Probability	<0.01	S	0.05	S	0.10	NS	0.50	NS

Table 2. Non-melanoma skin cancer incidence in population living in the vicinity of the power-plant burning the coal with high arsenic content and in the rest of the district (Female). (Bencko et al., 2005)

	1977-1981		1982-1986		1987-1991		1992-1996	
	EXP Cases (p-years)	REF Cases (p-years)	EXP Cases (p-years)	REF Cases (p-years)	EXP Cases (p-years)	REF Cases (p-years)	EXP Cases (p-years)	REF Cases (p-years)
Absolute number	46	118	32	134	22	165	25	205
Expected number	22.7		20.1		20.1		23.4	
Non-standardized rate	104.9	43.3	78.3	46.3	59.7	53.9	70.9	64.0
Age standardized rate	81.4	34.6	54.4	37.4	39.8	42.7	37.5	47.1
Person-years	(43 869)	(272 729)	(40 869)	(289 593)	(36 870)	(306 319)	(35 263)	(311 304)

Statistical parameters (Confidence interval (p = 0.05))

		Limit values		Limit values		Limit values		Limit values
RR	2.35	1.67 - 3.3	1.45	0.98 - 2.2	0.93	0.59 - 1.5	0.80	0.52 - 1.2
Mantel-Haensz. estimate	2.25	1.60 - 3.2	1.47	1.00 - 2.2	0.88	0.57 - 1.4	0.89	0.59 - 1.4
Chi - square	22.81		3.84		0.29		0.31	
Probability	<0.01	S	0.05	S	0.59	NS	0.58	NS

4. DISCUSSION

The age-standardized rate of NMSC appeared to exhibit an upward trend in the reference area during the last five years of the study. The individuals living in this area may have been exposed to low levels of arsenic over a prolonged period of time. These data suggest that exposure to low levels arsenic may have a cumulative effect on the incidence of non-melanoma skin cancer over time.

We did not observe any sex-linked bias in the incidence of NMSC in our study population. Current studies are focused on the long-term effects of arsenic exposure on human health for those having been exposed in occupational settings before the improvements to industrial safety measures in the 1960s and 1970s (Buchancová et al., 1998; Fabiánová et al., 2000).

The history of smoking habits was carefully evaluated for all study subjects. This provided us an opportunity to examine the potential between arsenic exposure and cigarette smoking in the induction of malignant lesions in different areas of the body (Welch, 1982; Jarup and Pershagen, 1991; Hertz-Picciotto et al., 1992). We have also considered the smoking habit of the general population (CINDY, 1990) and its relevance to the population we studied. Until now, we have not found any reliable statistical tool to perform relevant analysis that would enable us to assess whether a combination of

arsenic and smoking has been the cause of cancer in any of the cases in our lung cancer database. Part of the dilemma in this situation is that "soft" data (CINDY) which covers only a limited amount of the period of time being studied is being compared with the carefully registered and specific data in our database.

4.1 Confounding factors

Although debatable, cigarette smoking is not considered to be an important risk factor for NMSC (Nieuwenhuijsen et al., 2001; Pesch et al., 2002). The possibility that the study cohorts differed in terms of the exposure to ultraviolet radiation (Rossman, 1999; Seidl et al., 2001) is considered extremely unlikely since the study populations in both the more polluted, as well as the less polluted areas of the Prievidza district, comprise almost equal proportions of villagers and city dwellers.

5. CONCLUSION

There does not appear to have been an appreciable difference between the exposed and reference populations in the extent to which they were exposed to ultraviolet light. As such, this potential confounding variable does not appear to have influenced the demonstrated differences in incidence of NMSC in either of these populations. Our data demonstrates a positive correlation between human cumulative exposure to arsenic and incidence of NMSC. This adds further confirmation to the long-held clinical and epidemiological findings corroborating an association between non-melanoma skin cancer incidence and exposure to arsenic.

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CHAPTER 6

ECOCYTOGENETICS AS A BIOMONITORING MODEL FOR OCCUPATIONAL EXPOSURE

Aleksandra Fučić[1], Ariana Znaor[2], Ana Marija Jazbec[3], Miljenko Sedlar[2]
[1]Institute for Medical Research and Occupational Health, Zagreb, Croatia; [2]National Institute for Public Health, Zagreb, Croatia;[3] Faculty of Forestry, Zagreb, Croatia

Abstract: Biomonitoring has become a potent tool for preventive medical care and occupational health policy. Recent epidemiological results confirm the utility of chromosome aberrations as a biomarker for cancer risk. The present study included 1,200 subjects exposed to physical and chemical agents. As human health genotoxicity studies are time consuming and expensive, it is necessary to select populations with the highest health risk to be studied for biomonitoring. Our results suggest genotoxicological analyses should be performed for subjects exposed to the chemicals listed by the International Agency for Research on Cancer (IARC) as Group 1 carcinogens, radioisotopes, individuals that have been simultaneously exposed to ionizing radiation and ultrasound, and a subgroup of nuclear plant workers. The input of a new genotoxicological monitoring model, termed "cocytogenetics", is discussed.

Keywords: Chromosome aberration assay, Sister chromatid exchange frequency, Micronucleus assay, Occupational exposure, Ionizing radiation, Ultrasound, Policy, Cancer.

1. INTRODUCTION

During the last three decades, chromosome aberrations (CA) and sister chromatid exchange frequency (SCE) have been used in the biomonitoring of occupationally and environmentally exposed subjects. In the 1980s, the *in vitro* micronucleus assay (MN) was introduced, and this has also become a reliable method for detecting agents that preferentially damage cells by aneugenic

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mechanisms. Cytogenetic biomonitoring has the potential to detect damage due to complex radiochemical exposures that are of great interest in the present environmental and working settings. The literature regarding cytogenetic biomonitoring has confirmed the utility of CA as a predictor of increased cancer risk (Bonassi, 1995; 2004), while other genotoxicological methods are still being evaluated (Znaor, 2003).

In the Republic of Croatia, there has been systematic cytogenic follow up since the 1980s of subjects who have been occupationally exposed to ionizing radiation and selected chemical agents. In the present study, CA, SCE and micronuclei were measured in 1,200 subjects who were occupationally exposed to radioactive material or selected chemicals. The goal of the study was to determine which occupational exposures were associated with a significantly greater frequency of CA, SCE or micronuclei when compared to the control population, and to identify whether cytogenetic biomonitoring should be incorporated into regular health survillence of individuals in certain occupations.

2. SUBJECTS AND METHODS

Cytogenetic monitoring was performed by analyzing chromosome aberrations, sister chromatid exchanges and micronuclei in 1,200 subjects who were occupationally exposed to ionizing radiation, ultrasound, vinyl chloride monomer (VCM), ethylene oxide, formaldehyde, radioisotopes, or tobacco over a 14-year period. The control group consisted of 91 subjects not exposed to any known mutagens in the workplace or at home. Both men and women were included in the exposed and control groups. Ionizing radiation was monitored by film dosimeters. None of the subjects included in the study were exposed to more than 50 millisieverts (mSv) per year. The concentration of VCM was measured by gas chromatography, and exposures ranged between 1-200 ppm.

In the study, 542 subjects were occupationally exposed to X-rays, 63 subjects were exposed to antineoplastic drugs, 115 subjects were employed in jobs involving non-destructive testing (gamma radiation and ultrasound) in industry, 32 subjects were exposed to X-rays and ultrasound in the field of medicine, 134 subjects were exposed to radioisotopes and 36 subjects were maintenance workers in nuclear plants.

Samples of 0.5 ml of whole heparinized blood were added to 8 ml of F-10 medium (GIBCO) containing 20% calf serum (Biologial Industries). Lymphocytes were stimulated by phytoheamgglutinin (Murex) and incubated at 37^{o}C for 48 h. Colchicine (Sigma) was added after 45 h. The cultures were fixed and the slides prepared according to the International

Atomic Energy Agency method (IAEA, 1986). For the chromosome aberration assay, 200 well spread metaphases were analysed.

To determine SCE frequency, bromodeoxyuridine (Sigma) was added to produce a final concentration of 10 μg/ml. The cultures were harvested after 72h. One hundred second-division cells were scored according to the method of Perry and Wolff (1974).

For the analysis of MN frequency, cytochalasin B (Sigma) was added to lymphocyte cultures in the final concentration of 3 μg/ml 44h after the initiation of cultures. The slides were prepared according to the method of Fenech and Morley (1985). One thousand binucleated lymphocytes per subject were analyzed.

The data were analyzed using Poisson regression. The control group served as the baseline. Poisson regression was conducted for each type of chromosome aberration. All analyses were performed using the statistical software SAS 6.12.

3. RESULTS

Among the studied groups exposed to radiation, the highest frequency of dicentric and ring chromosomes (0.38%) was detected in subjects with a temporary assignment in the nuclear plant. Another group with a high deviation from the control values was the group of industrial radiographers exposed to gamma rays and ultrasound (Table 1). These groups showed a significant increase in chromosome breaks and fragments, and dicentric and

Table 1. Chromsome aberrations in groups occupationally exposed to different physical and chemical agents (bold denotes significant difference from control at $p < 0.05$).

Agent	Chromatid breaks (%)	Chromosome breaks + fragments (%)	Dicentric + ring chromosomes (%)
Gamma rays	*2.5*	*1.2*	*0.16*
Gamma rays + US*	*3.2*	*1.4*	*0.22*
X-rays	*2.1*	*1.2*	*0.12*
X-rays + US	1.8	1.1	*0.15*
Nuclear plant	1.9	*1.6*	*0.38*
Radioisotopes	1.9	*1.1*	*0.23*
Vinyl chloride monomer	*2.5*	*1.4*	*0.15*
Tobacco factory	1.7	*1.8*	*0.11*
Antineoplastic drugs	*2.3*	*1.1*	*0.15*
Control	1.5	0.6	0.01

*US = Ultrasound

ring chromosomes. Among subjects exposed to chemical agents, a significant increase in all categories of chromosome aberrations was seen in the subjects that were exposed to vinyl chloride monomer and antineoplastic drugs.

Among subjects exposed to radiation, the highest deviations from the control value were detected in the group exposed to gamma rays and ultrasound, while among subjects exposed to chemicals, the highest deviations were detected in subjects exposed to the vinyl chloride monomer (Table 2). The % micronuclei frequency was significantly higher in the group exposed to VCM than in the control group. The VCM-exposed group also had a significantly higher SCE frequency than the control group (Table 3).

Table 2. Distribution of Micronuclei frequency by physical and chemical agents (bold denotes significant difference from control at $p < 0.05$).

	Percent of Cells with micronuclei	Percent of Cells with 1, 2, 3, or 4 micronuclei per binucleated lymphocyte			
Agent	Total	1	2	3	4
Gamma rays + US	*7.5*	*6.6*	*0.87*	*0.08*	0.0
Gamma rays	*3.8*	*3.5*	0.28	*0.03*	0.0
X-rays	1.7	1.5	0.21	*0.06*	0.0
X-rays + US	*3.8*	*3.4*	*0.38*	*0.07*	0.0
Vinyl chloride monomer	*12.1*	*9.4*	1.5	*0.4*	0.07
Antineoplastic drugs	3.9	*3.6*	0.26	*0.02*	0.0
Control	1.3	1.1	0.2	0.0	0.0

*There were no cells in any of the exposure groups with 5 or 6 micronuclei per binucleated lymphocyte.

Table 3. Sister chromatid exchange frequency and range for different chemical agents (bold denotes significant difference from control at $p < 0.05$).

Agent	SCE/cell	Range/cell
Vinyl chloride monomer	*9.2*	4-27
NDT (solvents, dye)	5.6	2-18
Antineoplastic drugs	5.6	0-21
Control	5.9	0-7

4. DISCUSSION

Accumulation of genome damage occurs as a consequence of the natural process of aging (Lucas, 1999), or by the action of natural or anthropogenic physical or chemical agents. Clastogenic and aneugenic events lead to changes in chromosome number and/or structure. Accumulation of genome damage occurs as a consequence of natural process of aging (Lucas, 1999) or by action of natural or antropogenic physical and chemical agents. Clastogenic and aneugenic agents lead to changes in chromosome number and/or structure. Similar chromosomal abnormalities are present in almost all types of tumor cells. The correlation between genome damage and malignancy was described at the beginning of the last century (Boveri, 1902). The introduction of methods such as the chromosome aberrations assay, micronucleus assay, sister chromatid exchange frequency assay, comet assay and fluorescent in situ hybridization, have facilitated research in genetic toxicology, biodosimetry, and investigation of the correlation between the results from these methods and cancer incidence.

This study has evaluated genetic damage in subjects who were occupationally exposed to radiation and chemical agents. Methods were utilized to detect both clastogenic and aneugenic events. The groups that were occupationally exposed to gamma rays in nuclear plants and a combination of gamma rays and ultrasound in industrial radiography and medicine had the highest levels of genetic damage, even if the exposures were within recommended exposure limits. The group exposed to radioisotopes in medicine also showed significantly more genetic damage than the control group. Assessing the effects of chemical agents was much more complex because personal dosimeters for chemical agents were not available, or were too expensive. The group exposed to VCM had significantly higher CA, MN and SCE than the control group. In most cases, it appeared that CA and MN frequency were related, except where MN frequency was higher than CA. This suggests aneugenic action by the analyzed agent.

To conclude, cytogenetic monitoring should be included in the medical surveillance of subjects who have been exposed to chemicals classified by the International Agency for Research on Cancer (IARC) as group 1 carcinogens. This medical surveillance should be provided for individuals exposed to a combination of ionizing radiation and ultrasound, individuals exposed to radioisotopes, and individuals involved in selected activities during nuclear plant maintenance. Although the results of this study showed increased genetic damage in subjects occupationally exposed to X-rays, further research is necessary to identify specific occupations with a higher risk of genetic damage.

Methods from clinical cytogenetics and genetic toxicology may be used to help identify the etiology of malignancies that develop due to environmental exposures, and could help support the implementation of preventive measures in exposed populations. A new field of study, which could be termed "ecocytogenetics", should be developed. The goal of this field would be to identify individuals at risk for specific cancers prior to the appearance of clinical symptoms. This would be accomplished by identifying the association between specific environmental agents, neoplasias, and particular types of genetic damage to certain chromosomes (using fluorescent in situ hybriddization and proteomics). Ecocytogenetics would be used to detect clones and to suggest additional markers of subclinical effects that could be monitored in occupationally or environmentally exposed individuals. Experts from different disciplines would collaborate, facilitating the identification of links between the cancer markers that are identified in clinical cytogenetics, and the frequency of specific types of chromosome damage that are detected when individuals with specific environmental or occupational exposures are monitored. "Risk profiles" (Bartsch, 2000) could be established for individuals, and the consequences of specific exposures could be identified, taking into account interindividual variability.

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CHAPTER 7

CYTOGENETIC DAMAGE DETECTED IN LYMPHOCYTES OF DONORS FROM MAŁOPOLSKA REGION IN POLAND AND CANCER INCIDENCE IN THE FOLLOW-UP STUDIES

Antonina Cebulska-Wasilewska[1,2], Jadwiga Rachtan[3], Zofia Rudek[1], Zbigniew Drąg[4]

[1]Environmental and Radiation Biology Department, The H. Niewodniczański Institute of Nuclear Physics PAN, Radzikowskiego 152, 31-342 Kraków, Poland; [2]Chair of Epidemiology and Preventive Medicine, CM – Jagiellonian University, Kraków, Poland; [3]Cancer Epidemiology Department, Centre of Oncology, Maria Skłodowska-Curie Memorial Institute, Kraków Branch, Kraków, Poland; [4]Institute of Sociology, Jagiellonian University, Kraków, Poland

Abstract: There is evidence to support an association between an increased frequency of chromosomal aberrations and increased cancer incidence. The aim of this study was to determine whether the frequency of chromosome aberrations (CSA), sister chromatid exchanges (SCE) or micronuclei (MN) in peripheral blood lymphocytes in humans showed any association with cancer incidence. The study utilized data on CSA, SCE and MN collected between the years 1981 and 2001 in exposed individuals and matched controls (a total of 455 individuals). At the time of sampling, all study subjects were healthy (with no apparent symptoms of any known acute or chronic diseases). A random-effects model was used to take into account variability among laboratories in procedures, time periods and scorers. The results, obtained in a subgroup with a longer period of follow up observation, showed an increasing cancer incidence with increased level of chromosomal damage. A weaker association was observed for a subgroup for which the follow up period was appreciably shorter, and the number of reported cancer cases lower. The results, although preliminary, show an apparent association between increased cytogenetic damage detected in the lymphocytes of smokers and growing risk of cancer incidence during the latency period.

Key words: chromosome aberrations, occupational exposure, follow-up studies, cancer risk

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1. INTRODUCTION

The conceptual basis for using cytogenetic biomarkers in peripheral blood lymphocytes (PBL) has been the hypothesis that the extent of genetic damage in PBL reflects a probability of similar events in the precursor cells for carcinogenic processes in the target tissues. Epidemiological data regarding the association between chromosome aberrations and cancer incidence has recently become available. Liou et al. [1] suggested a significant association of the biomarkers with adverse health outcome. Gibson et al. [2] reported an increase in the incidence of lung cancer among workers of a steel foundry in Canada, while Bender et al. [3] detected an increase in chromosomal damage in coke oven workers. The association between chromosome aberration frequency and increased cancer incidence was originally detected in a collaborative project between ten North-European cytogenetic laboratories [4, 5] and an independent study performed in Italy on cancer mortality data, which confirmed those findings [6]. Recently Smerhovsky et al. [7] found a significant and strong association between the frequency of chromosomal aberrations and cancer incidence in a group of miners exposed to radon, where a 1% increase in frequency of chromosomal aberrations was followed by a 64% increase in risk of cancer ($p < 0.001$). However, this study did not confirm the association between an increased frequency of chromosome aberrations and radon exposure [7]. One study from Poland, although conducted with a much smaller group (60 study subjects), did suggest a significant association between exposure, cytogenetic damage and cancer [8].

The aim of this study was to determine whether data on chromosome aberrations that were collected between 1981 and 2001 showed any relation to cancer incidence in the same group as evaluated during the follow up observation. A random-effects model was used to take into account variability among various procedures, time periods and scorers.

2. MATERIALS AND METHODS

2.1 Population study

The analysis includes a group of donors from the Malopolska region for whom results of the cytogenetic studies have already been published. The study base consists of observations carried out in the groups of individuals exposed and controls examined for the presence in peripheral blood lymphocytes of chromosome aberrations, sister chromatid exchanges (SCE) or micronuclei (MN). The examination was performed in two cytogenetic

laboratories belonging to different scientific institutions in Kraków, Poland. The older studies were performed at the Institute of Systematic and Experimental Zoology, Polish Academy of Sciences [9-12]. More recent studies were done at the Department of Radiation and Environmental Biology, at the Institute of Nuclear Physics (now a part of the Polish Academy of Sciences) [13-22].

All subjects participated in the studies voluntarily. All participants taken into the recent studies were men living in Kraków or in its vicinity in the Małopolska region of Southern Poland. At the time of sampling, the majority of them considered themselves healthy (with no apparent symptoms of any known acute or chronic diseases). Subjects were originally selected for cytogenetic analysis because of their variability in response to occupational exposures to mutagens or potential carcinogens or as unexposed matched controls. Investigated groups included workers of the rolling-mill division in a metallurgical plant and workers of the coke chemistry department, both departments belonged to the largest industrial plant in Poland (the Lenin Steel Works, Kraków, Nowa Huta, recently known as the Sendzimir Steel Works, Poland). The other investigated groups were composed of petroleum industry workers, glasshouses owners, workers exposed occupationally to pesticides, and workers exposed to mercury vapours from a chlorine production department in a plant using mercury electrodes in electrophoresis. Study subjects who were occupationally exposed to mercury vapours were working in an area that was monitored for occupational exposure. The concentration of metallic mercury in the air was measured in fixed samples from two different areas of the workplace. The time-weighted average of metallic mercury concentration was 0.025 mg/m^3 (while the maximum permissible concentration of metallic mercury is also 0.025 mg/m^3). The internal dose was also periodically monitored for workers at that department. The mercury content in urine and blood was determined by use of atomic absorption spectrometer [23]. The detection limit for determination of mercury in urine was 10 μg/l, and in blood was 4 μg/l. The mean mercury concentration in urine was 108.70 ± 42.19 μg/l (range 32.8 – 172.88) and in blood was 19.05 ± 7.56 μg/l (range 4.7 – 29.6).

Controls were selected from inhabitants of Kraków and its vicinity, or were small field farmers around the Kraków area. These individuals were interviewed using the same questionnaire as was administered to exposed subjects. The inclusion and exclusion criteria for the controls were the same as for the exposed individuals, except that the controls were unaware of any prior occupational exposure to genotoxic agents. The controls were matched with the exposed workers on the basis of gender, age (± 5 years) and cigarette smoking habits. The majority of the control group consisted of skilled state workers, students, teachers or clerks with relatively similar socio-economic status.

2.2 Blood culturing and cytogenetic screening

The culturing method was based on procedures that are commonly used by radiological protection laboratories for estimating a radiation dose absorbed by subjects involved in radiation accidents [24-26]. Fresh venous blood samples were collected in heparinized tubes and were incubated at 37° C using RPMI 1640 medium supplemented with 20% fetal calf serum, anti-biotics, and a small amount of BrdU. Lymphocytes were stimulated with phytohemagglutinin PHA. Two hours before the end of culturing, 0.1 µ l/ml of colcemid solution was added to each culture. Then, cells were fixed and stained with fluorescence plus Giemsa solution for chromosome preparations following a standard procedure described elsewhere [26, 27]. Only cells with identically dark-stained chromatids showing first mitosis were analyzed for the presence of chromatid and chromosomal aberrations [28, 29].

The frequency of chromosome aberrations was screened in 100–500 good metaphase spreads. All types of chromosome damage were noted including gaps, breaks in chromosomes and chromatids, fragments and chromatid exchanges. For analysis of the association between cytogenetic damage detected in lymphocytes and cancer incidence in the follow up study, the frequency of chromosome (CSA) and chromatid (CTA) aberrations were analysed separately.

2.3 Sister Chromatid Exchanges (SCE)

Lymphocytes were cultured for 72 hours in the same culture medium that was used for the chromosome aberration cultures (with 8 µg/ml BrdU). Differential staining of chromatids was carried out according to a modified procedure of Wolf [26]. Air dried slides were treated with Hoechst 33258 solution immersed horizontally in distilled water and then irradiated with the sun, or UV lamp for 25 min from a distance of 25 cm. Fifty metaphases per examined individual were analyzed in order to determine SCE frequency.

2.4 Lymphocyte cultures for screening of binucleated cells and micronuclei (BNMN)

Cultures were set up according to standard cytogenetic procedure. Cells were stimulated by 1% of phytohaemagglutinin and incubated for 72 h at 37ºC. Cytochalasin-B at a final concentration of 6 µ g/ml [11, 20] was added to the cultures 44 h later to arrest cytokinesis. At 72 h of incubation, the cultures were harvested by centrifugation. A fixation procedure was repeated 3 times and the resulting cells were re-suspended in a small volume of fixa-tive solution and dropped onto clean slides. The slides were stained with 10% Giemsa in phosphate buffer (pH 6.8) for 10 min. To determine the frequency

of BNMN and the total number of MN in lymphocytes (MNL), a total of 1000 binucleated cells with well preserved cytoplasm (500 per replicate) were scored per subject on coded slides.

2.5 Chromosome aberrations screening

The frequency of aberrant lymphocytes was treated as a continuous variable. To account for possible differences in the mechanism of induction of chromosome aberrations in relation to the various types of exposure, and for comparability of the results with previously published studies, the subjects were also categorized into terciles. The data were analyzed using analysis of variance (ANOVA) in the SPSS software package.

2.6 Cancer incidence in the cohort

The information on the cancer incidence and the specific mortality in the cohort up to December 2003 was obtained from the Cancer Registry maintained by the Cancer Epidemiology Department, Centre of Oncology, Maria Skłodowska-Curie Memorial Institute, Cracow Branch, Kraków, Poland. In case of uncertainties (i.e., a change of address) the correctness of the person reported in the cancer register was verified by the date and place of birth with records maintained by employers or crosschecked in the Registry of Inhabitants. Cancer incidence (I_C) was calculated as the number of persons who were diagnosed with cancer per total number of years the individuals remained disease-free $x10^5$.

3. RESULTS AND DISCUSSION

Table 1 presents the average values of various cytogenetic biomarkers in 245 individuals investigated during the period of 1981-1991, while Table 2 presents these data for 211 subjects who were studied during 1992-2001. The values of the detected biomarkers are the average values for the groups obtained by the stratification of the whole group of donors first according to gender, then into groups according to level of expressed cytogenetic damage; (chromosome and chromatid = CSA + CTA) in the range of low, medium or high category. Differences between categories

Table 1. Characteristics of the male and female subgroups: average age and levels of biomarkers, number of cancer cases (CC) reported in the group by December 2003 (on average 16 years from sampling), investigated during the period between years 1981 – 1991 for chromosome aberrations frequencies, stratified according to gender and category (L = low, M = medium, H = high) of chromosomal damage.

Gender	C	N		Age	AbF	CSA	CTA	AbC	SCE	MN	CC
M	**L**	**79**	**Av.**	**41**	**0.0**	**0.0**	**0.0**	**0.8**	**9.4**	**1.5**	**2**
			±sd	10	0.0	0.0	0.0	1.0	2.1	0.7	
	M	**65**	**Av.**	**44**	**1.0**	**0.6**	**0.4**	**1.9**	**9.8**	**1.6**	**4**
			±sd	12	0.3	0.5	0.4	0.9	2.5	1.1	
	H	**60**	**Av.**	**42**	**2.9**	**2.0**	**1.0**	**4.3**	**10.9**	**1.9**	**5**
			±sd	10	1.9	1.4	1.1	3.2	4.8	0.9	
			Sign.	Ns	.01	.001	.01	.001	.01	Ns	-
F	**L**	**12**	**Av.**	**62**	**0.0**	**0.0**	**0.0**	**1.1**	**8.7**	**-**	**1**
			±sd	7	0.0	0.0	0.0	1.1	2.5	-	
	M	**11**	**Av.**	**59**	**0.7**	**0.4**	**0.3**	**1.0**	**8.0**	**-**	**2**
			±sd	5	0.0	0.4	0.4	0.8	2.1	-	
	H	**18**	**Av.**	**49**	**2.2**	**1.4**	**0.8**	**3.4**	**11.0**	**-**	**2**
			±sd	12	0.0	0.8	0.6	1.3	4.0	-	
			Sign.	.005	.001	.001	.001	.001	.04		-

C, category of aberration frequency (based on percentiles from distribution observed for the whole group investigated in that period); NoD, number of donors; AbF, aberration frequency in 100 cells (excluding chromatid and isochromatid gaps); CSA, chromosome and CTA chromatid aberration frequency per 100 cells, (AbF = CSA + CTA); AbC, percent of aberrant cells; SCE, sister chromatid exchanges frequency per cell; MN, number of micronuclei per 1000 binucleated cells; CC, cancer cases reported.

for all biomarkers were significant ($p < .01$ or .001) except MN, and for females all biomarkers differed significantly ($p < .04$ for SCE and $p < .001$ for others).

Comparison of levels of cytogenetic biomarkers observed in categorized subgroups reveals that in the presented studies, the frequency of micronuclei and sister chromatid exchanges are less sensitive biomarkers than others, and the frequency of CSA is the best biomarker. In general, average values of biomarkers are higher in the period of more recent samplings ($p < 0.001$ for all biomarkers except chromatid type of aberrations CTA). The strongest difference is observed for chromosome types of aberration frequency, that might be a result of methodological development, that allowed an estimate of aberration frequency on the basis of scoring aberrations only in the first confirmed mitosis. The average follow up times (as well as latency) for

those two data basis are also significantly different (16 years for subgroups presented in Table 1, versus 5.9 years for studies presented in Table 2).

Although reported results are the largest cytogenetic studies available in Poland, the studied group is rather small as well as number of cancer cases

Table 2. Characteristics of the male and female subgroups: average age and levels of biomarkers, number of cancer cases (CC) reported in the group by December 2003 (on average 5.9 years from sampling), investigated during the period between years 1992 – 2001 for chromosome aberrations frequencies, stratified according to gender and category (L = low, M = medium, H = high) of chromosomal damage.

Gender	C	N		Age	AbF	CSA	CTA	AbC	SCE	MN	CC
M	**L**	**60**	**Av.**	**38**	**1.0**	**0.1**	**0.0**	**1.3**	**6.8**	**1.3**	**3**
			±sd	12	0.3	0.2	0.2	1.1	1.1	0.8	
	M	**60**	**Av.**	**42**	**1.2**	**0.7**	**0.5**	**2.8**	**7.2**	**1.0**	**3**
			±sd	12	0.4	0.6	0.6	1.6	1.4	0.6	
	H	**60**	**Av.**	**49**	**3.7**	**3.0**	**0.6**	**5.4**	**7.5**	**1.1**	**2**
			±sd	18	2.2	1.9	1.2	2.9	1.4	0.7	
			Sign.	.000	.001	.001	.001	.001	.001	.ns	-
F	**L**	**10**	**Av.**	**42**	**0.7**	**0.6**	**0.1**	**2.2**	**6.9**	**-**	**0**
			±sd	6	0.5	0.5	0.2	1.5	1.4	-	
	M	**11**	**Av.**	**48**	**2.2**	**1.5**	**0.7**	**4.0**	**7.9**	**-**	**0**
			±sd	16	0.6	0.8	0.8	2.0	1.4	-	
	H	**10**	**Av.**	**44**	**5.3**	**3.8**	**1.5**	**7.1**	**7.7**	**-**	**0**
			±sd	11	1.7	2.2	2.3	2.4	1.6	-	
			Sign.	ns	.001	.001	.001	.001	ns		-

reported during the follow up period (24 cancer cases). In more recent samplings there were even no cancer cases reported in the female subgroup. At the time of the cancer incidence analyses, the cohort included data on 456 subjects, who contributed with 5168 person years of follow-up time. Among the reported cases, the dominant group (62.5%) consists of cancer in respiratory and intrathoracic organs (29.2% of all cases were lung cancer patients, CD 34.9 according to ICD-10 system) and in digestive organs.

Malignant neoplasms account for 16.0% of the whole group and was followed by single cases (4.2% each) of neoplasms in various organs (brain, lip and pharynx, thyroid, female genital organs). Tables 1 and 2 also present average ages at the time of sampling, and number of cancer cases reported in the follow up studies (2003).

The results of the analysis of various biomarkers for investigated subgroups have shown that CSA and percent of aberrant cells are the most sensitive among the biomarkers chosen. Therefore, analysis of possible association between cytogenetic damage and cancer incidence was done on the base of comparison of cancer incidence observed for the subgroup stratified to low, medium and high categories of CSA damage.

Table 3 presents the results of categorization of the investigated group first according to sampling period and gender, and then to frequency of chromosomal aberrations (CSA) and finally to smoking habit. Cut-off points for CSA are shown there, numbers of person years of the follow up, as well as incidence of cancer (I_C) calculated as the number of persons who were diagnosed with cancer per total number of years the individuals remained disease-free multiplied by 10^5. The I_C value for group of males from the first sampling is visibly increased with the category of chromosome aberrations, and although this association is not statistically significant ($p < 0.08$), the linearity has statistically significant ($p < 0.04$). Other subgroups (females in the first sampling and males in the second) show an increase in the I_C value in the medium category, compared with that of the low category; however, an increase is not followed in a higher category.

Values of I_C for females are higher than reported for male donors (3,645 for females versus 934 obtained on average for males from the first sampling, and 1,058 for males from the second sampling). This seems to be in agreement with regional survey by Rachtan et al. [31]. An expected average age at the time of cancer incidence check-out for males from our first sampling is 58 years, and for females 71 years old. Rachtan et al. [31] reported an I_C value that at the end of year 2000 for inhabitants of Malopolska Region showed respective ages (I_C) for females 575 >64y >1314, and for men 552>64y >2165.

As in the follow up studies, the number of reported lung cancer cases was highest among cancers reported, the comparison was made taking into account smoking as a possible confounder. Smerhovsky et al. [7] reported that lung cancer cases account for 24.3% of all cases reported, however in their studies 51.4% percent of lung cancer patients consisted of underground miners occupationally exposed to radon gas in one ore mine. In our study, no such occupation was reported, thus, smoking is suspected to be a main potential source

Table 3. Cancer incidence in the investigated groups with various categories of chromosomal (CSA) aberration frequency after stratification of all subjects to various samplings (group of follow up on average 16 and 5.9 years from samplings respectively), gender, and history of smoking habits (NSM, no smokers; SM, smokers).

					I_C[b] / CSA[%]			
Gender	Group	*n*	[a]PY	I_C[b]	Low	Medium	High	
					(1st tercil)	(2nd tercil)	(3rd tercil)	Sign
					< .50	.50≤CSA<1.33	≤ 1.33	
M	All [16]	204	3198.0	933.8	144.4	488.4	2785.6	.08[c]
	NSM	53	791.0	134.8	<1	<1	1388.9	ns
	SM	151	2406.9	1214.2	199.8	686.8	3150.0	ns
					< .67	.67≤CSA<1.0	≤ 1.00	
F	All [16]	41	647	3644.7	1388.9	11,666.7	<1	.12
	NSM	27	407	5264.5	1562.5	16,666.7	<1	ns
	SM	12	240	<1	<1	<1	<1	ns
					< .48	.48≤CSA<1.9	≤ 1.90	
M	All[5.9]	180	1058	1048.2	921.1	1587.3	705.1	ns
	NSM	99	574	202	<1	<1	666.7	ns
	SM	81	484	2095	2258.1	2998.2	756.7	ns
					< .94	.94≤ CSA<2.4	≤ 2.40	
F	All[5.9]	31	265	none	None	None	none	

PY = person years

[b]I_C = cancer incidence calculated as number of persons who were diagnosed with cancer per total number of years the individuals remained disease-free x10^5.

and confounder (100% of lung cancer cases are smokers). Comparison of cancer incidence observed in various CSA categories between subgroups of smokers and nonsmokers shows indeed a much stronger association and growing cancer incidence for smoking donors. Correlation between cytogenetic damage and growing lung cancer incidence was also reported earlier and a profound impact of smoking on health hazard confirmed [8,12].

Our results investigate the utility of CSA levels as measured in two different laboratories in Poland, during various periods and with slightly different

techniques. These studies found CSA levels to be an appropriate biomarker for showing an association between genetic damage and cancer rate. However, an additional effort to enlarge an existing group and standardization of age is needed to improve the utility of the results. Our results show an apparent association between increased cytogenetic damage detected in the lymphocytes of smokers and a growing risk of cancer incidence during latency period. From a public health outlook, increases in the frequency of intermediate end-points such as CSA, that are strongly suggestive of an increased risk of cancer, implies the validation as predictor of disease, intervention policies and actions in populations showing increased frequency of this biomarker are highly recommended [6].

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CHAPTER 8

AN IMPROVED METHOD FOR THE BIOLOGICAL MONITORING OF VOLATILE COMPOUNDS

Karla D. Thrall[1]
[1]*Center for Biological Monitoring & Modeling, Battelle, Pacific Northwest Division, Richland, WA, USA*

Abstract: Exposure assessment is a critical component in estimating health risk. The analysis of exhaled breath offers an ideal non-invasive matrix for measuring volatile biomarkers associated with the absorption, distribution, metabolism and elimination of chemicals under a variety of environmental conditions. A real-time, field-portable system was developed to directly analyze undiluted exhaled air from experimental animals and humans. The exhaled breath data were evaluated using a physiologically based pharmacokinetic model to estimate total exposure and internal target tissue dosimetry, and to describe kinetic changes. To date, the system has been used to conduct occupational exposure assessments and dermal bioavailability studies.

Key words: dermal bioavailability, occupational exposure assessment, exhaled breath, PBPK

1. INTRODUCTION

Historically, the use of breath analysis for understanding the body's physiological chemistry and health status dates back over 100 years, when Anstie (1874) reported the elimination of alcohol in human breath. The work by Pauling et al. (1971) describing approximately 250 different volatile organic compounds in human breath is generally heralded as the start of the "modern" era of breath analysis (Phillips, 1997). Since that time, the most established use of breath analysis has been for determination of breath alcohol levels, generally for medical and/or legal purposes (Wilson, 1986).

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Beginning in the 1970s, breath analysis for occupational monitoring of workers began to emerge, although its acceptance as a routine monitoring method has been slow (i.e., Campbell et al., 1985; Monster and Boersma, 1975). Traditional exhaled breath analysis techniques have involved collection of breath samples in Tedlar bags or stainless steel canisters, followed by laboratory analysis, generally by gas chromatography. Disadvantages of these techniques include the possibility that the collection device may alter the integrity of the sample, the time-consuming and costly analysis of the sample, and the delay between sample collection and analysis. To overcome these disadvantages, a breath-inlet device for a mass spectrometer was developed to allow for the continuous real-time analysis of undiluted exhaled air from experimental animals and humans (Thrall and Kenny, 1998; Thrall et al., 2001). The applications of this system in studies ranging from occupational exposure assessment to basic research are described here.

2. BREATH ANALYSIS SYSTEM

The breath monitoring system utilized in all the described studies consisted of an inlet device connecting a human volunteer directly with a mass spectrometer. Exhaled breath is passed through a heated large-diameter transfer line into a heated glass-mixing chamber (1.3-L volume). Breath samples enter the mixing chamber via a glass tube that bends off to one side, and exit the mixing chamber via a glass tube bending in the opposite direction, thus maximizing turbulence and mixing. The mass spectrometer continually withdraws air samples from the center of the mixing chamber at a calibrated rate of approximately 200 ml/min. Excess exhaled air is vented from the mixing chamber via a large bore-hole exit tube with negligible flow restriction.

For rodent studies, the animals were individually placed in small off-gassing chambers, as described by Gargas (1990). The animals were awake and could move freely while in the off-gassing chamber. Breathing air was supplied to each animal through the lid of the off-gassing chamber at a calibrated rate of approximately 200 ml/min. The mass spectrometer continually withdrew air samples from the off-gassing chamber, through a port in the lid, at the same rate. The concentration of compound in the chamber was used to represent exhalation.

3. DATA ANALYSIS

Exhaled breath data was related to total exposure and internal target tissue dose through the use of physiologically based pharmacokinetic (PBPK) models. These physiologically relevant models describe the body as a series of tissue compartments representing the probable route(s) of exposure, the metabolically active tissues, target organs, and excretion pathways. These models are typically developed and experimentally validated using common laboratory animals, then extrapolated to represent man. A series of differential equations are used to mathematically describe the absorption, tissue distribution, metabolism and elimination of a compound in the body. An experimentally validated PBPK model facilitates extrapolation across different routes of exposure, from high-to-low doses, and among animal species (Andersen et al., 1993). Thus, by monitoring exhaled breath for a particular compound, the estimated exposure and target tissue dose can be determined.

4. APPLICATION IN DERMAL STUDIES

Human studies were conducted under approval from the Battelle Memorial Institutional Review Board (IRB) in accordance with the terms and conditions of Federal Regulation 45 CFR 46 under the authority of Multiple Project Assurance M1221. Dermal exposures were conducted by submersion in tap water to neck level in a 397-L stainless steel hydrotherapy tub (Whitehall Manufacturing, Industry, CA) containing an initial target concentration of approximately 500 μg/L toluene. The target concentrations were selected to stay below the U.S. EPA Federal Drinking Water Guidelines of maximum contaminant levels (US EPA, 2000). The exhaled breath for each volunteer was continually monitored for 2-5 min prior to entry into the tub, during the 20-25 min exposure, and for 10-30 min post exposure. Water was sampled at 5-min intervals throughout the exposure and analyzed by a gas chromatograph headspace method. Water temperature was recorded and stayed relatively constant ($\pm$1ºC) throughout the study. A dermal PBPK model was used to describe the exhaled breath data by simulating the rate of change in the concentration of compound in the skin compartment to relate to the rate of penetration through the skin (the flux). The skin permeability coefficient for each volunteer was estimated based on the kinetics of absorption as described by the exhaled breath and found to range from 0.003 to 0.02 cm/hr. A single averaged permeability coefficient value of 0.012 $\pm$ 0.007 cm/hr was found to adequately describe all the individual dermal exposure data sets.

For rodents, dermal studies were conducted as described previously (Thrall and Woodstock, 2002). In brief, a dermal patch was attached to a shaved area on the lower back of the animal using a cyanoacrylate adhesive and allowed to dry overnight. The dermal exposure patch consisted of a 1.7-cm inner-diameter hand-blown glass cell (Northwest Technical Glass, Richland, WA) with a needle hole opening in the top to allow addition of the dosing solution. On the day of exposure, approximately 2 ml aqueous toluene was added to the exposure patch and animals placed in the off-gassing chambers. The permeability coefficient for dermal absorption of aqueous toluene was estimated using PBPK modeling to simulate the exhaled breath data (Figure 1). The average permeability coefficient was 0.074 $\pm$ 0.005 cm/hr, a value roughly 6 times greater than that determined in the human. The magnitude of this difference is consistent with the results of previous studies comparing rat and human toluene-vapor exposures, where the rat *in vivo* permeability coefficient of 0.72 cm/hr (McDougal et al., 1990) is roughly 5 times greater then the *in vivo* human value of 0.14 cm/hr (Kezic et al., 2000).

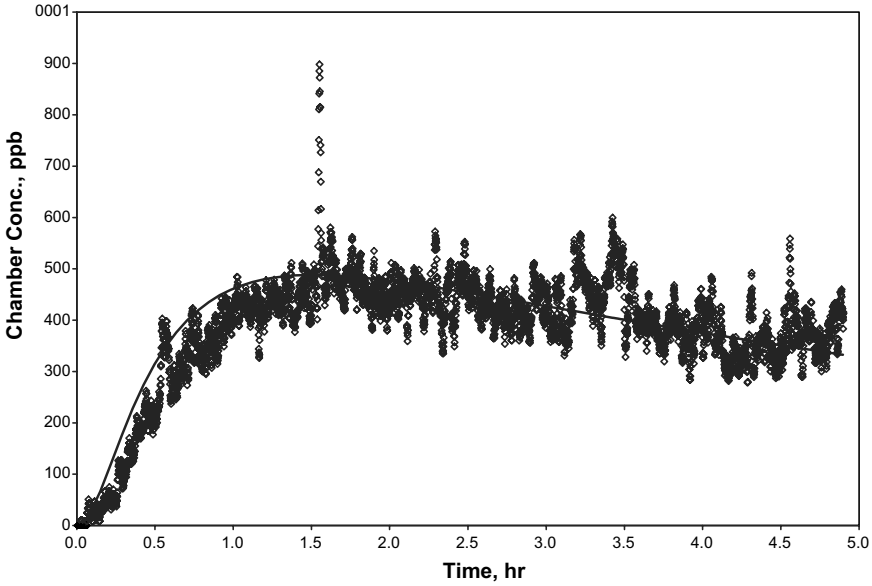

Figure 1. PBPK model prediction (line) and exhaled breath data (points), reflected as chamber concentration, for a rat exposed to a 2.08-ml volume of 0.51 mg/ml (4.41 mg/kg) aqueous toluene over a 4.91-cm^2 area of the back.

The U.S. EPA (1992) human permeability coefficient for aqueous toluene was estimated to be 1 cm/hr based on flux data from Dutkiewicz and Tyras (1968). In these previous human studies the amount of toluene

absorbed was quantified by measuring the loss of the compound from the donor solution without verifying steady-state conditions. Jepson and McDougal (1997) report that permeability may be overestimated by assuming that the rate of chemical loss from the exposure solution represents the average flux into the skin. Further, estimates of percutaneous absorption may be erroneous when standard Fick's law calculations of dermal flux are used without verifying that steady state was achieved (Jepson and McDougal, 1997). Therefore, it is particularly important for dermal absorption estimation methods to be able to account for unsteady-state dermal absorption (Roy et al., 1996). A PBPK model is ideally suited for estimating the actual permeability when the exposure concentration changes over time, such as described here.

The experiments on animals and human volunteers described here provide definitive data for assessing the absorption of aqueous toluene through skin and provide a basis for interspecies extrapolations. Furthermore, the utilization of sensitive, real-time instrumentation allows for controlled exposures to be conducted under realistic bath-water scenarios. Analysis of the resultant exhaled breath data, using a PBPK model, estimates an average whole-body permeability coefficient for dermal absorption of aqueous toluene to be more than 80 times lower than the U.S. EPA estimate.

5. APPLICATION IN EXPOSURE ASSESSMENT STUDIES

Field studies were conducted under approval from the IRB. As one example, a field study was conducted using the breath monitoring system to quantitate exposures to benzene and toluene at a waste incinerator plant. Breath samples were collected from volunteers prior to starting a particular job task, and again at the end of that task. Tasks ranged from 15 minutes to unload a waste drum, to 4+ hours in a control room; the frequency at which volunteers provided breath samples was dependent on the job task and the potential for exposure. Both toluene and benzene were simultaneously analyzed for concentration in the exhaled breath samples. Pre-activity breath samples were considered to reflect background concentrations, therefore job task-specific exposures were calculated as the difference between "post"- and "pre"-activity measurements. In total, breath samples were analyzed for 22 comparative (pre- versus post-job task) times (Figure 2). Every participant had some level of toluene and benzene in their exhaled breath in the "pre'-activity phase; this data is consistent with previous studies (Wallace 1989). The greatest pre- versus post-activity difference was observed in a volunteer (number 17) accidentally sprayed with fuel oil during a routine job; toluene

breath concentrations increased 59.6 ppb over pre-task levels. Overall, the results illustrate the utility of monitoring workers for exposures throughout the day, particularly when job-specific tasks may indicate a potential for exposure.

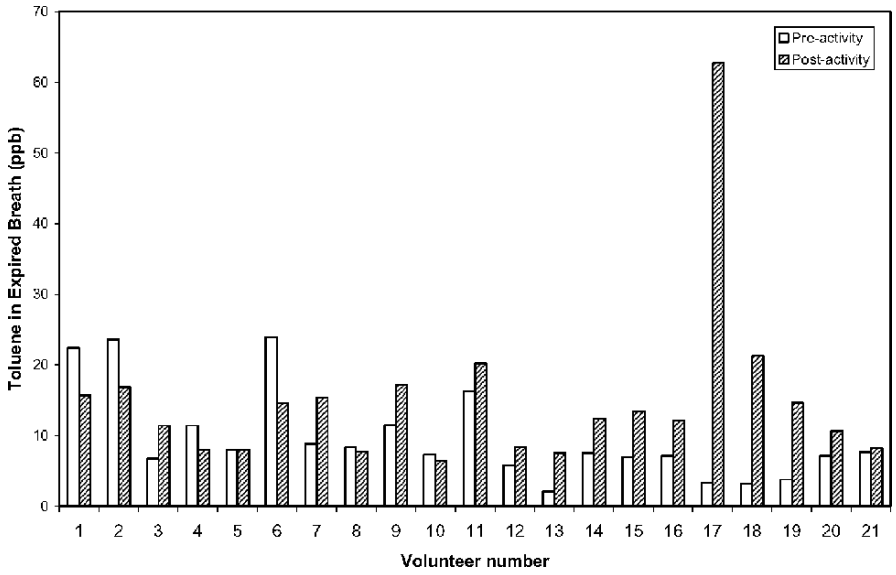

Figure 2. Comparison of pre-and post-activity measurements of toluene in the exhaled breath (in ppb) of volunteers participating in the field study at a waste incinerator.

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CHAPTER 9

CHANGES IN CADDIS LARVAE COMMUNITY COMPOSITION: EFFECT OF UNKNOWN CONTAMINANTS

Miklós Bálint,[1] Andrei Sárkány-Kiss,[1] and Mihály Braun,[2]
[1]Faculty of Biology-Geology, Babes-Bolyai University, Str. Clinicilor 5-7, Cluj-Napoca, Romania; [2] University of Debrecen, PO. 21, Debrecen, Hungary

Abstract: Caddis larvae (*Trichoptera, Insecta*) are well-known sentinel organisms used for bioindication. An investigation was carried out for mapping the effects of the heavy metal pollution originating from Baia Mare (Romania). Strong evidence was found for the efficient use of data regarding hydropsychid caddis larvae community structure as a biomarker in identification of unknown pollution. Caddis larvae appear to be cost effective tools for identifying contaminated environments because they are easier to identify compared with other benthic species.

Key words: Trichoptera, bioindication, heavy metals, Somes River, Baia Mare.

1. INTRODUCTION

The Someş is Romania's most important northwestern river. It is one of the most polluted tributaries of the Tisza river in Hungary (Sárkány-Kiss and Macalik, 1999). The river is contaminated by industrial and residential waste-water inflows, agricultural pesticides and fertilizers. The most hazardous contaminants are believed to be carried by the Lăpuş River from the mining and industrial sites around Baia Mare, Romania (Sárkány-Kiss and Macalik, 1999; Schultz, 2002). An investigation was carried out by the Department of Ecology of Babeş-Bolyai University between 2000 – 2003 to investigate the long-term effects of mining activities around Baia Mare and the long-term effects of a cyanide spill in 2000. The research was based on

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the use of benthic invertebrates as bioindicators, which have been shown previously to be effective in contaminant evaluation (e.g., Verneaux et al., 2004; Dyer et al., 2003; Vuori and Kukkonen, 1996; Tessier et al., 2000a,b; Iliopoulou-Georgudaki, 2003; Canivet, 2002; Neumann, 2003a). This paper discusses the use of caddis larvae as bioindicators (e.g., Highler and Tolkamp, 1982; Tessier et al., 2000a,b; Resh et al., 2000) to detect contaminants of known or unknown origin.

2. MATERIALS AND METHODS

Samples were collected along a 50 km-long section of the Someş river, from five sites upstream and five sites downstream of the inflow of the Lăpuş River (Figure 1), and from one site on the Lăpuş. Special care was taken to obtain a representative sample of the microhabitats of each sampling site by taking three samples from every site. The results from the site on the Lăpuş are not presented here. The samples discussed in this paper were collected in October 2001 and November 2002.

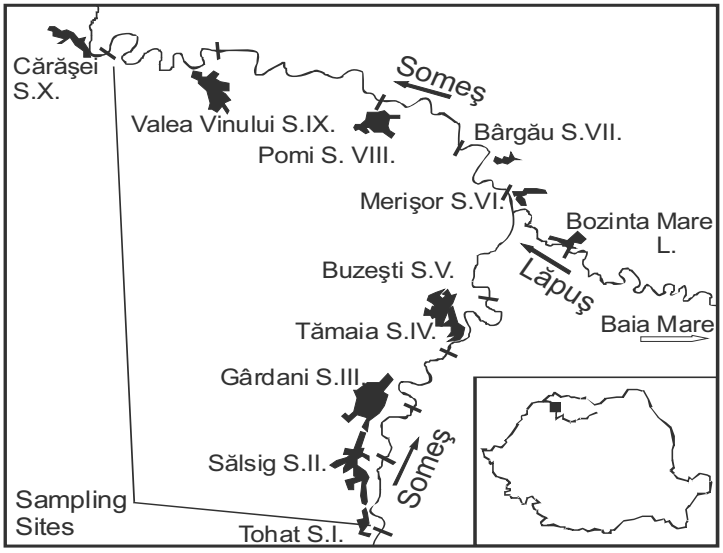

Figure 1. Sampling sites on the Someş River above and below of the influx of Lăpuş River.

The environmental factors that were measured included pH, conductivity, water and air temperature. The upper layer of silt was sampled at every site to estimate metal concentrations in the freshly deposited sediments. Metal content (Al, Cr, Fe, Li, Mn, Cu, Pb and Zn) was measured with ICP-AES.

Benthic samples were taken with a Surber over a surface area of 0.1 m^2, and were preserved and then identified to different taxonomic levels. Caddis larvae were identified to species level using keys (Waringer and Graf, 1997; Wallace et al., 2003). For data analysis, the Spearman rank-correlation and Mann-Whitney tests were used.

3. RESULTS

In both October 2001 and November 2002, an appreciable increase was observed in the Cu, Zn and Pb contents of the sediment after the influx of the

Table 1. Metal content in the Someş River sediment in October 2001 (values in mg/kg).

Sampling Station	Al	Cr	Cu	Fe	Mn	Pb	Zn
S.I.	7441.4	10.9	10.4	14568.2	808.6	9.4	90.2
S.II.	8968.3	0.1	8.8	16326.5	547.5	5.2	117.4
S.III.	9713.3	13.6	12.1	17626.3	1823.3	0.1	148.5
S.IV.	8855.4	22.5	14.2	15623.5	570.2	6.9	23.4
S.V.	9939.7	32.3	80.2	21482.2	686.2	57.9	83.7
S.VI.	5208.6	11.9	70.9	12661.1	444.8	56.1	639.2
S.VII.	5409.8	6.7	56.2	12574.3	327.5	49.4	303.6
S.VIII.	11432.5	15.3	64.1	21007.5	1136.5	26.7	337.6
S.IX.	9014.8	19.5	64.5	17778.7	861.7	47.0	414.0
S.X.	7139.1	15.3	329.5	22095.8	649.3	333.1	936.0

Table 2. Metal content in the Someş River sediment in November 2002 (values in mg/kg).

Sampling Station	Al	Cr	Cu	Fe	Mn	Pb	Zn
S.I.	14007.3	18.1	24.1	24430.5	1117.1	6.7	225.7
S.II.	3863.7	7.7	5.5	8335.8	234.2	1.0	48.4
S.III.	11521.6	16.8	17.9	21803.5	953.3	2.1	158.5
S.IV.	2375.1	5.6	3.1	5181.9	243.5	4.4	37.0
S.V.	8527.4	16.7	15.1	17252.8	692.4	4.8	124.6
S.VI.	4330.6	10.7	44.1	10916.8	544.1	37.6	378.7
S.VII. [a]	-	-	-	-	-	-	-
S.VIII. [a]	-	-	-	-	-	-	-
S.IX.	8962.7	12.5	94.2	19909.4	1083.0	62.8	953.9
S.X.	6340.6	7.4	67.6	15678.8	870.2	45.7	660.0

[a] *Samples were not collected due to unfavorable weather conditions.*

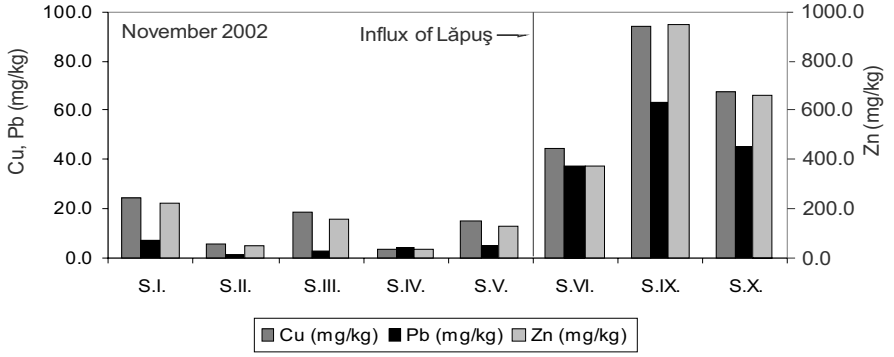

Figure 2. Increase in heavy metal content in the sediment of the Someş River, after the inflow of the Lăpuş river near Baia Mare, November 2002 (significance levels for Pb, Cu, Zn $p = 0.03$).

Lapus River. The increase of each of these metals was significant in 2002 (Figure 2), while in 2001 only the quantity of Zn was significantly increased (significance levels in 2001 were Cu, $p = 0.07$; Pb, $p = 0.07$; Zn, $p = 0.01$). No important change was observed in the metal content of freshly deposited sediments between 2001 and 2002 samples (Tables 1 and 2.).

3.1 Caddis larvae and benthic fauna

In October 2001, 549 caddis larvae were collected from the section of the Someş River that was investigated. These caddis larvae belonged to the

Table 3. Number of characteristic caddis larvae found on the sampling sites along the investigated section of Someş, estimated to m^2 (individuals/m^2).

	2001		2002
Sampling Station	*Hydropsyche contubernalis*	*Psychomyia pusilla*	*Hydropsyche contubernalis*
S.I.	927	63	27
S.II.	140	37	103
S.III.	65	15	3
S.IV.	10	5	0
S.V.	305	45	43
S.VI.	10	0	3
S.VII.	213	23	[a]
S.VIII.	70	7	[a]
S.IX.	5	0	33
S.X.	30	3	0

[a] *Samples were not collected due to unfavorable weather conditions.*

families Hydropsychidae: *Hydropsyche contubernalis* (McLachlan, 1865), *Cheumatopsyche lepida* (Pictet, 1834), Psychomyiidae: *Psychomyia pusilla* (Fabricius, 1781), and Leptoceridae: *Ceraclea dissimilis* (Stephens, 1836). Only one individual each of *Cheumatopsyche lepida* and *C. dissimilis* were identified. Thus, these latter two species were not considered to be important species in this area.

A significant negative correlation was found between the quantity of Zn in the sediment and the density of *P. pusilla* larvae (Spearman correlation coefficient = –0.681, p = 0.03). No correlation was found between the sediment's heavy metal concentrations and the densities of *H. contubernalis* larvae. As in the case of *P. pusilla,* the *H. contubernalis* larvae densities clearly decreased after the influx of the contaminated Lăpuş (Table 3).

During the 2002 November sampling session, a significant reduction was observed in the number of *Trichoptera* species and individuals along the whole section of the Someş which was being investigated; the only species found was *H. contubernalis,* of which there were 64 individuals. The decrease from 2001 to 2002 in the number of *H. contubernalis* larvae was significant at p = 0.04 (Figure 3).

4. DISCUSSION

H. contubernalis is a species known to be tolerant of moderate organic pollution and other types of pollutants (Dudgeon, 1992; Loch, 1996), which can be found in many of the Romanian rivers (Ciubuc, 1993). *P. pusilla* is

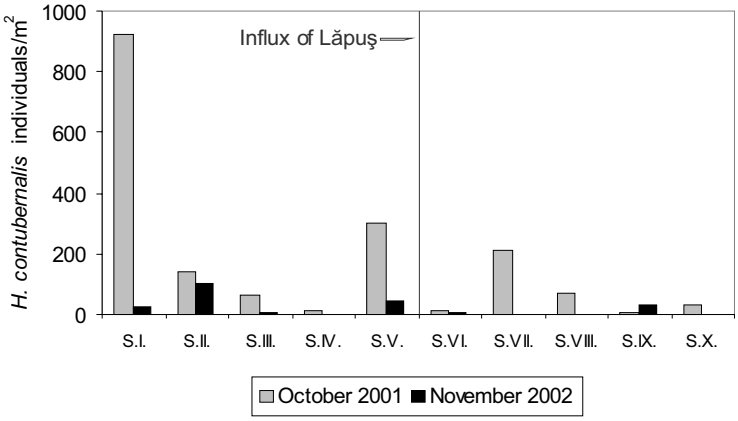

Figure 3. Significant decrease between 2001 and 2002 in the number of H. contubernalis larvae found on the investigated river section (p = 0.04).

usually found in silt in higher numbers, and can tolerate moderate levels of organic contamination. The number of caddis species and their densities were clearly reduced after the influx of the Lăpuş, which may be due to heavy metal contamination. Although the decrease in *H. contubernalis* density was not statistically significant in this study, the sensitivity of hydropsychid caddis larvae to different metals is well-documented (e.g., Vuori, 1996; Vuori and Kukkonen, 1996; Cain and Luoma, 1998, Maret et al., 2003).

Psychomyiidae species are usually less resistant to contamination than hydropsychids (Dudgeon, 1992). The complete elimination of *P. pusilla* in 2002, and the significant reduction in density of *H. contubernalis* larvae in 2002 compared to the levels seen in 2001, suggest that contamination affected the entire section of the Someş River that was investigated. The existence of contamination along this entire section of the river was unknown to us during the initial investigation period, although it was later confirmed. The contamination is believed to have originated from the Dej cellulose mill, located approximately 100 km upstream of the sampling sites. After further data analysis, a reduction was noted in the abundance of other taxa that are relatively sensitive to contaminants (*Plecoptera, Ephemeroptera*), and the densities of pollution-tolerant taxa (*Oligocheata, Chironomidae*) were greatly increased. The decrease in density of *Ephemeroptera* was appreciable, and the *Plecoptera* completely disappeared.

The changes that were seen in the density of contaminant-sensitive and contaminant-tolerant species could have resulted from changes in agricultural practices (e.g., increased pesticide use) (Wendt-Rasch et al., 1999). However, no intensification of agriculture was observed in the area. In the early 1980's a parallel case occurred on the lower Rhine, where only *H. contubernalis* were found in a contaminated area (Higler and Tolkamp, 1982).

These results indicate that caddis larvae identified to species level, especially hydropsychids, can be used as cost-effective bioindicators because these organisms are relatively easy to identify compared to other benthic invertebrates such as *Chironomidae*. Further investigation is needed regarding the sublethal responses of caddis larvae to chronic exposures in order to find reliable biomarkers for specific types of contaminants.

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CHAPTER 10

THE EFFECT OF ACRYLONITRILE ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS

Olena Beskid, Zdik Dušek, Irena Chvátalová, Zdena Lnenickova, Pavel Rössner, Radim J. Šrám

Institute of Experimental Medicine AS CR & Health Institute of Central Bohemia, Prague, Czech Republic

Abstract: The influence of acrylonitrile (ACN) on the level of aberrations was measured by conventional cytogenetic analysis (CCA) and fluorescence in situ hybridization (FISH) painting. The investigation was carried out on lymphocytes from a group of 60 chemical plant workers and 55 healthy volunteers. We observed a significant increase in % of aberrant cells (% AB.C.) measured by CCA in the exposed group (3.27 ± 1.91 %AB.C.) in comparison with controls (2.05 ± 1.53% AB.C, $P < 0.01$). However, there was no significant difference between these two groups in genomic frequency of translocations per 100 cells ($F_G/100$) measured by FISH (1.88 ± 1.52 in the exposed subjects vs. 1.63 ± 1.30 in controls). Smoking did not affect the level of aberrations. The level of breaks per cell evaluated by CCA decreased in the subjects with GSTM1 null genotype, while $F_G/100$ revealed an association with age, EPHX, MTHFR polymorphisms, and the levels of vitamins C and E.

Key words: acrylonitrile, chromosomal aberrations, FISH, genetic polymorphisms, vitamins

1. INTRODUCTION

Acrylonitrile ($H_2C{=}CH{-}C{\equiv}N$) is an important industrial compound that is used in the manufacture of synthetic fibers, plastic, elastomers and rubber. It is also often used in the production of fatty amines, ion exchange resins and fatty amine amides in cosmetics, adhesives, corrosion inhibitors and water-treatment resins. The International Agency for Research on Cancer (IARC)

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has classified acrylonitrile (ACN) as a possible human carcinogen (group 2B) (IARC, 1999). Due to the rising consumption of ACN in industry, further studies on the adverse effects of this compound are needed.

ACN has been shown to be carcinogenic in laboratory animals and has been linked to Zymbal's gland carcinoma, brain astrocytoma and forestomach tumors (Maltoni et al., 1977; Nagasawa et al., 2003). Epidemiological studies suggest that ACN may be a human carcinogen (O'Berg, 1980; O'Berg et al., 1985; Chen et al., 1987; Marsh et al., 2001; Scelo et al., 2004). On the other hand, several studies did not find ACN to be carcinogenic, mutagenic or teratogenic among occupationally exposed workers (Rothman, 1994; Swaen et al., 1998; Collins and Strother, 1999; Schulz et al., 2001; Czeizel et al., 2004; Starr et al., 2004). These discrepancies led to studies comparing ACN metabolism in animals and humans (Thier et al., 2000), and to the evaluation of different biomarkers of effect such as p53 and p21 expression (Rossner et al., 2002) or DNA strand breakage and sex chromosome aneuploidy in human spermatozoa (Xu et al., 2003). Though detailed reviews about ACN are available (Felter and Dollarhide, 1997; Whysner et al., 1998; Leonard et al., 1999), little is known about its early biological effects.

Chromosomal aberrations are biomarkers of early effect, and are considered to predict an increased risk of cancer (Bonassi et al., 2000; Smerhovsky et al., 2001). ACN has been reported to increase chromosomal aberrations as measured by conventional cytogenetic analysis (CCA) (Borba et al., 1996; Major et al., 1998; Tucek et al, 2002). However, there are no results available for stable aberrations measured by fluorescence in situ hybridization (FISH). The goal of this study was to (i) evaluate the effect of ACN on the level of unstable aberrations measured by CCA and stable aberrations measured by FISH, and (ii) to identify factors affecting chromosomal breakage.

2. MATERIALS AND METHODS

2.1 Subjects and sampling

The subjects in this study were 60 employees of a petrochemical plant where ACN is produced and polymerized [mean age 42 yrs (19-53) 48% of smokers], and 55 control subjects [mean age 30 yrs (20-61), 24% of smokers]. The average ACN exposure was 0.05-0.7 mg/m^3 in the work environment of the exposed subjects. Blood samples were collected in heparinized tubes at the end of the workshift by venipuncture and transferred to the laboratory.

2.2 Lymphocyte culture

Lymphocyte cultures were prepared from whole blood by adding RPMI 1640 medium supplemented with 20% calf serum and 1% phytohemagglutinin. Two identical cultures were prepared from each sample and cultivated at 37ºC for 48 h for conventional cytogenetic analysis and 72 h for FISH painting. Two hours before harvesting, colchicine was added at the final concentration of 1.25×10^{-6} M. The cells were collected and fixed according to standard procedures (Sorsa et al., 1994).

2.3 Conventional cytogenetic analysis (CCA)

The slides for the analysis were stained with 5% Giemsa solution (pH 6.8). Four categories of chromosomal aberrations were evaluated, chromatid and chromosome breaks, and chromatid and chromosome exchanges. A total of 100 well-spread metaphases per subject with 46 ± 1 centromere were examined on coded slides (Carrano and Natarajan, 1988).

2.4 FISH analysis

The cell suspensions were dropped on the cool, moistened slides and allowed to air-dry. Commercial probes for chromosome 1 (biotinilated) and 4 (FITC) (Cambio, Cambridge, UK) were prepared according to a modified manufacturer's protocol. Probes were placed on slides, sealed with rubber cement and incubated at 37ºC in the moisture chamber overnight. After hybridization, the slides were washed and mounted in antifade Vectashield (Vector) with DAPI (Sigma, St. Louis, MO, USA).

The aberrations were classified according to PAINT nomenclature (Tucker et al., 1995). One thousand metaphases per subject were analyzed and recorded by ISIS4.4.16 software (MetaSystems GmbH, Germany).

2.5 Genotypic analyses and evaluation of the vitamin C, A, E and folates levels in serum

GSTM1, GSTP1, GSTT1, EPXH, XPD, XRCC1, hOGG1, MTHFR and MS gene polymorphisms were determined by PCR-based restriction fragment length polymorphism (RFLP) assays.

A modified high performance liquid chromatography (HPLC) method was used for determining the level of vitamin C (Tanishima and Kita, 1993). The simultaneous measurement of the amount of vitamins A and E in serum was performed according to a modified procedure of Driskell et al. (1982).

2.6 Statistical analysis

Data from different study groups were compared by the nonparametric Mann-Whitney test. The influence of independent variables (exposure, smoking habits, age, genotypes, etc.) on the level of chromosomal aberrations was estimated by multiple regression analysis.

3. RESULTS

The results of the conventional cytogenetic analysis (CCA) indicated that ACN showed clastogenic activity (Fig. 1). When CCA was used, the percent of aberrant cells (%AB.C.) and breaks per cell (B/C) were significantly higher in the exposed group (3.25 and 0.036, respectively) than in the controls (2.05 and 0.021, respectively). However, the genomic frequency of translocations per 100 cells (F_G/100) and %AB.C. measured by FISH did not differ significantly between the exposed and control groups.

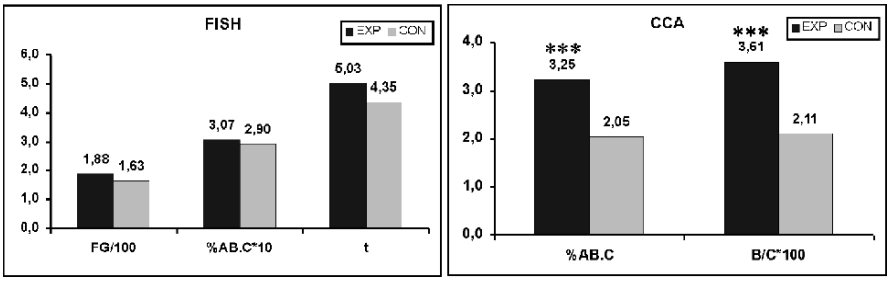

Figure 1. Clastogenic activity of acrylonitrile in exposed (EXP) and control (CON) subjects as assayed by fluorescence in situ hybridization (FISH) and conventional cytogenetic analysis (CCA).
*** $P < 0.01$; F_G/100, genomic frequency of translocations per 100 cells; %AB.C., percentage of aberrant cells; t, translocations per 1000 cells; B/C, breaks per cell.

The aberration frequencies measured by FISH among smokers in the exposed and control groups were not significantly higher than the aberration frequencies among their non-smoking counterparts (data not shown). No effect of smoking was found by CCA.

The FISH method measures stable chromosomal aberrations. With this method, age was an important confounding factor. Significant age-related dependence was found by regression analysis (the coefficient for increase was 0.056 for F_G/100 and 0.008 for %AB.C. per year; $P < 0.0001$) (Table 1).

To assess the influence of metabolic polymorphisms on cytogenetic biomarkers, the study subjects were genotyped for polymorphisms in GSTP1,

GSTT1, GSTM1, EPHX and MTHFR. To evaluate individual susceptibility, multiple regression analysis was performed (Fig. 2). Conventional staining demonstrated significantly more breakage of chromosome material in subjects with the GSTM1+ genotype than in subjects with the GSTM1 null genotype. However, analysis by FISH demonstrated that GSTM1-null individuals showed higher genomic frequency of translocations per 100 cells, and a higher percent of aberrant cells than individuals with the GSTM1+ genotype, although this difference was not significant.

Table 1. Results of multiple regression analysis of data from FISH and conventional cytogenetic analysis (CCA).

		Intercept (β_0)	GROUP		AGE	
				p		*P*
FISH	**F_G/100**	1.978	–0.426	0.0999	0.056	**0.0000**
	%AB.C.	0.340	–0.080	0.0528	0.008	**0.0000**
	t	5.282	–1.132	0.1031	0.150	**0.0000**
CCA	**%AB.C.**	1.995	1.327	**0.0004**	–0.099	0.46
	B/C	0.021	0.016	**0.0003**	–0.000	0.78

FISH, fluorescence in situ hybridization; CCA, conventional cytogenetic analysis; F_G/100, genomic frequency of translocations per 100 cells; %AB.C., percentage of aberrant cells; t, translocations per 1000 cells; B/C, breaks per cell.

Since the activity of EPHX depends on polymorphisms in both exons 3 and 4, it was necessary to analyze their combined effect. Statistical evaluation confirmed the generally accepted theory that subjects with fast EPHX activity had a lower frequency of chromosomal breakage when compared to subjects with slow and normal activity.

The parameters measured by FISH were also affected by MTHFR. The wild type Ala/Ala in the C677T codon was associated with increased chromosomal breakage in the control group, while heterozygotes with Ala/Val appeared to be more resistant.

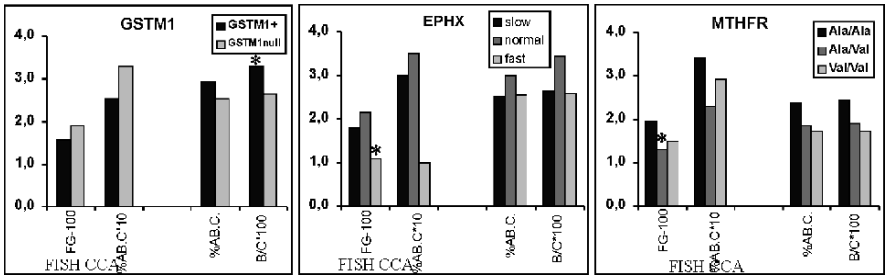

Figure 2. Impact of genetic polymorphisms on cytogenetic biomarkers

* $p < 0.05$; F_G/100, genomic frequency of translocations per 100 cells; %AB.C., percent of aberrant cells; B/C, breaks per cell.

There was no relationship between polymorphisms in the repair genes (XPD, XRCC1, MS and hOGG1), and the level of clastogenic damage.

A positive correlation was found between %AB.C. measured by FISH and plasma levels of vitamin E. Nevertheless, $F_G/100$ was inversely associated with the concentration of vitamin C ($P < 0.05$) (data not shown).

When these results were compared with results from an earlier study in the year 2000 (in which the concentration of ACN at the workplace was 0.05-0.3 mg/m^3), we found a statistically significant increase in CCA parameters with time. However, the level of stable aberrations measured by FISH did not change significantly (Table 2).

Table 2. Comparison of cytogenetic outcomes in two different sample periods.

	CCA		**FISH**		
	%AB.C.	***B/C***	***%AB.C.***	***$F_G/100$***	***t***
2000	2.03	0.022	0.37	2.11	5.66
2003	3.14	0.036	0.30	1.76	4.72
p-values	0.008	0.007	0.096	0.151	0.151

4. CONCLUSIONS

Our study indicates that occupational exposure to ACN (related to the level of ACN) significantly affected the level of chromosomal aberrations measured by conventional method. The frequency of translocations measured by FISH was age-dependent and correlated with EPHX and MTHFR polymorphisms and vitamins C and E levels. Chromosomal breaks evaluated by CCA decreased in subjects with GSTM1 null genotype. Smoking did not increase the clastogenic damage of ACN.

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CHAPTER 11

BIOMARKERS OF AIR POLLUTION EXPOSURE: FOLLOW-UP STUDY IN POLICEMEN IN PRAGUE

Blanka Binková, Jan Topinka, Olena Beskid, Irena Chvatalova, Zdena Lnenickova, Alena Milcova, Pavel Rossner, Oksana Sevastyanova and Radim J. Šrám

Institute of Experimental Medicine AS CR and Health Institute of Central Bohemia, Prague, Czech Republic

Abstract: The effect of exposure to polycyclic aromatic hydrocarbons (PAHs) adsorbed onto respirable air particles (<2.5 μm) on DNA adducts and chromosomal aberrations was studied in a group of city policemen (males, aged 22-50 years) spending >8 h outdoors. The results were compared to controls spending >90% of time indoors. The level of "like" benzo[a]pyrene (B[a]P)-derived DNA adducts was higher in the exposed group than in the controls (0.122 ± 0.036 vs. 0.099 ± 0.035 adducts/10^8 nucleotides, respectively, $P = 0.003$). "Like" B[a]P-derived DNA adducts have similar chromatographic mobility to the major B[a]P-derived DNA adduct, but their identity was not proven by co-chromatography with a standard. Using the fluorescence *in situ* hybridization technique and probes for chromosomes #1 and #4, the genomic frequencies of translocations calculated as $F_G/100$ were 1.72 and 1.24 for the exposed and control groups, respectively ($P < 0.05$). To evaluate the dynamics of the observed changes reported here, a prospective cohort study in similar populations (nonsmokers only) is currently underway.

Key words: air pollution, chromosomal aberrations, DNA adducts, FISH, genotypes, polycyclic aromatic hydrocarbons.

1. INTRODUCTION

Respirable ambient particulate matter (PM) comprises a complex mixture consisting of various chemicals. Epidemiological studies conducted in

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metropolitan areas have consistently demonstrated that exposure to PM is associated with increased mortality and/or morbidity, including respiratory and cardiovascular deaths, respiratory dysfunction, asthma, pneumonia and bronchitis (U.S. EPA, 1996). A consistent relationship between maternal exposure to fine particles during early gestation and intrauterine growth retardation (IUGR) was recently observed in a highly polluted district of Northern Bohemia (Dejmek et al., 1999). A possible explanation of this finding was that rather than particles, the sum of associated co-pollutants such as polycyclic aromatic hydrocarbons (PAHs) may interfere with fetal development (Topinka et al., 1997; Sram et al., 1999). Perera et al. (1998) showed in studies in Poland that ambient air pollution was significantly associated with the level of polycyclic aromatic hydrocarbon (PAH)-DNA adducts in white blood cells from both maternal and infant cohorts. Newborns with elevated DNA adducts in cord blood had significantly decreased length, birth weight and head circumference compared to newborns with lower DNA adduct levels. The results indicate that at least in some regions, PAHs are a major contributor to the genotoxicity and embryotoxicity of organic mixtures associated with air pollution.

To evaluate the hypothesis that PAHs are the major source of the genotoxicity of organic mixtures associated with air pollution, a molecular epidemiology study was performed in city policemen in Prague (Czech Republic). These policemen normally work on busy streets for 12-hour shifts. To examine the dynamics of the observed changes reported in this paper, a prospective cohort study is currently being conducted. Within this study, all the biomarkers of exposure and effect are analyzed every three months over a one-year period, to include periods with high (winter) and low (summer) levels of air pollution.

2. METHODS

2.1 Subjects and sampling

The study population consisted of 53 policemen (males) working in downtown Prague, who spent more than 8 hours outdoors daily (exposed group [EXP]). These individuals were matched with 52 healthy volunteers (control group [CON]) who spent >90% of their daily time indoors. The sampling of both groups was carried out in winter (February 6-20, 2001) when the highest air pollution levels were expected.

2.2 Exposure monitoring

To assess the differences in levels of exposure between the EXP and CON groups, personal exposure monitoring was conducted during the entire work shift using personal monitors for collection of respirable ambient particulate matter <2.5 μm (PM2.5). Blood and urine samples were collected at the end of the work shift.

In addition, the data generated by stationary air pollution monitoring stations, specifically Versatile Air Pollution Samplers (VAPS) located in Prague 5 – Smíchov (a high traffic-density area), and Prague 4 – Libuš (a low traffic-density area), provided long-term averages of pollutants from different periods of the study (Farmer et al., 2003).

2.3 Personal exposure to PAHs and internal markers

Quantitative chemical analysis of carcinogenic PAHs (c-PAHs) was conducted on the organic extract of PM2.5 collected by personal monitors. Cotinine, a major nicotine metabolite, was analyzed in urine using the RIA assay (Langone and Van Vunakis, 1982) and was compared to the tobacco smoke exposure reported in the life style questionnaire. Plasma levels of vitamins A, C and E were determined by high performance liquid chromatography (HPLC) (Driskell et al., 1982, Tanishina and Kita, 1993).

2.4 DNA adduct analysis by ^{32}P-postlabeling

DNA adduct levels in lymphocytes isolated from blood samples were evaluated using ^{32}P-postlabeling for bulky aromatic DNA adducts using the nuclease P1 enrichment procedure (Reddy and Randerath, 1986) and thin layer chromatography (TLC). The diagonal radioactive zone and distinct spots were evaluated using the same template for all chromatographic plates. A benzo[a]pyrene (B[a]P)-derived DNA adduct standard was analyzed in triplicate in each experiment to control for interassay variability and to normalize the calculated DNA adduct levels. To calculate DNA adduct levels and to control the purity of the DNA, the aliquots of DNA digest were analyzed for nucleotide content by HPLC. The data presented here are average values of the total level of DNA adducts and averages for “like” B[a]P-DNA adduct spots, analyzed in at least three independent postlabeling experiments.

2.5. Cytogenetic analysis (conventional technique and fluorescence *in situ* hybridization technique)

In addition to the conventional method for determining chromosomal aberrations in peripheral blood lymphocytes, the frequency of aberrations was determined by the fluorescence *in situ* hybridization technique (FISH). In contrast to the conventional method, FISH is particularly sensitive for detecting chromosomal translocations and insertions. These events are believed to be more relevant for carcinogenesis than the chromosomal breaks detected by conventional cytogenetic analysis. We used FISH with whole chromosome painting for chromosome #1 and #4 (Rubeš et al., 1998). Each aberration detected by chromosome painting was then categorized according to the developed nomenclature for aberrations (Tucker et al., 1995). All rearrangements of the painted chromosomes were involved and were registered as exchanges between painted and unpainted materials as well as exchanges among painted chromosomes (Sram et al., 2004).

2.6. Genetic susceptibility markers

Polymorphisms of metabolic genotypes (GSTM1, GSTP1, GSTT1, EPHX, CYP1A1-Msp-I, NAT2, MTHFR, MS), DNA repair genotypes (XRCC1 codon 399, XPD exon 6, XPD exon 23, hOGG1) and tumor supressor gene p53-MspI were determined by PCR-based RFLP assays.

2.7. Statistical analysis

Multivariate statistics was used to evaluate the association between air pollution and various biomarkers. The impact of metabolic and DNA repair gene polymorphisms on DNA adduct levels and cytogenetic markers was studied by multiple regression analysis. An unpaired Student's t-test was used to compare cytogenetic data for various groups of subjects.

3. RESULTS

During the sampling period (February 6-20, 2001), the particulate air pollution monitored by VAPS at two monitoring sites was as follows: PM10 ranged from 32 to 55 $\mu g/m^3$, PM2.5 was from 27 to 38 $\mu g/m^3$, and c-PAHs were between 18 and 22 ng/m^3. Based on the personal monitoring data, the policemen were exposed to significantly ($p < 0.01$) higher levels of c-PAHs during their work shifts than were the controls: 12.0 ± 11.1 ng/m^3 vs. $6.2 \pm$

3.5 ng/m^3, respectively. Plasma levels of vitamins A, C and E (markers of antioxidants status) did not differ between EXP and CON groups.

The total DNA adduct levels did not significantly differ between exposed and control subjects (0.92 ± 0.28 vs. 0.82 ± 0.23 adducts/10^8 nucleotides, respectively; p = 0.065; Fig. 1), whereas the level of "like" B[a]P-derived DNA adducts was significantly higher in the exposed group than in the control group (0.122 ±0.036 vs. 0.099 ± 0.035 adducts/10^8 nucleotides, respectively; p = 0.003; Fig. 2). Within both the exposed and control groups, there was a significant increase in both the total DNA adduct levels ($p < 0.05$) and "like" B[a]P-DNA adduct levels ($p < 0.01$) in smokers when compared to nonsmokers.

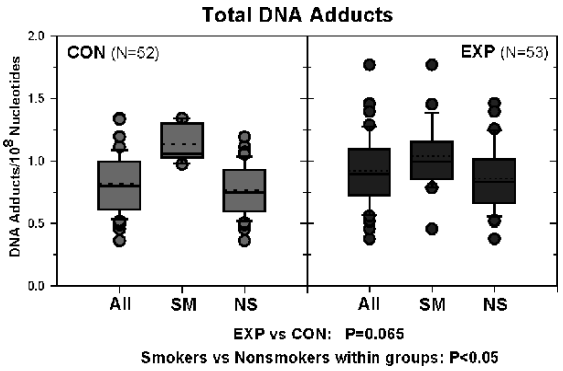

Figure 1. Total DNA adduct levels in lymphocytes of control subjects and city policemen.

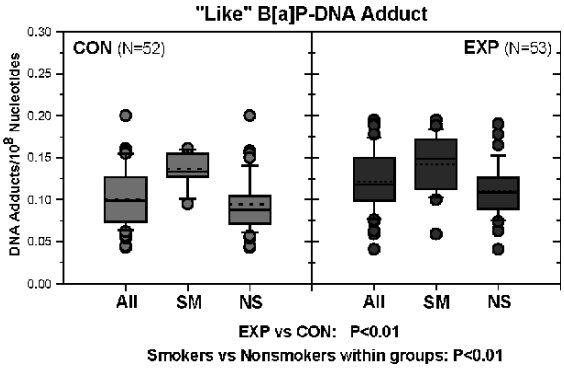

Figure 2. "Like" B[a]P-DNA adduct levels in lymphocytes of control subjects and city policemen.

No relationship was observed between DNA adduct levels and short-term exposure to PAHs as evaluated by the personal monitors during work shifts. The results of multivariate regression analysis suggested that smoking, vitamin C levels, polymorphisms in EPHX in exon 4, and XRCC1 and XPD in exon 23 were major indicators of total DNA adducts. Smoking, exposure to c-PAHs and polymorphisms in the XPD repair gene in exon 6 were significant indicators of "like"B[a]P-DNA adducts.

3.1 Cytogenetic analysis

There was a significantly increased frequency of translocations detected by FISH in policemen who were nonsmokers in the city of Prague (Tables 1 and 2). The frequency of translocations in the control group was significantly higher among smokers than among non-smokers ($p < 0.05$). The frequency of translocations was predicted by age, smoking, exposure to c-PAHs, B[a]P "like"-DNA adducts, folates and polymorphisms in CYP1A1, GSTM1, GSTP1, EPHX, p53 MspI and MTHFR.

As determined by conventional cytogenetics, the level of chromosomal aberrations (AB.C) in both the EXP and CON groups from Prague (2.28% and 1.94% AB.C, respectively) increased in comparison to the spontaneous levels in the Czech Republic (1.16% AB.C., 1,801 subjects, aged 20-59 years). This result indicates the need of further studies of reasons for such differences. Polymorphisms of CYP1A1, XPD in exon 6 and XPD in exon 23 were predictors of frequency of aberrant cells by the conventional method.

Table 1. Results of cytogenetic analysis by FISH.

GROUP		**F_G/100**	**% AB.C.**	**NCJ**	**B/1000**		
					Total	**t**	**rcp**
EXP	**All**	1.72	0.33	5.16	5.62	4.62	1.74
N = 53	**SM (N = 19)**	2.02	0.39	6.29	6.88	5.41	1.88
	NS (N = 34)	1.56	0.29	4.58	4.97	4.21	1.67
CON	**All**	1.25	0.24	3.42	3.86	3.35	1.21
N = 52	**SM (N = 7)**	2.05	0.40	5.50	6.33	5.50	2.17
	NS (N = 45)	1.14	0.22	3.12	3.51	3.05	1.07

EXP, exposed group; CON, control group; SM, smokers; NS, non-smokers; F_G/100, genomic frequency of translocations per 100 cells; % AB.C., % aberrant cells; NCJ-number of color junctions; t; translocations; rcp, reciprocal translocations.

Table 2. Statistical evaluation* of FISH data: *p*-values for comparison of various groups and subgroups.

GROUPS	F_G/100	% AB.C.	NCJ	Total	t	rcp
EXP vs CON	**0.04**	**0.02**	**0.01**	**0.01**	**0.04**	**0.05**
EXP SM vs CON SM	0.49	0.48	0.38	0.42	0.49	0.38
EXP SM vs EXP NS	0.17	0.09	0.11	0.08	0.17	0.35
CON SM vs CON NS	**0.03**	**0.01**	**0.03**	**0.02**	**0.03**	**0.03**
EXP NS vs CON NS	0.06	**0.03**	**0.03**	**0.03**	**0.05**	**0.04**

*Unpaired Student's t-test
F_G/100, genomic frequency of translocations per 100 cells; % AB.C., % aberrant cells; NCJ, number of color junctions; t, translocations; rcp, reciprocal translocations.

4. CONCLUSIONS

The results of DNA adduct analysis and FISH indicate that city policemen represent a group with higher genotoxic risk. FISH analysis appears to be a more sensitive method for detecting clastogenic activity than conventional cytogenetic analysis. In addition, it appears that polymorphisms in metabolic and DNA repair genes could be used to identify subjects with increased sensitivity to DNA damage caused by exposure to carcinogenic PAHs.

5. FOLLOW-UP STUDY

To confirm the results of the the present study and to see the dynamics of the observed changes, a follow-up prospective epidemiological study is being conducted. The study involves non-smoking policemen who work in downtown Prague (n = 120). Personal exposure monitoring of PM2.5 and PAHs (for 48 hours) as well as biological sampling are being repeated four times per person over the course of one year in order to obtain data for periods with different levels of air pollution (January, March, June, and September of 2004) and to analyze the variability in biomarkers.

ACKNOWLEDGMENTS

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CHAPTER 12

ASBESTOS EXPOSURE AND ITS HEALTH EFFECTS

Petra Gergelová[1], Margareta Šulcová[1,2], Marta Hurbánková[3]
[1]Trnava University, Faculty of Public Health and Social Care, Trnava, Slovakia;[2]Slovak Medical University, Faculty of Public Health, Bratislava, Slovakia; [3]Research base of Slovak Medical University- Institute of Preventive and Clinical Medicine, Bratislava, Slovakia

Abstract: Asbestos fibres represent a potential environmental and occupational health hazard for humans. Long-term exposure to asbestos can cause pleural plaques, asbestosis and oncological diseases. The aim of this in-depth study was to analyze the trend in the development of malignant mesothelioma in Slovakia between 1988 and 2000. The results show an apparent fluctuation in the occurrence of malignant mesothelioma in Slovakia; until 1997 there was an increasing number of cases each year but since then there has been an apparent decline. The greatest number of incidences of mesothelioma was reported in 1997, at 0.59/100 000 inhabitants. The reported incidences of occupational diseases related to asbestos exposure was also part of this study.

Key words: asbestos, mesothelioma, health effects, asbestosis, incidence, Slovakia

1. INTRODUCTION

Asbestos has been used and mined for at least 2500 years. The first systematic epidemiological studies on lung cancer and mesothelioma were published in the 1930s and 1940s. As Rantanen (1997) noted, these early findings did not affect the asbestos production or its use. Industry-wide actions to regulate its use were initiated in most countries about 40 years ago but not implemented in full scale until as late as the 1970s and 1980s. Vogel (2001) warns that, while it is impossible to put a precise figure on the number of victims claimed by asbestos in the 20th century, the death toll from cancers and pulmonary fibrosis is at least several hundred thousand. Šulcová et al. (1997) reported that due to a massive over-utilization of

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asbestos and asbestos contaminated materials (in the building industry especially but also in other professional activities), and because of the lengthy dormant period before the first symptoms of asbestos related diseases, we are now confronted with serious negative consequences and the incidence of malignant mesothelioma is still on the rise. Although the European Union and USA have banned the use of asbestos, its use continues; one fifth of the consumption taking place is in developing countries. The increase of its production in developing countries, Brazil and India for example, raises apprehensions of the potential increase in diseases and deaths related to asbestos in these countries (Rantanen, 1997). Nine European countries (Denmark, Finland, France, Germany, Italy, Netherlands, Norway, Sweden and Switzerland) have banned the use of all types of asbestos and the same criteria has also been accepted by the countries who have recently joined the EU (since may 2004). (Peto et al., 1999).

1.1 Asbestos

Asbestos is a generic term for a group of six naturally occuring fibrous minerals. The basic unit of asbestos-class minerals is silicate mixed in varying proportions with magnesium, iron, calcium, aluminum, and sodium or trace elements. There are two subdivisions of asbestos: the serpenine group containing only chrysotile, and the amphibole group containing several minerals (amosite, athophylite, tremolite, actolite, crocidolite). Chrisotile consists of bundles of curly fibrils, whereas the amphiboles tend to be more straight and rigid (Demers et al., 1997; Gochfeld, 1995).

1.1.1 Asbestos in commerce

The fire-resistant properties of asbestos have been known for millennia. It was not until the 1870s, fairly late in the industrial revolution, that asbestos came to have widespread commercial use. Its unique properties included resistance to fire, chemicals, and friction; these same properties, however, account for its unique toxicity (Gochfeld, 1995; Barnínec, 1999).

1.2 Respiratory effects of asbestos exposure

The respiratory, immunologic, cardiovascular, and gastrointestinal systems may be adversely affected by asbestos inhalation and by ingestion of food subsequent to exposure to asbestos (exposure may cause mucociliary removal from the respiratory tract). Inhalation of asbestos fibres may cause parenchymal and pleural asbestosis, mesothelioma, and carcinoma. Exposure to other carcinogenes, dose and duration of exposure, all may play a role in

disease development. Chronic low-level exposures to asbestos has been associated with lung cancer, mesothelioma, and pleural diseases, including pleural asbestosis. Higher doses are more likely to produce parenchymal asbestosis. Smoking and other toxins increase the risk of asbestos-associated lung cancer (Demers et al., 1997; Hurbánková, 1999; Hurbánková, 1998).

Table 1. Partial listing of commercial and building materials which may contain asbestos as a component.

Commercial	Homes and buildings
boilers and heating vessels	duct insulation
cement pipe	fire protection panels
clutch, brake, transmission components	sheet vinyl or floor tiles
roofing products	pipe or boiler insulation
paper products	shingles
textiles ect.	textured acoustical ceiling ect.

1.2.1 Mesothelioma

Mesothelioma are tumors arising from the thin membranes that surround Internal organs. Pleural and peritoneal mesotheliomas are rare in most unexposed populations but are definite indicators of asbestos exposure. Although all asbestos types can cause mesothelioma, several studies have suggested that, in humans, the amphibole mineral may be more likely to induce mesothelioma than the serpentine form (Demers et al., 1997). Peto et al. (1999) estimated that world incidence of malignant mesothelioma is 1-2 cases/million inhabitants, but during the last few decades the incidences have clearly increased in industrial countries to as high as 10-25 cases/million inhabitants at the beginning of the 1990s. Mesothelioma often begins with pleural effusion and/or chest pain, dyspnea, and weight loss. Many mesotheliomas have been misdiagnosed as lung cancer, because nosology emphasizes site rather than histology. The histological picture resembles fibrosarcoma. Gochfeld (1995) demonstrated that although all fiber types can cause mesothelioma, crocidolite seems to be the worst offender. The prognosis for patients with mesothelioma is poor; they seldom live longer than 12 to 18 months after diagnosis.

1.2.2 Asbestos in Slovakia

Šulcová et al. (1997) report that asbestos in Slovakia has been used in the making of building materials, in the production of asphalt isolation materials, as a filter material in chemical, pharmaceutical and food industries, as an insulating material in ship building and piping, as a fire retardant in

construction, and as a brake material. A serpentine mine in Dopsina, which is now closed, had an annual production of 80,000 tons of asbestos in 1991 and 24,500 tons in 1996. The use of asbestos in production of food, pharmaceuticals, buildings, brakelines, insulation in shipbuilding and crocidolite has been prohibited. The production of asbestos-containing materials ceased by the end of 1998 and the import of such materials is now legally prohibited The construction of new buildings using asbestos-containing material is not allowed.

2. MATERIALS AND METHODS

The information on reported mesotheliomas was obtained from The National Oncological Register, and we calculated the rate of incidences by the number of inhabitants listed in the Statistical Year-Book of the Slovak Republic (1991-2000). The number of reported occupational diseases related to asbestos exposure was taken from regularly updated material in "The course of occupational diseases in Slovak Republic from the year 1993 to 2000" (Krutý and Moricová, 2001).

3. RESULTS

The number of malignant mesothelioma in Slovakia during the last decade has increased and the incidence of this malignant disease suggested an increasing trend. It peaked in 1997 (32 new cases) after which it seems to fluctuate with a substantial decrease in 2000 (only 13 new cases). The lowest number of incidences was recorded in 1992 (10 new cases) *(*Table 2, Figure 1).

Figure 2 shows that the 32 new cases of mesothelioma in 1997 represents the highest incidence of this disease: 0.59 new cases/100 000 inhabitants. The lowest incidences of mesothilioma reported in 1992was 0.18/100,000.

In the Slovak region, the highest incidence was 1.53/100 000 inhabitants and was reported from the Nitra region in 1997, when 11 new cases of mesothelioma were also noted. *Figure 3* shows, that 17% of all mesotheliomas in 1994-2000 occured in the Nitra region. The Banska Bystrica region represents the lowest percentage of mesothelioma in Slovakia during this period (6%) (*Figure 3)*.

Table 2. Incidence (Inc.) and absolute number (Abs.) of malignant mesothelioma cases in 1994-2000 in Slovakia.

Year	Men		Women		Total	
	Abs. number	Inc/ 100 000	Abs. number	Inc/ 100 000	Abs. number	Inc/ 100 000
1994	4	0.2	12	0.4	16	0.29
1995	9	0.3	11	0.4	20	0.37
1996	22	0.8	3	0.1	25	0.46
1997	17	0.6	15	0.5	32	0.59
1998	9	0.3	8	0.3	17	0.31
1999	13	0.5	10	0.4	23	0.42
2000	6	0.2	7	0.3	13	0.24

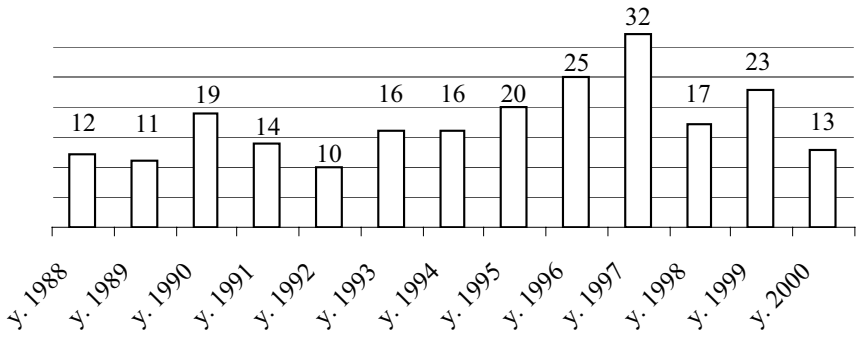

Figure 1. Absolute number of new cases of mesothelioma in 1988-2000 in Slovakia.

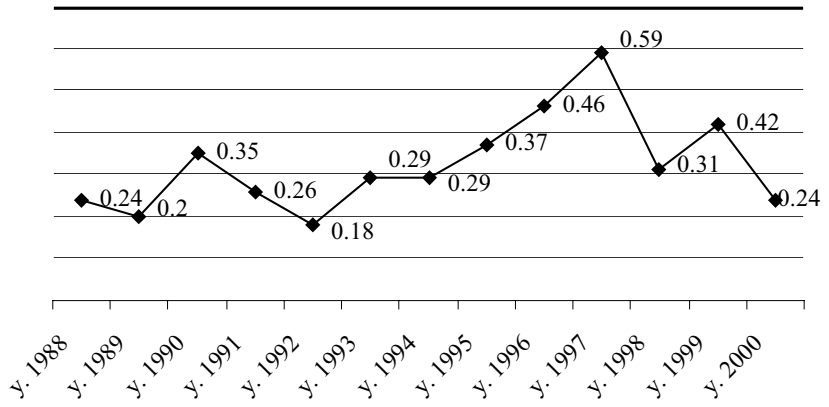

Figure 2. Incidence of mesothelioma in 1988-2000 in Slovakia per 100 000 inhabitants.

Figure 4 summarizes data on the number of diseases related to asbestos in occupationally exposed workers between 1980-2000. The highest number of asbestosis cases reported as an occupational disease was in 1998 (7 new cases), and the highest number of lung cancer cases related to asbestos exposure and characterized as an occupational disease were diagnosed in 1999 (4 new cases).

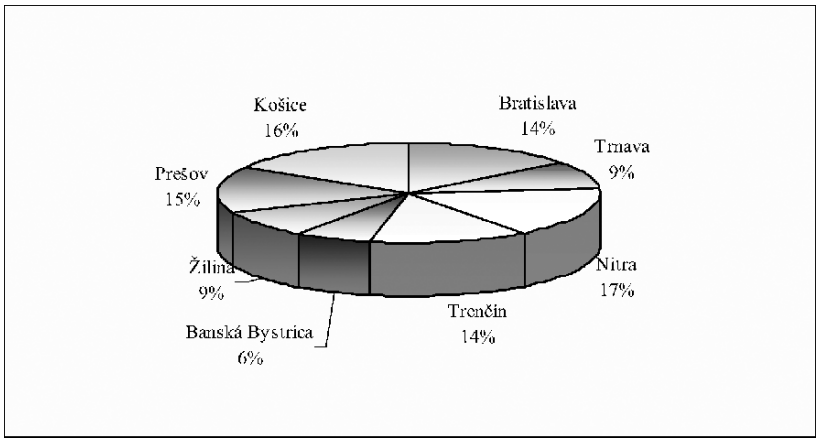

Figure 3. Percentage of Slovak regions on total number of mesothelioma in 1994-2000.

4. DISCUSSION

Rantanen (1997) warns that the global asbestosis epidemic is far from residing. It is a fact that we are going to face growing numbers of clinical cases in the future. A look at global consumption data and analysis of cancer rates in developing countries from the recent years to the present, lead us to expect that even though the level of protection and the working practices will reduce the asbestos-related exposure and morbidity, these diseases will continue to occur at the present rate at least throughout the first half of the21st century. The aim of our study was to provide data about the trend of incidences of malignant mesothelioma in Slovakia during the last decade. The results show an apparent fluctuation in the rate of occurrence of malignant mesothelioma in Slovakia; although there has appeared to be an increase up to the year 1997, it began to decline in the following years. The highest incidence of mesothelioma was reported in 1997, and was 0.59/100,000 inhabitants. In Great Britain, the incidence of mesothelioma is expected to continue to increase over next 20 years. Annual rates in Europe

range from around 8 per 100,000 in Scotland, England and the Netherlands, and to lower than 1 per 100,000 in Spain (0.96), Estonia (0.85), Poland (0.85) and Yugoslavia. Between 1978 and 1987, rates in men significantly increased in all countries (except Denmark). In the following 10 years, there was a deceleration in this trend, and a significant increase was detectable only in England and France (Peto et al., 1999).

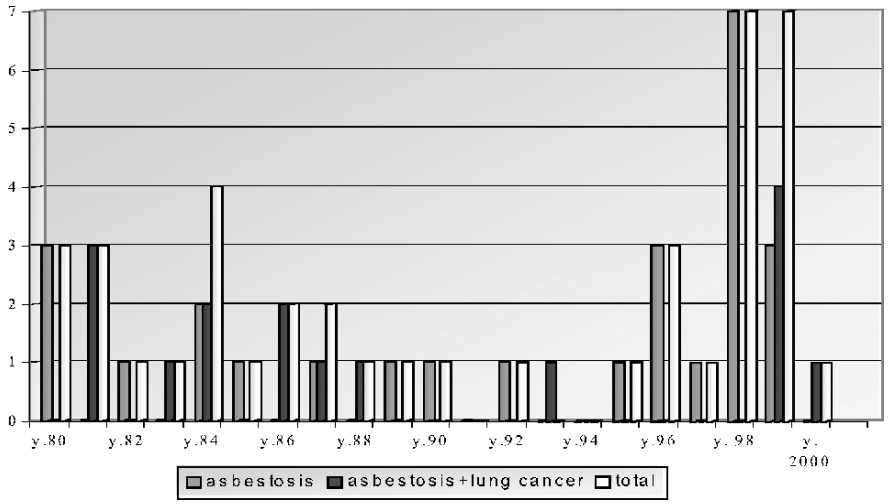

Figure 4. Number of occupational diseases related to asbestos exposure in Slovakia in 1988-2000.

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CHAPTER 13

ENVIRONMENTAL CONDITIONS AND HEALTH OUTCOMES FOR CHILDREN IN ARMENIA

Environment and Child Health in Armenia

Artak Khachatryan[1] and Anahit Aleksandryan[2]

[1]*Yerevan State Medical University, 2 Koryun Street, Yerevan, 375028 Republic of Armenia;*
[2]*Ministry of Nature Protection, Yerevan, Republic of Armenia*

Abstract: Children are vulnerable to environmental factors. The potential adverse health effects of chemical exposure in children depend on toxicity, dose, duration, and degree of contact. Considering the importance of milk and dairy products in children's lives, monitoring studies were performed. In milk samples from Echmiadzin (an agricultural district), polychlorinated biphenyls (PCBs) were present at concentrations ranging from 2.12 - 7.45 mcg/L, while in samples from Gugark and Alaverdi (industrial districts), PCBs were present at concentrations of 1.13 - 5.13 and 2.04 - 4.53 mcg/L, respectively. Persistent Organic Pollutants (POPs) were found in almost all samples of cheese from Alaverdi and Gugark districts. These data indicate that children in Armenia may be exposed to POPs through ingestion of milk and dairy products.

Keywords: Children's health, environmental exposure, Persistent Organic Pollutants (POPs), residual amounts, monitoring.

1. INTRODUCTION

It is apparent that environmental conditions can impact human health. Recent studies have linked chemical exposures with specific disease states (Barbieri et al., 2002; Integrated Risk Information System (IRIS) on Benzene, 2002; Toxicological Profile for Benzene, 1997; Hyland et al., 2003; Tadevosyan et al., 2003; Tadevosyan et al., 2004; Engel and Smith, 1994; Moore et al., 2002; Yih et al., 1997). Chemical exposures, both direct and indirect, may be as threatening to human health as infectious diseases. Long-term chemical exposures may have a serious impact on public health, especially in the cohorts of children (infants, children, adolescents).

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Children, developing fetuses, and pregnant women are the most vulnerable to the harmful effects of environmental chemicals. As stated by Carlson (1998), pediatric medicine shows that children are not just small adults; they are uniquely vulnerable. The resulting effects of exposure depend on chemical toxicity, dose, duration, and degree of contact with the chemical. Poverty, malnutrition, and other stressors can increase the susceptibility of children to harmful environmental factors.

The compounds of greatest concern in relation to children's health include the following:

- Persistent Organic Pollutants (POPs) (for example, polychlorinated biphenyls [PCBs], p,p'-Dichlorodiphenyltrichloroethane (DDT), etc.), which remain in the environment for an extended period and can cause reproductive disorders;
- pesticides, including accidental exposures in children and exposures to pesticides that have been banned elsewhere;
- metals and their derivatives, including lead, mercury, arsenic, cadmium, and chromium, which are widely used in modern society;
- household products, including kerosene, solvents, and pharmaceuticals, which children may accidentally ingest, leading to acute poisoning;
- hazardous waste sites, which present a potential threat to the health of children, especially those who live and scavenge in poor regions.

2. SPECIFIC AIMS

Milk is a basic and essential food for children that supplies the calcium needed for growth and development. The recommended daily intake of milk for children ages 4 to 8 is three glasses per day. The goal of this research was to determine the level of contaminants in milk from agricultural and industrial areas in Armenia. Monitoring studies were performed to determine the residual amounts of several persistent organic pollutants (POPs) in milk and dairy products (Aleksandryan et al., 2003a; 2003b; Aleksandryan et al., 2004a; 2004b).

3. MATERIALS AND METHODS

Samples of milk and cheese were taken from private enterprises (farms) of Aragatzotn, Ararat, Armavir, Lori and Syunik marzes (regions) of the

Republic of Armenia. Both full-fat and low-fat cheeses prepared from cow and sheep milk were taken.

Analysis of persistent organic pollutants (organochlorine pesticides and PCBs) in samples was accomplished using a gas-liquid chromatograph with an electron capture detector (Chromatograph "Tswet-106", with a 2 m glass column [i.d. 3 mm filled with chromaton N-AW with HMDS]). The carrier gas was nitrogen.

Table 1. Average persistent organic pollutant (POP) content in samples of milk from several regions (marzes) of Armenia in 2002-2003 (in mcg/L).

Sampling point location (marz/ region)	HCH			DDT and metabolites					
	α + β	γ	Σ HCH	DDT	DDE	DDD	Σ DDT	HCB	PCBs
Syunik Marz, Meghri	1.23 ± 0.44	0.38 ± 0.02	1.67	1.45 ± 0.61	0.097 ± –	1.11 ± 0.34	2.66	0.021 ± 0.13	NS
Lori Marz, Alaverdi	0.27 ± 2.8	0.31 ± –	0.58	0.81 ± 0.11	0.10 ± 0.08	0.14 ± 0.05	0.42	0.10 ± 0.04	5.15 ± 1.13
Lori Marz, Gugark	0.11 ± 0.09	0.06 ± 0.01	0.17	0.16 ± 0.14	0.03 ± 0.02	0.10 ± 0.06	0.29	0.025 ± 0.013	4.53 ± 2.04
Ararat Marz, Artashat	0.05 ± 0.02	2.25 ± 2.40	2.30	2.61 ± –	NR	NR	2.61	0.07 ± –	NS
Armavir Marz, Echmiadzin	0.17 ± –	0.23 ± 0.16	0.40	0.37 ± 0.53	NR	NR	0.37	NR	7.45 ± 2.12
Aragatsotn Marz, Ashtarak	0.02 ± –	0.08 ± 0.03	0.20	NR	NR	0.48 ± 0.37	0.48	NR	NS

HCH = hexachlorocyclohexane

DDD = p, p'-dichlorodiphenyldichloroethane

HCB = hexachlorobenzene

4. RESEARCH OUTCOMES

PCBs were detected in samples of milk from the Echmiadzin District (sub-region) of Armavir Region, which was selected as an example of an agricultural region (for comparison with industrial regions). The total PCB content in samples of milk from this area was 2.12 - 7.45 mcg/L. This was higher than expected when compared to the PCB content of milk samples from the industrial region of Lori marz (districts of Gugark and Alaverdi), where the PCB content in milk was 1.13 - 5.13 and 2.04 - 4.53 mcg/L, respectively.

These observations would appear to indicate that PCB contamination is more widespread than previously thought in the Republic of Armenia, and that PCB contamination is not limited to the regions where the electric power production facilities are located.

Table 2. Average persistent organic pollutant (POP) content in samples of cheese from different regions (marzes) of Armenia, 2002-2003 (in mcg/L).

Sampling point location (marz/ region)	HCH			DDT and metabolites					
	α+ β	γ		DDT	DDE	DDD	Σ DDT	HCB	PCBs
Syunik Marz, Meghri	3.18 ± 0.53	0.41 ± 0.17	3.59	7.11 ± 1.85	0.10 ± –	5.52± 1.45	12.73	0.66 ± 0.009	NS
Lori Marz, Alaverdi	1.08 ± 0.23	0.03 ± 0.02	1.11	0.19 ± 0.12	0.27 ± 0.0	0.18 ± 0.14	0.64	11.09 ± 2.80	11.94 ± 5.21
Lori Marz, Gugark	0.56 ± 0.4	0.07 ± 0.05	0.63	0.22 ± 0.05	0.36 ± 0.18	2.34 ± 4.08	2.92	4.63 ± 3.74	63.86 ± 7.89
Ararat Marz, Artashat	0.20 ± 0.09	2.65 ± 1.23	2.85	0.42 ± 0.11	NR	0.43 ± 0.03	0.85	0.10 ± –	NS
Armavir Marz, Echmiadzin	1.06 ± 1.46	2.77 ± 1.05	3.83	NR	NR	0.35± –	0.35	NR	NS
Aragatsotn Marz, Ashtarak	0.66 ± 0.12	5.17 ± 0.12	5.83	2.54 ± 3.61	0.35 ± 0.09	0.48 ± 0.04	3.37	4.22 ± 1.45	NS

HCH = hexachlorocyclohexane

DDD = p, p'-dichlorodiphenyldichloroethane

HCB = hexachlorobenzene

Various samples of cheeses made in the countryside and taken from these same regions were analyzed for POPs. Tables 1 and 2 present the POP content of milk and cheeses. POPs were found in almost all cheese samples.

Analysis of cheese samples for PCBs showed that PCBs were found only in samples collected from the districts of Alaverdi and Gugark in the Lori marz region. For comparison, the content of DDT and p, p'-dichlorodiphenyldichloroethylene (DDE) in milk and dairy products in 1970 is presented in Table 3. At this time point, among the samples that contained detectable levels of these two compounds, the average content of DDT + DDE was 0.24 ± 0.51 mg/kg (Monitoring of POPs in Armenia, 2003).

Table 3. DDT and DDE content in some milk and dairy products origin, Armenia, 1970, (in mg/kg).

Food stuffs	Pesticide	Number of regions involved in study	Average content in positive samples	Average content considering all samples	Pesticide Detection % in samples
Milk, dairy products	DDT+ DDE	15	0.24±0.51	0.15± 0.03	92.4

5. CONCLUSIONS

The data from this study indicate that children may be exposed to the above-mentioned chemicals through the ingestion of milk and other dairy products. Additional research is needed to identify and mitigate the potential health impacts of environmental hazards, in particular, POPs.

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CHAPTER 14

THE CARCINOGENIC RISK OF OCCUPATIONAL EXPOSURE TO QUARTZ DUST: BIOMONITORING RESULTS

Lubomir Dobiáš,[1] Hana Lehocká,[1,2,3] Ivona Závacká,[1] Jaromira Kůsová,[2] Tomas Adamus,[1] Hana Tomášková,[1]

[1]Medico-Social Faculty University of Ostrava, Czech Republic; [2]Institute of Health Ostrava, Czech Republic; [3]Medical Faculty University of Palacky, Olomouc, Czech Republic

Abstract: This study utilized biomonitoring to evaluate the genotoxic potential of quartz dust in the ambient air at the workplace in coal mines, quarries, and stone processing facilities. The data suggest that quartz dust is genotoxic, and is an important contributor to the genotoxicity of complex mixtures of fibrogenic respirable airborne particles in ambient air in these workplaces. In areas of quarrying and stone processing, the respirable dust particle concentration was between 0.18 – 2.16 mg/m^3, and quartz made up 30% of the dust. In coal mines the average concentration of respirabile dust was 2.68 mg/m^3; quartz made up only 3% of the dust or less. The frequency of chromosomal aberrations increased significantly in exposed groups, compared to the controls. The results indicate a significant increase in chromosomal aberrations (%AB.C.) among workers who were occupationally exposed to dust containing quartz (during the earliest period of exposure, about 30% of the highest acceptable exposure. The results show a non-linear dose-response for chromosomal aberrations at high exposure levels.

Key words: quartz, genotoxicity, carcinogenicity, chromosomal aberrations, biomonitoring, highest acceptable exposure (HAE), coal mines, stone processing

1. INTRODUCTION

The increasing exposure of humans to genotoxic contaminants in the environment is a complex public health problem. According to an IARC/WHO Monograph (IARC/WHO, 1997), crystalline silica and

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cristobalite are classified as Group 1 substances, i.e., substances that are carcinogenic to humans. Exposure to these substances has been linked to pulmonary disease and other types of malignant cancers. Research is needed to determine the emission, distribution and fate of genotoxicants in the environment, as well as human exposures to these compounds (Lewtas, 1990, Hunter, et al., 1997).

In the Czech Republic, more information is needed regarding the carcinogenic risk of quartz dust among workers in tunneling, metallurgy, and the ceramic industry. The present study evaluated selected biomarkers of exposure and effect among occupationally exposed workers (miners of black coal, and workers in quarrying and stone processing). The goal of this study was to assess the carcinogenic risk of quartz dust.

2. METHODS

2.1 Cytogenetic analysis of lymphocytes in peripheral blood

Cytogenetic methods were used to evaluate health effects among workers with lengthy occupational exposures. Cytogenetic analysis (CA) of structural chromosomal aberrations was conducted using cultures of peripheral blood lymphocytes. A modified conventional method (Hungerford, 1965) was used with short-term cultures for 52 hrs. All cells were in first division. CA of chromosomal aberrations was used for the groups of workers (miners, stonecutters) exposed to dust particles containing quartz. These workers were exposed to between 30% and 85-95% of the highest acceptable exposure (HAE). The matching control group was composed of individuals living in the same region who were not occupationally exposed. Every group (exposed and unexposed) included 25 cases, to give a total of 150 persons.

2.2 Urine collection and 1-hydroxypyrene determination

1-Hydroxypyrene (1-OH-P) is generally accepted as an indicator of human exposure to PAHs. Approximately 150 ml of post-shift urine was collected from the occupationally exposed individuals, and stored at - 20^{o}C in the dark within 24 hours of collection. In this study, this metabolite was detected in urine samples after enzymatic hydrolysis of the urine samples, followed by high performance liquid chromatography (HPLC) with fluorescence detection. Creatinine was also measured, and 1-OH-P was presented as a ratio to creatinine.

2.3 Air sampling procedures and analysis

In this study, the following parameters were monitored to evaluate the genotoxic risk of quartz dust: personal exposure (quartz dust as a fraction of total respirable airborne particles < 2.5 μm (PM 2.5), stationary exposure to airborne particulate matter, and exposure to polycyclic aromatic hydrocarbons (PAHs) in the particulate and semivolatile fraction. The personal dosimetry method was used to evaluate exposure to PAHs. This consisted of personal samplers (PEMs) with connected sampling heads for selective detection of the respirable dust fraction and the semivolatile phase of contaminants in the workplace.

The total mass of the sampled particulate fraction was determined gravimetrically. The chemical substances from the dust particles and the semivolatile fraction were extracted by dichloromethane and acetonitrile. Chemical analysis of PAHs was performed using HPLC with fluorescence detection.

2.4 Mutagenicity in urine

Post-shift urine from occupationally exposed workers was collected and preserved as described previously for 1-OH-P analysis. The mutagenicity of this urine was determined using a short-term bacterial test (Ames assay with metabolic activation (S9) and *Salmonella* strain TA98). The Genetox Manager version 2.21 Program (U.S. Environmental Protection Agency) was used to evaluate whether the samples were positive or not.

3. RESULTS AND CONCLUSIONS

Quartz appears to be an important genotoxic contaminant in the complex mixtures of fibrogenic respirable airborne particles of ambient air in the following workplaces: quarrying and stone processing (where the respirabile dust concentration is 0.18 – 2.16 mg/m^3, and quartz represents 30% of the dust); and coal mines (where the average respirable dust concentration is 2.68 mg/m^3; and the quartz-containing dust is 3% or lower).

The frequency of chromosomal aberrations (%AB.C.) was significantly higher in both of the exposed groups of miners (HAE 30%, 2.64 %AB.C.; and HAE 85%, 2.12 %AB.C.) and in the stonecutters (HAE 30%, 1.94 %AB.C.), than in the controls ($p < 0.01$). Chromosomal aberrations were also found to be significantly higher in miners and stonecutters than in coke oven workers with high exposure to carcinogenic PAHs. The differences were not significant between the different groups of miners and stonecutters (Fig. 1, 2, 3).

The results indicated a significant increase in chromosomal aberrations (%AB.C.) in the group of workers exposed to dust containing quartz during the early period of exposure (about 30% HAE). There was a non-linear dose-response at high dose levels. There did not appear to be a relationship between the level of cytogenetic damage and exposure to PAHs in the workplace, based on urinary 1-OH-P levels.

In this study, the genotoxicity of occupational exposure to dust containing a crystalline form of silica was assessed. The results indicate that the crystalline form of silica is a major factor determining the genotoxic potential and carcinogenic hazard of complex mixtures in the ambient air of coal mines as well as quarrying and stone processing facilities.

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CHAPTER 15

POLYCHLORINATED BIPHENYL-MEDIATED INFLAMMATORY SIGNALING: IMPLICATIONS IN ATHEROSCLEROSIS

Elizabeth Oesterling[1, 2], Zuzana Majkova[1, 2], Gudrun Reiterer[2, 3], Hongxia Guo[2, 3], Michal Toborek[3,4], Bernhard Hennig[1,2,3]

[1]Graduate Center for Toxicology, University of Kentucky, Lexington, KY, USA; [2]Molecular and Cell Nutrition Laboratory, College of Agriculture University of Kentucky, Lexington,KY, USA; [3]Graduate Center for Nutritional Sciences, University of Kentucky, Lexington,KY, USA; [4]Department of Surgery, University of Kentucky, Lexington,KY, USA

Abstract: Polychlorinated biphenyls (PCBs) are widespread environmental contaminants that can cause inflammation of the vascular endothelium leading to atherosclerotic events. Pro- and anti-inflammatory signaling pathways were studied following endothelial cell exposure to coplanar and non-coplanar PCBs. Both PCBs 77 and 153 induced inflammatory transcription factors such as nuclear factor-κB (NF-κB) or signal transducer and activator of transcription-3 (STAT-3) as well as downstream inflammatory genes such as COX-2 and IL-6. Many of pro-inflammatory genes and gene products are associated or localized into lipid rafts such as caveolae. Protection from PCB-induced inflammation was also studied using agonists of the anti-inflammatory transcription factors PPARs α and γ. Our data demonstrate a possible caveolae-dependent mechanism of PCB-mediated inflammation and the role of PPAR agonists in preventing PCB-induced endothelial activation and atherosclerotic events.

Key words: PCB, atherosclerosis, endothelial cells, inflammation, PPAR, caveolae.

1. INTRODUCTION

There is substantial evidence that cardiovascular diseases are linked to environmental pollution (reviewed in Hennig et al., 2002). For example, there was a significant increase in mortality from cardiovascular diseases among Swedish capacitor manufacturing workers exposed to polychlorinated

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biphenyls (PCBs) for at least five years, and most excess deaths were due to cardiovascular disease in power workers exposed to phenoxy herbicides and PCBs in waste transformer oil. Furthermore, an increase in cardiovascular disease was detected in studies of the population of Seveso, Italy, after an industrial accident in 1976.

Atherosclerotic lesions are thought to be initiated by vascular endothelial cell dysfunction. Because the endothelium is in immediate contact with the blood, endothelial cells are particularly susceptible to the effect of environmental contaminants and their downstream mediators present in the bloodstream. These risk factors induce certain cell signaling pathways leading to the activation of pro-inflammatory transcription factors such as nuclear factor-κB (NF-κB) or signal transducer and activator of transcription-3 (STAT-3) (Hennig et al., 2001; Peilot et al., 2000). NF-κB and STAT-3 control inflammatory genes in endothelial cells, including cyclooxygenase-2 (COX-2) and interleukin-6 (IL-6).

Peroxisome proliferation activated receptor (PPAR) α and γ agonists have been shown to be protective against these events by downregulating proinflammatory signaling pathways. PPARs have been shown to negatively interfere with NF-κB, STAT, and AP-1 signaling pathways and can therefore prevent the expression of inflammatory genes such as adhesion molecules and cytokines (Delerive et al., 1999). Pro-inflammatory environmental pollutants, such as PCBs, could act by antagonizing PPARs.

There is increasing evidence that caveolae play a critical role in atherosclerosis (Matveev et al. 2001; Frank et al., 2003). Caveolae are a subset of lipid rafts characterized by the presence of caveolin. Caveolae are abundantly found in endothelial cells and are thought to play a role in regulation of endothelial vesicular trafficking. Caveolae have been shown to be involved in uptake of lipids and possibly lipophilic xenobiotics such as PCBs.

2. MATERIALS AND METHODS

2.1 Cell culture and experimental media

Endothelial cells were isolated from porcine pulmonary arteries as described previously (Toborek et al. 1997). MCF-7 cells stably transfected with a luciferase gene driven by a triple repeat of the PPAR response element (PPRE) were utilized for selected experiments. Cells were subcultured in medium 199 (endothelial cells) and DMEM (MCF-7 cells) containing 10% (v/v) fetal bovine serum (FBS, HyClone Laboratories, Logan, UT) using standard techniques.

The experimental media contained 5% (v/v) FBS. PCB 77 (3,3',4,4'-tetrachlorobiphenyl) or 153 (2,2',4,4',5,5'-hexachlorobiphenyl) (3.4 μM) and PPAR agonists (10-25 μM) were added from a stock solution in dimethyl sulfoxide (DMSO). PCB concentrations used are calculated values from plasma levels reported after acute exposure (Wassermann et al., 1979). For most experimental settings, cells were treated with PPAR agonists for 6 -18 h, and with PCBs for 1.5 - 6 h, depending on the inflammatory end product examined.

2.2 Transcription factor (NF-κB, STAT-3 and PPAR) activation studies: electrophoretic mobility shift assay (EMSA)

Following overnight treatment with the PPAR α agonist fenofibrate or the PPARγ agonist thiazolidinedeione, endothelial cells were treated with PCB 77 or PCB 153. Nuclear proteins were incubated with ^{32}P-end labeled oligonucleotides specific for PPAR response element (PPRE), NF-κB, or STAT-3, and DNA binding was determined by gel shift assay. The transcription factor bands were confirmed by supershift assay.

2.3 IL-6, COX-2 expression studies

Total RNA was extracted from endothelial cells by the use of TRI reagent according to the manufacturer's protocol. Gene expression was determined through reverse transcription-polymerase chain reaction (RT-PCR) as described earlier (Lee et al. 2001). The following primers were employed in the PCRs; IL-6 forward: 5' AAT TCG GTA CAT CCT CGA CG 3', reverse: 5' GCG CAG AAT GAG ATG AGT TG 3'; COX-2 forward: 5' GGA GAG ACA GCA TAA ACT GC 3', reverse: 5' GTG TGT TAA ACT CAG CAG CA 3'; ß-actin forward: 5' GGG ACC TGA CCG ACT ACC TC 3', reverse: 5' GGG CGA TGA TCT TGA TCT TC 3'. Amplified PCR products were separated on 2% agarose gel, stained with SYBR gold and visualized by using phosphorimaging technology.

2.4 mRNA expression of lipid raft proteins

Time-dependent changes in mRNA levels of caveolae-associated genes, such as caveolin-1, annexin-II, and COX-2, were determined in endothelial cells exposed to PCB 77. Total cellular RNA was extracted followed by RT-PCR. The following primers were employed in the PCRs; caveolin-1 forward: 5' CCA TTC TCT CCT TCC TGC AC 3', reverse: 5' GCA TGT

TGA TGC GGA TAT TG 3'; annexin-II forward: 5' AGC AGC GCT TTC TGG TAG TC 3', reverse: 5' CAC GCG TGA TAA GGT CCT G 3'.

2.5 PPAR reporter gene studies

The human breast cancer epithelial cell line MCF-7 (a gift from M. Kilgore, University of Kentucky) was stably transfected with a luciferase reporter gene driven by a triple repeat of PPRE, and cells were exposed to PCBs.

2.6 Bovine serum albumin co-transportation studies

Endothelial cells were treated with PCB 153 or DMSO in media containing varying amounts of the plasma binding protein albumin. Total cellular RNA was extracted followed by RT-PCR using E-selectin as a model adhesion molecule leading to endothelial cell activation. The following primers were employed: E-selectin forward: 5' GAC TCG GGC AAG TGG AAT GAT GAG 3', reverse: 5' CAT CAC CAT TCT GAG GAT GGC CGA C 3'. PCR products were separated on 2% agarose gel, stained with SYBR gold and visualized by using phosphorimaging technology.

2.7 Statistical analysis

All experiments were confirmed at least 3 times. Data were quantified and analyzed using the Scion Image, Image Gauge and Sigma Stat software. Comparisons between treatments were made by one way ANOVA with post hoc comparisons of the means made by Tukey tests. Statistical probability of $p < 0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 PPAR protection from PCB-mediated transcription factor activation

Environmental contaminants such as PCBs are pro-inflammatory and can trigger cell signaling pathways leading to endothelial cell activation, which is proposed to be the initial step in the pathogenesis of atherosclerosis. We have shown previously that coplanar PCBs, such as PCB 77 can induce oxidative stress through the aryl hydrocarbon receptor (AhR)-cytochrome P450 1A1 (CYP1A1) pathway (Slim et al., 1999, Ramadass et al., 2003).

Reactive oxygen species (ROS) can subsequently trigger oxidative stress sensitive pro-inflammatory pathways including the transcription factors NF-κB and AP-1, as well as downstream genes such as vascular cell adhesion molecule-1 (VCAM-1), IL-6 and E-selectin (Hennig et al., 2002).

In the present study, both coplanar and non-coplanar PCBs induced matory pathways in endothelial cells and caused endothelial cell dysfunction. Although different pathways might be responsible for the PCB-mediated endothelial cell activation, both coplanar and non-coplanar PCBs appear to be pro-atherogenic. For example, DNA binding activity of the NF-κB was significantly induced by exposure to PCB 77. Cotreatment with the PPARγ agonist thiazolidinedione protected against PCB 77-induced increase in NF-κB binding activity. The signaling pathways of non-coplanar PCBs, such as PCB 153, are less well understood. We provide evidence that non-coplanar PCBs can induce pro-inflammatory pathways through an oxidative stress-independent mechanism. For example, PCB 153 strongly induced DNA binding activity of STAT-3. Cells pretreated (18 h) with the PPARα agonist fenofibrate or the PPARγ agonist thiazolidinedione showed a significant decrease in PCB 153-induced STAT-3 activation.

3.2 PPAR protection from PCB-mediated inflammatory gene expression

Little is known about protective mechanisms against PCB-induced inflamematory insults. Of great interest are PPARs, which are nuclear receptors that appear to possess potent anti-inflammatory signaling properties (Tham et al., 2003). Both PPARα and γ are expressed in the vasculature, including endothelial cells, and their agonists such as fibrates (for α) and glitazones (for γ) have repeatedly been shown to reduce inflammation in response to a variety of stimuli. In the current study, we demonstrate that both PPARα and γ agonists can protect against PCB 77 and PCB 153-mediated toxicity. We observed a marked reduction in PCB-induced DNA binding activity of NF-κB and STAT-3 when cells were pre-enriched with PPAR agonists. Accordingly, inflammatory genes responding to these transcription factors exhibited a similar pattern. PPARα and γ agonists significantly reduced COX-2 and IL-6 mRNA expression in response to PCBs. In fact, we detected maximal mRNA expression of COX-2 following exposure to PCB 77 and PCB 153 for 4 h. IL-6 expression was upregulated after a 6 h exposure to PCB 77. The expression of both PCB-induced genes could be significantly decreased by pre-treatment of cells with the PPARα agonist fenofibrate or the PPARγ agonists thiazolidinedione or troglitazone.

3.3 Downregulation of PPAR transcriptional activity

Our data also support our hypothesis that PCBs contribute to an endothelial inflammatory response in part by down-regulating PPAR signaling. Indeed, we demonstrated that PCB 77 can inhibit PPAR DNA binding activity. Our preliminary data suggest that PCBs decrease not only DNA binding, but also transcriptional activity. For example, both PCB 77 and PCB 153 reduced transcription of PPRE-driven luciferase in MCF-7 cells. Similar results were obtained with PCB 114 (2,3,4,4',5-pentachlorobiphenyl), a mixed type P450 inducer. The exact mechanisms of PCB-mediated inhibition of PPARs remain unknown and require further investigation.

3.4 mRNA expression of lipid raft proteins

Lipid rafts are regions in cellular membranes characterized by unique lipid and protein compositions. One subset of lipid rafts, marked by the cholesterol-binding protein caveolin, is recognized as caveolae. Caveolae are capable of internalization in a clathrin-independent manner and play a role in various signaling pathways. Another marker, annexin II, is a phospholipid-binding protein involved in signal transduction, and in the endothelium, annexin II is present predominantly in caveolae. Also, COX-2 can colocalize with caveolin-1 in a segregated caveolae compartment (Liou et al., 2001). Enhanced caveolae internalization may promote COX-2 enzyme activity. It is also known that caveolin-1 gene expression is increased following caveolae-mediated uptake of low density lipoproteins. We demonstrated that PCB 77 can increase expression of caveolin-1 and other caveolae-associated proteins, suggesting that PCBs can disrupt caveolae-associated signaling pathways and possibly utilize caveolae internalization as a mode of cellular uptake.

3.5 Bovine serum albumin co-transportation

PCBs, as extremely hydrophobic molecules, associate in the plasma primarily with albumin and lipoproteins. Gp60, the 60-kDa albumin-binding protein, is localized in caveolae within endothelial cell membranes, and disruption of caveolae or gp60 can abolish albumin uptake and transport in endothelial cells. Therefore, caveolae might be involved in the uptake of albumin and also albumin-associated lipophilic xenobiotics. We examined whether increasing concentrations of bovine serum albumin (BSA) in our treatment media could increase the PCB toxicity, presumably due to increased uptake by co-transport with BSA. Using E-selectin as a model adhesion molecule leading to activation, we demonstrated a direct

correlation between albumin concentration and PCB-induced adhesion molecule expression.

4. CONCLUSION

In summary, our data demonstrate the pro-inflammatory properties of both coplanar and non-coplanar PCBs. PCB 77 and PCB 153 can trigger inflammatory signaling pathways while inhibiting the anti-inflammatory properties of PPARs. Our data also suggest that PPARα and γ agonists can potently downregulate PCB-mediated toxicity. Furthermore, caveolae and associated proteins appear to provide a critical signaling platform through which PCBs and related environmental pollutants induce an endothelial inflammatory response.

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CHAPTER 16

BLOOD LEAD LEVELS AND HAND LEAD CONTAMINATION IN CHILDREN AGES 4-6 IN COPSA MICA, ROMANIA

Simona Surdu [1], Iulia Neamtiu [1], Eugen Gurzau [1], Iosif Kasler [1], David Carpenter [2]

[1] Environmental Health Center, Cluj-Napoca, Romania; [2] University of Albany, New York, NY, USA

Abstract: The area of Copsa Mica (Sibiu County, Romania) is well known because of its high lead concentration in the environment. A large portion of young children have been found to develop lead poisoning. We studied children ages 4-6 years. The blood lead levels were 43.89 µg/dL ± 13.61 µg/dL with an average value of 44.32 µg/dL ± 13.71 µg/dL in boys and 42.98 µg/dL ± 13.78 µg/dL in girls. Blood lead levels were measured using the Lead Care System. The lead concentration on children's hands was measured using KXRay Fluorescence (dust wipes). No correlation was found between blood lead levels and lead concentrations on children's hands. Blood lead levels were correlated with certain behaviors such as the time a child spends playing outdoors.

Key words: young children, blood lead levels, lead concentration on children's hands, behaviors

1. INTRODUCTION

Lead contamination is one of an extensive list of environmental hazards that have been identified in some areas of Eastern Europe (Gurazau et al., 1995a). The potentially harmful effects of exposure of young children to relatively low levels of lead are widely recognized (Center for Disease Control and Prevention, 1991). It has also been established that several areas exist in Eastern Europe where children have experienced moderate to severe

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lead poisoning due to the presence of environmental contamination (Billig et al., 1999). Policies and programs have been designed to reduce potential lead exposure. New strategies such as social marketing, that are designed to change the behaviors of the most susceptible population group, have been reported as success "stories"(Gurzau et al., 1995b).

Much research has been conducted in recent years to study children with moderately elevated and very high blood lead levels associated with lead contamination (Billig et al., 1999, Gurzau et al., 1995a, 1995b). There is a greater potential for adverse health effects from lead exposure in children because their intake of lead per unit body weight is higher than it is for adults. In addition, young children ingest soil and dust via hands and dirty toys, leading to an increased intake of lead, and the physiological rate of uptake in children is higher than it is in adults (Schwartz, 1994). Finally, young children are more susceptible to the effects of lead exposure since they are undergoing rapid development (Fergusson and Horwood, 1993, Mushak, 1992, Tong and McMichael, 1999, United States Environmental Protection Agency, 1986, World Health Organization, 1995).

Elevated lead levels continue to be a particular problem among socially and economically deprived children. People with limited economic resources are more likely to live in substandard housing, live near industries such as smelters, be exposed to lead dust brought home by lead workers, and be nutritionally deprived and therefore more susceptible (Wasserman et al., 1992).

Debate continues over the nature, magnitude and persistence of the adverse health effects of low-level exposure to environmental lead in humans (Tong, 1998) though there is less debate regarding the effects of high level exposure to environmental lead (Von Schirnding, 1999). However, there is good evidence that intervention strategies such as social marketing can decrease lead exposure and associated risks in very young children (Billig et al., 1999, Gurzau et al., 1995a, 1995b). Unfortunately, recent data show that some neuropsychological effects are largely irreversible (Bellinger et al., 1992, Davis, 1990, Dietrich et al., 1992, Needleman et al., 1990, Pocock et al., 1994, Ruff et al., 1993, Tong et al., 1998), so that intervention is needed in areas highly contaminated with lead.

2. OBJECTIVES

The main objective of this study was to investigate lead exposure in susceptible populations (children aged 4-6 years) living in the area impacted most significantly by a heavy metal smelter in the town of Copsa Mica (Sibiu County, Romania). Data were also collected to assess the relationship between blood lead levels and lead levels in dust collected from children's

hands, and to assess the relationship between blood lead levels and certain risk factors in lead exposure (education, behaviors, cleaning practices etc.).

3. METHODS

To assess the lead exposure in children living near the smelter in Copsa Mica, the following information was obtained: measurements of blood lead levels in children recruited from Kindergarten No. 1 in Copsa Mica; collection of dust samples from children's hands; and collection of information about the children's exposure to lead from a questionnaire filled out by each child's parents.

A letter of recruitment was given to the parent or guardian of each child who would potentially participate. After obtaining an informed consent, a questionnaire was administered to the parents or guardians of all study subjects. The questionnaire obtained information regarding potential risk factors for lead exposure including personal characteristics (age, sex), child residence, and traffic in the residential area and near the kindergarten. Questions were also asked related to behaviors that are important for lead exposure such as whether the children played with soil and put dirty toys in their mouths. Finally, questions were included regarding socio-economic factors such as parents' education and family income, and questions were asked about the parents' occupational exposure to lead.

3.1 Laboratory techniques

The technique used to measure blood lead levels was the stripped anodic voltametry technique which has 99% accuracy. The device used was a Lead Care System, produced in 2000. This device is used in the USA for screening and community risk assessment for lead exposure, and has been approved by the Romanian Ministery of Health. Children's hands were carefully washed, and then capillary blood samples were collected from children's fingers.

Hand dust samples were collected using dust wipes. Lead on dust wipes was measured using the KX Ray Fluorescence technique. X Ray dust wipe exposures were detected within 120 seconds, at which time the technique sensitivity was 1 sigma (0.001 ppm) and the accuracy was 99.99%. The device used was a K-X-ray Fluorescence 720SL, produced in 2002.

3.2 Statistical analyses

Statistical analyses were performed using the STATA statistical packages. Simple ^{2} tests and Student's *t*-test were applied for comparing variables. A number of variables (representing the potential risk factors in lead exposure) were entered into a stepwise linear regression multivariate estimation model, in which each variable was assessed and was considered significant in the model if the *p* value was <0.05. Variables considered to be confounders such as age, sex, parents' occupational exposure, and amount of traffic near the residence and school, were kept in the model. Socioeconomic status was based on the mothers' education and the family's income.

4. RESULTS

The study included 53 subjects aged 4-6 years. Sixteen (30%) of the subjects were girls and 37 (70%) were boys. Blood lead levels were measured in 50 subjects, and lead in the dust collected from the children's hands was measured in 23 subjects. The parents of all 53 subjects included in the study were asked to complete the questionnaires.

Blood lead levels in girls varied between 22.50 μg/dL and 65 μg/dL. The mean value (±standard deviation) was 42.98 μg/dL ± 13.78 μg/dL in girls and 44.32 μg/dL ± 13.71 μg/dL in boys. In the group of boys, the blood lead levels varied between 15.30 μg/dL and 65 μg/dL (Table 1). The mean blood lead levels were slightly higher in boys than in girls, although this difference was not statistically significant.

Table 1. Distribution of blood lead levels (μg/dL) by gender.

	No. of children	Mean (μg/dL)	Std. Dev.	Min. value*	Max. value*
Girls	16	42.98	13.78	22.50	65
Boys	34	44.32	13.71	15.30	65

*Min. value and max. value, minimum and maximum values.

None of the subjects had a blood lead level <10 μg/dL. Most of the subjects (20 children, or 58%) had blood lead levels between 35 and 60 μg/dL. Only 2 subjects had blood lead levels in the range of 10-20 μg/dL and 5 subjects had blood lead levels >60 μg/dL (Table 2).

Lead levels in the dust collected from girls' hands varied between 20 μg/single hand and 379.6 μg/single hand. The mean value and the standard deviation in girls were 134.76 ± 178.05μg/single hand and 112.21 ± 168.82 μg/single hand in boys. Among boys, lead levels in the dust collected from their hands varied between 20 μg/single hand and 511.2 μg/single hand (Table 3). Lead concentration mean values were higher in girls as compared

Table 2. Subjects' distribution by gender and category of blood lead levels.

Blood lead level (μg/dL)	Girls (no., %)*	Boys (no., %)*	Total (no., %)*
10-20	0 (0.00%)	2 (5.88%)	2 (4.00%)
20-35	6 (37.50%)	8 (23.53%)	14 (28%)
35-60	9 (56.25%)	20 (58.82%)	29 (58%)
>60	1 (6.25%)	4 (11.76%)	5 (10%)
Total	16 (100%)	34 (100%)	50 (100%)

* No, number of children; %, percent of the children in that column.

Table 3. Lead concentration in dust collected from children's hands (μg/single hand).

	No. of children	Mean (μg/hand*)	Std. Dev.	Min.	Max.
Girls	6	134.76	178.05	20	379.6
Boys	17	112.21	168.82	20	511.2

*One hand per child was sampled.

Table 4. Social economic factors.

Risk factors		Females Blood lead levels		Males Blood lead levels	
		No. of subjects	mean (SE**) (μ g/dl)	No. of subjects	mean (SE**) (μ g/dl)
Mother's education	Elementary school				
	Secondary school	3	55 (8.66)	6	51.6 (14.86)
	High-school/college	12	41.05 (13.66)	28	42.76 13.22)
	University	1	30 (0)		
Monthly income*	>5 millions lei*	10	39.6 (14.10)	11	47.54 11.76)
	2-5 millions lei	6	48.61 (12.29)	21	42.58 (13.84)
	1-2 millions lei				
	<1 million lei			1	65 (0)

* Values are in local currency; these numbers were determined prior to the change in the value of the local currency that took place in 2005.

**SE, standard error of the mean.

with boys this time, but the difference between the mean values in boys and girls were not statistically significant when tested using statistical *t* test.

There was no correlation between blood lead levels and hand dust lead levels among either the girls or the boys. The mean blood lead concentrations were not found to vary significantly in relation to the socio-economic risk factors taken into consideration in this study. Table 4 presents the mean blood lead values in relation to the mother's educational level and family income, stratified by the subject's gender.

Table 5. Household and kindergarten cleaning practices and children's blood lead levels.

Risk factors		Females Blood lead levels (μ g/dL)		Males Blood lead levels (μ g/dL)	
		No. of subjects	mean (SE*) in μg/dL	No. of subjects	mean (SE*) in μg/dL
Use of vacuum cleaner in child's dwelling	Daily	8	46.31 (14.72)	18	44.05 (14.06)
	Weekly	8	39.65 (12.85)	12	46.85 (12.09)
	Monthly			2	23.3 (11.31)
	Rarely				
	Never			2	52.5 (3.53)
Wet mopping in child's dwelling	Daily	10	42.06 (15.35)	23	42.40 (14.85)
	Weekly	6	44.51 (11.88)	11	48.32 (10.44)
	Monthly				
	Rarely				
	Never				
Use of vacuum cleaner at kindergarten	Daily	7	41.5 (14.83)	14	43.21 (15.52)
	Weekly	3	35.16 (13.87)	4	39.55 (10.69)
	Monthly				
	Rarely	4	53.75 (7.5)	8	48.5 (11.45)
	Never	2	38.35(16.47)	6	43.6 (17.51)
Wet mopping at kindergarten	Daily	15	43.64 (14)	29	42.31 (13.56)
	Weekly	1	33 (0)	3	59.93 (8.60)
	Monthly				
	Rarely			1	50 (0)
	Never				

*SE, standard error of the mean.

The mean blood lead concentrations were not found to vary significantly in relation to the cleaning practices that were evaluated as potential risk factors for lead exposure. Table 5 presents the mean blood lead values in relation to the frequency of the most important cleaning practices (vacuum cleaning and wet mopping the child's living areas and kindergarten).

The comparative analysis using a statistical *t*-test indicated a positive and statistically significant correlation between blood lead levels in boys and the time spent playing outdoors while at home. The boys who played outdoors less than 2 hours had lower blood lead levels than those who played outdoors 2-4 hours ($p = 0.028$), and also had lower levels than those who played outdoors more than 6 hours ($p = 0.003$). The mean value and standard deviation for boys spending less than 2 hours outdoors was 26.48 ± 10.56 as compared with 46.82 ± 16.83 for boys outdoors for more than 6 hours and 49.32 ± 8.88 for boys outdoors from 2 - 4 hours (Table 6).

Table 6. Children's behaviors and blood lead levels *(continued on next page).*

		Females Blood lead levels (μ g/dL)		Males Blood lead levels (μ g/dL)	
		No. of subjects	mean (SE**)	No. of subjects	mean (SE**)
Time spent/day playing outdoor while the child is at home	<2 hours	1	57.40 (0)	5	26.48 (10.56)*
	2-4 hours	2	26.85 (4.45)	8	46.82 (16.83)*
	4-6 hours	4	39.02 (15.72)	4	40.32 (11.36)
	>6 hours	9	46.72 (12.11)	17	49.32 (8.88)*
Time spent/day playing outdoor while the child is at the kindergarten	<10 minutes	5	39.58 (15.66)	6	38.75 (14.83)
	10-20 minutes	5	37.96 (18.64)	15	42.41 (16.12)
	20-40 minutes	5	50 (0)	12	49.01 (9.20)
	>40 minutes	1	50 (0)	1	50 (0)

*Statistically significant

**SE, standard error of the mean.

Table 6 (continued). Children's behaviors and blood lead levels.

		Females Blood lead levels (μ g/dL)		Males Blood lead levels (μ g/dL)	
		No. of subjects	mean (SE**)	No. of subjects	mean (SE**)
Playing with soil while the child is at home	Yes	12	43.63 (4.04)	24	46.2 (2.30)
	No	4	41.02 (7.50)	10	39.81 (5.77)
Playing with soil while the child is at kindergarten	Yes	3	34.36 (10.31)	10	41.34 (4.69)
	No	13	44.96 (3.52)	24	45.56 (2.72)
Playing with sand while the child is at home	Yes	13	44.69 (3.86)	22	46.85 (2.61)
	No	3	35.56 (7.27)	12	39.67 (4.47)
Playing with sand while the child is at kindergarten	Yes	9	42.2 (4.35)	17	46.7 (2.36)
	No	7	43.98 (5.91)	17	41.94 (4.06)
Putting dirty toys and dirty fingers in the mouth	Yes	10	41.97 (5.03)	25	45.21 (2.88)
	No	6	44.66 (4.25)	9	41.83 (4.00)

**SE, standard error of the mean.

The comparative analysis using statistical t-test of blood lead levels in children with parents who were occupationally exposed to lead and the blood lead level in children with parents who were not occupationally exposed to lead indicated that blood lead levels were higher in girls whose parents had no known occupational exposure to lead. In addition, the difference between the mean blood lead levels in these two groups was statistically significant ($p = 0.045$; 48.48 μg/dL ± 3.69 μg/dL as compared with 33.81 μg/dL ± 5.22 μg/dL) (Table 7).

The results of the stepwise multivariate linear regression estimations indicated a positive and statistically significant correlation ($p = 0.04$) between blood lead levels and some potential risk factors in lead exposure that were identified using the questionnaire. Such risk factors included spending time outside in the Copsa Mica area and playing outdoors while the children were at kindergarten. A negative and statistically significant correlation ($p = 0.03$) was found between blood lead levels and a socioeconomic risk factor – the mother's level of education. Other potential risk factors in lead exposure (see

tables 4-7) were not found to be statistically significant in correlation to the blood lead levels (Table 8).

Table 7. Other risk factors in lead exposure.

Risk Factors		Females Blood lead levels (µg/dL)		Males Blood lead levels (µg/dL)	
		No. of subjects	Mean (SE*)	No. of subjects	Mean (SE*)
Traffic at the child dwelling	Less than a car/minute	14	42.33 (3.47)	22	44.08 (2.66)
	More than a car/minute	2	47.5 (17.5)	12	44.75 (4.70)
Traffic at the kindergarten	Less than a car/minute	13	42.9 (4.07)	29	43.57 (2.31)
	More than a car/minute	3	43.33 (6.66)	4	52.5 (11.04)
Street cleaning at the child dwelling	Yes	7	47.57 (4.62)	22	44.57 (3.18)
	No	8	38.08 (5.28)	12	43.85 (3.39)
Occupational exposure	Yes	6	33.81 (5.22)	10	43.58 (4.42)
	No	10	48.48 (3.69)**	24	44.62 (2.83)

*SE, standard error of the mean.

Table 8. Risk factors statistically significant in lead exposure estimated by stepwise multivariate linear regression after adjusting for age, sex and other risk factors.

Blood lead level	Coef.	SE*	t	p	[95% Conf. Interval]
Spending time outside Copsa Mica area	10.20	4.81	2.11	0.04	0.26 20.15
Time spent playing outdoor while the child is at the kindergarten	6.11	2.94	2.07	0.04	0.02 12.20
Mother's education	–11.30	5.06	–2.23	0.03	–21.76 –0.84

*SE, standard error of the mean.

5. DISCUSSION AND CONCLUSIONS

High levels of lead in the blood have been an ongoing concern because lead is known to affect learning abilities and produce severe health problems. The CDC (Centers for Disease Control and Prevention, 1991) defines a threshold for the "harmful effects" level of lead at 10 micrograms per deciliter (mg/dL) of blood. In our study the mean blood lead level for girls was 43 µg/dL, while the mean lead level in hand rinse samples was 135 µg/single hand. In boys, the mean blood lead level was found to be 44 µg/dL, while the mean concentration detected in hand rinse samples was 112 µg/single hand.

This study did not observe a significant association between lead levels in dust samples collected from children's hands and the children's blood lead levels. On the other hand children's blood lead levels were positively correlated with the time spent playing outdoors at home and negatively correlated with their parents' occupational exposure.

The results of the stepwise multivariate linear regression estimations indicated that children's blood lead levels were positively correlated with some risk factors in lead exposure such as spending time outside in the Copsa Mica area and the time spent playing outdoors. It was negatively correlated with the mother's education level.

The small number of statistically significant correlations may be due to the small number of subjects included in the study. That is why we intend to enlarge the number of subjects in a subsequent stage of the study. In order to improve results we will assure quality control for the blood lead levels measured from capillary blood by measuring the levels in venous blood using atomic absorption spectrometry with 5% of the blood sample, collecting and analyzing dust samples from both hands of the subjects and repeating the dust and soil sampling from the children's environment.

One reason for uncertainty in this study was the methodological pitfalls that beset many crossectional studies – exposure was only measured once per time period. A single measure of the exposure biomarkers also provided a limited scope to answer questions about the variation of these biomarkers in time. Blood lead levels were measured in the capillary blood and although we carefully washed the children's fingers it might be possible for the blood samples to be contaminated with lead from the skin.

Questionnaire responses were also subject to observational biases because parents may have slightly different interpretations of the questions asked about their children. In spite of these limitations, questionnaire based studies in lead contaminated areas are quite common. In order to identify the major pathways with regard to lead poisoning in the very young children, it is necessary to evaluate attitudes and practices which may influence the exposure to lead from environment. There are still many questions to be

answered including individual factors affecting susceptibility and the relationship between lead on children's hands and blood lead levels.

Despite potential limitations, what makes this study distinct is the novelty of the issues it approached, especially in the East European country of Romania where the people are just beginning to become aware of this health problem. Further research in this field is needed. That is why we intend to extend this study, to assess and characterize in detail the exposure to lead through soil and dust (from outdoor and indoor environments, vegetables, hands) and the community's risk related to this exposure. In conclusion, this study established that children's blood lead levels in Copsa Mica area are considerably high. Our knowledge of this population would indicate that implementation of a social marketing program for risk communication and changing of behavior related to lead contamination in the susceptible population group (in our case, children aged 4-6 years), could decrease exposures and thus, the risk associated with this toxin.

In addition to monitoring contaminant levels in environmental media, the children's blood lead levels should be measured regularly along with indicators of lead toxicity such as IQ and growth rate. A concerted effort should be made to reduce lead exposure because of the insidious effects it can have on very young children.

The primary sources of blood lead are soil and household dust, but not air. Controlling exposure to soil and dust is more difficult than reducing air emissions and may require measures such as restricting children's access to soil, promoting personal hygiene practices such as requiring smelter workers to shower and change clothes before returning to their homes and promoting other dust control measures in houses such as frequent wet mopping.

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CHAPTER 17

ASSESSMENT OF FESCUE CULTIVARS FOR PHYTOSTABILIZATION EFFECTIVENESS

Tyler Lane[1] and Jacek Krzyzak[2]

[1]*Harvard School of Public Health, 677 Huntington Ave., Boston, MA, 02115, USA;*
[2]*Institute for Ecology of Industrial Areas, ul. Kossutha 6, 40-844 Katowice, Poland*

Abstract: Previous research has identified *Festuca* cultivars as good candidates for use as phytostabilizers in metal-contaminated soils. *Festuca* species combine good metal sorption properties in root tissues with high metal tolerance and proven cultivation methods. These advantages may allow *Festuca* use in remediation projects where funding is unavailable to support more expensive soil remediation methods. This project expands previous research by combining fescue cultivars with various soil additives in laboratory tests, and identifying the best cultivar/soil amendment combination for field applications. Following laboratory analysis, field test plots will be monitored over two growing seasons to assess biomass production and metal stabilization effectiveness.

Key words: Festuca, phytostabilization, phytochemostabilization, heavy metals, soil remediation

1. INTRODUCTION

Previous hydroponic and soil pot research investigations have identified a number of *Festuca rubra* and *Festuca arundinacea* cultivars suitable for binding and holding various heavy metals on or within their root tissue (Kucharski and Sas-Nowosielska, 2004a). *Festuca*'s ability to tolerate heavy metal-contaminated soils, combined with proven cultivation methods, renders it an excellent candidate for phytostabilization projects at metal-contaminated sites where alternative remediation methods may not be economically feasible.

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For phytostabilization projects, the primary goal is the immobilization of soil contaminants to prevent or reduce introduction of pollutants into exposure pathways. Most phytoremediation research, however, has been conducted using metal hyperaccumulating dicots, with the goal of translocating metals from soils to aerial plant tissues through phytoextraction (Baker and Brooks, 1989, Boyd and Martens, 1992, Brooks et al., 1998). In the current study, plants have been used to stabilize metals in soil rather than extract them. Although the end goals of phytoremediation and phytostabilization are vastly different, many of the biogeochemical root processes for moving available metals from soil to root tissues are similar in both processes.

Plant roots stabilize bioavailable soil metal fractions through several mechanisms. Precipitation at the rhizosphere of the solubilized fraction of heavy metals in the soil is the primary mechanism of most metal adsorption to root surfaces (Blaylock et al., 1997). Adsorption also occurs through the binding of free metal cations by pectins on root cell walls and in pectin and other polysaccharide combinations from root-secreted mucilage (Waisel et al., 1996).

Studies have found many natural plant hyperaccumulators tend to have a higher density of metal transporters at the root-cell plasma membrane (Pence et al., 2000). The higher density of metal transporters allows these plants to readily take up metal cations from the soil solution. Once metals are accumulated, hyperaccumulating plant species usually exhibit a rapid translocation of accumulated metals from roots to shoots (Kramer, 2000). Translocated metals are then stored in vacuoles of the epidermal or mesophyllic cells of the stem to decrease toxicity to the plant (Mathys, 1977; Ernst et al., 1992).

In highly contaminated soils these hyperaccumulating attributes are often fatal to the plant species, and therefore metal-tolerant phytostabilizers are a more viable remediation option. Further, unless a shoot treatment or isolation program is part of the phytoextraction process, the use of hyperaccumulators susceptible to herbivory should be avoided.

As a non-hyperaccumulating species, *Festuca* cultivars are much more suitable for phytostabilization processes. *Festuca* display several desirable phytostabilizing qualities, as they are highly metal-tolerant and the majority of cultivars appear to concentrate most accumulated metals in their root systems (Kucharski and Sas-Nowosielska, 2004a). In addition, *F. rubra* and *F. arundinacea* are indigenous in pasture lands and meadows throughout Europe, temperate Asia and through introduction in North America. *F. rubra* is generally cold-, salt- and drought-tolerant while *F. arundinacea* prefers calcareous, sandy soils and is also salt- and drought-resistant (Hubbard, 1984; Mossberg et al., 1992).

Although the mechanism is uncertain, like most monocots, root to shoot metal translocation does not readily occur in *Festuca* species. The lack of a translocation mechanism may be due to low concentrations of histidine in the xylem (Kramer et al., 1996, Kerkeb et al., 2003), but this inhibition in *Festuca* requires further investigation. In many cases, *Festuca* species have also developed a symbiotic relationship with endophytic fungi, which appears to aid in reducing insect and some herbivore grazing (Hahn et al., 2003). The low transport of metals into shoot tissues and symbiosis with endophytic fungi further reduce the possibility of metal introduction into the food chain, and increase the attractiveness of *Festuca* as a phytostabilizer.

2. METHODS

Soil pot tests were used to identify the best *Festuca* cultivar and soil amendment combination for use in field trials. Ten cultivars of fescue seed were procured from the Swiss company DLF-Trifolium (Roskilde, Denmark). Table 1 outlines the cultivars identified as viable metal accumulators during previous hydroponic laboratory studies (Kucharski and Sas-Nowosielska, 2004a).

Table 1. Ten fescue test cultivars.

Festuca rubra	*Festuca arundinacea*
1. – cultivar Pernille	1. – cultivar Cochise
2. – cultivar Gondolin	2. – cultivar Montserrat
3. – cultivar Leonora	3. – cultivar DP 50-9011
4. – cultivar Napoli	4. – cultivar Kora
5. – cultivar Carina	5. – cultivar Feline

The cultivar seeds were sown in pots filled with contaminated soil prepared from the Warynski zinc smelter site. The Warynski smelter is located near the town of Piekary Slaskie [pop. 66,000] and 15 km northwest of the major population center of Katowice [pop. 345,000] (Central Statistical Office, 1999). The site is owned by the Orzel Bialy Mining & Metallurgical Works, S.A. and over 1.3 million people live within a 15 km radius of this site. Zinc and lead ore smelters operated at the site from 1927 until 1990. During this activity period, the smelters produced approximately 3,500,000 tons of mixed lead (Pb), cadmium (Cd) and zinc (Zn) waste, deposited in piles spread across a 60 hectare site (Wcislo et al., 2001). Although limited recyclable smelting activity continues at the site, the Piekary Slaskie municipal authorities are interested in redeveloping the land for alternative industrial purposes.

The Warynski soil was analyzed for compositional content and mixed to ensure all test samples received identical nutrient/contaminant exposure. The results of the soil parameter determination are displayed in Table 2.

Table 2. Warynski soil characterization.

Sand	**Silt**	**Clay**	**OM[a] (mg/kg)**	**pH (H_2O)**	**PH (KCl)**	**EC (µ g/cm)**	**CEC[b] (cmol/kg)**
37.3%	56.3%	6.8%	8.52 ± 0.12	6.57 ± 0.07	6.71 ± 0.03	154.0 ± 11.0	6.67 ± 0.24

[a] Soil organic matter, measured by content loss on ignition.

[b] Cation Exchange Capacity, measured according to ISO 13536.

A thorough analysis of Warynski soil samples was performed to assess the potential plant exposure to three major metal contaminants, Pb, Cd and Zn. Tables 3 and 4 describe the major metal contaminant component of the Warynski soil, displaying available fractions and soil composition differentiated by depth.

Table 3. Warynski soil metal concentrations.

	Pb (mg/kg)	% of Total	**Zn** (mg/kg)	% of Total	**Cd** (mg/kg)	% of Total
Total Metal Concentration[a]	9712 ± 562	100.0	11498 ± 417	100.0	537 ± 23	100.0
Potentially Available[b]	6533 ± 91	69.0	7673 ± 105	73.0	365 ± 2.89	68.0
Bioavailable[c]	5.23 ± 0.13	3.3	363 ± 7.47	0.06	41.78 ± 0.69	8.0

[a] Total soil element concentrations determined in *aqua regia*.

[b] Extraction with 0.43 N HNO_3.

[c] Extraction with 0.01 M $CaCl_2$.

Table 4. Warynski major contaminant soil characterization by depth.

Depth (cm)	**EC** (µ g/cm)	**Pb** (mg/kg)	**Zn** (mg/kg)	**Cd** (mg/kg)
0-20	154 ± 11	8265 ± 1143	9673 ± 925	392 ± 45
20-40	125 ± 10	2890 ± 822	4854 ± 760	155 ± 23

Table 4 shows 65-70% of total metal contamination concentrating in the first 20 cm, indicating high accessibility for plant roots where phytostabilization would be most effective. Minor metal concentrations were analyzed (Table 5) to identify any additional elements that might be mobilized by soil amendment additions and act as potential confounders to plant toxicity.

Table 5. Warynski soil minor metal and semimetal concentrations.

	As[c] (mg/kg)	Cu[c] (mg/kg)	Cr[c] (mg/kg)	Ni[c] (mg/kg)	Hg[c] (mg/kg)
Total Metal Concentration[a]	211.57 ± 26.2	54.89 ± 4.49	14.37 ± 0.70	10.35 ± 1.60	1.74 ± 0.18
Bioavailable[b]	0.039 ± 0.005	0.002 ± 0.001	0.066 ± 0.02	0.060 ± 0.03	0.714 ± 0.04

[a] Total soil element concentrations determined in *aqua regia*.

[b] Extraction with 0.01 M $CaCl_2$.

[c] As, arsenic; Cu, copper; Cr, chromium; Ni, nickel; Hg, mercury

Source: Kucharski et al. (2004a).

3. EXPERIMENT

A total of 120 test pots were used to examine the effects of three soil additive mixtures to root and shoot growth of the *Festuca* cultivars. Due to the extremely high metal content of the Warynski soils, additive mixtures were necessary to provide a nutrient substrate for plant development. The use of additives in phytoremediation projects may therefore be more properly termed phytochemostabilization.

Three replicates for each of the ten cultivars were established for each control and each of the three additive combinations. Thus, each cultivar was tested using 1) three control pots, 2) three pots with 2.5% Superphosphate (SP) additive [$Ca(H_2PO_4)_2$], 3) three with 2.5% SP additive and 10% lignite, and 4) three with 20% lignite. Controls were potted in Warynski soil without any soil additives. Soil additive specifications were determined in previous tests (Kucharski et al., 2004b). Each pot contained 400 g of soil, including the addition of any soil additive combinations. Due to variance in seed sizes, *Festuca rubra* seeds were sown with 250 mg of seed per pot, while *Festuca arundinacea* seeds were sown with 500 mg seed per pot.

The test period ran for ten weeks in a growing room, controlling for light (11,000 lumens), temperature (24°C), humidity (65-95%), water intake, drainage, and air circulation. Plant monitoring was performed weekly, examining height, coloration, soil saturation and general growth characteristics. At the end of the test period, all plant samples were removed from the pots and separated from the soil. Each sample was dried and evaluated for biomass ratio (root to shoot), root density, mass, metal binding and heavy metal uptake (analysis pending). Comparative analysis was performed to identify the species and cultivar with the greatest root mass and ability to stabilize heavy metal-contaminated soil.

4. RESULTS AND DISCUSSION

Lacking any soil amendment additives, the control group displayed a stunted growth peak at three weeks followed by discoloring and decline by week eight. A lack of nutrients in the Warynski soil and high concentrations of metal toxicants combined to severely inhibit growth, possibly via decreased root elasticity (Lane and Martin, 1982, Barcelo et al., 1986), or synergistic toxicity (Kahle and Breckle, 1989). Further root analysis will be necessary to provide a definitive toxicant determination.

While the 2.5% SP additive provided additional soil nutrients, it also lowered the pH and thus further mobilized metals for binding. Growth was increased, especially in combination with the lignite additive, but at a cost of elevating the soluble metal content of the soil (Kucharski et al., 2004b). The 20% lignite soil additive provided all *Festuca* cultivars with the highest above-ground vegetative growth (see *Figure 1*). These results point to a reduction in the solubilized soil metal content, allowing for greater vegetative growth in both roots and shoots. Reducing soluble metal content by substituting lignite for SP fertilizers should further aid in soil stabilization remediation efforts.

Atomic absorption spectroscopy (AAS) determination of solubilized soil metal content found the highest metal sorption for the roots of cultivars Montserrat and Feline (see *Figure 2*).

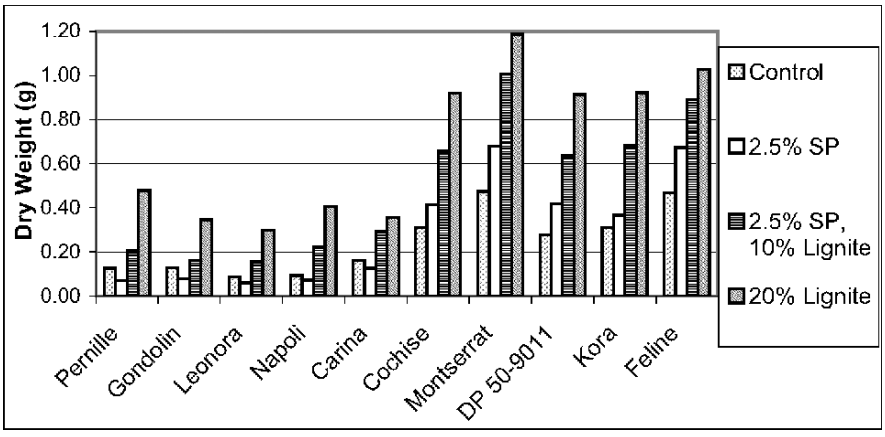

Figure 1. Above-Ground Tissue Comparison of *Festuca* Cultivars (at 10 weeks).

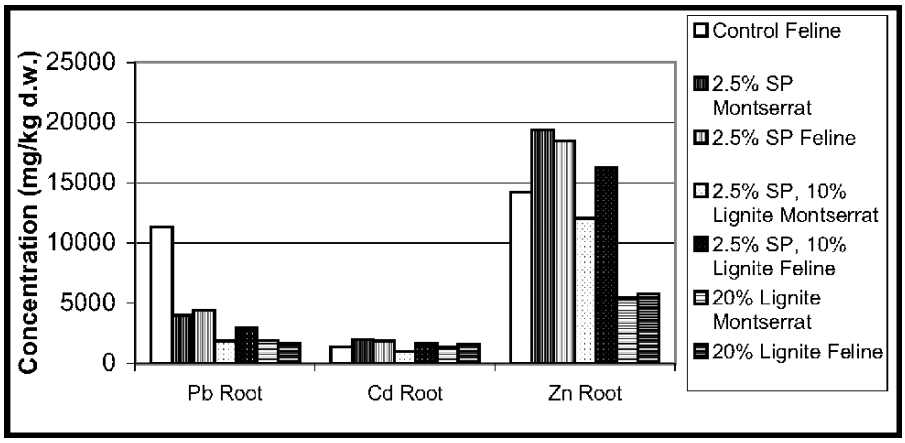

Figure 2. Selected *Festuca* Average Root Metal Concentrations.

5. CONCLUSION

The soil pot test results are the first step in a total phytostabilization assessment using *Festuca* grasses. The next steps will evaluate the Montserrat and Feline cultivar and amendment combinations under field trial conditions.

The laboratory growth results indicated that the Montserrat and Feline cultivars held the greatest biomass potential for future field trials, due to high metal sorption at the root and low root to shoot metal transfer ratios (see *Figure 3*). The 20% lignite soil additive produced the best vegetative growth, but due to cost considerations at the field trial scale, the 2.5% SP and 10% lignite mixture may be a more cost effective additive. These field trials will be conducted over two growing seasons at two sites with lower levels of metal contamination, the Cooperative Farm (Bytom, Poland) and the Warynski smelter sites. These tests will assess shoot biomass production and metal stabilization effectiveness bioenergy analysis. The final goal will be the development of a harvestable *Festuca* crop yield for burning in local energy production facilities. *Festuca* species were chosen due to their low root to shoot transport of sequestered metals, thus avoiding secondary mobilization of the metals during biomass burning. As an additional precaution, the selected energy production facility will have sufficient scrubbers to remove any remaining mobilized metal particles from the outlet stack. Thus, the use of *Festuca* as a phytostabilizer may generate a sufficient bioenergy crop to render this exposure reduction method self-sustainable at sites where remediation would not otherwise be economical.

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CHAPTER 18

HEALTH RISK ASSESSMENT IN CHILDREN OF THE ISYKKOL REGION OF THE KYRGYZ REPUBLIC

Ainash Sharshenova[1], Omor Kasymov[1], Michel Maignan[2], Anne-Laure Zufferey[2], Elvira Majikova[1], Almaz Sultashev[1], Zhaukharia Bezverkhnyaya[1], Gulbaram Arzygulova[1]

[1]Scientific and Production Centre for Preventive Medicine of the Ministry of Health, 34 Baitik Baatyr Street, Bishkek, Kyrgyz Republic; [2]University of Lausanne, Institute of Mineralogy and Geochemistry, Dorigny, BFSH2, Lausanne, CH-1015, Switzerland

Abstract: The health status of children living in the Isykkol Region, Kyrgyz Republic, has been studied and the main set of children's potential morbidity parameters has been identified. Morbidity indicators were analyzed at the level of Family Medicine Centres and Family Physician Groups for 1991-2001. For monitoring of children's health a database of morbidity data has been created for 5 districts of the Isykkol Region according to the ICD X revision. Electronic maps on noninfectious disease morbidity have been created (iron-deficient anemia, endemic goiter). These maps have been used to assess the health risk for children living in the regions of medium elevation.

Key words: children's health, morbidity indicators, classes of diseases, risk assessment, relative risk, family physician practices, environmental factors, regionalization, GIS maps.

1. INTRODUCTION

Integral health indicators can serve as criteria to provide a preliminary evaluation of the negative influence of the environment (Bykov and Revich, 1999; Onishchenko and Samoshkin, 2000*)*. Morbidity indicators are among integral indicators that can be used to establish the relationship between environmental exposures and potential adverse health effects. Health assessment

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of children in relation to environmental factors is of great interest for many countries including the Kyrgyz Republic. Studies by Sharshenova et al. (2000) and Majikova (2004) on the assessment of the health of the population taking into account the environmental impact were based on mortality data. Work on the population health risk due to environmental factors is almost absent.

This work was carried out in the framework of the project JRP: 7KSP J065715 "Creation of environmental health GIS for the Isykkol Region" supported by the Swiss SCOPES 2000-2003 Programme.

The study area includes the Isykkol Region (43,100 km^2) of Kyrgyzstan, around a lake with the same name. The Isykkol oblast is in the eastern part of the country and is divided into five districts, the "rayon". The largest segment of the population lives around the lake at an altitude between 1600 and 1700 meters. Few villages are located above 2000 meters. The total population of the Kyrgyz Republic as of January 1,2003, was approximately 5 million people including 420,624 in Isykkol Oblast.

The aim of this study was to investigate morbidity and to assess environmentally determined health risk in children of the Isykkol Region.

2. METHODS

The population health status was studied using the list of health indicators recommended by WHO (1994). The list includes classes of diseases that are potential health indicators related to environmental factors. The morbidity of children under 15 years of age for the period 1999-2001 was studied using data from reporting forms of 71-67 Family Physician Groups. Data on the number of population were obtained from the National Statistical Committee (2003) and those on morbidity of children from the Republican Medical Information Centre of the Kyrgyz Republic (2002). The analysis covered 21 classes of diseases according to ICD X (International Classification of Diseases) and the incidence per 100,000 children was determined. Children's environment and health was assessed using relative risk (RR) and 5-score ranking (Methodical Recommendations, 2001; 2003a, 2003b). Degrees of environmental dependence of health outcome according to the relative risk estimations were as follows: (0) "none," $0 < RR \leq 1$; (1) "small," $1 < RR \leq 1.5$; (2) "medium," $1.5 < RR \leq 2$; (3) "high," $2 < RR \leq 3.2$; (4) "very high," $3.2 < RR \leq 5$; (5) "almost complete," dependence $RR > 5$. This scale reflects the variation in the frequencies of disease and thus provides an indication for the relationship between the environment and a specific disease.

In this study statistical, geostatistical and spatial methods were used for analyzing epidemiological and environmental data from the Isykkol Region (Kanevsky and Maignan, 2004). The creation of maps was accomplished with GIS. The complete geostatistic study and results were obtained with Geostat Office, a scientific software package for geostatistical and machine learning analysis of data, the clustering and PCA study were realized with R languages (R Project for statistical computing) and the GIS project was created with ArcGis from ESRI (Environmental Systems Research Institute).

3. RESULTS AND DISCUSSION

The health of children was assessed in 5 ecologically different areas: Isykkol (IkD), Tyup (TpD), Aksu (AsD), Jetyoguz (JoD), and Ton (TnD) districts. Respiratory illnesses were the class of diseases observed at the highest incidence rate in all 5 districts (43% - 55%; 7094 - 13006 cases per 100,000 children). Second in frequency were diseases of blood and hemopoietic organs in IkD-Tp-JoD (6.5% - 13.7% - 15.9%; 1510-2777-2728/100,000), diseases of the endocrine system in AsD (14.9%; 3827/100,000), and parasitic diseases in TnD (10.3%; 1980/100,000). The incidence of infectious and parasitic diseases in the IkD, TpD, and AsD regions ranged from 6.0 to 7.7%, with an incidence rate per 100,000 of 1,554 in the IkD region, 1,375 in the TpD region, and 1,718 in the AsD region. In the JoD region, the diseases of the endocrine system were found to have an incidence rate of 9.0% or 1,475 per 100,000. The frequency of diseases of the blood and hematopoietic system was 6.8% or 1,469 per 100,000 in the TnD region. Additional details regarding the prevalence of disease in these regions are presented in Table 1.

Measurement of incidence rates indicates that the following diseases were completely related to the local environment: respiratory diseases, including bronchial asthma; diseases of blood and blood-forming organs, including iron-deficient anemia; endocrine diseases, including endemic goiter; genitourinary diseases, including kidney and urethra stones; certain perinatal conditions; congenital anomalies and developmental defects.

The risk for environmentally related morbidity was assessed for children in 5 districts of the Isykkol Region by a comparison to morbidity data for the whole district, region and republic. For the three years 308 cases were found in the category of environmentally dependent morbidity degrees from 1 to 5 in the Isykkol Region. Among the cases that fell into categories 3-5, there were 162 cases. Cases in categories 3-5 by district were as follows: 250/116 in the Tyup District, 130/32 – in the Aksu District, 221/107 – in the Jetyoguz District, and 125/46 – in the Ton District.

For the assessment of children's health risk the category 0 "none" was ignored and the categories 1 "small" and 2 "medium" were excluded from analysis because this morbidity of children appears to be more closely related to other factors, including socio-economical ones.

Table 2 provides the average number of cases falling into RR categories 3 "high" to 5 "almost complete" dependence of the disease on an environmental factor. Disease percentages are provided for the Family Physician Group (FPG), the specific districts, the Isykkol Region, and the Kyrgyz Republic. RR ranking showed that risk of children's morbidity ranged from high to almost complete environmental relatedness and averaged 13.9%, 17.5%, 15.8% as compared to the district-, region- and republic-wide levels, respectively.

Because category 5 is the most interesting in the evaluation of environmentally determined morbidity, additional analyses were performed for diseases falling under this category. Based on the ICD-X, 13 out of 21 classes of diseases and 6 subclasses of diseases were found to belong to this category. The number of forms varied in each district from 6 (Ton district) to 16 (Tyup district). The portions of the main classes under the category "almost complete" dependence of children's morbidity on the local environment factor are presented in Table 3.

Table 1. The prevalence of selected classes of diseases in children living in five districts of the Isykkol Region in 1999-2001 (per 100,000 child population).

Classes of diseases	ICD Codes	1999	2000	2001
Isykkol District				
Hematopoietic system	D50-D89	996.8	1170.5	1546.4
Endocrine system	E00-E90	258.1	407.5	486.2
Genitourinary system	N00-N99	470.6	194.3	384.3
Tyup District				
Hematopoietic system	D50-D89	4489.3	3126.9	2897.8
Endocrine system	E00-E90	1236.0	1296.5	1271.2
Genitourinary system	N00-N99	2498.8	1464.3	760.7
Aksu District				
Hematopoietic system	D50-D89	840.5	747.6	1215.5
Endocrine system	E00-E90	1481.1	2817.9	3097.3
Genitourinary system	N00-N99	302.4	350.3	549.1
Jetyoguz District				
Hematopoietic system	D50-D89	1189.9	1340.9	2648.8
Endocrine system	E00-E90	669.3	618.2	1499.8
Genitourinary system	N00-N99	388.4	363.4	555.2
Ton District				
Hematopoietic system	D50-D89	1319.7	1170.1	1301.1
Endocrine system	E00-E90	932.0	654.3	1002.5
Genitourinary system	N00-N99	453.6	364.9	476.4

Table 2. Comparison of cases in the RR category from 3 "high" to 5 "almost complete" relatedness of morbidity to the local environment factor.

Districts	Number of cases for the RR scores category/ %			Rank
	FPG/D	FPG/IKR	FPG/KR	
Isykkol	54 / 17.5	34 / 20	22.3 / 14.7	2
Tyup	38.7 / 15.4	32 / 15.5	25.0 / 15.8	3
Aksu	10.7 / 8.2	7 / 11.5	3.7 / 6.2	4
Jetyoguz	35.7 /16.1	26.3 / 20.2	36.3 / 22.4	1
Ton	15.3 / 12.2	17.3 / 20.2	20.3 / 19.9	2

Comparison to the RR indexes: FPG = Family Physician Group; D = District; IKR = Isykkol Region; KR = Kyrgyz Republic.

In the Isykkol, Aksu and Ton districts, respiratory diseases were classified as having "almost complete" environmental dependence with regards to children's morbidity. Repiratory diseases were ranked second in the Jetoguz and Tyup districts. Diseases originating in the perinatal period had the highest ranking for the Jetoguz district. For the Tyup district, diseases of the digestive system had the highest ranking for environmental dependence.

The incidence of morbidity in children was evaluated for specific regions within each district in relation to altitude, location near a lake or river, geological features and urban areas. The data were analyzed by Family Physician Groups and Centres of Family Medicine. Regional evaluations were prepared for four diseases. Table 4 presents a summary of localized areas of elevated disease and the region where they are located. These regions include North (N) for the Isykkol districts, South (S) for the

Table 3. The set of classes of diseases under category 5 "almost complete" environmental dependence in children (%)in each of the five districts.

		District				
Classes	Diseases	IkD	TpD	AsD	JoD	TnD
I	Infectious and parasitic diseases	25	9.1	6.3	2.4	8.3
II	Neoplasms		5.5	6.3	7.3	8.3
III	D. blood & the hematopoietic system	1.5	5.5	6.3	4.9	
IV	Diseases of endocrine system		9.1	6.3		
VI	Diseases of the nervous system	2.9	5.5		4.9	
IX	Diseases of the circulatory system	1.5	10.9		17.1	8.3
X	Diseases of the respiratory system	38.2	14.5	31.3	19.5	58.3
XI	Disease of the digestive system	10.3	18.2	12.5		
XIV	Diseases of the genitourinary system	11.8	3.6	25.0		
XVI	Certain conditions originating in the perinatal period		9.1	6.3	43.9	8.3
XVII	Congenital anomalies	1.5	1.8			
XVIII	Symptoms, signs, abnormalities not classified under other headings		7.3			
XIX	Injuries and poisonings	7.4				8.3
	Sum of the classes or diseases	9	12	8	7	6

Table 4. Summary of localized areas of disease incidence.

Years	Anemia	Endocrine Diseases	Respiratory Diseases.	Infectious and Parasitic Diseases
1999	Araket Taldysuu Balykchy t. E + W	 Pristan Karakol t. E	Tasma Toguzbulak Araket Karakol t. E + S	 Orgochor S
2000	Karakol t. Barskoon Balykchy t. E + S	Elaman Karakol t. Chelpek E	 Karakol t. E	Aral Karakol t. N + (E)
2001	Vostok Majak Lipenka Baltabay Intymak Tamga Sarykol Karakol t. Baykchy t. E + S	Elaman Pristan Chelpek Karakol t. E	Jergalan Chongoruktu E + N	Tamchy Bosuchuk Karakol t. N+ S+ E

Jetyoguz and Ton districts, East (E) for the Asku and Tyup districts, and West (W) for the Balykchy town region.

Potential sources of exposure that may have affected disease incidence include the coal mine in Jergalan, and thermal mineral water with radon in Aksu, Jetyoguz and Tyup district (near Tasma, Orgochor villages). These sources may also be a factor in the high rate of respiratory diseases in the East part of the oblast. The high rates of endocrine diseases may also be associated with the presence of natural thermal mineral springs with radon in the South and East of Isykkol Region. Poor quality of water supply in Tyup, Ton, Jetyoguz and Isykkol districts is a likely factor influencing the high rates of infectious and parasitic diseases in the North of the Region. The low socio-economic status of many residents in the Tyup and Ton districts is likely to increase their contact with the surrounding environment and environmental contaminants. These exposures may be a factor in the high frequency of children's morbidity observed for these two districts.

4. CONCLUSIONS

The prevalence of disease in children and the potential relationship of disease frequency with environmental conditions was evaluated for five districts within the Isykkol Region of the Kyrgyz Republic. The disease frequency for children was found to be highest in the Jetyoguz district,

followed by the Issykol, Ton, Tyup and Asku districts. The survey also identified the most prevalent diseases in children in each of the districts. A regional database for epidemiological and environmental data has been established. This includes creation of a database on children's morbidity in Access. GIS maps of the prevalence of diseases (e.g., endemic goiter, anemia) have also been created along with a ranking of specific areas within the region.

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CHAPTER 19

ARSENIC HEALTH RISK ASSESSMENT AND MOLECULAR EPIDEMIOLOGY PROJECT IN SLOVAKIA

Kvetoslava Koppová, Eleonora Fabiánová, Katarina Slotová, Pavlina Bartová, Marek Drímal
Regional Institute of Public Health, Banská Bystrica, Slovakia

Abstract: The Arsenic Health Risk Assessment and Molecular Epidemiology (ASHRAM) project is an international study funded by the European Commission and managed by an international consortium. For this project, areas in Slovakia, Romania and Hungary were selected for evaluation. The objectives of the study included: (1) to quantify the cancer risks in relation to arsenic ingestion via drinking water; (2) to assess the effect of inter–individual variation in arsenic metabolism and DNA repair on carcinogenic risk; and (3) to review current risk assessment models for arsenic and cancer, and to evaluate the impact of these models with regards to the adequacy of the current and proposed drinking water standards. The recruitment and interviewing of study participants, sample collection, and data from an arsenic exposure model are presented.

Key words: drinking water, food, arsenic ingestion, bladder, skin, kidney, cancer risk

1. INTRODUCTION

Arsenic can be found as a naturally occurring element in soil, air and water. Humans have been exposed to organic or inorganic forms of arsenic through a variety of pathways including inhalation of arsenic-contaminated dust in air, and ingestion of contaminated food or water. In many parts of the world, inorganic arsenic of geological origin is an important environmental contaminant. As a result of industrial and geological activities, arsenic can contaminate air, water, and soil. A variety of adverse health consequences have been associated with arsenic exposure. These include cardiovascular effects

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(Chen et al., 1996), neurological effects (Hindmarsh et al., 1977; Bolla et al., 1987; Brouwer et al., 1992), effects on children's mental health (Siripitaykunkitt et al., 1999), skin cancer, bladder cancer and kidney cancer (Bates et al., 1992; Chen et al., 1992; Chiou, 1995).

The World Health Organization has classified arsenic as a human carcinogen and has established a recommended upper limit in drinking water of 10 μg/l. This value is based on epidemiological studies which have evaluated the impact of arsenic exposure via drinking water on the risk of skin cancer in Taiwan (Yeh, 1963).

The Council Directive No.98/83/ES, dated 3rd November, 1998, concerning the quality of water intended for human consumption, has also fixed the upper limit value for arsenic content at 10 μg/l. Similarly, in the Slovak Republic, the maximum arsenic concentration in drinking water has been fixed by means of the Decree of the Ministry of Health of the Slovak Republic No.151/2004 of the Official Law Digest to the Slovak Republic at 10 μg/l.

Drinking water often contains low amounts of inorganic arsenic. Measured amounts between 0.1 and 0.3 μg/l are not uncommon. In various parts of world, elevated concentrations of arsenic of geological origin have been detected in drinking water. In Central Europe, elevated levels of arsenic in drinking water have been detected in Hungary (Bacs, Bekes, Csongrad, Jasz-Nagykun-Szolnok) and in Romania (Arad, Bihor). In the Slovak Republic, such areas exist in districts of Brezno, Banská Bystrica, Nové Zámky, Levice, Žarnovica, Žiar nad Hronom.

The Arsenic Health Risk Assessment and Molecular Epidemiology (ASHRAM) Project is an international study supported financially by a European Commission grant, within the Fifth Framework Programme the Quality of Life and Living Resources. It is managed by a team of EU experts, represented by the London School of Hygiene and Tropical Medicine in London, Great Britain; the Caroline Institute in Stockholm, Sweden; and Karl-Franz University in Graz, Austria. Slovakia, Hungary and Romania are the three countries responsible for investigation and research under this project. In the Slovak Republic, the responsible investigator and researcher is the Regional Institute of Public Health (RIPH) in the city of Banská Bystrica. The other investigation and research institutions include the RIPH of Nové Zámky, Levice, Nitra, and Žiar nad Hronom. Hospitals in the cities of Banská Bystrica, Nové Zámky, Levice, Nitra, Brezno, Nová Baňa are also involved in the project. The Project duration is for three years.

Recognising the widespread European exposure to low levels of arsenic, and the need for further scientific evidence on dose – response relationships, gene-environment interactions and mechanisms of action, this epidemiological study has following objectives:

- to quantify the cancer risks for the bladder, skin and kidney in relation to arsenic ingestion via drinking water in Hungary, Romania and Slovakia;
- to assess the effect of inter-individual variation in arsenic metabolism and DNA repair on carcinogenic risk;
- to characterise determinants of individual differences in arsenic specification in vivo; and
- to review, in the light of the new data, the current risk assessment for arsenic and cancer.

2. METHODS

The ASHRAM Project has been designed as a prospective epidemiologic case control study. Quantitative health risk assessment of the arsenic intake will be applied, including the genetic biomarkers of sensitivity and determination of arsenic metabolites in urine of case and control subjects. Information for each participant in this Project were obtained through two questionnaires. The first is the main questionnaire, which contains questions about basic demographic data, personal and family health history, working history, detailed findings related to source and consumption of drinking water and questions directed towards the lifestyle, including smoking and sun-exposure. The second questionnaire is a food frequency questionnaire, which evaluates nutritional habits. This consists of two parts, one which evaluates nutritional habits before 1989 and one which evaluates nutritional habits after 1989.

Subjects ages 30-79 years were recruited for the epidemiological study from new cases of skin cancer, bladder cancer and kidney cancer. Kidney cancer and bladder cancer cases were identified by clinicians at the Urology Department of each hospital. Cases with skin cancer were identified by histological examinations by pathologists.

Controls were matched by age, sex and residence. They must fulfill the diagnostic criteria (patients with appendicitis, abdominal hernias, duodenal ulcer, colelithiasis and fractures). Patients with malignant tumour disorders, diabetes or cardiovascular disease were not included as controls in the study. For each respondent that has been recruited for the study, after obtaining the written agreement of consent, the following activities were performed:

- Completion of the main and food frequency questionnaires by guided interview,
- Urine sampling for determination of arsenic (As) metabolites,
- Blood sampling for total As determination, DNA analysis, genetic polymorphism analysis, and selenium determination,

- Examination of hands of patients to evaluate possible case of keratosis and pigment changes,
- Identification of the subject's source of drinking water and collection of water samples, in order to determine the concentration of arsenic in the drinking water. Arsenic concentrations in drinking water were evaluated not only for current drinking water supplies but also, where possible, for previous drinking water supplies. Drinking water sources at the work place was also evaluated. Water source locations has been identified by global positioning systems (GPS) and the data were recorded onto maps.

All laboratory analyses were conducted using the appropriate QA/QC procedures.

3. RESULTS

To accomplish the goals of the ASHRAM PROJECT within the Slovak Republic, communities have been identified in which the inhabitants have been using drinking water from public water supplies with an arsenic content above 10 μg/l. These areas are located in the regions of Banská Bystrica and Nitra and include seven districts: Banská Bystrica, Brezno, Žiar nad Hronom, Žarnovica, Nitra, Levice and Nové Zámky (Figure 1).

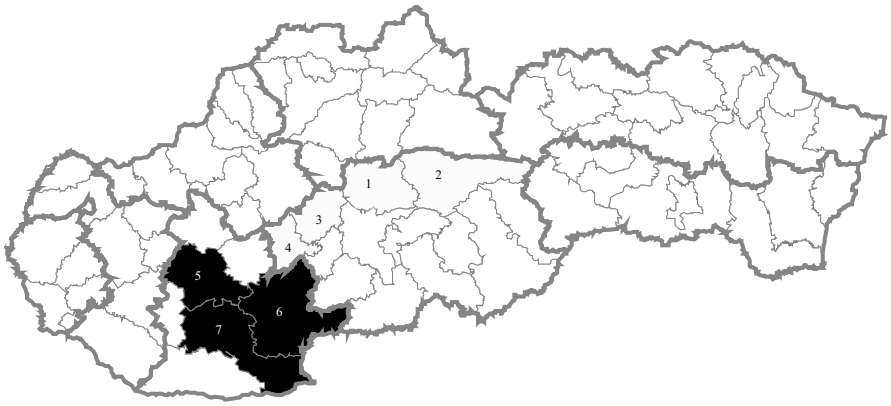

Figure 1. Regions and districts (1-7) selected for the ASHRAM study area in Slovakia.

The full phase of this Project was initiated in January, 2003. During this time, the study has focused on locating appropriate patients at the Urology and the Dermatology Departments of the hospitals. Subsequent activities have been focused on recruiting case and control subjects for participation in the study.

At the present time the recruitment of cases has been completed, with a total of 365 patients recruited. These include 230 patients diagnosed with skin cancer, 87 patients diagnosed with bladder cancer and 48 patients diagnosed with kidney cancer (see Table 1).

Following recruitment into the study, each of the patients completed the two questionnaires. In addition, biological samples and drinking water samples were obtained. For skin cancer patients, histological examination records were reviewed to establish both the type of tumor (i.e. basal cell or squamous cell), as well as the location of lesions on the patient's body. The distribution and location of skin cancers are summarized in Table 2.

A total of 230 subjects with skin cancer were recruited for the study. As many subjects had tumors at multiple sites, the total number of tumors in this population was 267. From the entire study population, a total of 245 lesions were classified as basal cell carcinoma, while 22 lesions were classified as squamous (spinocellular) carcinomas. Histological examinations for those patients having kidney or bladder tumors have also been processed.

Table 1 . Summary, by region and district, of cancer sites for case subjects recruited for the study in 2003–2004.

Region	District	Skin cancer	Bladder cancer	Kidney cancer
Nitra	Nitra	51	21	6
	Nove Zámky	58	25	3
	Levice	21	9	8
Banská Bystrica	Banská Bystrica	65	9	12
	Brezno	20	6	10
	Žiar nad Hronom	11	15	9
	Žarnovica	4	2	0
Cases excluded due to negative histology			–5	–2
Total		230	87	48

Table 2. Total number of skin cancer cases and histological classification of lesions in Banská Bystrica and Nitra regions for 2003–2004.

Region	No. of cases	BCC*	SCC**	No.of tumours Total
Banská Bystrica	100	103	9	112
Nitra	130	142	13	155
Total	230	245	22	267

*BCC, basal cell carcinoma.
**SCC, squamous (spinocellullar) carcinoma.

A total of 117 control subjects have been recruited for the study according to strict selection criteria. The diagnostic groups identified for control subjects is provided in Table 3. The majority of controls were diagnosed with colelithiasis, abdominal hernias and fractures.

The geographic distribution of case and control subjects within the Nitra and Banská Bystrica regions is provided in Table 4. Study participants were evenly distributed between the district or regional towns and small towns or villages. For the case subjects, half resided in district or regional towns and half in small towns or villages. For the control subjects, 31% resided in regional towns and 21% in district towns, while 48% were located in small towns or villages.

Table 3. Controls divided by diagnostic groups for each region.

Region	Diagnostic groups	Number
Nitra Total: 58	K 40-K 46	18
	K 80	25
	K 35	1
	K 26	1
	S 02-S 92	13
Banská Bystrica Total: 59	K 40-K 46	13
	K 80	8
	K 35-K 38	3
	K 26	3
	S 02-S 92	11
Total		117

*K40 – K46, abdominal hernias; K38, cholelithiasis; K35 – K38, appendicitis; K26, duodenal ulcers; S02 – S92, fractures (WHO, 1992).

Table 4. Distribution of case and control study participants by geographical residence.

	Cases		Controls	
	No.	%	No.	%
Regional towns	106	29	36	31
District towns	75	21	25	21
Small towns	18	5	2	2
Villages	165	45	54	46
Total	364	100	117	100

Following recruitment of case and control subjects, data were obtained to provide an estimate of As intake. The arsenic exposure assessment model will consider the following pathways of exposures:

1) Cumulative exposure via drinking water consumption. This will be determined based on measurement of As concentrations in drinking water as well as retrospective data. Ingestion rates will be estimated using data in questionnaires.
2) Other sources of arsenic exposure including intake via food will be determined using the results of As measurements from food collected for the project and from retrospective data. Data regarding nutritional habits will be obtained from the Main and Food Frequency Questionnaires and from the special sub-study based on 24-hour recalls.
3) Exposure in the work environment. Information concerning exposure to As at certain jobs, working habits, and work technology at these jobs will be obtained from the Main Questionnaire.

At the present time, the ASHRAM project in the Slovak Republic has completed subject recruitment and is in the process of collecting biological and environmental samples. Databases have been created to organize information from sampling and from questionnaires. Once all of the data have been evaluated, a seminar will be held in the Spring of 2005 in Hungary to present the results and review the conclusions.

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CHAPTER 20

A PUBLIC HEALTH APPROACH TO IDENTIFYING AND REDUCING LEAD EXPOSURES AT A MINING SITE

Susan Griffin[1], Paula Schmittdiel[1], and William Brattin[2]
[1]*U.S. Environmental Protection Agency, Region VIII, 999 18th Street, Denver, CO,USA;*
[2]*Syracuse Research Corporation, 999 18th Street, Denver, CO, USA*

Abstract: Eureka City, Utah, was the site of mining, milling, and smelting activities that resulted in contamination of soil with lead. The lead is mainly in the form of lead carbonate, which is readily bioavailable. Children in Eureka had elevated blood lead levels (24% above 10 μg/dL). Recreational exposures on waste piles were also associated with increased risk of elevated blood lead. To reduce lead exposures, contaminated residential soils were removed or capped, and homes with contaminated dust were provided HEPA vacuums. Education programs, home visits by the community nurse, and regular blood lead testing programs were developed. Blood lead levels in younger children have decreased in recent years.

Key words: lead exposure, risk assessment, public health evaluation, mining sites

1. INTRODUCTION

Lead is a common environmental contaminant at mining and smelting sites in the Western United States (Davies, 1988). When residential communities are located adjacent to or near these sites, both children and adults can be exposed to lead via the incidental ingestion of contaminated soil and mine waste, inhalation of airborne particulates, and ingestion of contaminated drinking water. Young children less than 6 years of age are particularly sensitive to the adverse effects of lead because of their rapidly developing neurological systems, increased absorption of lead from the gastrointestinal tract, and more frequent mouthing behavior (CDC, 1991). Children and adults can also be exposed to lead from non-mining related sources, such as

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lead-based paint, diet, and occupational activities (CDC, 1991). When evaluating a mining and smelting site for remediation under the U.S. Environmental Protection Agency (USEPA) Superfund program, it is important to identify and characterize both the mining and non-mining related lead sources as well as key behavioral factors within the populace which may increase or mitigate exposures to these sources.

2. SITE DESCRIPTION AND HISTORY

The town of Eureka City was a gold and silver mining area in central Utah from 1871-1965 (USEPA, 2002). Mining and milling activities took place along the southern and western boundary of Eureka City, adjacent to current residential areas. As a result, large waste piles and soil were contaminated with lead, arsenic, copper, and mercury. In 2000, Eureka City had a population of 767 with 91 children less than 6 years of age. The younger children played in residential yards contaminated with metals and the older children and adults rode motorized vehicles and bicycles on or near the former mine sites. Although several contaminants were evaluated during the investigation, this paper is focused on lead as the primary contaminant of concern. Exposure pathways identified for evaluation included ingestion of soil and indoor dust, inhalation of air-borne particulates, ingestion of drinking water, and ingestion of lead-based paint.

3. ENVIRONMENTAL DATA COLLECTION AND RESULTS

In August 2000, the USEPA sampled the surface soil, indoor house dust, tap water, and interior and exterior paint in residential homes for inorganics typically associated with mining and smelting processes (USEPA, 2002). Soil and air particulates were sampled in the mining areas adjacent to the town.

Surface soil samples were collected from 0-2 inches in depth. Each sample was a composite of surface soil from 4 to 6 locations. All soil samples were sieved and soil passing through a 250μm mesh sieve was analyzed for inorganics using X-ray Fluorescence Spectroscopy (XRF). The < 250 μm fraction was analyzed because metals have been shown to be more concentrated in the soil fractions that have smaller particle sizes at smelting and mining sites (Davis et al., 1996; Walker and Griffin, 1998) and these smaller particles more readily adhere to hands, making them more available for incidental ingestion (Driver et al., 1989). Seventeen of the surface soil

samples, spanning a range of lead concentrations, were chosen for geochemical speciation by electron microprobe analysis. The same seventeen samples were also tested for bioaccessibility using an *in vitro* system that is designed to measure the relative solubility of a chemical under specified laboratory test conditions.

Indoor dust samples were collected using an HVS3 vacuum and analyzed for inorganics via Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES) (USEPA, 2002). First draw tap water was collected and analyzed for metals. The town of Eureka City receives water from a municipal supply system that undergoes testing for pollutants each year. Since levels of lead in the supplied water were below levels of detection, only testing to detect contaminants in the water distribution systems were conducted. Interior and exterior paint were analyzed for lead via XRF. Sampling for air particulates was conducted using a high volume PM-10 sampler.

Table 1. Environmental Sampling Results for Lead in Eureka City.

Media	Sample Size	Range	Arithmetic Average
Residential soil (mg/kg)	N = 4211	18-25,000	1239
Non-residential soil (mg/kg)	N = 265	3-82,700	16,366
Indoor dust (mg/kg)	N = 57	193-2010	707
Drinking water (ug/L)	N = 54	2-14	4
Ambient Air ($\mu g/m^3$)	N/A	< 0.03	< 0.03
Paint – Interior (mg/cm^2)	N = 54	0.01-1.70	0.15
Paint – Exterior (mg/cm^2)	N = 54	0.01-1.40	0.25

The results of the surface soil sampling are shown in Table 1. Lead concentrations in the residential yard soils ranged from 18-25,000 mg/kg, with an arithmetic average of 1239 mg/kg. Typically, the USEPA recommends that residential soil with lead levels exceeding 400 mg/kg be evaluated for risk. Concentrations in the soils collected from areas adjacent to the town ranged from 3-82,700 mg/kg with an arithmetic average of 16,366 mg/kg.

Geochemical analysis of the soil samples showed that lead occurs primarily as cerussite (lead carbonate), a relatively soluble form of lead. An example of the speciation results are shown in Figure 1. In most samples, the majority of lead-bearing particles were 5-100 μm in diameter. *In vitro* bioaccessibility of lead ranged from 60 to 89%. Although *in vitro* bioaccessibility is not identical to absorption *in vivo*, preliminary results have shown that the results obtained using *in vitro* methods are well correlated with results observed *in vivo* (USEPA 2004). Thus, the *in vitro* results suggest that *in vivo* bioavailability at this site may be somewhat higher than EPA's default value (60%).

Results of the indoor dust sampling for lead are also shown in Table 1. Lead concentrations ranged from 193 to 2010 mg/kg with an arithmetic average of 707 ppm. Similar to the soil lead levels, the indoor dust lead levels exceeded the USEPA's 400 mg/kg level of concern. Lead concentrations in drinking water averaged 4 μg/L, well below the national drinking water action level of 15 μg/L. Air lead concentrations were below the analytical detection limit of 0.03 $\mu g/m^3$. As shown in Table 1, levels of lead in house paint were also low. Average concentrations in interior and exterior paint were 0.15 and 0.25 mg/cm^2, respectively. In comparison, the national standard for lead in paint is 1.0 mg/cm^2.

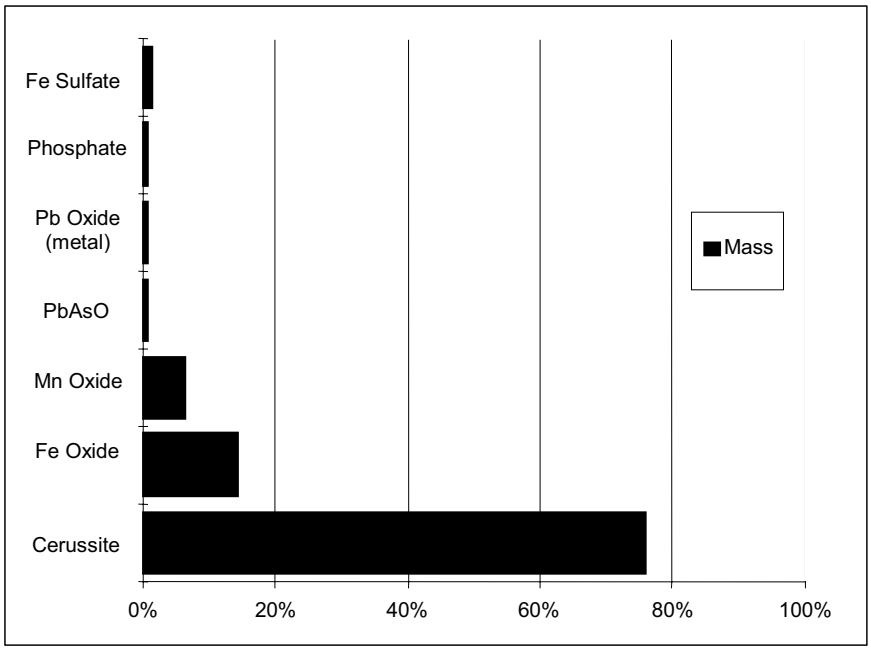

Figure 1. Geochemical Speciation of Soil Lead in Eureka, Percent by Mass.

4. BLOOD LEAD DATA COLLECTION AND RESULTS

In August 2000, the Utah Department of Health, in coordination with the Central Utah Health Department, conducted blood lead testing in Eureka City (ATSDR, 2002). Blood was collected via a fingerstick technique. If results were elevated above 10 μg/dL, which is the blood lead level of concern for neurological effects in young children (CDC, 1991), a follow-up test was conducted using venipuncture. The results of the blood lead testing are shown in Table 2. Of the children less than 6 years of age, 22% (13/55) had blood lead levels which exceeded 10 μg/dL. In comparison, only 1.7% of the children less than 6 years old in the state of Utah had elevated blood lead levels. For children 6-17 years of age in Eureka City, 16% (8/73) had blood lead levels greater than 10 μg/dL. In the state of Utah, only 4.2% within the same age group had elevated levels.

Table 2. Year 2000 Results of Blood Lead Testing in Eureka City.

Resident Status	Participants		# of persons with a BLL ≥ 10 g/dL	Prevalence of elevated BLL %	Geometric Mean of BLLs (g/dL)	BLL Range (g/L)
	Age	*n*				
Eureka City 2000	0-72 months	59	13	22.0	6.7	1.6-34.2
	6-17 years	81	13	16.0	5.0	0.9-32.5
UBLR 2000	0-72 months	3526	59	1.7	2.6	0.0-34.2
	6-17 years	545	23	4.2	2.2	0.2-44.0

UBLR = Utah Blood Lead Registry.
BLL = blood lead level.
n = number of children tested.

5. INVESTIGATIONS TO IDENTIFY SPECIFIC RISK FACTORS

The blood lead data were used to investigate the relationship between measured blood lead levels and elevations of lead in residential environmental media. For example, the blood lead level of each child was plotted on a graph versus the lead concentration in a particular media (e.g., soil, dust, water) in that child's yard or home and linear regression was used to find the line of best fit. Using this technique, no statistically signify-cant correlations were found between measured blood lead levels and

environmental lead concentrations in the home or yard. Also, no significant correlation was found between measured blood lead levels and blood lead levels predicted by the Integrated Exposure Uptake Biokinetic (IEUBK) Model. This is not unexpected, considering that the IEUBK Model is primarily driven by lead concentrations of soil and dust within a child's home or yard.

In conjunction with the blood lead testing, a questionnaire was administered to better understand occupational, demographic, and behavioral factors that may have been impacting exposures to lead. The relationship between the measured blood lead levels and participation in various activities included in the survey were examined by calculating an odds ratio for each behavior. Statistical significance of the odds ratio was tested by calculating a 95% confidence interval using either the Cornfields method, the Exact method, or Woolf's method (ATSDR, 2002). Statistically significant odds ratios were found for motorcycle and all-terrain vehicle (ATV) riding, mouthing toys, and ingesting dirt.

The strong statistically significant association between elevated blood lead levels and motorcycle and ATV use was intriguing. Following discussions between the USEPA project manager and the local school science teacher, a school science project was initiated. Personal air samplers were provided to the children. They were instructed to wear the air samplers and go about their regular routines for one week. The results from that experiment were unexpected and are shown in Table 3. The PM-10 air particulate concentrations of lead ranged from < 0.3 to 89 μg/m^3 with an arithmetic average of 8.4 μg/m^3. The National Ambient Air Quality Standard for lead in the U.S. is 1.5 μg/m^3. The results were surprising because the air lead concentrations measured by the site-wide PM-10 high volume air samplers were below 0.03 μg/m^3.

Table 3. Personal Air Sampling Results for Lead in Fifteen Children.

Range	Arithmetic Mean	PM-10 Air Sample Results	NAAQS*
< 0.3 – 89 g/m^3	8.4 g/m^3	< 0.03 g/m^3	1.5 g/m^3

*NAAQS = National Ambient Air Quality Standard for Lead.

What this suggests is that the children are creating their own micro-environments for lead exposure that aren't necessarily measured by standard sampling protocols. This phenomenon has also been observed with asbestos at the USEPA Superfund site in Libby, Montana. At this site, remediation

workers were provided with personal air samplers and instructed to perform routine housecleaning chores. The air levels of asbestos measured in personal air samplers tended to be higher than levels measured in the same house using stationary air monitors (Miller 2004).

To investigate the impact of these intermittent inhalation exposures on blood lead levels, we input these "microenvironment" air lead concentrations into the International Commission of Radiation Protection (ICRP) model. The ICRP model is a physiologically-based pharmacokinetic model for predicting the disposition of radionuclides in the body, including radio-isotopes of lead (Leggett, 1993). FORTRAN code for the ICRP model was provided by Dr. Joel Pounds of Pacific Northwest National Laboratory and the source code was modified to allow unique daily air lead intakes in each simulation (Khoury and Diamond, 2003). The modified ICRP model can predict short-term blood lead concentrations following acute and intermittent inhalation exposures. This is an advantage over the IEUBK model which relies on annual average air lead concentrations as inputs, thereby suppressing acute upward excursions in blood lead concentration that might occur in response to short term elevations in air lead levels. The key inputs to the ICRP model are shown in Table 4. Soil and indoor dust lead concentrations were assumed to be 1488 and 707 mg/kg, respectively, the average of the entire site.

Table 4. Input Values to the ICRP Model.

Exposure Variable	Input Value
Soil Lead (mg/kg)	1488
Dust Lead (mg/kg)	707
Outside	5 hours/day
Air Exposure duration	2.5 hours/day
Air exposure frequency 1	1 day per 3 days
Air exposure frequency 2	100 days per 365 days
Age (years)	7-14
Air lead (g/m^3)	0.5, 21, or 89

Daily air lead exposures were a combination of the intermittent recreational exposures (as shown in Table 3) and a baseline of 0.1 μg/m^3. Based on the responses to survey questions on frequency of bicycle and motorized vehicle use, it was estimated that recreational exposures would occur for 2.5 hours every 3 days for 7-14 year olds. The model results for 0.5, 21, and 89 μg/m^3 are shown in Figures 2A, 2B, and 2C, respectively. As seen, the model results suggest that the intermittent and highly elevated air lead exposures during recreational activities, such as motorcycle and ATV riding, could easily result in upward excursions of elevated blood lead levels.

6. DISCUSSION AND CONCLUSIONS

Blood lead testing showed elevated levels (i.e., greater than 10 μg/dL) for a number of the children living in Eureka City. Environmental sampling results showed that both soil and indoor dust contain highly elevated levels of lead. Lead in the soil was present as fine particles (5-100 μm) primarily in the form of cerussite (lead carbonate), a relatively soluble form of lead. The small particle size suggests that the soil would readily adhere to hands and toys, and be inhaled easily. If the soil is ingested, the lead would be expected to be absorbed fairly rapidly from the gastrointestinal tract into the bloodstream. However, statistical correlations between measured blood lead levels and concentrations of environmental lead in each child's home are poor, suggesting that ingestion of lead in soil or dust from the immediate home and yard may not be the dominant exposure pathway in all cases. Data from personal air samplers suggest that children may be creating their own microenvironments of high lead exposure depending on the activities they engage in. Accurate assessment of these true exposure environments is problematic. Up until now, we believed that area wide air sampling provided an accurate indication of lead exposure via the inhalation pathway. However, the information we obtained from personal air samplers suggests otherwise. It seems probable that elevated blood lead levels in Eureka City are a combination of individual behaviors and the presence of highly elevated levels of relatively soluble lead in soils and air particulates. A successful remediation strategy must recognize and address all of these factors, not just one.

In Eureka City, a remedial program was developed which addressed both the environmental and the behavioral issues. The mine waste piles adjacent to the town are being removed and capped to reduce direct contact (from recreational activities) and prevent particulates from becoming airborne. Since motorcycle and ATV use is highly popular and a significant risk factor for elevated blood lead levels, a motocross track is being built in a clean soil area, as an alternative recreation site. Contaminated soil is being removed from individual yards and replaced with clean fill and sod to reduce direct contact for younger children. HEPA vacuums are being loaned to homeowners to cleanup indoor dust and reduce indoor lead levels. Finally, the Utah Department of Health and the Central Utah Health Department instituted a semiannual blood lead testing program, combined with education in the schools and home visits by the County Health nurses. The construction activities have only recently begun, so it is too early to determine the effectiveness of these actions. To date, the blood lead levels of older children (7-18 years) have remained elevated, but completion of these construction activities should start to result in decreases. The blood

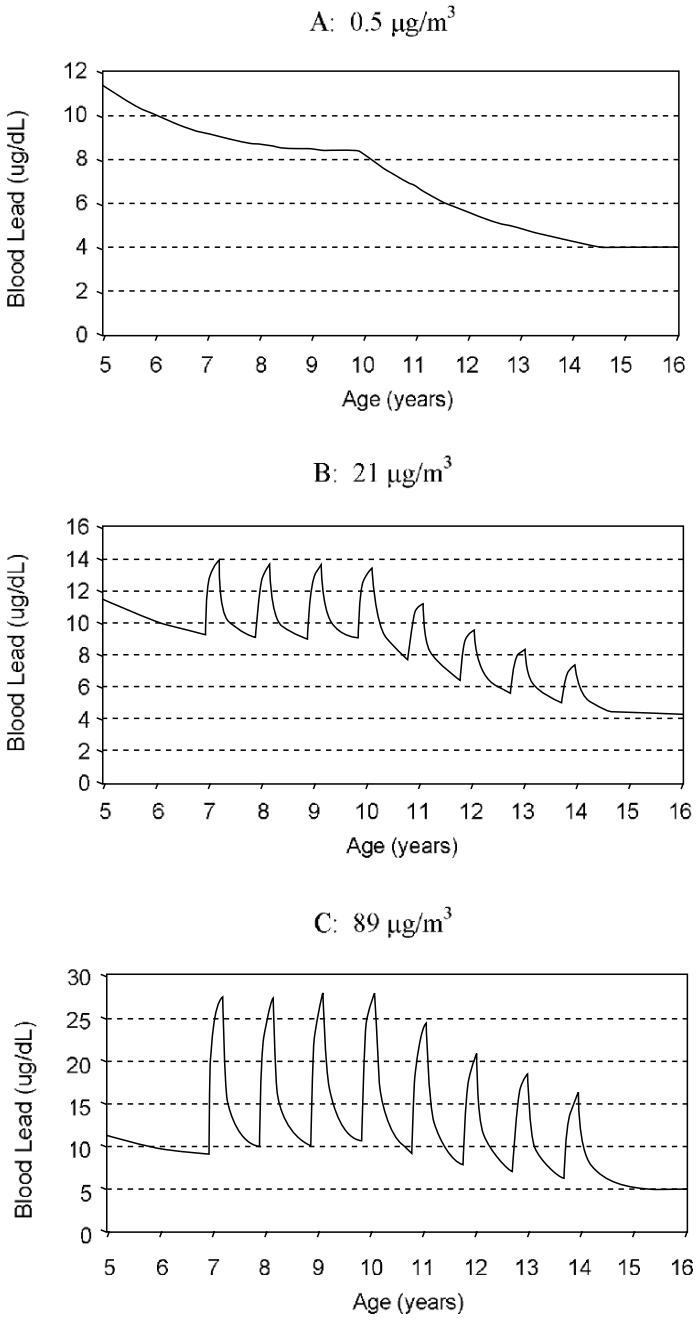

Figure 2. ICRP Modeling of Blood Lead Levels from Intermittent Air Lead Exposures.

lead testing and education programs have been in effect for the last two years. Blood lead levels for children less than six years of age have decreased from 22% with levels greater than 10 μg/dL to approximately 10%. The parents of younger children are more likely to be reached by knowledge of elevated blood lead levels and educational material and take actions to mitigate their child's exposure. We will continue to monitor blood lead levels in Eureka City to gauge the effectiveness of these remedial actions.

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CHAPTER 21

COMBINED EFFECT OF SELECTED INDUSTRIAL FIBROUS DUSTS AND TOBACCO SMOKE ON THE RESPIRATORY TRACT

Combined effect of mineral fibers and tobacco smoke

Marta Hurbánková, Milan Beno, Silvia Cerná, Sona Wimmerová, Zuzana Kováciková, Soterios Kyrtopoulos

[1] Research Base of the Slovak Medical University - Institute of Preventive and Clinical Medicine, Respiratory Toxicology, Bratislava, Slovak Republic; [2] Institute of Biological Research and Biotechnology, National Hellenic Research Foundation, Athens, Greece

Abstract: Rats were exposed via inhalation using a nose-only device to amosite and wollastonite fibers at two concentrations (30 and 60 mg/m^3), 1 hour every 2 days and cigarette smoke from 3 cigarettes/day. The animals were sacrificed after 6 months' exposure. Bronchoalveolar lavage (BAL) was performed and inflammatory and cytotoxic parameters were examined. Following exposure to amosite, the inflammatory parameters showed the greatest change in rats in the 60mg/m^3 dose groups, with or without tobacco smoke exposure. The cytotoxicity of amosite was strongly influenced by tobacco smoke. Amosite produced greater inflammatory and cytotoxic effects in all groups than wollastonite, an asbestos substitute.

Keywords: combined effect, Fibrous dusts, Tobacco smoke, Bronchoalveolar lavage, Inflammation, Cytotoxicity

1. INTRODUCTION

The effects of industrial fibrous dusts on the respiratory system represent a potential environmental and occupational health hazard for humans. Long term exposure to asbestos can cause pleural plaques, asbestosis and oncological diseases. Research is needed to characterize the health effects of fibrous materials, including wollastonite (an asbestos substitute), and to assess the health effects associated with exposure to multiple hazardous materials. This study examined the dose-response relationships in male Fisher 344 rats following inhalation of amosite (a form of asbestos) or wollastonite, alone or

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combined with daily exposure to tobacco smoke. The aim of this study was to assess the combined effect of amosite or wollastonite (an asbestos substitute) and cigarette smoke on selected inflammatory and cytotoxic parameters.

2. MATERIALS AND METHODS

Male Fisher 344 rats were supplied by Charles River Company (Germany). Following two weeks of quarantine, prior to the initiation of the exposures, the animals weighed 191.2 ± 10.4 grams (mean ± standard deviation). All animals were maintained under non-infectious laboratory conditions at 22± 2°C, 45% relative humidity, under natural light and normal light/dark photoperiodicity and in air-conditioned rooms using the unit WOLF KG 100 (WOLF - Clima Technic, GmbH, Mainburg, Germany).

The animals were fed standard commercial laboratory pellets ST1 (TOP - Dovo, Horne Dubove, Slovak Republic) and water *ad libitum*. The period of exposure lasted 175 days (6 months). Animals inhaled amosite asbestos or wollastonite fibers in a nose-only inhalation device (In-Tox, USA). Wollastonite fibers (Table 1) and amosite, an amphibole mineral that is one form of asbestos (Table 2), are naturally occurring silicate inorganic fibers. Wollastonite is used as a substitute for asbestos.

Dust aerosol was produced at two dosages: 30 mg/m^3 air or 60 mg/m^3 air for one hour per exposure. The animal groups were exposed to dusts every second day, including all holidays. No exposures were conducted on Saturday and Sunday. Six groups, each of 11 animals, were exposed to the following: Group 1, 60 mg/m^3 amosite fibers for one hour every two days, combined with exposure to mainstream smoke from three cigarettes daily; Group 2, 60 mg/m^3 amosite fibers for one hour every two days; Group 3, 30 mg/m^3 amosite fibers for one hour every two days, combined with exposure to mainstream smoke from three cigarettes daily; Group 4, 30 mg/m^3 amosite fibers for one hour every two days; Group 5, exposure to mainstream smoke from three cigarettes daily plus immobilization stress as for animals exposed to dust; Group 6, immobilization stress as for animals exposed to dust.

Standard research cigarettes of the 1R1 type (Tobacco and Health Research Institute - THRI, Lexington, KY, USA) were used in all experiments. A whole-body actively ventilated exposure chamber was used, with a cigarette smoke generator and pumps (THRI, Lexington, KY, USA).

Table 1. Diameter, length and percentage of fibers in wollastonite dust after sedimentation.

Diameter [μ m]	**%**
= 1	47
< 1	22
< 3	21
= 3	6
> 3	4
Length [μ m]	**%**
1 – 10	48
11 – 30	40
> 30	12

All groups exposed to tobacco smoke inhaled diluted mainstream tobacco smoke at the target concentration of 30 mg of total particulate matter (TPM)/m^3 air for one hour daily (an exposure requiring the combustion of three cigarettes).

Six months after beginning the inhalation exposures, the animals were anesthetized with thiopental (150 mg/kg animal), and exsanguinated by cutting the vena cava caudalis. Bronchoalveolar lavage (BAL) was performed. The following BAL parameters were examined: a) inflammatory response biomarkers, including total cell count/ml BAL fluid; alveolar macrophage (AM) count/ml BAL fluid; and differential cell count (AM, lymphocytes [Ly], and granulocytes [Gr]); and b) cytotoxic parameters, including the phagocytic activity of AM; the viability of AM; lactate dehydrogenase activity (in the cell-free lavage fluid); acid phosphatase activity (in the cell-free lavage fluid and in the BAL cell suspension); and the cathepsin D activity (in the cell-free lavage fluid and in the BAL suspension). Additional details of the methods used in this study are provided in Hurbánková and Kaiglová (1999) and Černá et al. (2004). The results were statistically evaluated using Mann Whitney's test.

Table 2. Length, diameter and percentage of fibers in amosite dust.

Length [μ m]	%	Diameter [μ m]
< 20	5	
20 – 30	75	0.71
> 30	20	

3. RESULTS

Table 3 presents the measurements of inflammatory response following exposure to amosite fibers alone, or amosite fibers and tobacco smoke. For

Table 3. Inflammatory response parameters in bronchoalveolar lavage fluid (BALF) following inhalation exposure to amosite with or without tobacco smoke (tob. sm.).

	Control	Fibers alone		Tobacco smoke alone	Fibers + tobacco smoke (tob. sm.)	
		30 mg/m^3	60 mg/m^3		tob. sm + fibers 30 mg/m^3	tob. sm. + fibers 60mg/m^3
n	7	7	7	7	7	6
Total cell count/ ml BALF 10^3/ml)	216.67 ± 11.30	223.00 ± 4.36	240.00 ± 7.75	241.43 ± 27.51	261.0 ± 23.31	283,00 ± 7,68 ↑ **
AM count/ml BALF (10^3/ml)	158.57 ± 8.14	154.28 ± 7.59	143.57 ± 7.13	144.28 ± 21.89	137.50 ± 17.31	114,17 ± 6,76 ↓ **
Lymphocytes (%)	2.86 ± 0.26	4.57 ± 0.61 ↑ *	7.57 ± 1.27 ↑**	3.43 ± 0.42	4.43 ± 0.61	7,00 ± 0,69 ↑ ***
AM %	96.71 ± 0.36	94.57 ± 0.65 ↓ *	92.29 ± 1.36 ↓ *	95.14 ± 0.46 ↓ *	94.14 ± 0.68 ↓ **	90,71 ± 0,48 ↓ ***
PMN[a]%	0.43 ± 0.30	0.85 ± 0.26	1.57 ± 0.30 ↑ *	1.43 ± 0.48	1.43 ± 0.20 ↑ *	2,29 ± 0,47 ↑ **
Immature forms of AM (%)	31.71 ± 3.31	44.0 ± 4.38 ↑ *	43.14 ± 6.27	50.43 ± 2.29 ↑ **	46.71 ± 3.64 ↑ *	51,86 ± 4,63 ↑ **
Multinuclear cells (%)	0.485 ± 0.096	0.686 ± 0.06	0.971 ± 0.1 ↑**	0.457 ± 0.084	0.686 ± 0.122	0,714 ± 0,156

Values are presented as mean ± SEM. Comparison of exposed group with the control group (without any exposure); *p < 0.05, **p < 0.01, ***p < 0.001; ↑: increase against control group; ↓: decrease against control group. Abbreviations: tob. sm., tobacco smoke; BALF, bronchoalveolar lavage fluid; PMN, polymorphonuclears; AM, alveolar macrophages.
[a]PMN = polumorphonuclears.

the animals treated with amosite fibers alone, a significant increase was observed in the percentage of lymphocytes, PMN, and multinuclear cells, while a significant decrease was observed in the percent alveolar macrophage (AM). For animals that were exposed to tobacco smoke alone, there was a significant decrease in % AM and a significant increase in the percent of immature forms of AM. Most effects appeared to be exacerbated when the animals were exposed to fibers and tobacco smoke. There was a significant

Table 4. Cytotoxic parameters in bronchoalveolar lavage fluid (BALF) following inhalation exposure to amosite with or without tobacco smoke (tob. sm.).

	Control	Fibers alone		Tobacco smoke alone	Fibers + tobacco smoke (tob. sm.)	
		30 mg/m^3	60 mg/m^3		tob. sm. + fibers (30 mg/m^3)	tob. sm. + fibers (60 mg/m^3)
n	7	7	7	7	7	6
Phagocytic activity of AM (%)	69.71 ± 1.87	70.57 ± 1.99	64.86 ± 3.52	47.4 ± 5.63 ↓**	53.14 ± 4.27 ↓**	61.85 ± 1.84 ↓*
Viability of living AM (%)	86.29 ± 1.27	87.29 ± 0.68	81.00 ± 1.05 ↓*	83.86 ± 0.26	85.86 ± 0.50	79.80 ± 2.13 ↓*
LDH μkat/g protein	4.81 ± 0.86	4.43 ± 0.19	4.22 ± 0.26	4.09 ± 0.73	4.14 ± 0.78	3.31 ± 0.24
ACP nkat/g protein	65.77 ± 10.57	67.76 ± 6.02	49.45 ± 3.76	57.72 ± 4.25	52.52 ± 4.38	46.52 ± 7.17
ACP nkat/10^6 cells	0.18 ± 0.013	0.18 ± 0.032	0.25 ± 0.039	0.17 ± 0.020	0.22 ± 0.027	0.27 ± 0.055
Cathepsin D U_{tyr}/mg protein	77.70 ± 7.59	78.02 ± 11.28	83.63 ± 5.53	104.75 ± 9.31	105.42 ± 7.17 ↑*	116.38 ± 12.73
Cathepsin D U_{tyr} /10^6 cells	419.14 ± 32.25	455.53 ± 38.79	535.44 ± 33.47 ↑*	604.27 ± 26.88 ↑ **	681.63 ± 27.57 ↑**	779.58 ± 47.04 ↑ **

Values are presented as mean ± SEM. Comparison of exposed groups with the control group (without any exposure): *p < 0.05, **p < 0.01, ***p < 0.001; ↑: increase against control group, ↓: decrease against control group; (1) enzyme activity expressed as μmol of p-nitrophenol/hour/mg protein. Abbreviations: AM, alveolar macrophages; LDH, lactate dehydrogenase; ACP, acid phosphatase; U_{tyr}, μg of thyrosine released per hour.

decrease in the count of alveolar macrophage per mL BALF and in the percent AM. The only treatment where a significant increase was observed in total cell count/mL BALF was the highest combined fiber and smoke exposure. At the highest combined exposure, significant increases were also observed for % lymphocytes, % PMN and % immature forms of AM.

The observations of cytotoxic parameters in bronchoalveolar lavage fluid (BALF) are provided in Table 4. For animals exposed to amosite fibers alone, only AM viability and Cathepsin D levels were affected. The percent

viability of living AM was significantly decreased, while Cathepsin D levels per 10^6 cells were significantly increased. For animals that were exposed to tobacco smoke alone, phagocytic activity was significantly decreased and Cathepsin D levels/10^6 cells were significantly increased. For animals with the combined exposure, phagocytic activity was increased in comparison to the levels seen when animals were exposed to tobacco smoke alone, although a significant reduction in activity was observed at all exposure

Table 5. Inflammatory response parameters in bronchoalveolar lavage fluid (BALF) following inhalation exposure to wollastonite with or without tobacco smoke (tob. sm.).

	fibers alone			fibers/tobacco smoke		
		30 mg/m3	60 mg/m3	tobacco smoke alone	tob. sm. + fibers 30 mg/m3	tob. sm. + fibers 60 mg/m3
n	7	7	7	7	7	6
Total cell count/ ml BALF (10^3/ml)	218.33 ± 7.27	225.83 ± 6.247	236.00 ± 15.12	227.00 ± 12.51	231.00 ± 15.36	255.00 ± 27.47
AM count/ml BALF (10^3/ml)	152.86 ± 10.17	135.71 ± 12.23	172.14 ± 22.44	172.86 ± 20.09	158.57 ± 15.26	175.83 ± 25.67
Ly %	2.57 ± 0.84	3.14 ± 0.76	3.00 ± 0.92	3.00 ± 0.31	2.86 ± 10.51	3.33 ± 0.33
AM %	84.28 ± 12.57	96.14 ± 0.96	95.71 ± 0.99	96.00 ± 0.58	96.00 ± 0.82	95.33 ± 0.42
PMN %	0.57 ± 0.20	0.71 ± 0.29	1.28 ± 0.29	1.00 ± 0.38	1.14 ± 0.40	1.33 ± 0.42
Immature forms of AM (%)	20.57 ± 2.19	25.14 ± 1.87	26.29 ± 2.29	33.57 ± 3.16 ↑ **	34.86 ± 1.06 ↑ **	48.67 ± 2.04 ↑ **
Multinuclear cells %	0.26 ± 0.057	0.4 ± 0.0.14	0,4 ± 0.31	0.77 ± 0.09 ↑ **	0.4 ± 10.09	0.33 ± 0.07

Values represent means ± SEM; Comparison of exposed groups with control group (without any exposure): *p < 0.05, **p < 0.01, ***p < 0.001; ↑: increase against compared group., ↓: decrease against compared group; abbreviations: AM - alveolar macrophages; BAL - bronchoalveolar lavage; Ly – lymphocytes; PMN- polymorphonuclear cells.

levels. Cathepsin D levels/10^6 cells appeared to be the only cytotoxic parameter that was significantly increased by the combined exposure. The control value for Cathepsin D was 419 μg thyrosine/10^6 cells, while exposure to 60 mg/m^3 only yielded 535 μg thyrosine/10^6 cells, and the

combined high dose exposure yielded 779 μg thyrosine/10^6 cells. Cathepsin D levels/10^6 cells were significantly increased for animals with tobacco exposure alone, as well as for the high dose fiber only and both combined doses.

Table 5 shows inflammatory BAL parameters after inhalation of wollastonite fibers alone or in the combination with tobacco smoke. After exposure to wollastonite only there were no changes in the examined parameters. In the group "tobacco smoke alone" statistically significant increas in immature AM forms and multinuclear cells (%). Combined significant effect was seen in immature forms of AM in both doses only.

Table 6 presents the measurement of cytotoxic parameters following exposure to wollastonite, or wollastonite and tobacco smoke. For animals exposed to wollastonite alone significantly increased levels of Catepsin D ($U_{tyr}/10^6$ cells) in both doses were investigated; tobacco smoke alone significantly decreased phagocytic activity of AM only and statistically significant combined effect was found in the levels of Cathepsin D ($U_{tyr}/10^6$ cells) after exposure to both doses.

4. DISCUSSION

Increased numbers of BALF cells, as a result of an inflammatory response following exposure to asbestos or other particles, have been observed in numerous studies (Morimoto and Tanaka, 2001; Greim et al., 2001; Hurbankova and Kaiglova, 1999). In our study, a significantly increased number of BALF cells, in comparison with the control group, was observed in the smoker plus 60 mg/m^3 fiber group (by 11.4%) as well as in the corresponding-dose, non-smoker group (by about 16%). This increase could be ascribed to the increase of lymphocyte population proportions. These changes were accompanied by an inverse change in the AM count in BALF, which significantly decreased in the same group exposed to combined higher dust plus cigarette smoke. A very similar but shorter exposure only to cigarette smoke has been reported to lead to a higher (35%) difference in ALF cell counts in comparison with the control values (Ishihara et al., 1997).

The higher than control values of the proportions of PMN and percent of lymphocytes in the bronchoalveolar lavage fluid (BALF) indicate the presence of inflammation in the lung at sacrifice. The magnitude of the increase of these parameters was dose-dependent.

Table 6. Cytotoxic parameters in bronchoalveolar lavage fluid (BALF) following inhalation exposure to wollastonite with or without tobacco smoke (tob. sm.).

	Fibers alone			Fibers / tobacco smoke		
	Control	30 mg/m3	60 mg/m3	Tobacco smoke alone	tob. sm. + fibers 30 mg/m3	tob. sm. + fibers 60 mg/m3
n	7	7	7	7	7	6
Phagocytic activity of AM % of AM	56.80 ± 3.43	49.2 ± 2.87	47.57 ± 4.0	41.60 ± 1.69 ↓ *	43.16 ± 5.74	52.33 ± 4.17
Viabilty % of living AM	89.29 ± 1.43	87.14 ± 1.47	86,57 ± 1,34	87.28 ± 1.13	87.43 ± 21.28	85.67 ± 1.07
LDH µ kat/g prot	3.66 ± 0.58	3.98 ± 0.77	4.30 ± 0.74	5.59 ± 0.54	5.96 ± 20.91	4.45 ± 0.76
ACP nkat/g	57.65 ± 7.8	60.26 ± 7.05	59.73 ± 8.31	84.35 ± 13.21	92.86 ± 11.52 ↑ *	63.29 ± 18.13
ACP Nkat/10^6 cells	0.17 ± 0.011	0.17 ± 0.021	0.16 ± 0.01	0.17 ± 0.019	0.18 ± 0.015	0.18 ± 0.026
Cathepsin D U_{tyr}./mg prot.	63.54 ± 9.67	68.15 ± 9.51	73.17 ± 11.84	82.92 ± 2.36	70.9 ± 9.95	71.11 ± 8.48
Cathepsin D U_{tyr}./10^6 cells	288.25 ± 28.55	403.06 ± 28.90 ↑*	403.79 ± 29.41 ↑*	325.57 ± 9.93	419.20 ± 46.27 ↑ *	437.54 ± 41.64 ↑ *

Values represent means ± SEM; Comparison of exposed groups with the control group (without any exposure): *p < 0.05, **p < 0.01, ***p < 0.001;↑: increase against compared group; ↓: decrease against compared group; enzyme activity expressed as µ mol of p-nitrophenol/hour/mg protein; Abbreviations: LDH - lactate dehydrogenase; ACP - acid posphatase; $U_{tyr:}$ µ g of thyrosine released in an hour time.

AM are the predominant cells present in BALF, and changes in their number or function are important factors in determining the lung inflammatory response and characterizing the pathogenesis of such a response. A decrease in macrophage number or phagocytic capacity may result in the reduction of the clearance of inhaled materials and thus can lead to an increase in the effective dose of the potentially injurious agent (Dziedzic et al., 1993). A significant reduction in the number of AM after intra-tracheal instillation of amosite has also been observed in our previous experiments (Hurbankova and Kaiglova, 1999).

In association with inflammatory changes, a dose-dependent increase in the proportion of multinuclear cells (MNC) was found in the BALF as well as in the lung tissue suspensions. MNC were increased after exposure (separate or in combination) to tobacco smoke as well as both fiber concentrations although the difference was significant only at the high dose without smoking. Similarly, in comparison with the control, there were

increased immature forms of AM in all exposed groups. A strong dose dependent decrease in AM viability (higher dose with and without smoking) as well as phagocytic activity of AM (all group with smoking) was found in this experiment. This consistent with a previously described effect of asbestos (Hurbánková and Kaiglová, 1999). Increases in LDH and ACP activity in extracellular fluids are generally accepted as good markers of cell or tissue injury, and can be used for evaluation of cytotoxicity. We did not find significant changes in LDH or ACP activity in our experiment. Cathepsin D activity after amosite inhalation was significantly changed. These results are consistent with those of Sjörstrand et al. (1989).

Wollastonite inhalation confirmed the lower cytotoxicity in comparison with asbestos. Significant changes were found only by measurement of cathepsin D activity in BAL cells and increased levels of immature forms in combined groups as well as multinuclear cell percentage in the group exposed only to tobacco smoke.

5. CONCLUSIONS

Tobacco smoke alone induced changes in inflammatory parameters, suggesting that smoking might play an important role in inflammatory processes. Tobacco smoke alone also caused some changes in the cytotoxic parameters, and intensified the harmful effect of amosite exposure. Of the groups exposed to amosite, the greatest change in inflammatory parameters was seen in the group exposed to a high fiber ($60mg/m^3$) level of amosite plus tobacco smoke. There was also a weak dose-dependent effect on inflammatory parameters following exposure to the amosite fibers alone at $30mg/ m^3$ and $60mg/ m^3$ (Table 7).

Table 7. Dose dependence after inhalation exposure.

Fiber	Inflammatory parameters		Cytotoxic parameters	
	Without tobacco smoke	With tobacco smoke	Without tobacco smoke	With tobacco smoke
Amosite	+	+	±	± *
Wollastonite	–	±	±	±

+ = dose-dependence was statistically significant.
– = no dose-dependence (no trends or statistical significance observed).
* = similarity of extent of parameter changes after $30mg/m^3$ and $60mg/m^3$ of exposure.

Changes in inflammatory parameters showed no dose dependence in groups exposed to wollastonite without tobacco smoke (Table 7). Changes in

these parameters were very weak in groups exposed to both wollastonite and tobacco smoke. Mild dose dependent changes in cytotoxic parameters were observed in groups exposed to wollastonite without or with tobacco smoke. In animals exposed to wollastonite, the influence of tobacco smoke on cytotoxic parameters was not explicit.

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CHAPTER 22

DUSTS CONTAINING QUARTZ AND CARCINOGENICITY RISK IN MINES

Epidemiological study

Hana Tomášková [1, 2], Zdenek Jirak [1, 2], Milena Menzlova [1], Frantisek Beska [3], Vladislava Zavadilova [1], Katerina Cimova [1], Marek Buzga [1]

[1]*University of Ostrava, Medico-Social Faculty, Syllabova 19, Ostrava, Czech Republic;*
[2]*Institute of Public Health Ostrava, Partyzanske nam. 7, Ostrava, Czech Republic;*
[3]*University Hospital Ostrava, 17. listopadu 1780, Ostrava, Czech Republic*

Abstract: The risk of malignant tumors and lung cancer was monitored in a population of 7,774 ex-miners who had worked in black-coal mines. The incidence of all malignant tumors (n = 264) was comparable to incidence in the Czech Republic. Lung cancer represented 18% of all malignant tumors. About 10% of miners were found to have a light form of coal workers´ pneumoconiosis. The population studied included 55% smokers, 31% non-smokers and 14% ex-smokers. The incidence of lung cancer significantly increased with age (p = 0.01) and 98% of miners with lung cancer were smokers or ex-smokers.

Key words: black-coal miners, malignant tumor, lung cancer, pneumoconiosis, quartz, respirable dust

1. INTRODUCTION

In 1997, the International Agency for Research on Cancer (IARC) classified dust containing crystalline silica and its thermal modifications, cristobalite and tridymite, as Group 1 human carcinogens. The results of many epidemiological studies confirm a statistically significant increase in lung cancer among workers that were occupationally exposed to mineral dusts.

Two large meta-analyses by American (Smith et al., 1995) and Japanese (Tsuda et al., 1997) investigators observed a statistically significant increase in the risk of lung cancer among workers diagnosed with silicosis. Numerous epidemiologic studies have observed that excluding coal miners'' pneumoconiosis, workers diagnosed with silicosis are at increased risk of lung cancer. Smith et al. (1995) observed a significant increase (RR = 2.2) of lung

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cancer in the population they evaluated. Similar results were also observed in a Japanese study (Tsuda et al., 1997). The carcinogenic effect of quartz has been observed consistently in studies of workers in stone quarries, ore mines, foundries, steel works and less frequently in workers in gravel pits as well as the ceramics and glass industries (Lynge et al., 1990; Carta et al., 2001; Meijers et al., 1990; Partanen et al., 1995; Smith et al., 1995; Tsuda et al., 1997; Ulm et al., 2001). Apart from quartz, exposure to other compounds such as polyaromatic hydrocarbons can play an important role in the origin of lung cancer among workers in foundries and steel works.

The increased risk of lung cancer in persons with risk of silicosis has been confirmed in several epidemiological studies. In contrast, studies of populations of black-coal miners do not confirm this risk (Kohout, 1989; Latza et al., 2000; Mehnert et al., 1990; Meijers et al., 1991; Morfeld et al., 1997; Strazinski, 1995; Swaen et al., 1995).

2. METHODS

The population evaluated for the current study included a total of 7,774 examiners, from mines in the Ostrava-Karvina coal region (OKR), who started working in the OKR between 1950 and 1987. These miners worked for at least 8 years in the mining profession, and were exposed to at least 70% of the highest acceptable exposure (HAE). HAE was determined by Jirak (1992) and was expressed as the cumulative dose of respirable dust, which is connected with the probability of incidence of an initial form of pneumoconiosis in 5% of the exposed miners.

The highest acceptable cumulative dose of respirable dust was determined, based on risk factors of the individual mines for coal dust, to be in the range of 99-169 g for coal extraction, and in the range of 58-91 g for drifting. The highest acceptable cumulative dose of respirable quartz fraction for both types of mining working-places ranged from 1.5-3.6 g.

The average concentration of respirable dust fraction in coal extraction was 4.32 mg/m^3 with an average quartz content of 1.9 ± 0.8%. In drifting, the average concentration of respirable dust was 2.2 mg/m^3 with an average quartz content of 3.8 ± 2.2%. However, in individual workplaces, the respirable dust fraction reached concentrations up to six times above the highest acceptable concentration of respirable dust fraction (HACr). HACr at the level of 2.0 mg/m^3 was measured for the respirable dust fraction during coal extraction; while the level of 1.0 mg/m^3 was measured for the respirable dust fraction from drifting.

Data regarding the miners recruited for the study was obtained from the health documentation in the Department of Occupational Medicine. This included information regarding date of birth, profession, cumulative dust dose, percentage of the highest acceptable exposure to dust (% of HAE), first and last year of work in the OKR, smoking habits (i.e. non-smoker, smoker, ex-smoker), and presence of pneumoconiosis disease. Data were also obtained from the Regional Cancer Register to establish the incidence of malignant diseases, site and type of tumor, date of diagnosis, and date of death.

The relative risk (RR) for malignant diseases in miners compared to general population was calculated as a ratio of the age-specific cumulative incidences over an 11-year period (1990–2000). RR was calculated for cancer of the stomach, colon, and lung, as well as for all malignant diseases. Data about the general population was obtained from the Institute of Czech Health Statistics.

Conditional logistic regression for matched case-control groups was used for the analysis of the relationship between lung cancer and duration of exposure in a mine, smoking habits, and occurrance of pneumoconiosis or silicosis. The data for miners was matched by year of birth. Records for a total of 5,183 miners were evaluated. Proportions in tables were evaluated by chi^2-test and Fischer´s exact test ($\alpha = 0.05$). Program Stata, version 8, was used for the data analysis.

3. RESULTS

In the population studied, the average age when subjects began their employment in the mine was 20 ± 3.4 (s.d.) years. The average age at the time of leaving the mine was 43 ± 5.9 (s.d.) years and the total duration of exposure in mine was 23 ± 5.9 (s.d.) years. The sample included 55% smokers, 14% ex-smokers and 31% non-smokers. Coal workers´ pneumoconiosis was diagnosed in about 10% of the persons (n = 751). Forty percent of miners worked in coal extraction and 24% as drifters.

During the period from 1990 to 2001, a total of 264 new malignant diseases were registered. The sites at which malignant diseases were diagnosed are shown in Figure 1 and the characteristics of miners with malignant diseases are provided in Table 1.

There were no specific malignant diseases for which the relative risk in miners was significantly higher than in the general population (Table 2). The age-specific and crude RR were higher for cancer of the stomach in the age group 45-49 (RR = 1.17, 95% CI: 0.376–3.635). The RR for cancer of the colon in the age group 60-64 was 1.05 (95% CI: 0.396–2.782); while

the RR for cancer of the lung in the age group 35-39 was 1.25 (95% CI: 0.176–8.899).

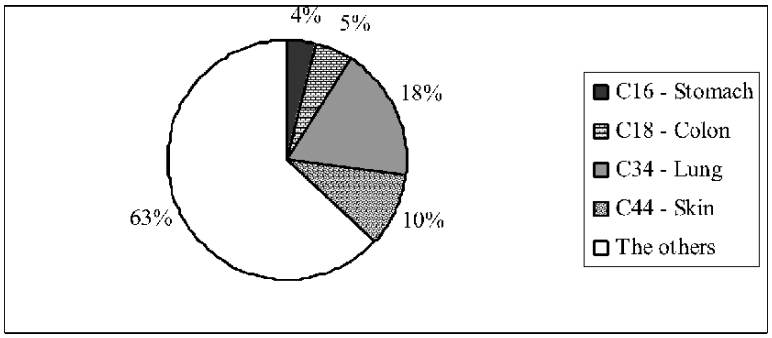

Figure 1. Summary of sites of origin for malignant disease in miners registered during the period of 1990 to 2001.

Table 1. Characteristics of miners with malignant tumorous diseases by site.

Characteristics (years)	**Count**	**Arithmetic mean**	**Min.**	**Max.**
Stomach				
Age at diagnosis (dg.)	10	51.5	42	61
Time from end of work to dg.	10	6.2	2	10
Duration of exposure in mine	10	24.6	14	31
Colon				
Age (dg)	14	55.5	41	63
Time from end of work to dg.	14	7.6	0	13
Duration of exposure in mine	14	27.4	20	35
Lung				
Age (dg)	47	53.3	37	66
Time from end of work to dg.	47	6.6	0	13
Duration of exposure in mine	47	26.7	10	36
Skin				
Age (dg)	29	53.4	37	66
Time from end of work to dg.	29	6.9	0	13
Total period of work in mine	29	27.5	15	39
All others				
Age (dg)	164	52.7	34	65
Time from end of work to dg.	164	5.9	– 4	15
Duration of exposure in mine	164	25.8	13	38

Table 2. Relative risk of malignant diseases by site.

Age (years)	Relative risk Miners/General population			
	Stomach	Colon	Lung	All
25 – 29	-	-	-	-
30 – 34	-	-	-	0.53
35 – 39	-	-	1.25	0.57
40 – 44	0.92	0.67	0.51	0.53
45 – 49	1.17	0.28	0.93	0.76
50 – 54	0.85	0.31	0.49	0.66
55 – 59	0.54	0.87	0.39	0.71
60 – 64	0.52	1.05	0.73	0.70
65 – 69	-	-	0.46	0.28
Total	0.37	0.46	0.55	0.53

Table 3. Lung cancer and duration of exposure, smoking habits, pneumoconiosis and type of profession.

	Occurrence of lung cancer				P
Duration of exposure in mine (years)	Yes		No		
	N	Col. %	N	Col. %	
8 – 20	4	7.8	900	17.5	0.061
21 – 25	13	25.5	1,718	33.5	
26 – 30	22	43.1	1,715	33.4	
31 and more	12	23.5	799	15.6	
Smoking habit					
Non-smokers	1	2.0	1,499	29.3	0.000
Ex-smokers	7	13.7	894	17.5	
Smokers	43	84.3	2,724	53.2	
Pneumoconiosis					
Yes	10	20.0	603	12.1	0.089
No	40	80.0	4,382	87.9	
Type of profession					
Drifters	17	33.3	1,170	22.8	0.106
Coal extraction workers	20	39.2	1,908	37.2	
The others	14	27.5	2,050	40.0	

The incidence of lung cancer was found to increase significantly with age (p = 0.01). A significant difference was not observed for miners with lung cancer in relation with duration of exposure in the mine, work activity

or the occurrence of pneumoconiosis (Table 3). However, a significant effect was observed for smoking habits (84.3 % of miners with lung cancer were current smokers). These results are consistant with the results from a model stratified by year of birth. The odds ratio was 24.2 (95% CI 3.3 – 176.5) in smokers, and 10.6 (95% CI 1.3 – 86.3) among ex-smokers in comparison with non-smokers.

4. CONCLUSION AND DISCUSSION

The incidence of all malignant tumors, including lung cancer, in underground workers in the OKR mines was found to be comparable to the incidence of these diseases in general population of the Czech Republic.

In the population of black-coal miners recruited for the current study, 18% of the total number of malignant diseases were diagnosed as lung cancer. This occurrence is comparable to the value stated by Kohout (1989) of 16.9% in the miners of West Bohemian coal mines. Spacilova et al. (1988) found lung cancer in 17% of persons with complicated silicosis in the period 1964–1987.

In the monitored sample of miners, about 10% of the persons were found to have a light form of coal workers' pneumoconiosis. The sample population included 55% smokers, 31% non-smokers and 14% ex-smokers.

The incidence of lung cancer was found to increase significantly with age (p = 0.01). In addition, 98% of miners with lung cancer were current smokers or ex-smokers. In comparison with non-smokers, the odds ratio in smokers was 24.2 (95% CI 3.3 - 176.5) and the odds ratio in ex-smokers was 10.6 (95% CI 1.3 - 86.3).

In the population of workers recruited for the current study, the relationship between the incidence of lung cancer and coal workers' pneumoconiosis, total duration of exposure in mine and work activity was not significant.

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CHAPTER 23

ENERGY MANAGEMENT OF WASTE CLEAN-UP SITES, AVOIDING SECONDARY AIR IMPACTS TO HUMAN HEALTH

Katarina Mahutova[1] and Jan Pavlovic[2]
[1]*Pacific Northwest Pollution Prevention Resource Center, Seattle, USA;*
[2]*Faculty of Informatics, Masaryk University, Brno, Czech Republic*

Abstract: Hazardous waste cleanup sites create health hazards by the nature of their contamination. In addition, they adversely impact human health by air emissions that may be generated during the site characterization and remediation processes. These emissions can be substantial, especially those associated with energy utilization. The Waste Site Energy Calculator is a web-based tool that estimates energy requirements of individual remedial technologies at waste clean-up sites. It computes the air emissions produced by remedial processes, Global Warming Potential, and presents the energy efficiency of remedial technologies as implemented on a site-specific basis.

Keywords: energy management, remedial process, global warming, calculator, java

1. INTRODUCTION

Energy requirements during remediation and assessment at waste sites and the potential for energy savings are significant. The situation in the United States provides an example.

The environmental industry in the United States is a 220 billion dollar industry, approximately 2% of U.S. economy. The waste sector represents nearly 40% of this total. By translating this economic activity into energy requirements, and by assuming that energy efficiency improvements are adopted by the U.S. waste industry at a rate of 0.01% per year, for example, with an average efficiency improvement of 25%, energy savings may approach 100

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billion BTU/year (105,500 billion J/year). This is the equivalent of 26,000 cars removed from the road per year, energy provided to 3000 homes per year, or greenhouse gas emissions reductions of 3000 tonnes per year.

Waste clean-up sites are complex and the process of cleaning them up should be carefully planned so additional environmental problems are not created.

There is significant potential within the waste clean-up sector to manage energy consumption during characterization and remedial activities. Benefits include the reduction of green house gas emissions, avoidance of secondary impact to human health, and enhancement of waste minimization and energy recovery.

The Waste Site Energy Calculator is a web based tool that estimates the energy requirements of individual remedial technologies at waste clean-up sites, and calculates the potential for energy conservation. The calculator also estimates CO_2 and NO_x emission production, Global Warming Potential and their environmental equivalents like cars off the road per year, homes powered per year and carbon sequestration by trees. It is also designed to help foster markets for new environmentally conscious remedial technologies.

This paper is divided into two parts. The first part describes the features of the calculator and the second part provides insights into the programming of the Waste Site Energy Calculator.

2. ENERGY MANAGEMENT AT WASTE CLEAN-UP SITES

It is recognized that cleanup actions at waste sites often have significant energy requirements over many years. Very few sites include efficient energy management in the original design and operation plans in spite of the fact that sites offer a significant potential to save and/or produce energy.

The Waste Site Energy Calculator is a response to the lack of assessment tools to determine the energy requirements, pollution and greenhouse gas potentials at waste cleanup sites and to evaluate the energy efficiency of remedial technologies. The Waste Site Energy Calculator is a tool to assist site managers in assessing the energy implications of remedial actions proposed or completed at waste sites.

The Calculator is a web-based software tool that can be used for decision making, site management, and educational purposes in relation to promoting energy efficiency at waste sites. This tool helps site managers to answer the question “How much can I save /reduce energy consumption?”

Other features of the Calculator include:

- Computation of the energy use of selected remedial technologies and comparison to energy requirements of other relevant technologies (treating the same contaminant in the same media) (Figure 1)
- Computation of the energy efficiency at a cleanup site by choosing from optimization/energy efficient options related to individual technologies (e.g., energy substitution)
- Computation of the air emissions produced by different remedial processes
- Estimation of potential pollution prevention achievable by continuous improvement of the remedial process
- Computation of Global Warming Potential
- Computation of reduction of the Global Warming Potential
- Presentation of energy efficiency at remedial sites by using environmental equivalents.
- Energy Substitution

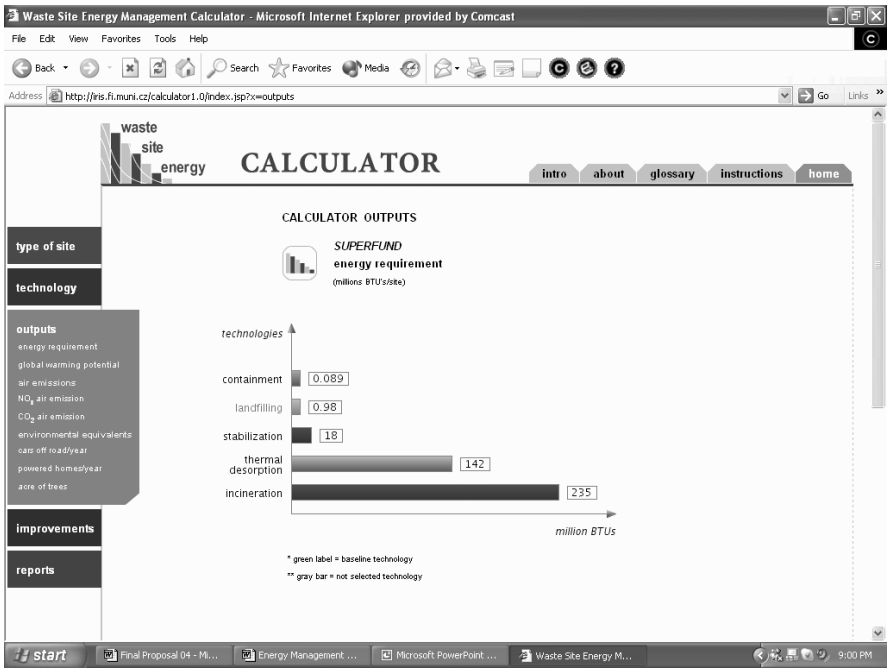

Figure 1. Calculator Output on Energy Requirements of Individual Technologies.

The current version of the calculator is Version 1.0 and is designed for ten soil treatment technologies and five groups of contaminants. It is structured to allow the user to view and compare the energy requirements of individual technologies with default data. If users are interested in a site with remedy specific information, basic data input is required in user defined boxes.

Output data is available in English or in SI (metric) units and can be depicted in graphic and/or text form. Total or partial summaries of the output data can be printed or saved as TXT, PDF, or HTML files.

3. INFORMATION TECHNOLOGY ANALYSIS

Selection of the particular technology that was selected for the calculator was one of the most important issues in the whole project. There are numerous criteria that the technology needed to meet. Most of them are related to the programming techniques.

Security: Since the product is readily available to all users, the level of security is of maximum importance. The security restrictions are not applied primarily to the calculator itself but to the environment in which it operates.

Accessibility: A primary design criterion for the calculator is easy accessibility. It is designed to be comprehensible, to offer help, tutorials, and appropriate graphical layout and outputs.

Development flexibility: The very idea of the calculator bears a number of implementation possibilities. Therefore the basic version of the calculator has to be designed to facilitate upgrades and alterations, this being the main reason of failure of many similar projects. To upgrade them wouldn't simply pay off.

There are a few programming languages usable in the calculator: Microsoft VBA, Borland Delphi and Sun Java. Table 1 presents a comparison of programming languages.

Table 1. Attributes of Candidate Programming Languages.

	Security	Simplicity	Flexibility	Platf. Independent
VBA	–	–	–	–
Delphi	+	+	+	–
Java	+	+	+	+

The Java language [Java] was found to be the most suitable one. It is one of the top contemporary programming languages and it provides easy application of many components, which leads to cheaper and faster development of the calculator.

Considering the accessibility of the Internet for the final user, the calculator is designed as a web portal. This allows the user to access the calculator irrespective of the efficiency of his hardware, operating system and software.

4. GOALS DEFINITION

The accurate planning of all phases, control points and testing was essential for the successful completion of the whole project. The Jeff Crow (http://java.sun.com) method, summarized in Figure 2, was used for the planning of this project.

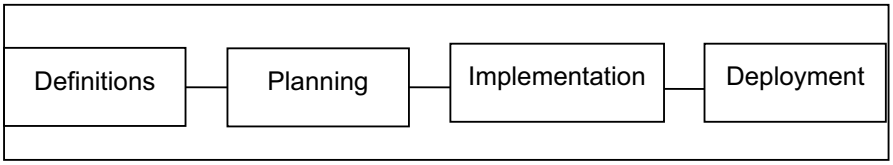

Figure 2. The Jeff Crow Method.

First, it was necessary to learn about the whole problem, the idea on which the calculator is based and to obtain agreement on the contents and the particular form of the communication with the final user. In the next step the characteristics and implementation time had to be decided.

Brainstorming was established as a very useful method when all project members participated in discussions and presented their comments. Since most of the topics have had visual form we could use a display board with colored pens. That is why we were able to catch easily all ideas about the calculator. The advantage is that project members were able to present their suggestions and the result was immediately visible. Screenshots of each board were made before clearing. All pictures were then available for future work. This method is very effective and technically simple.

5. PLANNING

We choose Work Breakdown Structure (WBS), the Float chart diagram version, as a basic planning method. Each task of the whole process is set

into the Gantt diagram. It is very useful to use some planning software. At the present time, there are only a few such products available. Free products that are readily available include Kickstart (http://www.projectkickstart.com) and Planner (http://www.planner.org).

Planner is completely sufficient for planning of small or medium projects. It enables insertion of whole progress phase including the time schedule and dependencies: Finish-to-start, Start-to-finish and Finish-to-finish. Another big advantage is the risk management possibility. It is wise to compute critical path (CP) in the project as well. It defines tasks whose delay will affect completion of other tasks in the project. We can also set the milestones in our project that can present alpha and beta versions of products. Thanks to these milestones you can release pre-version for testing and find problems in a very early stage. Once problems have been identified, correction is cheaper and faster.

Before the start of the implementation phase, it is good to model the basic structure of the program. Since Java is a fully objective language, we can use Unified Modeling Language (UML) diagrams. We used the modeling case Together (http://www.borland.com) from Borland. For simple modeling it is also possible to use the open source program Bluej (http://www.bluej.org). Both tools enable generation of a program skeleton of all objects and methods after the modeling process is complete.

6. IMPLEMENTATION

The whole program is divided into two main parts. These include a kernel that computes all mathematical equations and a graphical interface. Such division gives us the possibility of simple modification from a server-based to a client-based version in later phases of development. The kernel stays the same and only the graphical user interface (GUI) will replace the web interface. The whole application will be implemented in Java WebStart (http://java.sun.com/webstart). Users will be able to download the application from the web and launch it on their computers.

Server based design has several advantages. The immediate actualization is an example. The kernel of application runs as a Java Servlet. Java Server Pages (JSP) generates the graphical interface. The server part of the system runs on the opensource JSP/Servlet containing Tomcat (http://jakarta.apache.org) from the Apache organization forge. The visual form in the user web browser is a combination of XHTML and CSS (http://www.w3.org). This enables separation of visual and logical information. Thus, the design can be simply changed without modifying the source code as well.

CSS styles are optimized for web browsers based on Mozilla or Konqueror, which runs on the same kernel as the most popular web browser Saffary on common Apple computers in USA. And last but not least, on the web browser Internet Explorer. Although the code is valid according to the W3 consortium, there were some problems with IE, which does not implement some of CSS 2.0 basic features.

JSP code is divided into Java Beans components, which make the code more effective. XHTML is generated using Taglibs, which allow using of macros, which bring effectiveness as well. All configuration data as well as user configuration are stored in XML files. There are several frameworks for working with XML. We chose dom4j (http://www.dom4j.org).

At the present time, it is necessary to use some IDE for programming. We selected opensource IDE eclipse (http://www.eclipse.com) supported by investment from the IBM Company.

7. CONCLUSION

The Waste Site Energy Calculator is a broad tool providing capabilities in an effective way for waste site managers to manage energy requirements at remedial sites. This calculator is easy to use thanks to modern information technology used for programming of this software-based instrument. The Calculator can by used by anyone interested in visualizing energy savings and emission prevention. The Calculator can be found on following web site: http://iris.fi.muni.cz/calculator1.0/

ACKNOWLEDGMENTS

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CHAPTER 24

FIELD STUDY AND MODELED TRANSPORT OF CHLORTOLURON IN DIFFERENT SOIL TYPES OF THE CZECH REPUBLIC

Martin Kočárek, Radka Kodešová, Josef Kozák, Ondřej Drábek, Oldřich Vacek and Karel Němeček

Czech Univ. of Agriculture, Dept. of Soil Science and Geology., Kamýcká 129, 16521 Prague, Czech Republic

Abstract: Chlortoluron transport was studied in five different soil types in five locations in the Czech Republic. There were considerable differences in herbicide transport with varying soil types and locations. The BPS mathematical model (Kozák and Vacek, 1996) was used to simulate chlortoluron transport. The chlortoluron concentrations that were predicted by the model were similar to the measured data except in the Greyic Phaozem, where preferential flow may have greatly influenced solution transport.

Key words: pesticide, chlortoluron, solution transport, field study, model.

1. INTRODUCTION

Soil and groundwater contamination from pesticides used in agriculture is a common environmental problem worldwide. Chlortoluron, the herbicide tested in this study, is widely used on cereal and poppy crops. Zander et al. (1999) detected chlortoluron in 15% of 2,403 groundwater samples in western Germany. Chlortoluron concentrations in 7% of samples were greater than 0.1 μg/L, the maximum limit value according to the German drinking water standard.

Various simulation models have been developed for assessing groundwater vulnerability to contamination, resource management, and design of monitoring programs. The chlortoluron transport in several soil types of the Czech Republic was studied experimentally and modeled with the BPS code

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(Kočárek et al., 2003, 2005). HYDRUS-1D (Šimůnek et al., 1998) was used to simulate chlortoluron transport that was experimentally studied in chernozem (Kodešová et al., 2004, 2005).

Here we present the results of field and simulation model studies at five locations in the Czech Republic. Chlortoluron transport was studied in five different soil types in five different locations. The BPS (Behavior of Pesticides in Soil) mathematical model (Kozák and Vacek, 1996) was utilized to simulate herbicide transport.

2. MATERIALS AND METHODS

Experiments were carried out in five locations in the Czech Republic, with a Haplic Luvisol 1 (in Hněvčeves), a Haplic Luvisol 2 (in Kostelec nad Orlicí), a Haplic Cambisol (in Humpolec), a Dystic Cambisol (in Vysoké nad Jizerou), and a Greyic Phaozem (in Čáslav). The elevations above the sea level were: Hněvčeves 300 m a.s.l., Kostelec nad Orlicí 280 m a.s.l., Humpolec 530 m a.s.l., Vysoké nad Jizerou 680 m a.s.l., and Čáslav 270 m a.s.l. A 1g/L solution of chlortoluron was applied to 2 x 2 m experimental plots on 5th May 2004. Two liters of fresh water were used to wash the herbicide down the plants and ensure the solution inflow into the soil. At each 2 cm increment in depth, to a total depth of 30 cm, soil samples were taken from three locations in each experimental plot. These samples were taken at days 0, 5, 12, 21, and 35 following chlortoluron application.

The soil samples were analyzed in the laboratory to determine the chlortoluron distribution in the soil profiles. The soil samples were dried, ground and sieved through a 1-mm sieve. The total amount of chlortoluron in each soil sample was determined as follows. Five g of dry soil were placed into a centrifuge tube. Five ml of methanol were added, and the centrifuge tube was placed for 15 hours into a shaking apparatus. Next, the soil sample was centrifuged 30 minutes at 12,000 rotations per minute. The chlortoluron concentration in the methanol extract was determined using High Performance Liquid Chromatography (HPLC). The total amount of chlortoluron in the soil sample was expressed as the total amount of solute per mass unit of dry soil (ppm). The average value from three samples per layer was calculated.

The BPS mathematical model (Kozák and Vacek, 1996) was used to simulate herbicide transport in these soils. Daily precipitation was measured at each location. The cumulative rainfall during the 35 days monitoring period at different locations was 63 mm in Hněvčeves, 75 mm in Kostelec nad Orlicí, 96 mm in Humpolec, 104 mm in Vysoké nad Jizerou, and 92 mm in Čáslav. The average daily temperature was 14.4 C^{o} in Hněvčeves, 13.1 C^{o} in Kostelec nad Orlicí, 10.8 C^{o} in Humpolec, 11.8 C^{o} in Vysoké nad Jizerou,

and 13.7 C^{o} in Čáslav. Evapotranspiration was estimated using the BPS model. The BPS soil database contains mean values for the properties of each soil type (Kozák et al., 1996), and these values were used in the model. Soil hydraulic properties, sorption and half-life degradation were estimated using the pedotransfer functions and rules (see Kozák and Vacek, 1996).

3. RESULTS AND DISCUSSION

Figures 1A – 5A present the chlortoluron distribution that was measured in the five soil profiles using high performance liquid chromatography (HPLC). There were considerable differences in herbicide transport between varying

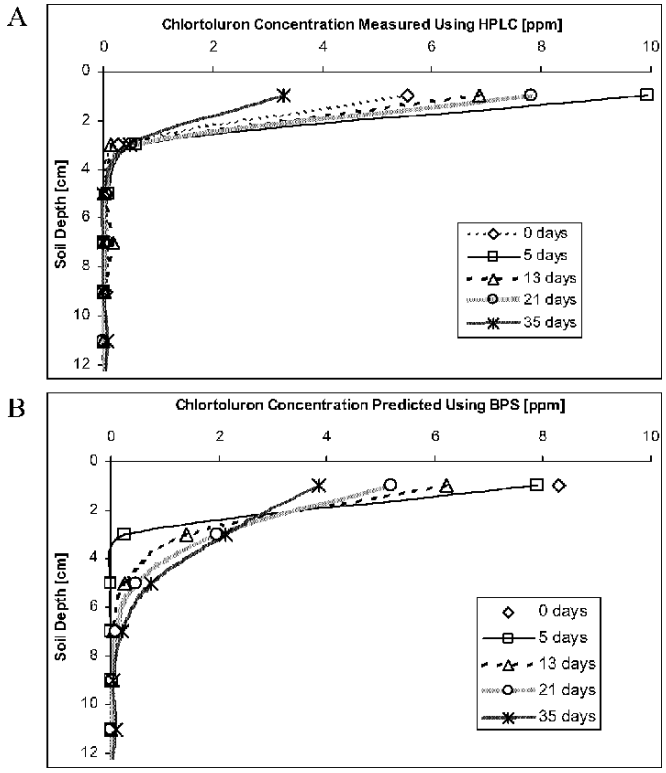

Figure 1. Observed (A) and simulated (B) chlortoluron distribution in the soil profile of a Haplic Luvisol 1.

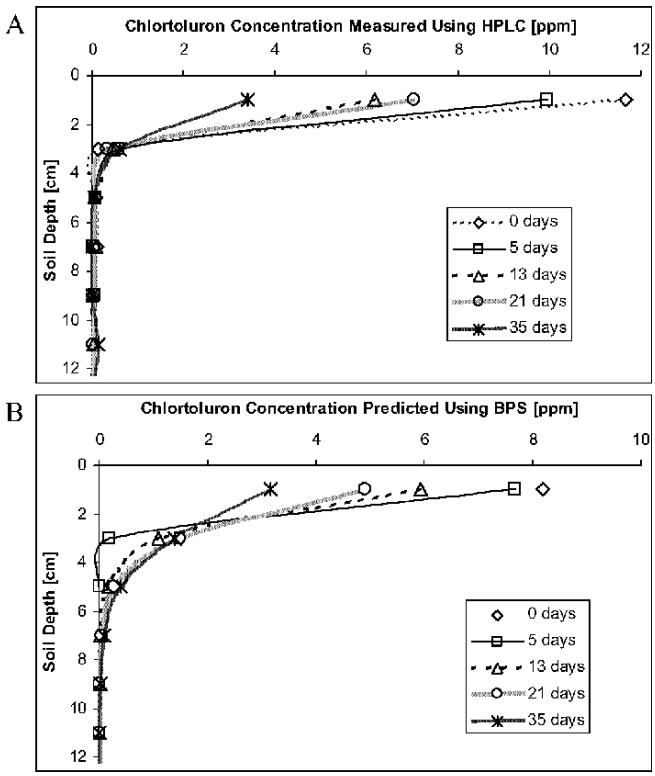

Figure 2. Observed (A) and simulated (B) chlortoluron distribution in the soil profile of a Haplic Luvisol 2.

soil types. Chlortoluron mobility in the soils increased as follows: Haplic Luvisol 1 = Haplic Luvisol 2 < Haplic Cambisol < Dystic Cambisol < Greyic Phaozem. The herbicide moved very little in both Luvisols (Haplic Luvisol 1 and Haplic Luvisol 2). Herbicide transport in the other three soil profiles appeared to be influenced by preferential flow. Preferential flow probably occurred in the Dystic Cambisol and the Haplic Cambisol due to the higher sand and gravel content of these soils and high variability of aggregate density. The Dystic Cambisol contained 5% clay, 69% silt, 26% sand, and 32% gravel, while the Haplic Cambisol content was 5% clay, 49% silt, 46% sand, and 6% gravel. Preferential flow in the Greyic Phaeozem has occurred due to volume changes caused by higher content of clay and silt (clay 21%, silt 66%, sand 13%, gravel 0%) and influence of living organisms. The saturated hydraulic conductivities of the Greyic Phaeozem showed the greatest variability ($2.8\ 10^{-3} – 9.6\ 10^{-6}$ cm/s).

It should be mentioned that some of the measured concentrations and consequently total chlortoluron content in the soil profiles observed 21 days after the herbicide application were higher than the values detected at day 0. The heterogeneity of the herbicide distribution may have been due to preferential flow and then relatively slow solute penetration into the soil matrix. Sample collection at three locations per experimental plot reduced the effect of uneven herbicide distribution on the soil surface.

Figures 1B – 5B present the simulated chlortoluron concentrations obtained using the BPS model. The simulated and actual herbicide transport results were similar for the Haplic Luvisol 1, the Haplic Luvisol 2, the Haplic Cambisol and the Dystic Cambisol, although the model slightly overestimated herbicide transport in the Luvisols and underestimated it in the Dystic Cambisol and the Haplic Cambisol. The model was not as successful in predicting herbicide movement in the Greyic Phaozem. It appears that preferential flow has influenced solution transport to a greater extent. The BPS model cannot describe the preferential flow. Special models have to be used to predict solute transport affected by preferential flow as was shown in Kodešová et al. (2005).

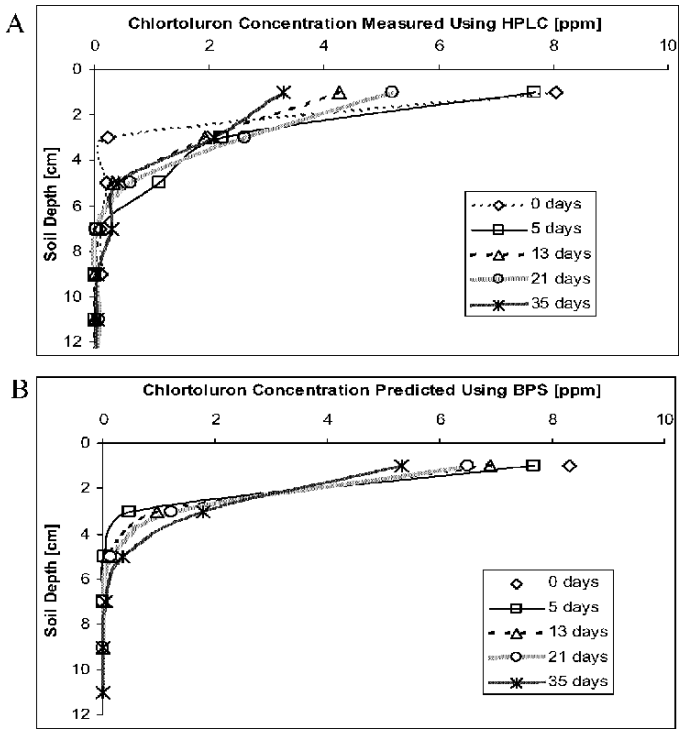

Figure 3. Observed (A) and simulated (B) chlortoluron distribution in the soil profile of a Haplic Cambisol.

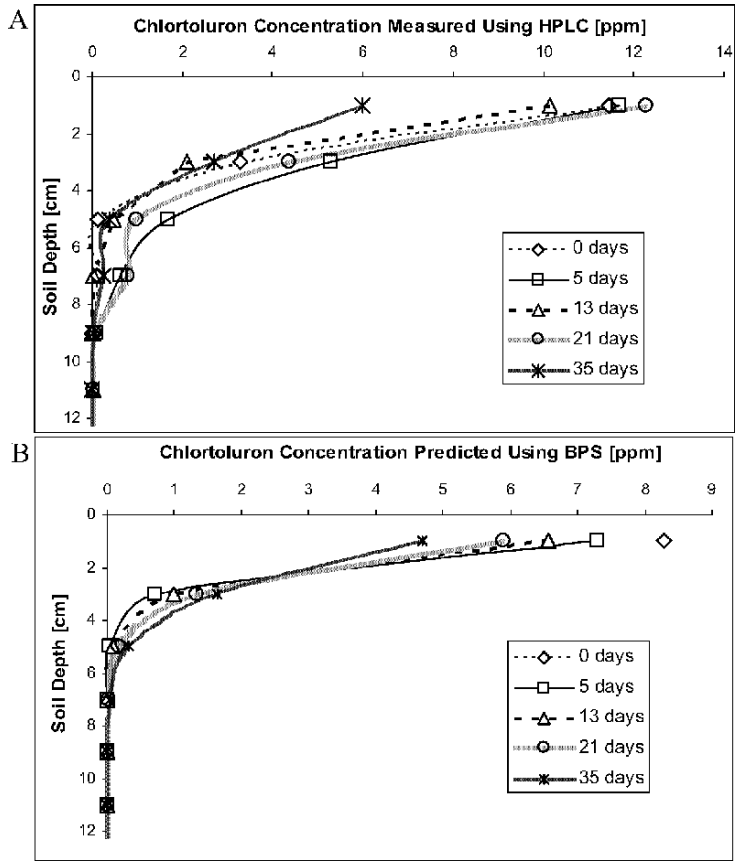

Figure 4. Observed (A) and simulated (B) chlortoluron distribution in the soil profile of a Dystic Cambisol.

4. CONCLUSIONS

The results of this study show considerable differences in chlortoluron transport between soil types. Preferential flow appeared to play an appreciable role in herbicide transport in several soils. The BPS model with the soil database was relatively accurate at estimating chlortoluron transport. However, further development of the model, including methods for estimating soil hydraulic properties, would be useful. In addition, further studies to compare modeled results to field studies should be conducted.

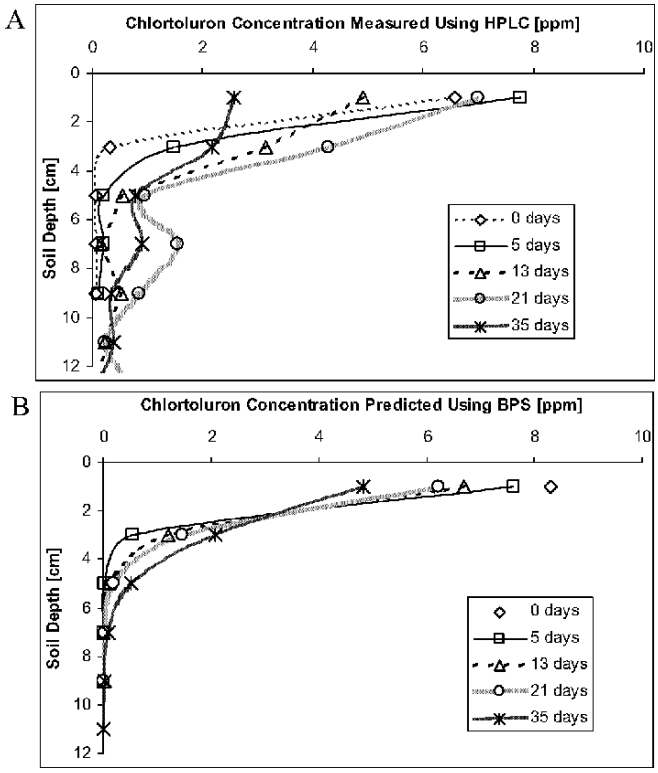

Figure 5. Observed (A) and simulated (B) chlortoluron distribution in the soil profile of a Greyic Phaozem.

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CHAPTER 25

METHODS FOR DETERMINATION OF SOIL HYDRAULIC PROPERTIES

Radka Kodešová[1] and Molly M. Gribb[2]
[1]Assistant Professor, Czech Univ. of Agriculture, Dept. of Soil Science and Geology., Kamýcká 129, 16521 Prague, Czech Republic; [2] Professor, Boise State Univ., Dept. of Civil Engineering, 1910 University Drive, Boise, Idaho 83725, USA

Abstract: The results of different procedures for the determination of soil hydraulic properties (soil-water retention and unsaturated hydraulic conductivity curves) are presented. Tests were performed for three uniform sandy soils at adjacent sites. Differences in results from the various tests are attributable to a number of factors including volume of soil tested, test conditions, dimensionality of imposed flow, method of analysis, and inherent soil variability. Each test method provided important information about the soil hydraulic properties. The advantages of in-situ transient flow experiments coupled with an inverse numerical solution method are discussed.

Key words: Cone permeameter test, Guelph permeameter test, falling head test, multistep outflow test, pressure plate test, capillary rice test, unsaturated soil hydraulic properties, inverse solution, field studies.

1. INTRODUCTION

Soil and groundwater contamination by various hazardous substances is a worldwide environmental problem. Contaminant concentrations in environmental media can be monitored. However, environmental monitoring is quite expensive and time consuming. Various simulation models have been developed for assessment of groundwater vulnerability to contamination, resource management, and design of monitoring programs. In order to apply numerical models, the hydraulic properties of the soils and solute transport parameters must be determined. The soil-water retention and hydraulic conductivity curves are basic soil hydraulic properties describing water behavior in soils. These parameters have a significant impact on contaminant

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migration in soils. The soil-water retention curve, $\theta(\psi)$, is typically defined as the relationship between the volumetric water content,θ, and matric suction head, ψ. The hydraulic conductivity curve, $K(\psi)$ or $K(\theta)$, is the relationship between hydraulic conductivity, K, and the matric suction head or volumetric water content. Information about $\theta(\psi)$ and $K(\psi)$ can be obtained directly from laboratory or field measurements, or indirectly using parameter optimization techniques.

Here we present results obtained with various laboratory and field methods for three sites composed of sandy soil and discuss the differences among the results. The methods represented in this study include pressure plate (PP) tests to measure drying $\theta(\psi)$ curves, capillary rise (CR) experiments to measure wetting $\theta(\psi)$ curves, in-situ Guelph permeameter (GP) and falling head permeability (FH) tests to measure K_s, the saturated hydraulic conductivity of the soil, and cone permeameter (CP) and multistep outflow/inflow (MSO) tests that were analyzed using parameter optimization techniques to simultaneously obtain estimates of the wetting and drying $\theta(\psi)$ and $K(\psi)$ curves. All results (except the CR data) are discussed in detail by Gribb et al. (2004). Selected results are presented here.

2. MATERIALS AND METHODS

Experiments were carried out at three sites (S1, S2 and S3) in Poinsett State Park, South Carolina, USA. This area is composed of interbedded, unconsolidated sands and clays of the Atlantic Coastal Plain. Soil profiles consisted of two layers of sandy soil. The top horizons contained little organic material and were approximately 10 cm deep. The tests in this study were conducted in the lower horizons. Upon excavation, the soils appeared to be very homogeneous with few macropores or other features that would impart significant variability to measured soil properties.

The series of testing and sampling events at each site was as follows. First, one Guelph permeameter test (Soil Moisture Equipment Corp., Santa Barbara, CA) was conducted at each corner of a 1.0-m square area. Later, soil anchors would be installed in these holes to secure the reaction frame used to insert the cone permeameter to testing depths. Following the Guelph permeameter tests, a 2.5-cm I.D. soil core sampler was inserted on each side of the square test area halfway between the Guelph permeameter holes. Soil specimens of known volume were removed from the barrel of the sampler, and later the volumetric water content of the specimens was determined in the laboratory. The cone permeameter was then inserted in the core sampler hole. Following cone permeameter tests (Kodešová et al., 1999), undisturbed soil specimens were taken at equivalent depths, adjacent to the cone

permeameter tensiometer rings. These specimens were subjected to falling head (Klute and Dirksen 1986), pressure plate (ASTM D 2325), and multistep outflow/inflow tests (van Dam et al., 1994) in the laboratory. Disturbed samples were taken for particle size analysis and the construction of capillary rise experiments (Lambe 1951).

The quasi-steady state inflow rates obtained from the Guelph permeameter tests were inputs to a semi-analytical solution to find K_s values. Darcy's equation was used to determine K_s values from the falling head tests. To describe $\theta(\psi)$ and $K(\psi)$ curves the analytical van Genuchten (1980) functions were employed in this work, where the statistical pore-size distribution model of Mualem (1976) was used to obtain the expression for $K(\psi)$. Unknown parameters of the analytical functions are found by fitting data directly using RETC (van Genuchten et al., 1991) (for the capillary rise and pressure plate tests), or via parameter estimation using HYDRUS-1D (Šimůnek et al., 1998a) (for the multistep outflow/inflow tests), or HYDRUS-2D (Šimůnek et al., 1999) (for the cone permeameter tests). Numerical inversion of cone permeameter transient flow data is described in detail by Kodešová et al. (1999).

3. RESULTS AND DISCUSSION

The normalized, or effective soil-water retention curves, $\theta_e(\psi)$, obtained from PP, CR, CP and MSO inflow and outflow tests for Site 1 (S1) are shown in Figure 1 (results for Sites 2 (S2) and 3 (S3) are not shown). As expected, the main drying curves obtained from the PP and MSO tests consistently formed the upper bounds for soil-water retention at all sites. The CR curves generally set the lower bounds for water retention at each site, as expected, with some deviations. At S2 and S3 some of the MSO scanning wetting curves are partially or completely lower than the CR curves. Finally, most of the scanning wetting MSO (with the exception of those at S2) and the wetting and drying CP curves, lie within the bounds of the main wetting and drying curves, with the exception of the CP curves at S2 which drop below the other curves between 3 and 6 kPa. The shape of the MSO, PP, CR, and CP curves were most similar to each other at S1. At S2, the MSO, PP, and CR curves were similar to each other, while the CP curves were steeper in slope. Finally, at S3, the CR and CP curves were similar in shape, and steeper than the MSO and PP curves, which were similar in shape as for S1 and S2.

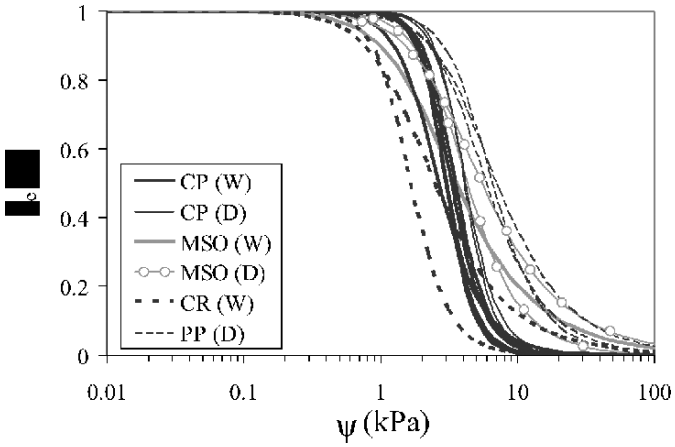

Figure 1. Soil-water Retention Curves for S1 (W = wetting, D = drying).

Comparing the general shape of the various soil-water retention curves, there is a noticeable difference in slope between the CP curves and the laboratory-derived MSO, CR and PP curves. This difference may be attributed to the relatively rapid flows that occurred during the CP experiments. The relatively sharp wetting front moving into the soil resulted in rapid changes in matric suction at the tensiometer rings. As a result, information about soil-water retention over the range of measured matric suction heads during the experiments (between the initial conditions before the wetting front reached the tensiometer ring locations, and after the wetting front passed the tensiometer rings) was limited, and therefore, the influence of this information on the inverse solution was also limited. The MSO, CR and PP soil-water retention curves were all obtained from experiments in which the flow processes occurred over a longer period, resulting in the more gradual shapes of these curves. In addition, more detailed information about the soil-water conditions was available from these experiments as compared to the CP tests since steady-state, or near steady-state conditions were achieved for intermediate points along the retention curve for these test methods. The PP and drying MSO tests also involved wider ranges of matric suctions than the drying portion of the CP tests, which may account for the greater hysteresis observed between the MSO wetting and drying curves than for the CP wetting and drying curves (Figure 2). Šimůnek et al. (1998b) also noted these general differences between curves obtained from parameter estimation of in-situ tension disk infiltration data, and curves obtained from pressure plate and multistep outflow experiments. In addition to the possible °reasons for this behavior listed above, Šimůnek et al. (1998b) suggested

that the numerous assumptions and simplifications used to analyze flow data can cause the simulated system to differ from the actual system. These assumptions include those inherent in Richards' equation, the ability of the assumed hydraulic functions to accurately represent the soil behavior, as well as the assumptions of homogeneity, isotropy, and uniform initial conditions of the field site.

The relative hydraulic conductivity curves, $K_r(\psi)=K(\psi)/K_s$ are not shown. In all cases the $K_r(\psi)$ curves reflected the shape of the $\theta_e(\psi)$ curves obtained with each method due to the coupled nature of equations describing soil hydraulic properties. In general, similar conclusions can be made about the behavior of these $K_r(\psi)$ curves as were made for the $\theta_e(\psi)$ curves above.

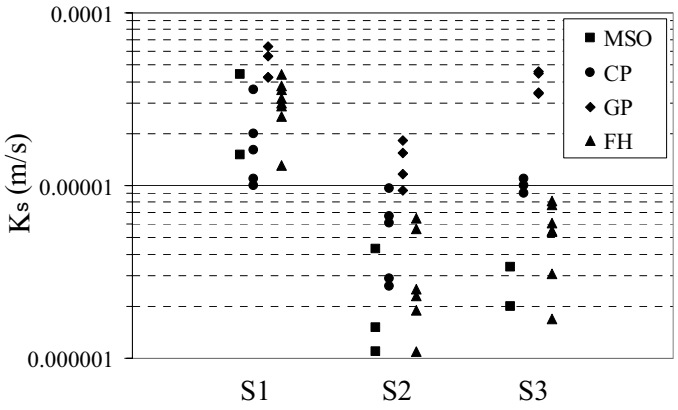

Figure 2. K_s Values at S1, S2, S3 Obtained Using Different Methods (Gribb et al., 2004).

The saturated hydraulic conductivity (K_s) values for the three sites obtained with Guelph permeameter (K_{GP}), falling head (K_{FH}), cone permeameter (K_{CP}), and multistep outflow/inflow (K_{MSO}) tests are presented in Figure 2. K_{GP} values were generally higher than K_{CP}, K_{FH}, and K_{MSO} values at all three sites. K_{CP} values were similar to K_{FH} and K_{MSO} values at S1, higher or equal to K_{FH} and K_{MSO} values at S2, and higher than K_{FH} and K_{MSO} values at S3. K_{MSO} values fell within the range of measured K_{FH} values at all three sites. Two reasons may account for the observation that K_{GP} values were greater than K_{CP} values at the study sites. Due to higher applied pressure heads (CP tests) more air become entrapped and therefore lower the permeability of the soil was obtained. The greater volume of soil impacted and possible inclusion of more permeable zones (GP tests) may also influence the K_s values. The various assumptions and methods of analysis

associated with the different test methods may also be responsible for the observed differences in values. Hydraulic conductivity values measured in the laboratory on small specimens might be lower than in-situ values due to accidental disturbance of macropores, smaller volumes, and preferential sampling that avoids roots, rocks and wormholes. In addition, the GP and CP tests yield a K_s value based on radial and vertical flow. The vertical hydraulic conductivity is often less than the horizontal hydraulic conductivity in naturally occurring soils.

4. CONCLUSIONS

The results of this study show the variability in saturated hydraulic conductivity values and soil-water retention and hydraulic conductivity curves that can result when using different test methods. Some of this variability is due to factors such as variable soil properties, specimen size and orientation, and hysteresis in the soil hydraulic properties. Other sources of variability include inherent differences in the test methods such as the dimensionality of the flow regime imparted, the rate at which water is imbibed or drained from the specimen, the range of water contents over which the test is conducted, operator error, and the method of analysis. Based on this limited set of tests performed on sandy soils, variability inherent in the soil porous system and that due to hysteresis appear to be more significant than variability due to use of the different test methods. However, this conclusion is only valid for the tests' results presented here. Comparison of results of similar tests for structural soils or soils with a higher degree of variability may show different trends, and variability due to the differences in the test methods may be more important in such soils. In general, in-situ tests are recommended. As in the case of the cone permeameter test, measurements are performed directly in the field without major disturbance of the soil profile. Initial and boundary conditions are properly defined. Experimental data are obtained during a relatively short time period. Both soil hydraulic properties (the soil-water retention and unsaturated hydraulic conductivity curves) are determined simultaneously from one experiment. The resulting soil hydraulic properties define larger soil specimens and the method may also be applicable at greater depths than other methods. However, methods for determination of soil hydraulic properties must be selected with the desired end use in mind. For example, the dimensionality, scale, type of flow, and source of water or contaminants must be taken into account in the case of numerical modeling. The variability in the measured or estimated soil hydraulic properties must be

considered when evaluating the results of numerical simulations obtained with inputs of these properties.

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CHAPTER 26

URBAN SOILS: A PART OF MAN'S ENVIRONMENT

Vít Penížek and Marcela Rohošková
Czech University of Agriculture, Dept. of Soil Science and Geology., Kamýcká 129, 16521 Prague, Czech Republic

Abstract: Soils in urban areas are an important part of man's environment. The nature and extent of contamination in soil influences both quality of life and the health of people living in these areas. For this reason, knowledge about soils in urban ecosystems is essential for environmental-impact assessments. This paper explains the definition of "urban soils" and presents a short overview of approaches to classification of these soils. The functions and typical anthropogenic parameters that influence soil contamination in different urban areas including traffic, industry or urban greenery are also described. The paper will also review some of the ways that altered soil can impact human health. Finally, methods that could be used to apply this information in urban planning and development to make urban soils a part of a healthy environment are discussed.

Key words: urban soils, anthropogenic soils, soil classification

1. INTRODUCTION

Soils are a part of the environment that influences both quality of life and the health of people living in these areas. The definition of "urban soils" covers a group of soils that may be affected by a wide range of anthropogenic impacts. The composition and concentration of anthropogenic contaminants vary greatly by time and space. Contamination of urban soils can impact both the structure and fertility of soil. Soils that are influenced by human activity are generally called "anthropogenic soils". One subgroup of these soils is "urban soils". The name "urban soils" is used for strongly impacted or even changed (man-made) soils in urban areas. This widely used definition is not exact, because soils in urban areas include a wide range of

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soils. This includes soils that may be slightly affected such as arable land or forest land, soils that have been strongly impacted such as horticulture soils or allotments, and finally, areas where soils are totally changed or created by man.

2. URBAN SOILS

A range of man's activities, including industrial and agricultural activities, can permanently alter the structure and chemical composition of the surface horizon of soil. Some activities may result in more significant changes in the soils (Table 1). These changes take place in different parts of the soil profile and the intensity of these changes differs throughout the soil profile as well (Figure 1). The type and intensity of these changes are defined by the nature of the human activities. Important factors that influence changes in soil characteristics include the type, intensity and time when these processes take place.

The type of activity and intensity depend on the use of the land and may change over time. The original use of a soil as a medium for food production has, to a large extent, been replaced by many other uses in urban areas. The use of soils in these areas is more versatile and changes with the demands of society (Stadtboden Arbeitkreis, 2002). The final structure and chemical characteristics of soil are a result of past activities. As an urban area grows and the needs of the adjacent population changes, soil may first be used for agriculture, and then converted to a city park and then used as a site for a factory. Each of these activities alters the structure and chemical characteristics of the soil. In addition, each of these changes alters the potential for soil contaminants to impact human health.

Table 1. Characteristics of anthropogenic soils.

• changed physical properties (e.g., compaction, structure, low aeration)
• changed chemical properties (e.g., usually elevated pH, contamination)
• changed biological properties (alterations to the size and diversity of microorganism populations)
• high heterogeneity (horizontal and vertical)
• changed condition (higher temperature, lowered groundwater, air deposition…)
• man-made and re-deposited natural materials
• changed or newly created soil profile (layers instead of diagnostic horizons)
• young age

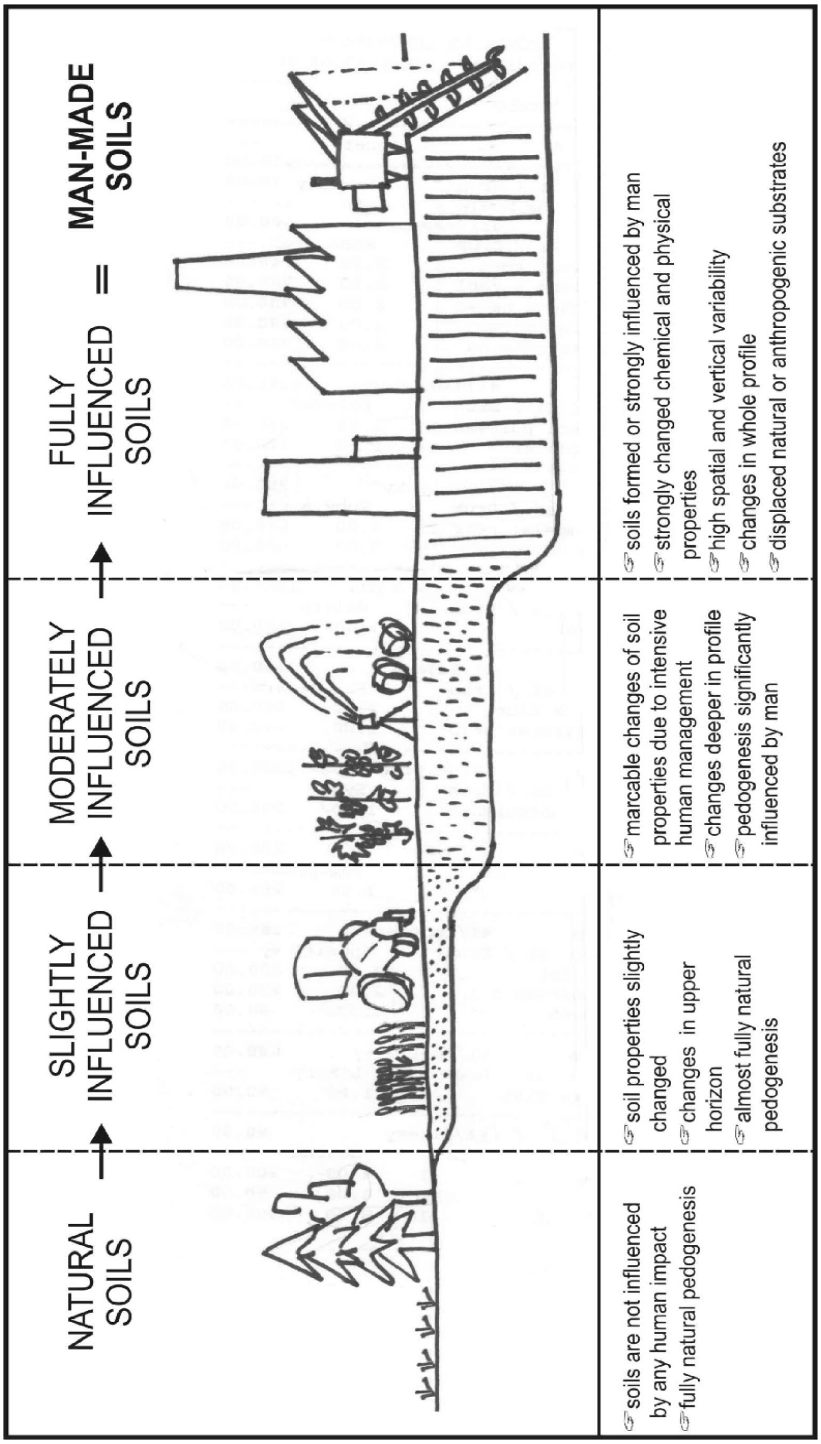

Figure 1. Levels of anthropogenic effects on soils.

Compaction may reduce particle size and increase dust dispersion, while contamination from solvents or leaking underground storage tanks may impact subsurface gas or groundwater. Chemical and physical alteration of the soil may produce a material with very different characteristics in comparison to native soils. These alterations result from the wide range of uses that soils have in the urban environment. Some of the typical uses for urban soils are listed in Table 2.

Table 2. Different uses of soils in urban areas.

- greenery (alleys, parks, woods, etc.)
- construction of buildings and roadways
- play and sport grounds
- recreation areas
- gardens, vegetable allotments, etc.
- cemeteries
- archeological places
- manufacturing and petrochemical industries

3. CLASSIFICATION OF SOILS IN URBAN AREAS

Soils are classified based on characteristic features and properties that are a result of pedogenesis. Soils of urban areas that are only slightly influenced by human activities, as agriculture or forest land in the suburb, can be classified as a natural soil and the anthropogenic influence can be considered at a lower level. In urban areas, it is important to consider properties of soils which are heavily changed by man or newly created. If a soil has been significantly impacted by industrial or other man-made activities, it may be difficult to classify using standard methods. These soils are generally described as man-made soils.

There are two general categories of man-made soils. These include materials that are newly created from synthetic materials and materials that consist of thick layers of fill that have been placed over the top of native soil. Man-made soils that were newly created by displacement of native materials can be further subdivided into two types: 1) soils formed from materials that were derived from the original soil profile. These materials retain some of the properties developed during pedogenesis, while some properties may also be changed by human activities (e.g., disturbance, transporting, storing, deposition); and, 2) soils created from natural materials that were not a part of soil profile and are newly exposed to pedogenic process (e.g., spoil, dredging). These newly created soils lack the structure and chemical

properties of native soil. Because these materials do not have the same properties as the original soil, they should not be regarded as native soils. Approaches to classification of these soils vary significantly. The World Reference Base for Soil Resources (WRB) (FAO, 1998) and Soil Taxonomy (USDA-NRCS, 1999) classify these soils as natural and the anthropogenic influence is taken in consideration on lower taxonomic units. In other classifications, the anthropogenic influence is considered on high classification levels (Czech, Slovak or Australian classification systems).

The second category includes soils that were created from non-natural (anthropogenic) materials such as household waste, garbage, ashes, building rubble, tar, etc., or from mixtures where these materials are a significant component. Very intensive human influences such as building activities, waste disposal and atmospheric deposition create very specific soil characteristics. Because these soils are usually very young, their classification is based on the features of the substrate from which these soils developed. Classification of these soils in WRB and Soil Taxonomy, as internationally accepted soil classifications, is limited. WRB includes these soils to natural soil classes at the highest classification level (Regosols, Leptosols and Arenosols). The anthropogenic influence is considered on lower units. Recently, it has been suggested that WRB does not properly accommodate soils of urban and industrial areas. Therefore, there are proposals to modify WRB and Soil Taxonomy to provide new classification of this very specific group of soils. Overview of classification of man-made urban soils in selected national and international classification systems is provided in Table 3.

4. INTERACTION SOIL-MAN

As man influences the soil, so, too, does soil influence man. Soil is able to buffer many of the detrimental effects due to man's activities, but the buffering capacity is limited. The soil and its properties are usually degraded in urban areas. This degradation of physical and chemical properties may lead to a decrease in the buffering and filtering capacity of soil. The physical and chemical properties of soil are often impaired in urban areas. If these impairments increase the potential for human contact with environmental chemicals, the impairments may, thus, adversely affect human health. For example, if man's activities increase the permeability of soil, contaminants may more rapidly migrate to groundwater where they may be ingested by humans. A variety of activities can impair the native properties of soils. Fossil fuel combustion and manufacturing activities may increase the input of metals and hydrocarbons to the soil. Construction activities may compact

Table 3. Classification of man-made urban soils.

Classification systems:	**Newly created soils from natural displaced materials:**		**Soils created of non-natural materials as household waste, garbage, ashes, building rubble, tar etc.**	*References:*
	1) that were a part of soil profile in the past	**2) that were not a part of soil profile as (mine spoil, dredging etc.)**		
WRB	Regosols (Leptosols, Arenosols) Anthropic (in Regosols only)	Regosols (Leptosols, Arenosols) Anthropic (in Regosols only)	Regosols (Leptosols, Arenosols) Anthropic (in Regosols only)	*FAO, 1998*
Soil Taxonomy	Entisols Orth-	Entisols Orth-	Entisols Orth-	*Sencindiver and Ammons, 2000*
Australian	Anthroposols	Anthroposols Dredgic, Spolic, Scalpic	Anthroposols Garbic, Urbic	*Isbell, 2002*
Czech	Anthrosols Anthro(po)zems	Anthrosols Anthro(po)zems	Anthrosols Anthro(po)zems Urbic, Contaminated, Reductic	*Nemecek et al., 2001*
German	classification based on substrates	classification based on substrates	classification based on substrates (Lithosol, Reductosol, Structosol, Nekrosol)	*Burghardt, 2000*
Russian	Technogenic surface formation Quazizems Replantozems, Urbiquazizems	Technogenic surface formation Naturfabricats Abraliths, Lihtostrats, Organostrats	Technogenic surface formation Artifabricats Artiindustrats, Artiurbistrats, Artifimostrats	*Tonkonogov et al., 2001*
Slovak	Anthrozems Recultivated, Modal	Anthrozems Initial, Recultivated	Anthrozems Modal, Initial Urbic, Dumpic	*SSCRI, 2000*
UK	Man-made Soils	Man-made Soils	Man-made Soils, Terrestrial Raw Soils	*Dudal et al., 2002*

soil and alter its structure. The severity of these impairments depends on the magnitude and duration of the specific activity.

Soil that has been impacted by man's activities may have both direct and indirect affects on human health. Direct effects occur when contaminants in soil are ingested or dermally absorbed. Indirect effects include translocation of contaminants into plants and subsequent consumption by humans. This problem is widespread in middle Europe with a long term tradition of these allotments in Germany, Czech Republic or Slovakia. Another example of indirect influence is changing the microclimate of the urban areas. Soils with degraded physical properties are unable to function as a tool for reducing temperature or increasing air moisture during the summer months or the soils are not able to provide enough water for vegetation. Impairment of soil properties causes an indirect effect on human health when soil is compacted or sealed, resulting in decreased infiltration and increased potential for flooding.

5. CONCLUSIONS

Soils in urban areas are an important part of the environment. In native soils, the sand, silt and clay content influences permeability, wind dispersion, and the ability to support plant growth. Urban soils are represented by a wide range of materials with different levels of anthropogenic influence. These impacts cause various changes in soil properties as well as the spatial heterogeneity of soil. Impacts that alter soil structure and texture can affect groundwater recharge and protection. Man's activities may also alter the biochemical processes of soil including buffering, filtering as well as transformation or storage of contaminants and nutrients. Impacts that reduce soil organic content or alter the microbial population may affect the rate of decomposition of organic contaminants.

The accurate classification of soil after it has been impacted by man's activities is an important aspect of understanding the potential for soil to impact human health. Soils that have been impacted by man's activities may be difficult to classify due to the presence of non-native materials and high spatial variability of both the surface, and often lower horizons of soil. Following alteration due to man's activities, soil pedogenic features may be difficult or impossible to classify. At present, a uniform system for classify-cation of impacted soils does not exist. However, increased interest in a system for classifying urban soils has been developing in recent years at national and international levels.

Human health risk assessments, as well as environmental impact assessments, are often driven by the type and extent of soil contamination.

Alteration of the physical characteristics of urban soils may also affect risk or impact assessments. Accurate information to describe the vertical and lateral extent of contamination is essential for an accurate estimate of risk. Knowledge of the features and genesis of urban soil, and the role of soil in the urban ecosystem, are also important factors. With accurate characterization and appropriate remediation, soil can be a component of a healthy environment.

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CHAPTER 27

CHILDREN, HEALTH AND THEIR ENVIRONMENT

Kirby C. Donnelly
School of Rural Public Health, Texas A&M University System Health Science Center, College Station, TX, USA

Abstract: A number of studies have confirmed that children are sensitive receptors with regards to exposure to hazardous chemicals in the environment. The vulnerability of a child is affected by a number of factors ranging from differences in pharmacokinetics that increase retention to behavioral differences that may increase the potential for exposure. This manuscript reports on several parallel studies designed to gain a better understanding of genetic and environmental factors affecting sensitivity in children. Data are being collected to identify chemical contaminants in the environment, and to measure contaminant concentrations in biological samples. The major contaminants in a child's environment will be determined through the measurement of organic and inorganic constituents, as appropriate, in house dust and indoor air. Biomarkers of exposure are being measured in blood and urine; and, DNA adducts quantified in serum and placenta. Study populations include children living in a rural community in south Texas, children born with congenital abnormalities in Shanxi Province, China, and populations in Azerbaijan, living in an industrialized and a rural area. Preliminary results indicate that contaminant concentrations in environmental media may not serve as an accurate predictor of exposures. Biomarker studies exhibit dramatic differences in exposure in each of the populations. In addition, genetic studies suggest that certain individuals within a population may be predisposed to disease due to a limited capability to metabolize and eliminate hazardous chemicals. Future studies will expand the size of the populations monitored, and provide longitudinal data on a subset of individuals within the populations.

Key words: children, health, pesticides, environment

1. INTRODUCTION

The smile of a small child is infectious. Regardless of one's mood or disposition, when one encounters one of these young individuals, grinning

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from ear to ear, the most common response is to return the smile to the sender. Thus, it is obvious that a foremost concern for parents should be to ensure that their children are raised in a healthy environment. Certainly, physical injuries are the major cause of adverse effects in young children, as well as in young adults. However, it is also apparent that the environment may affect the health of children. Many areas exist in the United States as well as in Central and Eastern Europe where children have the opportunity to be in frequent contact with contaminated media. Yet, limited information exists to determine the impact of exposure to hazardous environmental chemicals on the health of children.

Children are not small adults. The transformation of a neonate into an adult is a remarkable process. As stated by Dr. Ken Olden (former Director of the National Institute of Environmental Health Sciences) "A little kid goes from a single cell to a laughing, sociable, intelligent, friendly human being over the course of two years; that's dramatic growth and development". It is clear that a child is more sensitive to chemical exposures for several reasons. A 10 kg child receiving a comparable exposure as a 100 kg adult would receive a 10-fold higher dose per unit body weight. In addition, many systems are still developing during early years in a child. Metabolic and immune systems develop extensively during the first year of life (Blake et al., 2005; Holsapple et al., 2004; Makri et al., 2004). The changes to the gastrointestinal system that occur as children grow older influence uptake of several different classes of chemicals (Sreedharan and Mehta, 2004). The diet of a small child includes a higher percentage of fruits and vegetables than adults, which may be another source of exposure. Perhaps most importantly, the exposure of children to environmental chemicals is altered by behaviors as they grow older. As children get older they begin to walk, which gives them access to many exposures they did not have as a newborn. In addition, small children are more likely to put their hands, toys, and other non-food objects in their mouth when they are small. This hand-to-mouth behavior may put a child at increased risk of exposure to environmental chemicals.

The health of a child is a delicate balance between many factors. It is important that children eat a balanced diet to maintain good health; in addition, certain genetic characteristics may predispose a child to disease, or render them more sensitive to environmental exposures. The timing of a chemical exposure may also influence the type of adverse health effect that is observed. For example, lead exposure in children is associated with learning difficulties and growth retardation, while in adults lead exposures are most often associated with hypertension (Bellinger, 2004; Dietert et al., 2004; Gidlow, 2004).

Children are affected by environmental exposures. The World Health Organization (2004) estimates that more than 5,000,000 children die each

year due to environmentally related diseases. While much of this disease may be associated with infectious agents, the extent of chemical-specific disease is unknown. In the United States, childhood brain and nervous cancers were found to increase by 53% from 1973-1995, the incidence of autism was found to double between 1966 and 1997, and asthma in children increased by more than 72%. The contribution of environmental chemicals to these diseases is unknown. Some of the major environmental threats to children's health include lead, pesticides, air pollution, and environmental tobacco smoke. This manuscript describes on-going studies in the United States as well as in Eastern Europe and Asia to investigate sources of exposure in children. While much of the data in this manuscript are preliminary, a great deal has been learned regarding appropriate sampling methodologies and potential sources of exposure.

2. METHODS

Study Populations. Three cohorts have been enrolled to monitor environmental exposures. For a childhood exposure to pesticides study, children between the ages of 6 and 48 months were recruited in several *colonias*, or unconsolidated communities, in the Texas-Mexico border region. A total of 70 children have been recruited from three different communities for this study. A second study is being conducted in Azerbaijan to investigate environmental exposures in rural and urban populations. A total of 60 families have been enrolled in both areas. Each family includes at least one member over 50 years of age, two family members between 20 and 50 years of age, and two family members that are less than 20 years old. Finally, a study in Shanxi Province, China is investigating environmental factors influencing the incidence of birth defects. In this study, case subjects include children who are born with a neural tube defect in any of 14 different hospitals in Shanxi Province; controls are recruited from subjects born with no visible birth defect who were conceived at approximately the same time as the case subjects. To date, 30 case subjects and 10 controls have been enrolled for the study.

Environmental Exposures. Potential sources of environmental exposures differ for each of these populations. Many of the children in the Texas-Mexico study live in housing that puts them in frequent contact with the surrounding environment. As many of these houses are in close proximity to agricultural fields, it was assumed that agricultural pesticides would represent a major source of exposure. Analysis of soil and house dust has detected the presence of organophosphate and organochlorine (OCs) pesticides, although the concentration of organophosphates was generally an

order of magnitude greater than the OCs in the house dust samples (Carrillo-Zuniga et al., 2004). For the children living in Azerbaijan, families in the urban population live adjacent to a large synthetic rubber plant. Several large chemical industries and a wastewater treatment plant are also located near the community. Analysis of environmental samples, including soil, surface water and sediment, indicate that the major contaminants in the environment include organochlorines (i.e., PCBs, DDT, hexachlorobenzene), polycyclic aromatic hydrocarbons, and mercury. In Shanxi Province, China, coal is used both for cooking and for heat. Previous studies have identified PAHs as a major contaminant in the air in many of these houses (Agiu et al., 2004).

Environmental Monitoring. Contaminant levels in the environment were measured in samples of soil, surface water and sediment, as well as inside houses using samples of floor dust, window dust and hand rinses. Soil, sediment and dust samples were extracted with hexane:acetone using an Accelerated Solvent Extractor (ASE) (Dionex, Sunnyvale, CA). Aqueous samples were extracted using a C-18 Column (Waters Corporation, Milford Massachusetts) and acetone.

Biological Monitoring. The selection of biomarkers for each population was based on prior data quantifying environmental contaminants. In the Texas-Mexico study, water soluble organophosphate compounds were detected in urine. However, for the studies in China and Azerbaijan, the more lipid soluble PAHs are more readily detected in veinous blood; although measurement of 1-hydroxypyrene in urine has also been used to measure PAH exposures. For the population in Azerbaijan where metal exposure was a concern, samples of hair were also collected. For the birth defect study in China, serum was collected from parents and placenta from case and control subjects to measure PAHs and DNA adducts.

Human Subjects. Study protocols were reviewed by the Texas A&M University Institutional Review Board (IRB) for each of these research projects. Each participant over the age of 18 years was administered an Informed Consent Form prior to initiation of the study. A parent or guardian was asked to complete the Informed Consent Form for each child under the age of 18 years. In addition, results from each program are presented to the participants at a community meeting, or through a written report.

Postlabeling. Bulky DNA adducts were analyzed by nuclease P1-enhanced ^{32}P-postlabelling as described by Reddy and Randerath (1986). DNA (10 μg) was digested to normal (Np) and modified (Xp, where X = adduct or I-compound moiety) deoxyribonucleoside 3'-monophosphates by micrococcol endonuclease and spleen phosphodiesterase. The modified nucleosides (Xp) were enriched by treatment of the digest with nuclease P1, which degrades the normal nucleotides (Np) to deoxyribonucleosides (N)

and inorganic phosphate (P_I), but leaves the modified nucleotides intact. Only modified nucleotides (Xp), but not N, are substrates for the subsequent labeling, leading to their conversion to 5'-^{32}P-labeled deoxyribonucleoside 3',5'-bisphosphates (*pXp, where * = ^{32}P-label) in the presence of [γ-^{32}P]ATP and polynucleotide kinase. The labeled products (*pXp) were separated by multidirectional TLC and quantified as described previously (Randerath et al., 1967; 1989). [γ-^{32}P]ATP employed in the labeling reaction can be prepared from carrier-free $^{32}P_I$ and ADP. For the determination of relative adduct labeling (RAL) values, the specific activity of the ATP preparation was determined by ^{32}P-labeling of a known amount of deoxyadenosine 3'-monophsphate as described in Reddy and Randerath (1986).

3. RESULTS

A broad range of chemicals have been identified in environmental samples from the Texas-Mexico border region (Garcia et al., 2000; Carrillo-Zuniga et al., 2004). The most common chemicals detected in environmental media were organochlorine pesticides and plasticizers (phthalates and bis-phenol)(Garcia et al., 2002). Although the organophosphate (OP) insecticides were detected less frequently in outdoor soil, the OPs were detected at much higher (generally at least 10X >) concentrations than the organochlorines in house dust. None of the pesticides detected in house dust are considered appropriate for home use. In addition, the variability of data between homes suggests the presence of multiple sources of pesticide exposure. Data from monitoring exposure in children ages 6 to 48 months suggests that the paraoxonase gene (PON-1) may have a significant influence on pesticide elimination. For three children with the fast polymorphism in PON, hand rinse concentrations ranging from 1.0–15 $\mu g/cm^2$ resulted in urinary pesticide levels ranging from 0.22 to 8.6 ppb. Whereas for children with the slow form of PON, hand rinse concentrations ranging from 15 to 98 $\mu g/cm^2$ resulted in urinary levels ranging from .01 to 0.14 ppb. Additional studies are on-going to expand the study population and investigate the impact of other OP metabolizing enzymes on pesticide excretion.

The incidence of neural tube defects in rural high risk areas of Shanxi Province, China is 14/1,000; while, in low risk areas the incidence is only 0.5/1,000 (Agio, 2005). The major contaminant detected in house dust in the high risk area was polycyclic aromatic hydrocarbons (PAHs). Total PAH concentrations in house dust ranged from 50 to 823 mg/m^2, while the concentration of carcinogenic PAHs in house dust ranged from 12 to 107 ng/m^2. The Institute for Reproductive and Children's Health at the Beijing

Health Science Center (Agio, 2005) determined that concentrations of benzo(a)-pyrene in indoor air were up to 10 times higher in the winter than the summer months. Research by Agio and researchers at the Peiking Institute for Reproductive and Children's Health also indicate that the incidence of birth defects is higher in children that were conceived during the winter months when compared with children conceived in the summer.

Studies in Azerbaijan have determined that elevated PAH concentrations exist in houses in both the rural and urban areas. The source of the PAHs in each of these regions is likely to be very different. However, it is also apparent that families living in houses with elevated concentrations of PAHs in house dust also have elevated concentrations of PAHs in serum. The PAH concentration in serum in the urban population ranged from 27 to 947 ppb, while in the rural population, PAHs in serum ranged from 8 to 172 ppb.

Serum PAHs appear to result in an increased level of DNA adduct in lymphocytes. The results presented in Figure 1 indicate that significantly lower level of total DNA adducts were detected in serum samples collected from residents of the rural area in comparison to the urban population. Note that in men from the rural area this difference was not significant (data not shown). Preliminary information from questionnaires suggest tobacco smoking may contribute to the total DNA adducts detected in serum samples

Table 1. Serum concentrations of polycyclic aromatic hydrocarbons in adults in rural and urban areas of Azerbaijan (concentrations in ppb) from men living in the rural area.

	Urban Population		Rural Population	
	Men	Women	Men	Women
Minimum Value	31	27	21	8
Median Value	148	118	67	92
Maximum Value	947	402	113	172
Standard Deviation	371	133	39	58
Subjects	N = 6	N = 9	N = 4	N = 6

4. DISCUSSION & CONCLUSIONS

There is no question that children represent a sensitive subset of the human population. Due to behavioral and pharmacokinetic differences, children may have higher levels of exposure and retention of environmental contaminants. In addition, children, as organisms in a rapid stage of growth and development, may be more susceptible to the adverse health effects of environmental exposures. Studies in this and other laboratories have demonstrated that childhood exposures may be elevated in rural communities.

Children in the Texas-Mexico border region were observed to be exposed to elevated pesticide levels. Although the source was not clearly identified, data suggest that both pesticide drift and household misuse of pesticides may be a factor. In Azerbaijan, children were observed "mining" a landfill with their family to supplement income. Data from two communities in Azerbaijan observed a relationship between PAHs in house

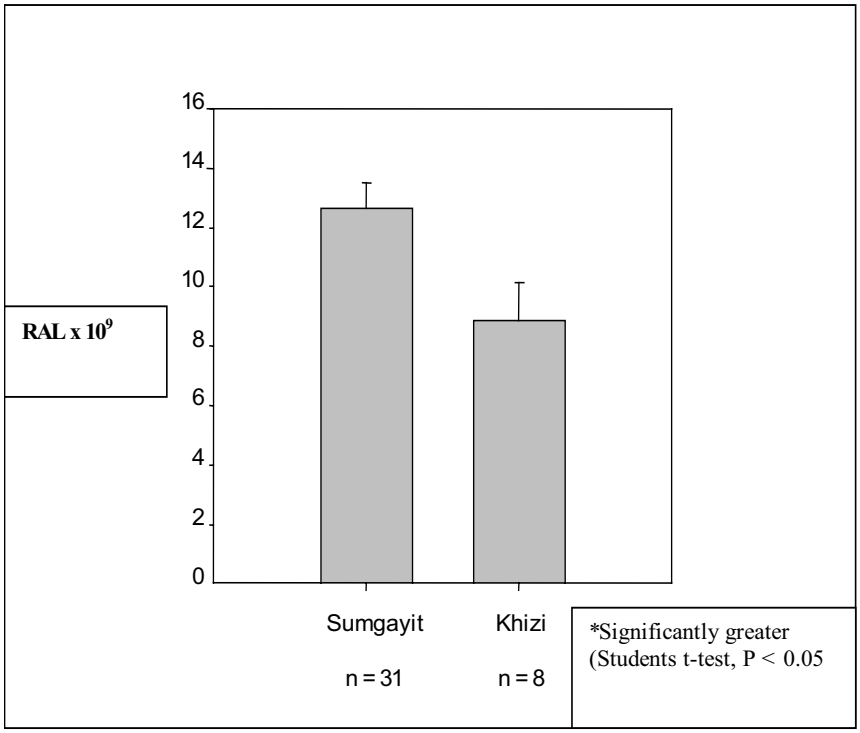

Figure 1. Mean total DNA adducts detected in lymphocytes from residents living in urban (Sumgayit) and rural (Khizi) areas of Azerbaijan.

dust and DNA adducts in serum. In China, elevated concentrations of PAHs have been detected in the kitchens and bedrooms of households in regions where the incidence of birth defects is elevated. Living conditions in these households suggest that the children in these families may be in close contact with the surrounding environment.

The health status of a young child is influenced by genetic, nutritional and environmental factors. Lifestyle factors, including exposure to second hand smoke and alcohol, may also impact children's health. Recent studies suggest that genetic polymorphisms affect pesticide retention, and thus the potential for adverse health effects. Behavioral changes such as proper use of pesticides, venting heaters and stoves, and even more frequent washing of

hands may significantly reduce a child's exposure to environmental chemicals. Working with families in these communities to ensure they are aware of these factors is an important first step in improving community health. Investments in the health of children today are likely to lead to significant dividends in reduced morality in the adults of the future.

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CHAPTER 28

NEW KNOWLEDGE ABOUT THE IMPACT OF ENVIRONMENTAL EXPOSURE TO PAHs

Radim J. Šrám, Blanka Binkova, Jan Dejmek, Irena Chvatalova, Alena Milcova, Ivo Solansky, Zdena Lnenickova and Jan Topinka
Institute of Experimental Medicine AS CR & Health Institute of Central Bohemia, Prague, Czech Republic

Abstract: Organic compounds adsorbed to air particles (PM_{10}) induced DNA adducts and embryotoxicity in *in vitro* and *in vivo* studies. Carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) induced 45-50% of all DNA adducts caused by organic matter bound to PM_{10}. Placental bulky DNA adducts were affected by air pollution, smoking, genotypes and vitamin C. Higher DNA adduct levels were observed in nonsmoking mothers delivering children with intrauterine growth retardation (IUGR). An increased risk of IUGR was established in the pregnancy outcome study for mothers exposed to c-PAHs >15 ng/m^3 during the first month of gestation. All these results indicate that c-PAHs represent a very important group of air pollutants.

Key words: air pollution, genotoxicity of PM_{10}, DNA adducts, genetic polymorphisms, pregnancy outcome, IUGR

1. INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are formed during the incomplete combustion pyrolysis of organic materials such as oil, gas, coal and wood (WHO, 2000). Cigarette smoke is an important contributor to indoor air pollution. The most extensively studied PAH is benzo[a]pyrene (BaP). BaP is sometimes used as an indicator for complex mixtures of PAHs. Risk analysis has traditionally focused on the carcinogenic properties of BaP. The WHO (2000) has estimated that a lifetime exposure to 0.012 ng/m^3 of BaP results in a lung cancer risk of 1 x 10^{-6}, a level of risk that is frequently considered the maximum acceptable level of risk due to non-occupational

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exposures. Ambient concentrations in urban areas (especially in busy streets) significantly exceed this level, suggesting that PAHs may contribute to the increased lung cancer incidence associated with particulate matter.

They comprise a portion of organic matter adsorbed onto respirable air particles (< 2.5 μm), and some of these compounds exhibit carcinogenic and/or mutagenic properties. Prospective cohort studies have shown that prolonged exposure to particulate air pollution may be associated with increased rate of morbidity and mortality from respiratory and cardiovascular diseases in the general population (Dockery et al., 1993; Pope et al., 1995; Pope et al., 2002).

Several recent studies have suggested that exposure to PAHs may have effects other than cancer. Fetuses in particular are considered to be highly susceptible to a variety of toxicants because of their exposure pattern and physiologic immaturity (Perera et al., 1999; Sram, 1999). Their developing organ systems can be more vulnerable to environmental toxicants during critical windows due to higher rates of cell proliferation or changing metabolic capabilities also the precision with which genes, etc., must be coordinated (Calabrese, 1995). As a result, prenatal exposure to environmental pollution can result in some adverse reproductive outcomes.

2. TEPLICE PROGRAM

The Teplice Program, consisting of air pollution monitoring and health effects studies, was developed as a collaborative research program with the U.S. Environmental Protection Agency (USEPA) (Sram et al., 1996). Simultaneously, the CEC included the Teplice Program within the project PHARE II "Impact of environmental pollution on the health of population". This support enabled participating Czech laboratories to buy new and updated equipment. Therefore, all studies were carried out on a contemporary scientific level from the beginning of the Teplice Program. Collaboration with the USEPA allowed several studies to be carried out for the first time in the Czech Republic. First, air pollution monitoring was initiated. For the health effects studies, projects were prepared to study pregnancy outcomes, quality of human sperm, respiratory and neurobehavioral function in children, and biomarkers of exposure to carcinogens. In particular, collaboration with the USEPA brought expertise and training in modern methods to Czech scientists (Moldan, 2001).

The most significant results connected to the Pregnancy Outcome project and related projects are discussed.

3. GENOTOXICITY AND EMBRYOTOXICITY OF URBAN AIR PARTICULATE MATTER

In order to assess the possible health risks associated with a complex mixture of hundreds of organic compounds adsorbed to air particles, biomarker-directed fractionation was used to evaluate the biological activities of different chemical compound classes (Binkova et al., 1998). Urban air particles <10μm (PM_{10}) were collected using an Andersen Hi-Vol air sampler (Graseby Andersen, Atlanta, GA) in the both district cities, Teplice and Prachatice, during the winter (October-March) and summer (April-September) seasons during the years 1993-1994. The principal aim of this study was to compare the DNA binding activities of these compound classes using an in vitro assay coupled with ^{32}P-postlabeling and an embryotoxicity assay using Chick Embryotoxicity Screening Test (CHEST) (Binkova et al., 1999). DNA adducts were determined by ^{32}P-postlabeling analysis using nuclease P1 and butanol extraction procedures. HPLC analysis of ^{32}P-postlabeled DNA adducts was used for qualitative measurement to identify some of the major DNA adducts that originated in the *in vitro* assay from crude extracts and/or their fractions. Using the CHEST assay, embryotoxicity was defined as the sum of dead and malformed embryos and expressed (calculated) as ED 50 (a dose inducing in 50% of exposed embryos malformation and/or death) (Binkova et al., 1998).

Both the assays evaluating DNA binding activities and embryotoxicity showed that the different organic compound classes behave in a qualitatively similar manner. For both assays, the highest toxicity was observed for the neutral fractions from which the aromatic subfractions were the most toxic for both the localities and seasons. These subfractions contained mainly PAHs and their methyl-derivatives as was confirmed by GC-MS analysis. These results are in agreement with the other studies showing that PAHs account for most of mutagenic activity of the neutral fraction of urban air (Cerna et al., 2000; Topinka et al., 2000). A good correlation between DNA adduct levels formed after applying the S9 metabolic activation system and the ED 50 was observed for all of the different complex mixtures of organic compounds tested ($r = 0.773$, $P < 0.001$) (Fig.1).

The major adduct spots were analyzed by HPLC analysis of ^{32}P-labeled DNA adducts. DNA adduct maps and HPLC profiles were similar for the samples from both the districts and seasons. The major DNA adducts resulting from the crude extracts were identical to those derived from the aromatic fractions. We tentatively identified presumably diolepoxide-derived adducts from: 9-OH-benzo[a]pyrene (9-OH-B[a]P), benzo[a]pyrene-r-7,t-8-dihydrodiol-t-9,10-epoxide[+] (anti-BPDE), benzo[b]fluoranthene (B[b]F), benzo[j]fluoranthene (B[j]F), benzo[k]fluoranthene (B[k]F), chrysene (CHRY), benzo[a]anthracene (B[a]A), and indeno[1,2,3cd]pyrene (I[cd]P)

(Fig. 2). The results confirmed the similarities of the major ubiquitous emission sources of organic compounds in both districts, which are presumably residential home heating and motor vehicles in winter seasons and likely motor vehicles in the summer period. All analyses indicate that carcinogenic PAHs (c-PAHs, benz[a]anthracene, benzo[b]fluoranthene, benzo[k]-fluoranthene, benzo[g,h,I]perylene, benzo[a]pyrene, chrysene, dibenz[a,h]-anthracene, and indeno[1,2,3-c,d]pyrene) are responsible for much of the genotoxic activity, contributing to approximately 50% of all DNA adducts induced by complex mixtures of organic compounds bound on respirable particles in ambient air.

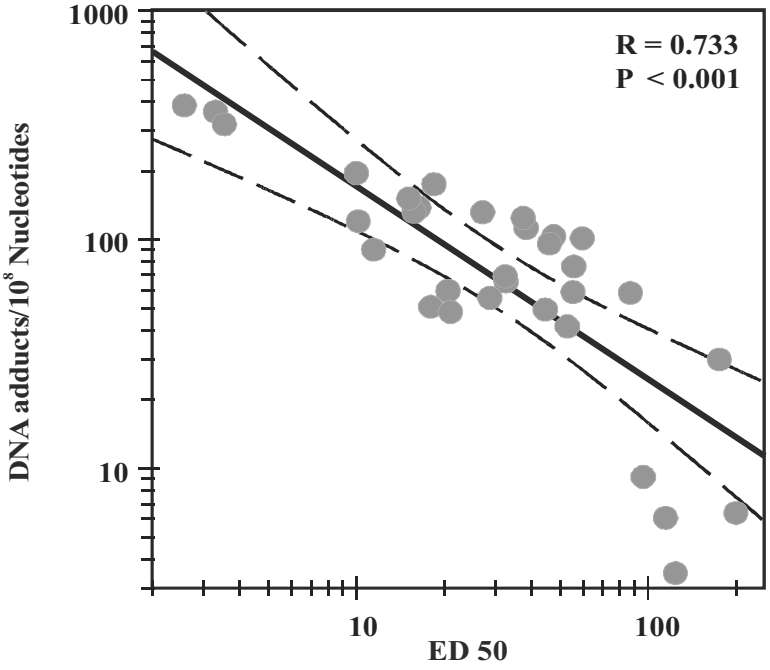

Figure 1. Relationship between DNA adducts *in vitro* and ED 50.

4. DNA ADDUCTS

A study of the effect of environmental pollution on placenta bulky DNA adducts by the butanol extraction enrichment procedure of ^{32}P-postlabeling was done. In the group of 158 mothers, the total DNA adduct levels in mothers living in Teplice were $2.12 \pm 1.46/10^8$ nucleotides compared to mothers in Prachatice $1.48 \pm 1.09/10^8$ nucleotides ($P = 0.04$). This difference was statistically significant (Fig. 3).

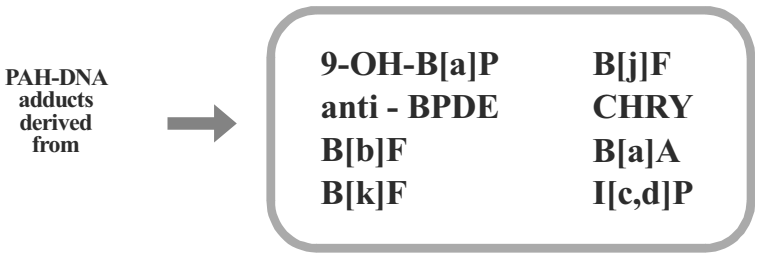

Figure 2. Contribution of the major PAH-DNA adducts to the total DNA adduct level from urban samples.

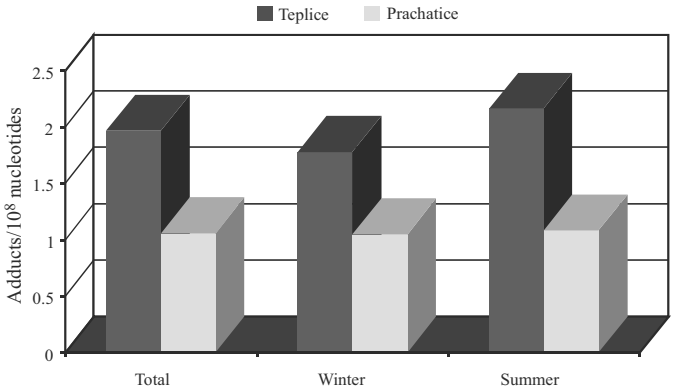

Figure 3. Total DNA adduct levels in placenta: effect of district and season.

Elevated DNA adduct levels were also found in smoking mothers compared to nonsmoking mothers (3.21 ± 1.39 vs. $1.32 \pm 0.88/10^8$ nucleotides, $P < 0.001$). Significant district and seasonal differences were first observed in subgroups with the GSTM1-null genotype (Fig. 4) (Topinka et al., 1997).

Multiple regression models with a stepwise procedure were used to evaluate the interaction of all possible variables. DNA adducts were utilized as outcome response variables. Air pollutants, smoking status, genotypes, antioxidant vitamin levels, and IUGR were used as the predictor variables. Multivariate regression analysis indicated that DNA adduct levels in placentas were affected by a number of factors including the concentration of carcinogenic PAHs during the last month of pregnancy, active and passive smoking, vitamin C levels, and GSTM1, NAT2, EPHX and CYP1A1 genotypes. DNA adducts were also increased in the placentas of newborns with intrauterine growth retardation (IUGR) (Sram et al., 1999). There was an inverse relationship between vitamin C levels and DNA adducts ($b = -0.649$,

$P < 0.05$), and also a relationship between the effect of passive smoking and DNA adducts ($b = 0.031$, $P < 0.01$).

The results of multiple regression analysis indicate that DNA adducts together with analysis of genotypes are sensitive biomarkers of exposure.

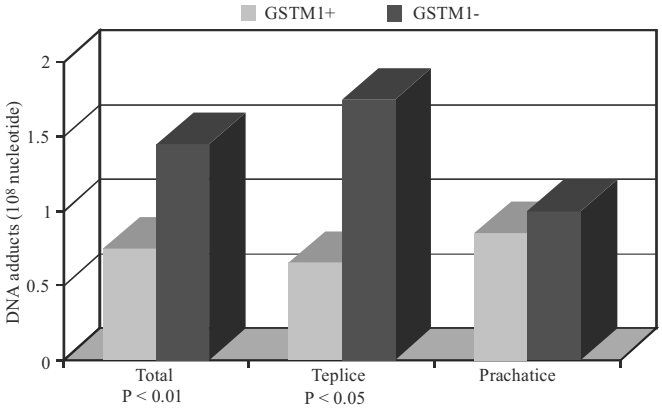

Figure 4. Effect of GSTM1 genotype on DNA adducts in nonsmokers. Where do heterozygotes fall?

5. PREGNANCY OUTCOME

In a cohort study conducted in the Teplice and Prachatice districts of the Czech Republic, information on all singleton term births was collected via self-administered maternal questionnaires and medical records. Intrauterine growth retardation (IUGR), defined as infants below the 10th percentile of birth weight for gestational age and gender, was selected as outcome measure. Odds ratios of IUGR were adjusted using logistic regression models for parental age, education, marital status, parity, recurrent abortion, alcohol and smoking habits, parental employment and season. The IUGR adjusted odds ratios (AOR) were calculated for three concentration intervals of each pollutant, low (reference level), medium, and high. These concentration intervals were close to tertiles. The pollutants measured were particulate matter (PM) including PM less than 10 μm (PM_{10}) and fine particles less than 2.5 μm ($PM_{2.5}$), PAHs and its carcinogenic fraction (c-PAHs), sulfur dioxide, nitrous oxides and ozone. AORs for continuous pollution data were also estimated to examine the exposure-response relationship. Each mother's exposure to a particular pollutant was estimated for each gestational month starting from the estimated date of conception.

Initially, a relationship between PM and fetal growth was found by analyzing data collected during the first two years of this study in the highly polluted district of Teplice (Dejmek, et al., 1999). A significantly increased risk of giving birth to a child with IUGR was found for mothers exposed to PM_{10} levels $> 40\ \mu g/m^3$ or $PM_{2.5} > 37\ \mu g/m^3$ during the first month of gestation. For each $10\ \mu g/m^3$ increase in PM_{10} the AOR of IUGR was 1.25 (CI 1.08-1.56); a similar, but weaker association was also observed for $PM_{2.5}$. No association of IUGR risk with PM concentrations was found in any later gestational month. These findings again suggest an exposure-response relationship between fetal growth and PM levels during the first month of gestation. No association was observed between IUGR risk and sulfur dioxide, nitrous oxides or ozone.

The influence of PM fine particles on fetal growth was later reanalyzed and reported in a four-year data set (Dejmek et al., 2000). A total of 3,349 pregnancies with 9.6% IUGR births were enrolled in the Teplice sample. In the Prachatice sample, there were 1505 pregnancies with 8.2% IUGR births. These results from the 4-year data set confirmed the preliminary results reported for Teplice (Dejmek et al., 2000) reported earlier (Sram, 2001). The IUGR risk was 1.41 (CI 1.03-2.02) for medium levels and 2.10 (CI 1.42-3.23) for high levels of PM_{10} during the first month of gestation (Fig. 5). Analysis of continuous pollution data again confirmed the exposure-response relationship: for each $10\ \mu g/m^3$ increase of PM_{10} in the first gestational month, the AOR was increased by 1.19 (CI 1.06-1.33). In contrast, no significant association between particulate matter and IUGR risk in any period of gestation was observed in the Prachatice data set.

Regardless of consistent and convincing results, no biologically plausible explanation for the impact of respirable particle mass on fetal growth has yet been found. An alternate explanation for the above findings is that rather than particle mass, associated chemical constituents or co-pollutants such as PAHs may interfere with fetal development. Moreover, most of these compounds are usually adsorbed on the surface of fine particles.

This possibility was tested directly in the following analysis of the association between carcinogenic PAHs (c-PAHs) and IUGR risk using the four-year samples from Teplice and Prachatice described above. A highly significant increase in IUGR risk was found in the Teplice data for exposures to c-PAHs during only the first gestational month. Adjusted ORs for medium levels was 1.67 (CI 1.06-2.39) and for high levels was 2.16 (CI 1.27-3.63) (Fig. 6). This relationship was strongly exposure-response related with an AOR of 1.22 (CI 1.07-1.39) per $10 ng/m^3$ increase in c-PAHs. In contrast to previous findings regarding particle mass in Prachatice, the association between c-PAHs and IUGR was close to that found in Teplice. Again, the only consistent c-PAH/IUGR association in Prachatice was observed in the first gestational month. The AOR for medium levels of c-PAHs in Prachatice

was 1.63 (CI 0.87-3.06) and for high levels was 2.39 (CI 1.01-5.65). Continuous data analysis also confirmed this exposure-response relationship.

This finding supports the hypothesis that the elevation of IUGR risk is related to c-PAHs. Based on these hypotheses, the association of particles with IUGR risk in Teplice be due to the high correlation of c-PAHs and PM in this area (Dejmek et al., 2000). This finding is consistent with the idea of a primary role for c-PAHs in fetal growth modulation.

The findings from (Sram et al., 1999*)* support the role of PAH in reproductive effects. . PAH-DNA adduct levels in placentas of nonsmoking mothers were associated with exposure to c-PAHs and DNA adducts in placentas of IUGR infants were significantly increased (Sram et al., 1999). The hypothesis that c-PAHs play a primary role in pregnancy outcome is also supported by recently published experimental results. A direct effect of PAH was found on receptors for epidermal growth factor (EGF) in placental cells (Guyda, 1991*)*. This observation has been recently confirmed (Zhang

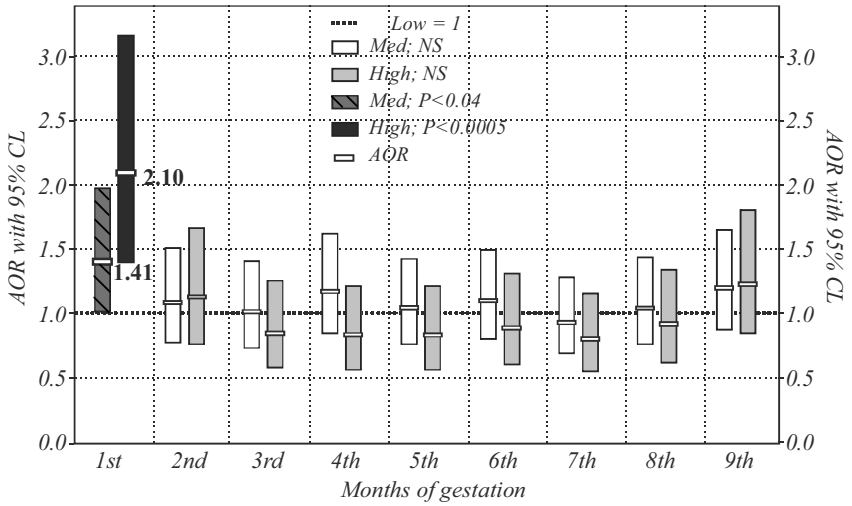

Figure 5. The risk of IUGR by PM 10 during years 1994-1998 in Teplice.

et al., 1995), who have shown that benzo[a]pyrene and other carcinogenic PAHs mediate a loss of EGF receptors and alter early trophoblast proliferation as well as endocrine function.

Thus, the first gestational month seems to be the critical period for the association of PAH contamination with fetal growth. The timing of this association is in agreement with the current hypothesis, that IUGR pathogenesis is triggered by an abnormal reaction between trophoblast and uterine tissues in the first weeks of pregnancy (Duvekot et al., 1995*)*. Fine particles and c-PAHs levels were associated with IUGR risk.

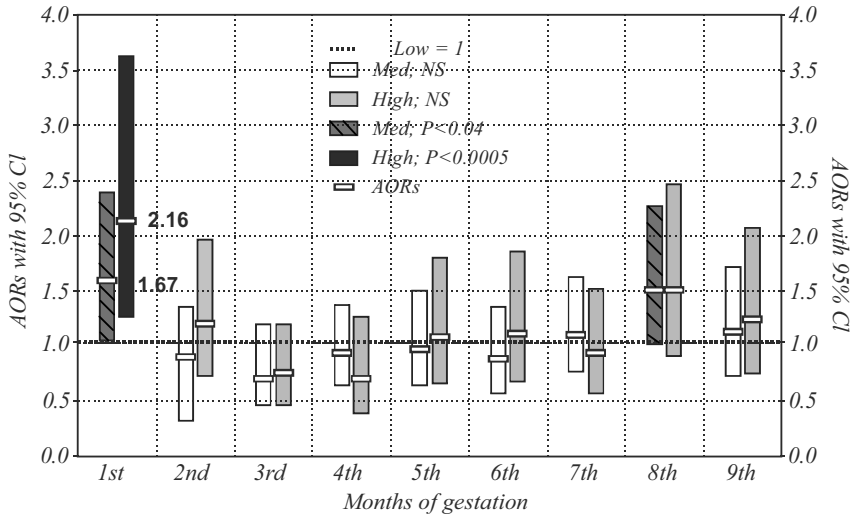

Figure 6. The risk of IUGR by c-PAHs during years 1994-1998 in Teplice.

6. DISCUSSION

The molecular epidemiological studies suggest biological mechanisms for the effect of air pollution on birth outcomes. It has been shown that the levels of DNA adducts are positively related to risk of IUGR (Sram et al., 1999; Dejmek et al., 2000), low birth weight, low birth length, small head circumference (Perera et al., 1998; Perera et al., 1999), and hypoxanthine-guanine phosphoribosyl-transferase locus (HPRT) mutation frequency in infants (Perera et al., 2002). These observations were supported by *in vitro* studies of the genotoxic and embryotoxic activities of organic extracts and their fractions from air particles (PM_{10}) collected in Teplice and in a control district during the years 1993-1994 (Binkova et al., 1999). Organic compounds adsorbed to air particles (PM_{10}) induced DNA adducts and embryotoxicity in *in vitro* and *in vivo* studies. The carcinogenic polycyclic aromatic hydrocarbons (c-PAHs) were mostly responsible for the genotoxic activity, contributing to 45-50% of all DNA adducts induced by these complex mixtures.

PAHs and/or their metabolites may bind to the aryl hydrocarbon receptor (AhR) and accumulate in the nucleus of cells, resulting in increased rates of mutagenesis. Binding of PAHs to the AhR may result in anti-estrogenic activity through increased metabolism and the depletion of endogenous estrogens (Carpenter et al., 2002), thus disrupting the endocrine system by altering steroid function. Bui et al. (1986) have hypothesized that BaP exposure may interfere with uterine growth during pregnancy because of its

anti-estrogenic effects that lead to disruption of the endocrine system. Fetal toxicity may be further caused by DNA damage resulting in activation of apoptotic pathways (Nicol et al., 1995) or to binding to receptors for placental growth factors resulting in decreased exchange of oxygen and nutrients (Dejmek et al., 2000).

The finding of higher DNA adduct levels in the infant compared to the mother suggests greater susceptibility of the developing fetus to DNA damage (Perera et al., 1999). With respect to IUGR, it appears that the increased risk is principally due to exposure to carcinogenic PAHs. This finding is consistent with the idea of a primary role for c-PAHs in foetal growth modulation (Ridgon and Renneis, 1964; MacKenzie and Angevine, 1981; Guyda 1991; Zhang et al., 1995). Perera et al. (2003) labelled PAHs as significant independent determinants of birth outcomes.

In addition, there appears to be an interaction between exposure to PAH and genotype in the production of DNA adducts (Whyatt et al., 2001). The placental bulky DNA adducts have been studied in relation to metabolic genotypes CYP1A1, EPHX, GSTM1 and NAT2, and plasma levels of cotinine and vitamins A, C, and E. DNA adducts were determined by the ^{32}P-postlabeling assay. Changes in DNA adduct levels in placentas were associated with differences in air pollution, smoking, genotypes, and vitamin C levels. Higher levels of DNA adducts were observed in nonsmoking mothers delivering children with IUGR (Topinka et al., 1997; Sram et al., 1999). While the specific steps of these pathways need to be further clarified, the effects of air pollution on birth outcomes are biologically plausible.

7. CONCLUSION

The effect of PAHs on reproduction represents a new field of study, supported by molecular and epidemiological studies. PAHs represent a biologically significant group of compounds, and appear to be responsible for the deleterious effect of respirable particles (< $PM_{2.5}$).

Data on the impact of carcinogenic PAHs on pregnancy outcome have only been collected during the last decade. These data significantly enhance the scientific knowledge base and must be incorporated into current risk assessment procedures to improve children's health in contaminated regions worldwide.

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CHAPTER 29

MONITORING OF ORGANOHALOGENS BODY BURDEN OF THE CZECH POPULATION

Milena Černá[1,2], Jiri Šmíd[1], Andrea Batáriová[1,2], Ruzena Kubínová[1]
[1]National Institute of Public Health, Šrobárova 48, 10042 Prague, Czech Republic; [2]Charles Univ., 3rd Med. Faculty, Ruská 87, Prague, Czech Republic

Abstract: This study reports the levels of indicator PCB, DDT, and HCBs in the human milk of women living in the Czech Republic. From 1994 to 2003 more than 3400 milk samples were collected, extracted and analyzed. A questionnaire was used to obtain information regarding personal characteristics and life style factors. Data expressed on a fat basis as median values showed significant time-trend decline for sum of indicator PCBs 138 + 153 + 180 from 806 ng/g fat (1994) to 445 ng/g fat (2003), as well as for HCB (from 427 ng/g fat in 1994 to 44 ng/g fat in 2003) and for DDT sum (from 1075 ng/g fat in 1994 to 288 ng/g fat in 2003). These data indicate that the body burden of organochlorines in women in the Czech Republic has decreased since 1994.

Key words: organohalogens, biological monitoring, human milk, Czech population

1. INTRODUCTION

Persistent chlorinated organic compounds (DDT, HCB, PCBs, PCDDs, PCDFs) are widespread environmental pollutants; and, being fat soluble, they tend to accumulate in animals including humans. Organochlorines have varying harmful effects on human health (Safe, 1994). Monitoring of organochlorine compounds in human milk is often used to investigate levels of contamination and to follow temporal trends (Furst et al., 1994). The Czech Republic is one of several Eastern European countries with a relative body burden of PCBs due to their production in the former Czechoslovakia and intensive industrial use in the past (WHO/ECEH, 1996; Bencko et al., 1998; Černá and Bencko, 1999). Therefore, monitoring of PCBs and other organochlorine compounds was included in the nationwide Environmental

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Health Monitoring System implemented in the Czech Republic in 1994 (Kliment et al., 1997).

2. MATERIAL AND METHODS

2.1 Sampling

From 1994 to 2003, a total of 3544 breast milk samples (one hundred or less per year and locality) were collected by manual expression from nursing mothers living in four regions of the Czech Republic; two of the regions were more industrial, the two other more rural and recreational. The women participating in the study were randomly sampled from the general population. All samples were obtained on a voluntary basis during the first week after delivery. Written informed content was obtained from each subject. A questionnaire was used to obtain information regarding personal characteristics and life style factors. The milk samples were collected in acetone-cleaned glass bottles in volumes of 30 to 50 ml each. Samples were stored at –18°C until analysis.

2.2 Laboratory procedures

The amount of indicator PCBs (IUPAC nos. 28, 52, 101, 118, 138, 153, and 180) and selected chlorinated pesticides in breast milk was determined in a laboratory accredited by the Czech Accreditation Institute. Briefly, after extraction in n-hexane and clean-up with florisil column adsorption, the analyses of milk samples were performed by chromatography or gel permeation chromatography on Bio Beads SX3 by HRGC on Capillary Column DB-5 and electron capture detector. Confirmation was performed using a mass selective spectrometric detector (Ion trap) connected with gas chromatography according to AOAC Official Methods of Analysis 983.21. Detection limits for specific analytes included: 5.0 ng/g fat for PCB 028, 052, 101; 3.0 ng/g fat for PCB 138 and 153; 13 ng/g fat for PCB 180; 1.0 ng/g fat for HCB; 2.0 ng/g fat for p, p´DDT and p, p´ DDE.

2.3 Statistics

All statistical analyses were performed using the Statistica 6.1. Descriptive statistics were computed to characterize the distribution of organochlorine

concentrations in milk samples. Potential correlations between residue levels in human milk and questionnaire data were tested using Spearman's Rank.

3. RESULTS AND DISCUSSION

Table 1 summarizes the concentrations of the dominant indicator PCB congeners 138, 153, and 180 for individual years. A significant correlation among these congeners and existing information in the literature (Rylander et al., 1998) prompted us to use the indicator congener 153 to be representative of total PCB exposure. A comparison of median values for PCB congeners indicates that concentration levels of congener 153 in 2003 are approximately 50% lower than 10 years ago. This decline may be attributed in part to the prohibition of PCBs in 1984. The levels of PCBs varied considerably between the individual donors and revealed differences that appeared to be related to the location where the donor resided. The concentrations of the lower chlorinated congeners were very low and mostly below the detection limits. The mean age of women under study was 26.2 years ranging from 16 to 45 years. The levels of dominant indicator congeners revealed a significant age-related increase ($p < 0.0001$) (Table 2). Neither parity nor smoking or other lifestyle factors recorded in the questionnaire were associated with PCB levels in breast milk.

Reference values for PCB 153 for the Czech human milk was assessed based on the results obtained in this study. The reference value for the years 1994/95 (700 ng/g fat) was about twice as high (Černá et al., 2003) as for human milk in German populations for the similar time period 1990 – 1992 (340 ng/g fat) (Ewers et al., 1999), whereas the reference value was comparable to German values (330 ng/g fat) assessed in 2002/03. Thus the concentration levels of PCBs measured in the Czech human milk samples document the delay of several years in decreasing worldwide trends in PCB exposure. Also the results of the 3rd round of the WHO-coordinated study in the year 2000 showed that the level of sum indicator PCBs in pooled milk samples from three Czech localities were the highest from all participating countries (Malisch and van Leeuwen, 2003). For samples collected in the current study, the total PCB values for congeners 138, 153 and 180 for 2000 (Table 1) were approximately 1.7 times the median value of 502 ng/g fat observed in the WHO-coordinated study (DGF, 1988). The high levels of exposure observed in the Czech population has also been seen in other studies (Čajka and Hajšlová, 2003, Bencko et al., 2004).

Table 1. Concentrations of dominant indicator PCB indicators in human milk (ng/g fat) - median (bolded) and ranges of 10th to 90th percentiles (in parenthesis).

Years	PCB 138	PCB 153	PCB 180
1994, n = 280	**190** (97 – 358)	**352** (183 –629)	**255** (131 –539)
1995, n = 395	**178** (70 – 396)	**323** (182 – 526)	**248** (130 – 429)
1996, n=285	**163** (69 – 321)	**201** (109 – 368)	**125** (65 – 241)
1997, n=391	**142** (83 – 265)	**165** (96 – 306)	**110** (58 – 210)
1998, n = 385	**130** (77 – 244)	**165** (97 – 293)	**127** (70 – 243)
1999, n = 389	**140** (76 – 271)	**164** (88 – 358)	**131** (69 – 276)
2000, n = 407	**118** (35 – 309)	**168** (53 – 400)	**98** (27 – 249)
2001, n=389	**100** (52 – 188)	**139** (70 – 273)	**78** (41 – 162)
2002, n = 367	**175** (91 – 316)	**137** (67 – 256)	**149** (79 – 248)
2003, n = 253	**132** (63 – 235)	**174** (89 – 301)	**139** (71 – 238)

Table 2. Concentrations of dominant indicator PCB indicators in human milk (ng/g fat) of primiparae according to age.

Age	n	Median	Ranges of 10th to 90th percentiles
< 20	124	116	57.5 - 227
20 - < 25	689	143	74.1 - 278
25 - < 30	521	171	89.8 - 316
> = 30	83	195	104 - 422

Concentrations of DDE, DDT sum and HCB detected in breast milk samples obtained for this study are shown in Table 3. Both, DDE (dominant representative of the DDTs components), and HCB were detected in all human milk samples. Related to the medians, the concentration level of DDT sum is approximately 75% lower than 1994. The median HCB level detected in human milk was reduced by 90% during the same period. A decrease in concentrations of DDT and HCB in milk samples was observed from as early as 1980. This decrease appears to have resulted from a ban on DDT (in 1974) and HCB (in 1982) that took place in the former Czechoslovakia. It is anticipated that this reduction would continue over

time. Measurements of total DDT and HCB observed in this study are similar to concentration levels observed in other European countries (Ewers et al., 1999, Fürst et al., 1994, Harris et al., 1999, Pohl and Tylenda, 2000). The DDE:DDT ratio does not indicate recent exposure to DDT, but more likely the historical exposure to DDT and/or the environmental exposure to the breakdown product DDE. Similar to the trend observed for PCBs, individual variability in levels of organochlorine pesticides appeared to reflect local differences resulting from variations in environmental pollution.

Table 3. Concentrations of DDE, total DDT, and HCB in human milk (ng/g fat) - median (bolded) and ranges of 10^{th} to 90^{th} percentiles (in parenthesis).

	HCB	DDT sum	DDE
1994, n = 280	**427** (175 – 1031)	**1075** (588 – 2311)	Not tested
1995, n = 395	**360** (153 – 924)	**923** (474 – 1892)	Not tested
1996, n = 285	**244** (95 – 531)	**508** (267 – 898)	**455** (217 – 838)
1997, n = 391	**175** (68 – 452)	**434** (236 – 841)	**417** (211 – 805)
1998, n=385	**168** (73 – 447)	**431** (268 – 743)	**360** (218 – 644)
1999, n = 389	**127** (51 – 387)	**325** (179 – 590)	**303** (165 – 550)
2000, n = 407	**120** (47 – 334)	**233** (50 – 823)	**209** (42 – 820)
2001, n = 389	**122** (49 – 269)	**421** (207 – 745)	**372** (186 – 668)
2002, n = 367	**89** (39 – 190)	**380** (220 – 720)	**360** (210 – 674)
2003, n = 253	**44** (23 – 98)	**288** (151 – 464)	**261** (136 – 420)

4. CONCLUSION

PCB concentrations in samples of human milk from the Czech Republic were found to consistently decrease from 1994-2002. In spite of these reduced levels of PCBs in milk, the concentrations measured in 2002 remained elevated in comparison with levels observed in milk from other European countries. In contrast, concentrations of total DDT, DDE and HCB measured in the human milk collected for this study are comparable to

results from other studies in Europe and northern America. Continued monitoring of organochlorine compounds in human milk or blood is important to monitor current population exposures to these compounds. More work remains to be done in characterizing the health risks that may be attributable to current levels of exposure. The reduction of these compounds in the environment will remain a major public health task for the future.

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