Ambient Air Pollutants in Upper Silesia: Partial Chemical Composition and Biological Activity

Mieczysław Chorąży,[1] Jan Szeliga,[1] Małgorzata Stróżyk,[1] and Bernard Cimander[2]

[1]Department of Tumor Biology, Institute of Oncology, Gliwice, Poland; [2]District Sanitary-Epidemiological Station, Katowice, Poland

The air monitoring system in Upper Silesia has provided abundant data on airborne pollutants. Air quality in this region is bad: a concentration of several gases, volatile compounds, metals, and complex mixtures of organic compounds carried by small particulate matter exceeds both daily and yearly admissible levels. About 250 individual polycyclic aromatic hydrocarbonds (PAHs) were identified in airborne pollutants, and hundreds of not identified compounds are seen on gas chromatographic profiles as minor peaks. Among PAHs are present compounds with known carcinogenic potency for humans. Seasonal variation with distinctly lower concentration of pollutants in summer than in winter was noticed. Fifteen PAHs including benzo[*a*]pyrene (B[*a*]P) determined by GC-MS method in 20 measuring points showed constant relative proportions. Thus B[*a*]P could be used as a representative compound for other PAHs. In urban areas, a core of Silesia values for B[*a*]P concentration ranged from 60 to 90 ng/m^3 in winter to 5 to 20 μg/m in summer. Mutagenicity tested on Salmonella strains showed seasonal variation with distinctly higher values in winter. Environmentally exposed humans showed a higher level of PAH-DNA adducts in WBC than the control population from rural area. Total organic extract of small particulate matter exhibited both direct and indirect mutagenic activity, induced formation of micronuclei in bone marrow cells of BALB/c mice, induced chromosomal rearrangements, and increased sister chromatid exchange index. — Environ Health Perspect 102(Suppl 4):61–66 (1994).

Key words: air pollution, carcinogens, chromosomal damage, mutagens, PAH, PAH-DNA adducts, particulate matter, sister chromatid exchange

Introduction

In the frequently cited paper of Doll and Peto (*1*), it is stated that in respect to air:

> gross pollution is now largely, if not entirely, eliminated from the American environment except in the immediate vicinity of old chemical waste dumps.

This statement certainly does not relate to the status of air pollution in the area of Upper Silesia (district of Katowice) in south Poland. This area, encompassing 6500 km^2 and inhabited by 4 million people, is considered an area of ecologic disaster.

Enormously dense concentrations of industry (mainly coal mines, metallurgical works, smelters, coke ovens, and chemical factories), high consumption of coal for heating, and heavy automobile traffic are the main sources of air pollutants. The emission of only gases and small particulate material amounts to over 2 million tons per year.

The amount of solid wastes produced by industry in 1990 was 72,443,000 tons, out of which 21,800,000 tons were deposited on waste dumps. It is estimated that about 640 million tons of solid industrial wastes are deposited on the industrial-owned terrain, which gives a figure of 54,000 tons/km^2 on average. Total waste dumps in Upper Silesia amount to over 2 billion tons. Close to 100% of rivers whose sources are in Silesia are polluted to such an extent that the water cannot be used even for industrial purposes. These few figures cited from the bulletin of the Central Statistical Office (*2*) give only an approximate picture of the environmental devastation in Silesia.

In this report, we present an analysis of airborne particulate matter and summarize some biological effects exerted by organic extracts of particulate matter on living cells.

Materials and Methods

Monitoring System of Air Pollution

The monitoring system developed by the District Sanitary-Epidemiological Station in Katowice (*3*) consists of 39 measuring stations operating on a 6 × 24 hr/m basis located mainly in the core of Silesia and of 750 measuring points for collection of freely sedimenting airborne matter. Measuring points are located at selected points over the whole district in the network of 2 × 2 km squares.

Measuring stations are equipped with Staplex-type samples to collect airborne suspended matter aspiring high volume of air (up to 2000 m^3/24 hr) and aspirators for trapping of gaseous pollutants. Suspended matter was collected on fiberglass filters by the Staplex Company. The following pollutants routinely are estimated in measuring stations: CO, CO_2, SO_2, NO_2, phenol, formaldehyde, ammonia, volatile hydrocarbons, fluoride, and suspended matter. Suspended matter was analyzed for Pb, Zn, Cd, Cr, Mn, Fe, Cu, Ni, tar substances (as cyclohexane extract), benzo[*a*]pyrene (B[*a*]P), and perylene.

This paper was presented at the Symposium on Risk Assessment of Urban Air: Emissions, Exposure, Risk Identification and Risk Quantitation held 31 May–3 June 1992 in Stockholm, Sweden.

The authors appreciate the assistance of David de Marini and Herman Autrup who read the manuscript and provided remarks. Jolanta Wiatrak provided skillful technical help in gas chromatography analysis. Biological data presented in this report are the result of research performed by Ewa Grzybowska, Jadwiga Michalska, Grazyna Motykiewicz, and Joanna Pendzich, staff members of the Department of Tumor Biology, Institute of Oncology, Gliwice, Poland. This work has been supported by Polish National Cancer Program CPBR 11.5, grant 84, and United Nations developmental program no. POL/82/003/L/01/14.

Address correspondance to Mieczysław Chorąży, Department of Tumor Biology, Institute of Oncology, 44-100 Gliwice, Poland. Telephone 48 32 31 10 61. Fax 48 32 31 35 12.

Table 1. Concentration range of average yearly pollutants in Upper Silesia in 1988 to 1990 compared to admissible level.

Substance	Concentration range	Unit	Admissible level
Suspended matter	66–253	$\mu g/m^3$	50
CO	4.4–7	mg/m^3	0.12
CO_2	0.76–0.94	g/m^3	
SO_2	21–97	$\mu g/m^3$	0.2
NO_2	25–134	$\mu g/m^3$	50
Phenol	5.2–27	$\mu g/m^3$	2.5
Fluoride	0.8–1.9	$\mu g/m^3$	1.6
Formaldehyde	2.6–44	$\mu g/m^3$	3.8
Ammonia	18–79	$\mu g/m^3$	51
Volatile hydrocarbons	3.4–4.8	mg/m^3	
Pb	0.11–1.51	$\mu g/m^3$	0.2
Zn	0.19–7.6	$\mu g/m^3$	
Cd	2.1–72	ng/m^3	10
Mn	0.03–0.21	$\mu g/m^3$	1
Fe (as Fe_2O_3)	2–24	$\mu g/m^3$	
Cu	0.3–4.4	$\mu g/m^3$	0.6
Cr	2.1–44	ng/m^3	400
Ni	2.3–59	ng/m^3	25
Tar substances	7–32	$\mu g/m^3$	
Benzo[*a*]pyrene [a]	15–125	ng/m^3	1
Perylene [a]	3–37	ng/m^3	
Free sedimenting matter	35–466	g/m^2	200
Sediment of Pb	2–2822	mg/m^2	100
Sediment of Cd	0–87	mg/m^2	10

[a] Determined by spectrophotometry (UV-VIS); values elevated as compared to gas chromatography.

Table 2. Maximal daily concentrations and frequency of maximal daily concentrations exceeding daily allowable level in Upper Silesia, winter of 1988.

Substance	Concentration	Unit	2D24 [b]	fD24, % [c]
Suspended matter	1170	$\mu g/m^3$	240	97
CO	25	mg/m^3	2	100
CO_2	19	g/m^3		
SO_2	798	$\mu g/m^3$	400	10
NO_2	519	$\mu g/m^3$	300	29
Fluoride	17	$\mu g/m^3$	20	0.5
Phenol	220	$\mu g/m^3$	20	86
Formaldehyde	326	$\mu g/m^3$	40	87
Ammonia	427	$\mu g/m^3$	400	3
Volatile hydrocarbons	12	mg/m^3		
Pb	21	$\mu g/m^3$	2	43
Zn	64	$\mu g/m^3$		
Cd	1.2	$\mu g/m^3$	0.44	5.4
Mn	1.33	$\mu g/m^3$	8	0
Fe (as Fe_2O_3)	92	$\mu g/m^3$		
Cu	106	$\mu g/m^3$	10	38
Cr	0.46	$\mu g/m^3$	4.0	0
Ni	1.3	$\mu g/m^3$	0.2	12
Tar substances	217	$\mu g/m^3$		
Benzo[*a*]pyrene [d]	950	ng/m^3	10	100
Perylene [d]	270	ng/m^3		

[a] Data from one measuring point from center of Upper Silesia, winter 1988. [b] 2D24. admissible level for single maximal daily concentrations. [c] Frequency (% of 24-hr time) of daily concentrations exceeding admissible level. [d] Determined by spectrophotometry (UV-VIS); values elevated as compared to gas chromatography.

Analysis of Suspended Matter

For advanced chemical analysis of organic pollutants, suspended particulate matter was collected in winter (December–February) and in summer (July–September) at 20 measuring stations located in the heavily industrialized core of Silesia and also at a periphery of the region. Two homologous sets of samples from winter and summer season (to be called winter and summer samples, respectively) were prepared. The samples were extracted in a Soxhlet apparatus with cyclohexane at least for 8-hr in order to obtain quantitative recovery. The solvent was completely removed to dryness by rotary evaporation, and the extraction yields (by weight) were determined. Samples of exctracts were stored under gaseous nitrogen at –20°C.

PAH fractions were separated by liquid-liquid partitioning between cyclohexane and dimethylformamide-water (9:1, v:v) according to the method of Bjorseth (*4*), and were used for quantitative analysis of 15 major polycyclic aromatic hydrocarbons (PAHs).

Sequential Elution Solvent Chromatography

To study the biological effects of organic pollutants, extracts were separated into 8 fractions by sequential elution solvent chromatography (*5,6*). This scheme used silica gel and a solvent sequence that provided a systematic variation in the Hildebrand solubility parameters and the specific solubility parameters. The details of the method were described previously (*7*). The collected fractions were evaporated to dryness by rotary evaporation at 50°C, weighted, and stored under gaseous nitrogen at –20°C until use.

Gas Chromatography-Mass Spectrometry Analysis

Gas chromatography (GC) analyses were carried out on a Hewlett-Packard 5890A gas chromatograph connected with HP-5970B mass selective detector (MSD, electron energy 70 eV), or flame ionization detector (FID). Both GC instruments were equipped with Ultra-2 columns (Hewlett-Packard) 25 m × 0.2 mm (for MSD) or 25 m × 0.32 mm (for FID). The GC conditions were as follows: injection port temperature 310°C; column temperature programmed from 40 to 310 at 4°/min with initial 2-min and final 15-min isothermal periods; detector temperature (FID), 310°C; carrier gas helium, 35 cm/sec; splitless (FID) or split (MSD) 1:30. Compound identification was based primarily on matching the measured mass spectra with those obtained from injected standards, from reference library of GC-MSD system (NBS/NIH/EPA/MSDC Data Base), and from retention indices obtained according to the methods described in (*8*). Quantitation of PAHs was carried out by comparing the peak areas in the FID chromatograms with 2,2′-dinaphtyl as an internal standard.

Biological Tests

Mutagenic, genotoxic and clastogenic activity of extracts from airborne suspended

matter was studied mainly with total extracts and also with major fractions obtained by the silica gel column chromatography (SESC) procedure. The detailed description of each test and references can be found in our previous publications cited in "Results and Discussion."

Total benzene extracts of small particulate matter or SESC fractions were evaporated to dryness in a rotary evaporator, and the dry substance was redissolved in DMSO. The concentration of pollutants used for biological tests was adjusted for the volume of air (in cubic meters).

The *Salmonella typhimurium* mutagenicity assays (Ames test) were used as an initial step for assessment of mutagenic effects of airborne pollutants. The other tests used were: formation of micronuclei (mouse bone marrow), structural damage to chromosomes, induction of sister chromatid exchange, and effect on cell cycle (hamster cell line V79). PAH-DNA adduct level was determined in peripheral WBC of exposed population.

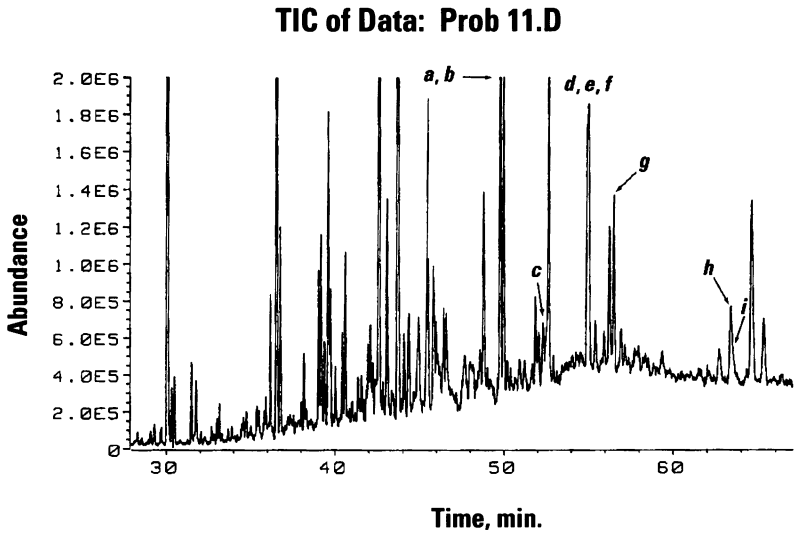

Figure 1. Gas chromatography profile of silica gel column chromatography fraction no. 2 containing mainly PAHs. Arrows indicate identified major components with known carcinogenic activity: (*a*) cyclopenta[*cd*]pyrene, (*b*) benzo[*a*]anthracene, (*c*) 4-methylchrysene, (*d*) benzo[*b*]fluoranthene, (*e*) benzo[*j*]fluoranthene, (*f*) benzo[*k*]fluoranthene, (*g*) benzo[*a*]pyrene, (*h*) indeno[1,2,3-*cd*]pyrene, and (*i*) dibenzo[*a,h*]anthracene.

Results and Discussion

Major Air Pollutants

Table 1 shows the yearly average levels of major pollutants found in ambient air in Silesia in 1988 to 1990 (*3*). The concentration of various pollutants shows a wide range of variation depending on the location of measuring stations or points. The lowest values were noted in the less industrialized periphery of the district, the highest in the core of the district and around individual plants. The yearly average concentrations of a row of listed substances at many locations were above or at the upper limits of the admissible yearly level (Da). This is clearly demonstrated for suspended matter, CO, SO_2, NO_2, formaldehyde, phenol, ammonia, Pb, and Cd in suspended matter and free sedimenting matter. Free sedimenting matter contained also high excess of Pb and Cd. Examples of maximal daily concentrations and the daily frequency for single maximal daily concentration exceeding allowable level are shown in Table 2. For several substances listed in Table 2, high daily concentrations exceeding admissible level and high frequencies were noted.

Fractions Obtained by SESC

Benzene extracts applied to a silica gel column and eluted successively with organic solvents as described in (*5,6*) yielded six major and two minor fractions. Fraction 1 contained saturated hydrocarbons (up to 35 C in the chain). Fraction 2 contained PAHs and was further analyzed by GC-MS method. Fraction 3 contained polar aromatics, nonbasic N, S, O, heterocyclics and was partially analyzed by GC-MS. Remaining fractions contained monophenols (fraction 7), basic nitrogen heterocyclics (fraction 5) and polyphenols (fraction 7), and other minor components. They were not submitted as yet to more detailed analysis, but they were evaluated for mutagenic and genotoxic effects.

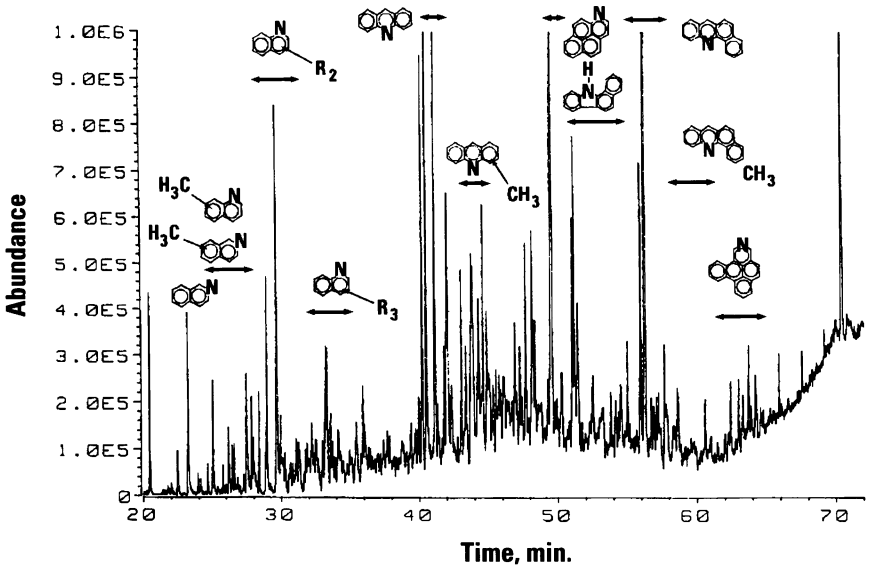

Figure 2. Gas chromatography profile of PAHs containing *N*-heteroatom in the ring. Over 100 individual components are identified.

Gas Chromatography and Mass Spectrometry Analysis of Polycyclic Aromatic Hydrocarbons

GC analysis of fractions 2 and 5 obtained by chromatography SECS revealed complexity of PAH (Figures 1, 2) components. Hundreds of major and hundreds of minor peaks were visible on routinely obtained chromatograms in winter and summer samples from the core region of Silesia. Until now, close to 250 PAHs and PAHs with *N*

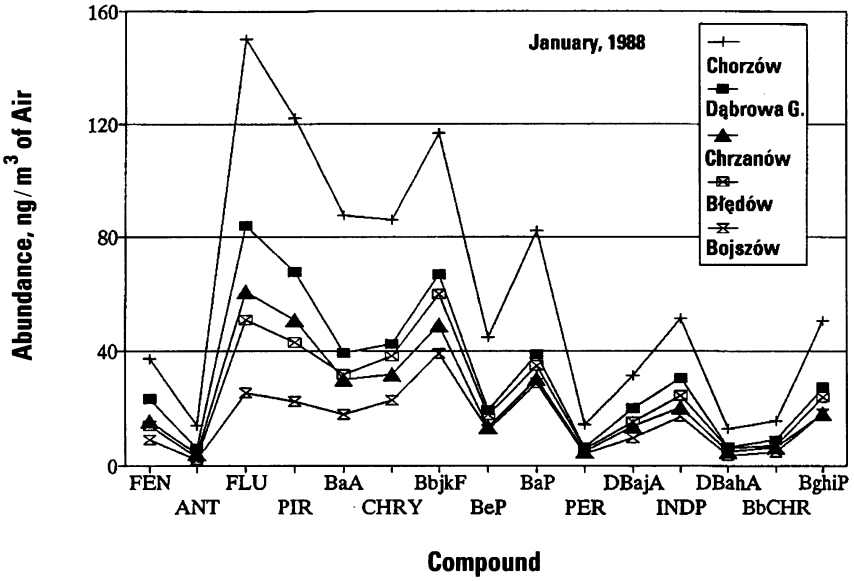

Figure 3. The distribution of 15 major polycyclic aromatic hydrocarbons (PAHs) in winter samples collected in highly polluted towns (Chorzów, Dąbrowa Górnicza, and Chrzanów) and two less polluted settlements (Błędow and Bojszow). Note the stable proportions among a given PAH at various measuring stations.

in the ring were identified. Among these were present compounds listed by the International Agency for Research on Cancer (IARC) (*9*) as potent carcinogens (Figure 1). Fifteen major PAHs were quantitatively estimated at 20 measuring points. Figure 3 shows concentrations of these compounds found in winter samples in three highly polluted towns (Chorzow, Dabrowa Gornicza, and Chrzanow) and two less-polluted settlements (Bledow and Bojszow). The relative proportions for each PAH studied was similar in different measuring points. The lack of variability in the proportions of PAHs in different towns of Silesia makes it reasonable to measure only B[*a*]P as a representative compound for other PAHs. This also means that sources emitting pollutants to air are using the same type of crude energetic material. In Silesia, black coal is preferentially used both for generating electricity in power stations and for industrial and domestic purposes. This supposition is further confirmed by the ratio of concentration of indeno[1,2,3-*c,d*]pyrene (INDP) to benzo[*g,h,i*]perylene (B[*ghi*]P), which is equal to approximately 0.9 (Figure 3), a value characteristic for products derived from incomplete combustion of black coal (*10*). Black coal is a major source of energy in Poland (Figure 4).

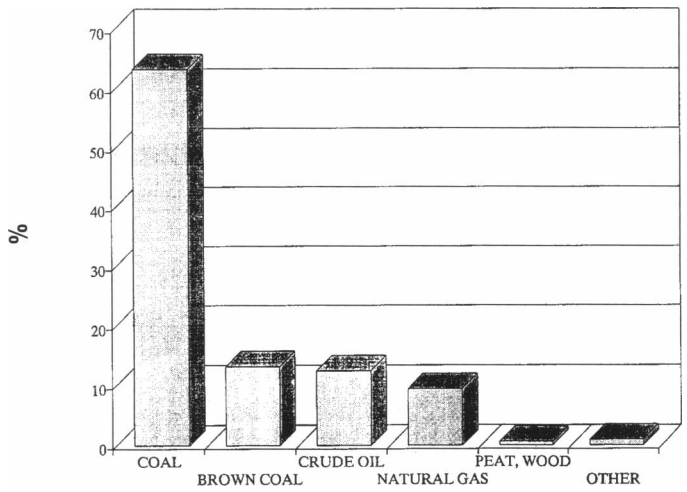

Figure 4. Relative consumption of fuel in Poland, 1990 (*2*).

The concentration of B[*a*]P estimated by GC was controlled in selected points for a period of 3 years. Distinct seasonal variations in the concentration of B[*a*]P were noticed: up to eight times higher concentrations of B[*a*]P in winter than in summer were found for all measuring points. An example of seasonal variations for two industrial towns in Silesia is shown on Figure 5*A* and 5*B*. Similar winter/summer variations for PAHs (factor of 3–5 times) were noticed in Sweden (*11*).

We suppose that this phenomenon could be explained by intensive use of coal for heating in the winter, but additional factors also should be considered (e.g., obvious differences in the meteorological parameters, temperature, humidity, intensity of sun radiation, etc.). In Ruda Slaska, a town belonging to the core agglomeration in Silesia, a gradual decrease of BP concentration in 1988, 1989, and 1990 (Figure 5B) may have resulted from the combined effect of decreasing industrial activities and elimination of some antiquated factories (e.g., cokery) in the area.

In general, the quality of air in Silesia is extremely bad, especially in the agglomeration of towns in the central industrial region, which is inhabited by over two million people. Any component in gaseous, vapor, or particulate phases of ambient air found in Silesia considerably exceeds the levels found, for example, in American or in European cities (*11,12*). Yearly average of B[*a*]P concentrations at many measuring points in Silesia exceed by a factor of 3–5 the concentration of B[*a*]P found in a room of 36 m^3 with a single air change per hour after smoking 40 cigarettes during an 8 hr day (*13*). Although components other than B[*a*]P and PAHs in tobacco smoke (e.g., nitrosonicotine) could be responsible for biological effect (e.g., lung cancer), this illustrates a dramatic burden of air pollution to humans in Silesia compared to the risk of smoking.

Biological Effects of Airborne Pollutants

Mutagenic activity of pollutants was tested in strains TA98 and TA100 of Salmonella with or without rat liver S9 microsomal fraction. Winter samples (from 20 measuring points) exhibited higher mutagenic activity than did summer samples. This is especially evident for direct mutagens detected by strain TA100. However, summer samples contained high levels of indirect mutagens, which, after enzymatic activation with S9 mix, showed mutagenic potency comparable with winter nonacti-

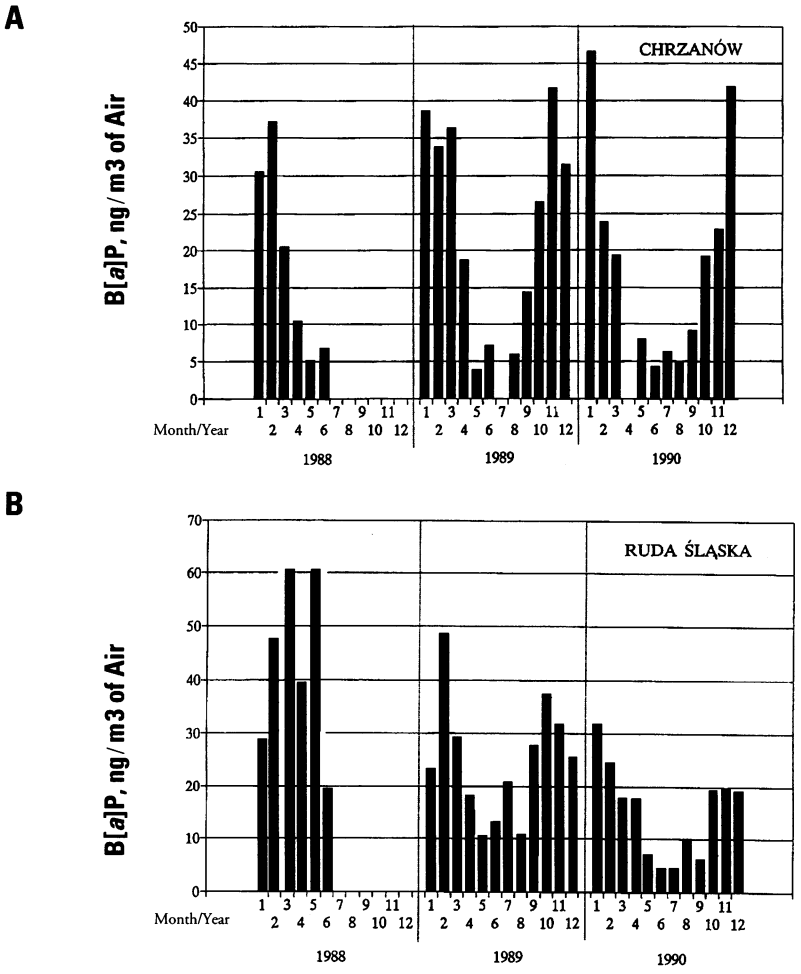

Figure 5A, B. Benzo[*a*]pyrene (B[*a*]P) concentrations in two industrialized towns in Upper Silesia: Chrzanów (*A*), and Ruda Ślaska (*B*). Note distinct seasonal variation in B[*a*]P concentration. B[*a*]P was determined by gas chromatography.

vated (*14*). Out of the eight SESC fractions, only fractions 2, 3, 4, 5, and 6 showed distinct mutagenic activity (*7,15*). The activation of SESC fractions with S9 mix was pronounced for fraction 2 (PAHs) for summer samples and to a lesser extent for winter samples, which showed toxic effects in Salmonella after enzymatic activation.

Observed levels of mutagenicity detected by strain TA98 was several-fold higher with Silesian samples as compared to samples from Sweden tested with the same strain (*11*).

Total extract induced the formation of micronuclei in polychromatic erythrocytes in bone marrow of BALB/c mice. Both crude extract and SESC fraction 2 in a course of 48-hr or 36-hr after intraperitoneal injection induced the formation of micronuclei (*16*). Various structural (*7*) and numerical (*17*) aberrations were observed in metaphase chromosomes of exponentially growing V79 cells exposed either to crude total extract of particulates or to SESC fractions 2, 3, 4, and 5 (*17*). Aberrations included endoreduplications and chromosomal and chromatid gaps, chromatid breaks, chromatid interchange, dicentrics, and rings. The clastogenic effects of both crude extract and the SESC fractions were reflected by higher numbers of structural aberrations of metaphase chromosomes. Endoreduplication was characteristic for SESC fraction 3 after 24-hr of exposure. The clastogenic effect was more pronounced with prolonged time of treatment (*15*).

Crude extract and SESC fractions (2,3, and 4 comprising about 67% of the total extract by weight) caused a decrease of the anaphase-telephase to metaphase (AT/M) ratio in V79 cells. Chromosomes arrested at metaphase were more compact and formed irregular metaphase plates. In recovery experiments of cells arrested at metaphase, significant numerical chromosomal aberrations were found (*17*).

Coke oven workers, the environmentally exposed group and control (rural area) group were studied for the level of sister chromatid exchange (SCE), chromosomal aberrations, and PAH-DNA adduct level in peripheral white blood cells. The environmentally exposed group had higher levels of SCE in cultured lymphocytes/cell as compared to the countryside control group. This was true both for matched smokers and nonsmokers, although the number of SCE in smokers in each group exceeded the SCE level as compared to non smokers (*18*). Chromosomal aberrations including gaps and a number of high-frequency cells (a subpopulation of lymphocytes with over 18 SCE/cell) were significantly higher in the environmentally exposed group. These data show that cytogenetic end points should be useful tools for evaluating of impact of environmental pollution exposure on human health.

The level of PAH-DNA adducts in the WBC showed interindividual variability both in coke-oven workers and in men environmentally exposed. The average level of adducts was highest in coke oven workers (*19,20*), but environmentally exposed men also exceeded significantly the level in a control group (*19,21*). Seasonal variation in the level of PAH-DNA adducts was noted in these three groups (*22*).

This progress report shows a serious burden of genetic damage imposed by environmental pollution in Silesia. Several described damages found in chromosomes and DNA could be directly related to cancer and possibly to other human malconditions. The possibility of transgeneration transmission of genetic damage (*23*) in Silesia cannot be excluded.

The cancer mortality maps in Silesia (B Zemla, unpublished data) for many cancer sites show an evident cluster type of distribution, with highest mortality indices in most highly polluted areas. Recent epidemiological studies aiming at finding the causal relation between air pollution and lung cancer (*24*) indicate an excessive risk for lung cancer posed by urban air pollution, with diesel exhaust being suspected as a cancer risk factor in urban areas (*25*). The highly complex nature of pollutants in Silesia presents a possible hazard also for pregnancies, newborns, and children.

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