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Immunological Effects of Environmental Exposure to NO_2 and NO. Results of Our Study

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Abstract

The immunological effects of environmental and/or occupational exposure to NO_2 and NO in air as polluting gases have been examined in groups of 16 men. The determination of NO_2 and NO concentrations in ambient air in the work enviroment as well as in ambient air in residential areas of these men was performed, always by use of an Amaya-Sugiura passive sampling spectrophotometric method. Mean concentration of NO_2 in ambient air in residential areas was 0.0210 mg x m^{-3} (0.0070 to 0.0470). NO_2 and NO mean concentrations in ambient air in the work enviroment were 0.0867 mg x m^{-3} (0.0165 to 0.1960) and 0.0614 mg x m^{-3} (0.0220 to 0.1090), respectively. For the determination of T-cell and (CD19+)B-cell populations Behring monoclonal antibodies were used in indirect immunofluorescence tests. The serum levels of immunoglobulins: G, A, M, E; C3c and C4 complement components; total circulating immunological complexes (CIC) as well as acute phase proteins: C-reactive protein (CRP), haptoglobin, ceruloplasmin and transferrin were determined by nephelometry. The stimulation T-cell line exposed to NO_2 and NO was evidenced by an increased number of (CD3+)T-cells, by about twice ($p < 0.001$) increased number of (CD4+)T-helper cells, and by an increased number of (CD8+)T-suppressor cells. The higher increase in count of (CD4+)T-helper cells than (CD8+)T-suppressor cells population caused an increased value of (CD+4)T-heIper/(CD8+)T-suppressor ratio by about 25% ($p < 0.01$) in the men exposed to NO_2 and NO. No changes were observed in the number of (CD19+)B-cells nor in the (CD3+)T/(CD8+)T-suppressor ratio. In men exposed to NO_2 and NO, elevation of IgG serum concentration by a 17.7% ($p < 0.01$) was evidenced as well as decreases of C3c by 18.6% ($p < 0.001$) and C4 by 35% ($p < 0.001$), whereas total CIC in serum was doubled ($p < 0.001$). Significant positive correlations between concentrations of NO_2 in air and numbers of total lymphocytes, (CD3+)-cells, (CD4+)T-helper, (CD8+)T-suppressor cells or IgG (/r/ from 0.31 to 0.71) as well as significant negative correlations between concentrations in air of NO_2 and IgE, C3c, CRP or haptoglobin (/r/ from -0.49 to -0.31) were calculated. Moreover, significant positive correlation between NO concentrations in air in work place and counts of (CD3+)T-, (CD8+)T-suppressor, (CD19+)B-cells and levels in serum of C4, haptoglobin and ceruloplasmin (A7 from 0.33 to 0.63) as well as significant negative correlations between NO concentrations in air in work place and serum levels of IgG, IgA and IgM (/r/ from -0.67 to -0.47) were also observed. In conclusion, environmental exposure to NO_2 and NO can modify in the peripheral blood of humans the parameters of cell-mediated and/or humoral immunity.

Keywords: Nitrous dioxide (NO_2), nitric oxide (NO), environment, occupational exposure, immunity

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Introduction

The NO/ozone system plays a key role in the chemistry of the atmosphere, particularly in terms of the formation of dinitrogen pentoxide and its reaction with water to form nitric acid. Increases in the atmospheric concentration of nitric oxide, an important trace gas, have evoked concern because of its role in stratospheric ozone level regulation, greenhouse warming, and acid rain formation. Three major contributors to atmospheric NO_X were identified: industrial sources, motor vehicles, and soil emissions. The data revealed that most of the NO_X emitted into the atmosphere was emitted as nitric oxide. Nitric oxide is produced in soils by nitrification, denitrification and the oxidation of ammonium to nitrate and nitrite, plus other ways, including automobile or power plant combustion [1, 2, 3, 4].

About 80-90% of inspired nitrogen dioxide is absorbed in humans and is excreted in urine as nitrate. Also about 80-90% of inhaled nitric oxide is absorbed in humans and rats, and most of the gas enters the blood unreacted [5]. Vasodilation is a sensitive target mechanism for NO action and this effect is evident *in vitro* at nanomolar or subnanomolar concentrations. A 5 ppm dose is established for acute vascular effects in animals and 10 ppm can be treated as the corresponding value for effects in humans [6]. Pharmacological and toxicologic research on effects of human and animal exposure to nitric oxide via inhalation are still being conducted [6]. Short-term inhalation of gaseous NO has been reported to decrease pulmonary artery pressure and improve oxygen transfer in patients with pulmonary hypertension [7, 8, 9].

Nitric oxide is well known not only as a toxic molecule and pollutant but at concentrations of aroud 10^{-8} mol x 1^{-1} it plays fundamental roles in physiology and biochemistry. Endogenous nitric oxide is synthesized from the amino acid L-arginine by constitutive and inducible NO syntheses. Formation of NO in the body is essential for control and regulation of the cardiovascular system (vasodilatation, regulation of myocardial perfusion, ischemia-reperfusion, hypertension-hypotension, atherosclerosis, platelet adhesion), defence against foreign cells (cell-cell communication within the immune system) as well as in the nervous system as a central and peripheral neuronal messenger and memory formation [10, 11, 12, 13, 14]. However, chronic pathological production of NO in the body may be a risk of carcinogenic cell transformation, because of the ability of NO to nitrosylate nucleic acids and break DNA strands and tissue damage after increased formation of the peroxynitrite (ONOO) radical [15, 16].

The aim of this study was to assess changes in the peripheral blood of men with environmental exposure to NO_2 and NO some of cell-mediated and humoral immunity parameters as well as correlations between air concentrations of these gases in residental and work places and values of determined parameters.

Material and Methods

The study was carried out in 16 males, aged 27 to 57 years old (X = 36.8 ± SD = 9.9) with occupational exposure to NO_2 and NO from 7 to 38 years (X =16.3 + 9.8). The subjects worked in nitric acid production in three 8 h shift work. Of these, 11 (69%) were smokers.

The control group comprised 36 males aged 28 - 55 years (X = 46.0 ± 8.7) not exposed to any chemical compounds or harmful physical influences; 29 (81%) were smokers. They worked also in three-shift system in the same plant but without occupational exposure to nitric compounds. All workers were subjected to medical examination for ruling out those with diseases which might have affected their immune status. Alcoholics, and those regularly taking drugs and convalescents after infectious diseases were disqualified.

The determination of NO_2 and NO concentrations in ambient air in the work enviroment as well as in ambient air in the residential areas of these men was performed, always by use of an Amaya-Sugiura passive sampling spectrophotometric method of nitrogen dioxide determination in ambient air with triethanolamine as absorbent after reaction with Saltzman reagent and in Krochmal et al. modification [17, 18, 19, 20, 21].

Blood samples for determining immunology and parameters were obtained from the veins during the morning hours to avoid external contamination. For the determination of T-cell and (CD19+)B-cell populations Behring (Behringwerke AG Diagnostica, Marburg, West Germany) monoclonal antibodies were used in indirect immunofluorescence tests. Heparinized venous blood was diluted 1 + 1 with normal saline, and overlaid on Ficoll/Paque (Pharmacia LKB Biotechnology, USA). This two-phase system was centrifuged at 400 g for 30 minutes at 18-20°C. The lymphocytes from the interphase were washed twice

Table 1. Effects of exposure to NO2 and NO in the population of leucocytes, total lymphocytes and T-cells.

Group			Leucocytes ($\times 10^9$/l)	Lymphocytes			
				Total		(CD3+) T-cells	
				a.n.	%	a.n.	%
Control	(C)	$\bar{X}$ =	6.47	2.05	33.0	1.33	64.7
n = 36		± SD =	1.59	0.56	7.0	0.43	8.5
Exposed to NO_2 and NO	(I)			XXX	XXX	XXX	X
n = 16		$\bar{X}$ =	7.30	2.96	41.1	2.00	67.4
		± SD =	1.80	0.77	6.6	0.57	6.8

a.n. - absolute number (x 10" cells/1), n - number of exposed men
Statistical significance: I:C: X - $p < 0.05$; XXX - $p < 0.001$

Table 2. Effects of exposure to NO_2 and NO in the population of T-cells (T-helper and T-suppressor), (CD16+)NK-cells and T-helper/T-suppressor cells ratio.

Group		Lymphocytes						CD3+/ /CD8+	CD4+/ /CD8+
		(CD4+) T-		(CD8+) T-		(CD16+) NK			
		a.n.	%	a.n.	%	a.n.	%		
Control (C)	$\bar{X}$ =	0.84	41.30	0.47	22.7	0.26	12.7	2.96	1.90
n = 36	± SD =	0.30	7.84	0.18	4.9	0.14	3.1	0.73	0.60
Exposed to NO_2 and NO (I)		XXX	XXX	XXX		X	XXX		XX
n = 16	$\bar{X}$ =	1.78	59.60	0.78	26.5	0.18	5.8	2.68	2.37
	± SD =	0.60	10.40	0.28	6.5	0.14	3.7	0.75	0.68

a.n. - absolute number (x 10^9 cells/1), n - number of exposed men
Statistical significance: I:C: X - $p < 0.05$; XXX - $p < 0.001$

with normal saline to remove platelets, and suspended for obtaining a concentration of 5 x 10^6 lymphocytes per cm^{-3}. (CD3+)T-cells were determined using monoclonal antibodies from Behring BMA 030. (CD4+)T-helper were found by means of monoclonal antibodies from Behring BMA 040, (CD8+)T-suppressor cells - Behring BMA 081, (CD16+)NK-cells Behring BMA 070 and (CD19+)B-cells monoclonal antibodies Behring BMA 0130 were used, always following the suggestions of the producer (Behring). A 50 mm^{-3} volume of cell suspension was incubated for 30 min at +4°C with 10 mm^{-3} of proper monoclonal antibodies. Then, after washing the cells with phosphate buffered saline (PBS) and sodium azide (pH 7.6) (according to Pharmacia, USA), antiimmunoglobulin rabbit serum labelled with fluorescein isothiocyanate (Behring) was added. After 30 min of incubation at +4°C the cells were washed with PBS and after supernatant removal they were suspended in 0.1 cm^{-3} of PBS. The percentage proportions of T-cell subpopulations were calculated with a fluorescence microscope (VEB Carl Zeiss Jena, E. Germany). The absolute number of CD 3+, CD 4+, CD 8+ and CD 16+ lymphocytes was calculated from the total white blood cell count (determined by chamber method), and from the differential white blood cell count. The serum levels of immunoglobulins: G, A, M, E; C3c and C4 complement components; total circulating immunological complexes (CIC); as well as acute phase proteins (C-reactive protein - CRP, haptoglobin, ceruloplasmin and transferrin) were determined by nephelometry (Nephelometer type 100, Behring, Germany), all with using standards, controls and sera of Behring, according to producer's instructions.

The obtained results were subjected to normal distribution analysis by Shapiro-Wilk Gaussian decomposition test. Thereafter, the results indicating normal distribution, without or after logarithmic conversion, were subjected to statistical analysis by Cochran-Cox C test or Student's *t* test. Analysis of variance (ANOVA) amongst comparison between the exposed groups were also calculated. In addition, the correlation coefficients /r/ were calculated between the analyzed immunological parameters and the NO2 or NO concentrations in ambient air in the work and also in the residental environments as well as time-weighted average NO_2 concentrations.

Results

The investigated group of 16 men exposed to NO_2 and NO and also men of the control group were regarded as healthy. The socioeconomic status of all workers in this study was similar. Air analyses in ambient air in residential areas have demonstrated that mean concentration of NO_2 was 0.0210 ± 0.0125 mg x m^{-3} (in range 0.007 to 0.047). NO_2 and NO mean concentrations in ambient air in the work enviroment were 0.0867 ± 0.0585 mg x m^{-3} (in range 0.0165 to 0.1960) and 0.0614 ± 0.0263 mg x m^{-3} (in range 0.0220 to 0.1090) respectively. The time weighted averages calculated for both residential and occupational exposures to NO_2 was about 0.043 ± 0.026 mg m^{-3} . Moreover, the degree of residental and occupational exposures to SO_2 (expressed as concentrations of SO2) was in the range 0.21 to 2.73 mgx m^{-3} (X = 0.6875 ± 0.6369) and 0.390 to 1.985 mg x m^{-3} (X = 1.196 ± 0.588), respectively and were lower and significantly different ($p < 0.05$) than in the residental and occupational enviroments of subjects working in sulphuric acid production.

Table 3. Effects of exposure to NO_2 and NO in the population of leucocytes, total lymphocytes and (CD 19+) B-cells.

Group		Leucocytes (× 10^9/1)	Lymphocytes			
			Total		(CD19+) B-cells	
			a.n.	%	a.n.	%
Control (C)	$\bar{X}$ =	6.47	2.05	33.0	0.17	8.30
n = 36	± SD =	1.59	0.56	7.0	0.10	2.51
Exposed to NO_2 and NO (I)			XXX	XXX		XX
n = 16	$\bar{X}$ =	7.30	2.96	41.1	0.17	5.84
	± SD =	1.80	0.77	6.6	0.10	2.86

a.n. - absolute number (x 10 cells/1), n - number of exposed men
Statistical significance: I:C: XX - $p < 0.01$; XXX - $p < 0.001$

Table 4. Effects of exposure to NO_2 and NO in the concentration of serum immunoglobulins G, A, M (g/1) and E (IU/ml).

Group	IgG	IgA	IgM	IgE
Control (C) $\bar{X}$ =	11.92	2.33	1.77	< 100
n = 36 ± SD =	2.92	0.78	0.71	
Exposed to (I)	XX			
NO_2 and NO $\bar{X}$ =	14.03	2.30	1.65	< 100
n = 16 ± SD =	2.48	0.67	0.63	

n - number of exposed men
Statistical significance: I:C: XX - $p < 0.01$

The number of total lymphocytes in men exposed to NO_2 and NO was increased by a 44% ($p < 0.001$) (Tab.l). Stimulation T-cell line in men exposed to NO_2 and NO was evidenced by a 50% ($p < 0.001$) increased number of (CD3+)T-cells, by about twice ($p < 0.001$) increased number of (CD4+)T-helper cells and by 66% ($p < 0.001$) increased number of (CD8+)T-suppressor cells (Tab.2). The higher increase in count of (CD4+)T-helper cells than (CD8+)T-suppressor cells population caused the increased value of the (CD+4)T-helper/(CD8+)T-suppressor ratio by about 25% ($p < 0.01$) in the men exposed to NO_2 and NO. In contrast, decreased number of (CD16+)NK cells by about twice ($p < 0.001$) in men exposed to NO_2 and NO was also observed. No changes were observed in the number of (CD19+)B-cells as well as in **the** (CD3+)T/(CD8+)T-suppressor ratio (Tab. 2 and Tab. 3). In the group exposed to NO_2 and NO elevation of IgG serum concentration by a 17.7% ($p < 0.01$) was evidenced as a well as a decrease of C3c by 18.6% ($p < 0.001$) and C4 by 35% ($p < 0.001$), whereas total CIC in serum was elevated by about two times ($p < 0.001$). Also, mean concentration of haptoglobin in serum was elevated in men exposed by about 50% ($p < 0.001$). No changes were observed in the serum levels of IgA, IgM, IgE as well as CRP, ceruloplasmin and transferrin.

Significant positive correlations between concentrations of NO_2 in air and numbers of total lymphocytes, (CD3+)-cells, (CD4+)T-helper, (CD8+)T-suppressor cells or IgG (A7 in range: 0.31 to 0.71) as well as significant negative correlations between concentrations in air of NO_2 and IgE, C3c, CRP or haptoglobin (/r/ in range: -0.49 to -0.31) were calculated (Tab. 6). Moreover, a significant positive correlation between NO concentrations in air in the work place and counts of (CD3+)T-, (CD8+)T-suppressor, (CD19+)B-cells and levels in serum of C4, haptoglobin and ceruloplasmin (/r/ in range: 0.33 to 0.63) as well as significant negative correlations between NO concentrations in air in work place and serum levels of IgG, IgA and IgM (/r/ in range: -0.67 to -0.47) were also observed (Tab. 6).

Discussion and Conclusions

The effects of toxic action of most air pollutants usually do not lead to specific diseases. But, depending on the pollutant, the concentration, and the duration of exposure, they affect some organs as mainly the mucous membranes and respiratory organs, which are more sensitivity to irritant gases. The consequences are more frequent inflammations, diminished lung function, increased susceptibility to respiratory infection, and a higher incidence of chronic bronchitis. These disorders and diseases are also influenced by other factors, such as immune deficiency, allergies or occupational exposure to pollutants [22, 23]. The mechanism of importance in sensitization and subsequent triggering by chemicals appears to be an epithelial release of mediators, chemotactic factors, the release of oxidants with increase activity of mononuclear cells, polymorphonuclear cells, eosinophils and can occur with immune complexes. Pulmonary alveolar macrophages are usually engaged in response to irritants and can release chemotactic factors and other mediators after stimulation by irritants [24].

In experimental models in mice, inflammation induced by single and repeated acute NO_2 exposure increases in lavage cells, protein, and cytotoxicity were observed. Acute exposures induced significant increases in lavageable macrophages, epithelial cells, and polymorphonuclear leukocytes [25]. Also, in rats exposed to 14.4 ppm of NO_2 and 0.8 ppm of ozone for 6 h/d, 7 d/week mast cells were present in increased numbers and epithelial cell alterations correlated with inflammatory and fibrotic changes over the treatment period [26]. In studies of children, a significant relationship was observed between traffic-related NO_2 and the prevalence of asthma and symptoms [27].

Nitric oxide may play a key role not only as vasodilatator but also in cell to cell communications within the immune system. Safety and the effects of inhaled nitric oxide (NO) in human studies was evaluated mainly in children with persistent pulmonary hypertension of the newborn or in patients with acute respiratory distress syndrome [9,28]. Immune effects of NO inhalation in humans, especially during long-term occupational exposure to NO, has not been studied so far.

Table 5. Effects of exposure to NO_2 and NO in the concentration of C3c and C4 (g/l), CIC (μg/l), CRP (g/1), haptoglobin, ceruloplasmin and transferrin (g/1) in serum.

Group	C3c	C4	CIC	CRP	Haptoglobin	Ceruloplasmin	Transferrin
Control (C) $\bar{X}$ =	97.3	37.2	0.90	< 0.01	153.0	27.3	285.9
n = 36 ± SD =	12.8	8.7	0.97		40.0	4.1	85.6
Exposed to NO_2 and NO (I)	XXX	XXX	XXX		XXX		
$\bar{X}$ =	79.0	24.0	2.97	< 0.01	231.0	27.0	288.0
n = 16 ± SD =	18.0	8.0	1.72		102.0	4.0	68.0

n - number of exposed men
Statistical significance: I:C: XXX - $p < 0.001$

Table 6. Correlation coefficients /r/ between parameters of immunity and: acute phase proteins immunity and
A. Concentrations of NO_2 (mg • m^{-3}) in ambient air in the residential place,
B. Concentrations of NO_2 (mg • m^{-3}) in ambient air in the work place,
C. Time-weighted average concentrations of NO2 (mg • m^{-3}) during 24 h of exposure
D. Concentrations of NO (mg • m^{-3}) in ambient air in the work place

/r/	WBC a.n.	Lymphocytes a.n.	Lymphocytes %	CD3+ a.n.	CD3+ %	CD4+ a.n.	CD4+ %	CD8+ a.n.	CD8+ %	CD3+/ /CD8+	CD4+/ /CD8+	CD16+ a.n.	CD16+ %
A	-0.35	-0.12	0.28	0.06	0.48	0.18	0.44	-0.07	0.07	0.08	0.28	0.01	0.19
B	-0.14	0.31	0.71	0.46	0.51	0.42	0.28	0.46	0.34	-0.18	-0.17	0.20	0.17
C	-0.23	0.21	0.67	0.38	0.57	0.39	0.36	0.34	0.31	-0.12	-0.07	0.16	0.19
D	0.19	0.06	-0.03	0.11	0.33	0.05	0.09	0.19	0.37	-0.30	-0.32	-0.16	-0.18

/r/	CD19+ a.n.	CD19+ %	IgG (g/l)	IgA (g/l)	IgM (g/l)	IgE (IU/ml)	C3c (g/l)	C4 (g/l)	CIC (µg/ml)	CRP (g/l)	Haptoglobin (g/l)	Transferrin (g/l)	Ceruloplasmin (g/l)
A	-0.26	-0.20	0.34	-0.11	-0.06	-0.35	-0.43	0.09	-0.17	-0.31	-0.42	-0.11	-0.16
B	0.09	0.05	0.45	0.12	0.10	-0.39	-0.44	-0.19	0.13	-0.21	-0.31	-0.03	-0.26
C	0	-0.01	0.46	0.06	0.06	-0.42	-0.49	-0.12	0.05	-0.27	-0.37	-0.06	-0.27
D	0.51	0.63	-0.47	-0.67	-0.54	-0.30	0.15	0.63	-0.39	0.30	0.37	0.04	0.58

The results of our present study have indicated a stimulatory effect of exposure to NO_2 and NO of the peripheral blood T-cell populations with elevation of IgG serum, total CIC, and haptoglobin levels, and decreases of C3c and C4 levels. The values of high positive correlation coefficients /r/ between NO_2 concentrations in the ambient air and parameters of cell-mediated immunity and IgG have also indicated on stimulation by NO_2 this type of immunity. Moreover, the values /r/ between NO_2 concentrations in the ambient air and IgG in range from 0.34 to 0.46 with exposure to NO_2 -dependent decrease of C3c have probably been connected with the proinflammatory action of NO_2. In contrast, the correlation coefficients /r/ between concentrations NO in the ambient air and determined parameters of immunity, (CD19+)B-cells and immunoglobulins can indicate on affect of absorbed egzogenous NO mainly on humoral immunity, alone or together with endogenous NO produced during the inflammatory proces. In conclusion, environmental exposure to NO_2 and NO can, in humans, modify the parameters of cell-mediated and/or humoral immunity.

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References

1. BADR O., PROBERT S.D. Appl. Energy **44,** 197, **1993.**
2. United Nations Environmental Programme, Environmental Data Report, 1995/1996, The Alden Press, Oxford, **1997.**
3. The Environment in Europe: A Global Perspective, National Institute of Public Health and Environmental Protection, RIVM, Bilthoven, **1992.**
4. ROSSI N.J. Pollut. Eng. **27,** 50, **1995.**
5. EWETZ L. Scand. J. Work. Environ. Health, **19,** 21, **1993.**
6. GUSTAFSSON L.E. Scand. J. Work. Environ. Health **19,** 44, **1993.**
7. BODY S.C., HARTIGAN P.M. Resp. Care Clin. N. Am. **3, 411, 1997.**
8. AIMASHEVA N.P., ZENINA T.A., MANUKHINA E.B., MIKOIAN V.D., KUBRINA L.N., VANIN A.F., MALYSHEV **I.Iu.** Izv. Akad Nauk Ser. Biol. **5,** 634, **1997.**
9. DELLINGER R.P. ZIMMERMAN J.L. TAYLOR R.W., STRAUBE R.C., HAUSNER D.L., CRINER G.J., DAVIS Jr K., HYERS T.M., PAPADAKOS P. Crit. Care Med. **26,** 15, **1998.**
10. MONCADA S., PALMER R.M.J., HIGGS E.A. Pharmacol. Rev. **43,** 109, **1991.**
11. FEELISCH M., OSTROWSKI J., NOACK E. J. Cardiovasc. Pharmacol. **14,** 13, **1989.**
12. GRYGLEWSKI R.J., BOTTING R.M., VANE J.R. Hyperten sion **12,** 530, **1988.**
13. GRYGLEWSKI R.J. Tromb. Haemorrh. Disorders **1/2, 1, 1990.**
14. DRAPIER J.C. Nitric oxide: brain and immune systems. Ed. MONCADA S., NISTICO G., HIGGS E.A. (red.). Portland Press, London & Chapel Hill, pp 205-212, **1993.**
15. KONOREV E.A., TARPEY M.M., JOSEPH J., BAKER J.E., KALYANARAMAN B. Free Radic. Biol. Med. **18,** 169, **1995.**
16. BECKMAN J.S., BECKMAN T.W., CHEN J., MARSHALL P.A., FREEMAN B.A. Proc. Natl. Acad. Sci. USA **87,** 1620, **1990.**
17. KROCHMAL D., KALINA A. Environ. Pollution **96,** 401, **1997.**
18. KROCHMAL D., KALINA A. Atmospheric Environ. **31,** 3473, **1997.**
19. KROCHMAL D., GORSKI L. Fresenius J. Anal. Chem. **340,** 220, **1991.**
20. KROCHMAL D. International Conference "EURO-ECO 97", Krakow, AGH, pp.55-59, **1997.**
21. BROWN **R.H.** Pure Appl. Chem. 65, 1859, **1993.**
22. WANNER H.U. Experientia **49,** 754, **1993.**
23. YANG S.C., YANG S.P. In: Health and Toxicology. Ed. Gulf Publishing Company, Houston, USA, pp.47-52, **1997.**

24. BYLIN O., HEDENSTIERNA G., LINDVALL T., SUNDIN B. Eur. Resp. J. 1, 606, 1988.
25. KLEEBERGER S.R., LIU-YI Z., JAKOB G.J. Inhal. Toxicol. 9,601, 1997.
26. FARMAN C.A., PINKERTON K.E., WITSCHI H., LAST J.A. Inhal. Toxicol. 9, 647, 1997.
27. STUDNICKA M., HACKLE., PISCHINGER J., FANGMEYER C, HASCHKE N., KUHR J., URBANEK R., NEUMANN M., FRISCHER T. Eur. Resp. J. 10, 2275, 1997.
28. FUJIKAWA S., YANG L., WAFFARN F., LERNER M. J. Am. Acad. Audiol. 8, 263, 1997.