RESEARCH COMMUNICATION

CYP1A1 Inducing Potential of Airborne Particulate Extracts Collected during a 25-year Period (1975-2000)

Chikako Kiyohara[1], Shigekatsu Sakai[2], Tomoko Akutagawa[2], Yutaka Matsumoto[3]

Abstract

Samples of airborne particles from Sapporo, the capital of Hokkaido, Japan, were collected between 1975 and 2000. Major polycyclic aromatic hydrocarbons (PAHs) included in the extracts of airborne particles were investigated for their mutagenicity and potential for inducing drug-metabolizing enzyme cytochrome P450 (CYP)1A1, which is considered to be responsible for the activation of PAHs in airborne particle extracts, as well as in cigarette smoke, to carcinogens and is associated with risk of several cancers. There was a dose-related increase in CYP1A1 activity in human lymphoblastoid cells after exposure to airborne particulates containing PAHs. The mutagenicity of the airborne particles collected in summer was lowest and for those collected in spring was lower than in autumn or winter. Likewise, the winter sample had the strongest CYP1A1 inducing potential while the summer sample had the weakest. CYP1A1 inducing potency was strongly related to the amount of benzo(k)fluorathene (Spearman's rank correlation coefficient (γ) = 0.97), benzo[a]pyrene γ = 0.96), benzo[g,h,i]perylene (γ = 0.94), benz[a]anthracene (γ = 0.93), chrysene (γ = 0.93) in the extracts during the 25-year period, while the enzyme activity was measurably related to the amount of pyrene (γ = 0.64) and fluorathene (γ = 0.54). During the 25-year period, CYP1A1 inducing potential decreased every year together with a decrease in PAHs in the airborne particle extracts. CYP1A1 inducing potential may be one of the most convenient biomarkers with which to estimate the overall carcinogenicity/mutagenicity of airborne particle extracts.

Key Words: CYP1A1 - airborne particles - polycyclic aromatic hydrocarbons - *Salmonella*/microsome assay

Asian Pacific J Cancer Prev, **6**, 153-158

Introduction

Airborne particles may constitute a health risk to man. For example, diesel emissions have been shown to contribute to the incidence of lung cancer and have been classified as probably carcinogenic to humans (IARC monograph, 1988). Short-term tests for genotoxicity has shown not only diesel exhaust, but also particles from different outdoor and indoor sources be genotoxic. These particles include those derived from industrial sources, smoking, open fires and other heating processes and more specific sources like rubber and waste incineration (Heussen et al., 1991).

Polycyclic aromatic hydrocarbons (PAHs) that are released into the atmosphere may have health consequences that can be compounded by their nitro-PAH atmospheric transformation products. The available literature suggests that some of the atmospheric nitro-PAH daughter products, such as 2-nitrofluorene and 4-nitropyrene, may increase the overall environmental health risk associated with PAHs (Moller et al., 1993; Atkinson and Arey, 1994; Yaffe et al., 2001). Therefore, an important issue is whether there is merit in considering atmospheric transformation products of air toxins when conducting environmental health-risk analyses. To illustrate the above issue, a comparative analysis of the potential risk that may be imposed by PAHs should be carried out using appropriate samples.

CYP1A1 is a membrane-bound monooxygenase system located in most tissues of the body. In mice, CYP1A1 inducibility (CYP1A1 activity in 3-methylcholanthrene (MC)-treated mice/CYP1A1 activity in olive oil (vehicle)-treated mice) is under the control of the Ah locus, and certain inbred strains of mice are susceptible to CYP1A1 induction by MC treatment (Ah responsive strains), whereas other strains are not (Ah non-responsive strains). A strong

[1]Department of Preventive Medicine, Division of Social Medicine, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan [2]Hokkaido Research Institute for Environmental Sciences, North 19, West 12, Kita-ku, Sapporo 060-0819, Japan [3]Department of Public Health, Graduate School of Medicine, Hokkaido University, North 15, West 7, Kita-ku, Sapporo 060-8638, Japan All correspondence should be addressed to: Dr. Chikako Kiyohara, Department of Preventive Medicine, Division of Social Medicine, Graduate School of Medical Sciences, Kyushu University, Maidashi 3-1-1, Higashi-ku, Fukuoka 812-8582, Japan Tel: 81 (0)92 642 6113 Fax: 81 (0)92 642 6115 E-mail: chikako@phealth.med.kyushu-u.ac.jp

correlation was observed between CYP1A1 inducibility and tumor incidence in mice (Kouri, 1973; Kouri et al., 1974). Also human studies suggested that higher CYP1A1 inducing potency has shown an increased risk of lung cancer (Kellermann et al., 1973; Kiyohara et al., 1998). Since CYP1A1 is considered to be responsible in humans for the activation of benzo[a]pyrene (B[a]P) and other PAHs in airborne particles, such as cigarette smoke, to carcinogens, it may also be important in not only the causation of lung cancer but also poor health conditions.

To evaluate the usefulness of CYP1A1 inducing potency as a convenient biomarker, we examined the relationship among seven PAHs (benzo[a]pyrene, benzo[g,h,i]perylene, benz[a]anthracene, chrysene, benzo[k]fluoranthene, pyrene and fluoranthene) and long-term changes of CYP1A1 inducing potency and the amount of individual PAHs during the 25-year period using airborne particle extracts collected between 1975 and 2000 in Sapporo, the capital of Hokkaido, Japan.

Materials and Methods

Sample Collection and Preparation

Samples of airborne particulate matter were colleted approximately 10 m above ground level on the roof of the laboratory, which is situated in a residential area about 2 km northwest of the center of Sapporo. About 4500 vehicles per day passed near the laboratory. High-volume samples of 24-hour periods were collected on a glass- or quartz-fiber every day for 5-15 continuous days per month. The filters were tightly wrapped with aluminum foil and stored at -20 ^{o}C. Each filter sample, corresponding to 1500-2400 m^3 of air, was combined on a quarterly basis for each year. Each combined sample was extracted with dichloromethane. Dissolved in acetone, the extracted samples in acetone were stored at -20 ^{o}C. Details were shown elsewhere (Matsumato et al., 1998).

PAH Analysis

Analyses of B[a]P and six PAHs were performed by high-pressure liquid chromatography with a fluorescence detector (Matsumoto et al., 1998). The six other PAHs measured were: benzo[g,h,i]perylene (B[g,h,i]P), benz[a]anthracene (B[a]A), chrysene (Chr), benzo[k]fluoranthene (B[k]F), pyrene (Pyr) and fluoranthene (Flu).

Assay of CYP1A1 Activity

Human lymphoblastoid cells were established by the method described by Katsuki and Hinuma (1976) and cultured in RPMI-1640 medium containing 20% heat-inactivated fetal bovine serum; penicillin, 100 IU/ml; and streptomycin, 100 μg/ml. The cells were seeded at a density of approximately 3 x 10^5 cells/ml and the cultures were grown at 37 ^{o}C in an atmosphere of fully humidified air with 5% CO_2. Ten μl of airborne particulate extracts (10 m^3/μl in acetone) was added to the cell culture (to obtain induced CYP1A1 activity). In a control culture (to measure the non-induced CYP1A1 activity), the solvent (acetone) alone was added (10 μl/10 ml culture medium). For positive control of induction of CYP1A1 activity, 10 μl of 3-methylcholanthrene (MC) in acetone was added to the cell culture (to obtain the MC-induced CYP1A1 activity), yielding a final concentration of 2.5 μM. Incubation was continued for an additional 48 hours. Cells from the culture flasks with a viability of 90% or more were harvested and washed twice with 0.05 M Tris-HCl buffer supplemented with 0.2 M sucrose and 3 mM $MgCl_2$. CYP1A1 activity was determined as follows. Reaction mixture (total volume 1.0 ml) consisted of the above-mentioned Tris-HCl buffer (pH8.5), NADPH (1.7 mM), NADH (1.3 mM), 2-4 x 10^6 viable B[a]P (0.1 mM) as a substrate in 50 μl of acetone (Kiyohara et al., 1990). The incubation was carried out at 37 ^{o}C for 50 min. The reaction was stopped by addition of 1.0 ml of ice-cold acetone, and then the mixture in each tube was extracted with 3.5 ml of n-hexane. The organic phase was extracted with 1.0 ml of 1N NaOH. CYP1A1 activity was measured with fluorescence of the NaOH layer and expressed as pmol equivalents of 3-hydroxy B[a]P formed per min per 10^6 viable cells. Each assay was performed 4 times and the quadruplicate results normally varied less than 5%. CYP1A1 inducing potency (treated/non-treated), which is under genetic control, was calculated to wipe out the inter-interindividual or inter-experimental fluctuations.

Mutagenicity Test

Mutagenicity of the samples was examined by the preincubation procedure of the Ames *Salmonella* mutagenicity assay (Yahagi et al., 1977). Each sample was assayed twice. Assays were performed with *Salmonella typhimurium* TA98 and TA100 (kindly supplied by Dr B.N. Ames, University of California, Berkeley, CA). Details were shown elsewhere (Matsumoto et al., 1998).

Statistical Analysis

Spearman's rank correlation test was used to analyze the correlation among the concentrations of PAHs. The correlation between the CYP1A1 inducing potency and the concentrations of PAHs was also evaluated by Spearman's rank correlation analysis. All the statistical analyses were performed with the computer program STATA Version 8 (STATA Corporation, College Station, TX). All *P* values are two-sided and *P* values less than 0.05 were considered statistically significant.

Results

The effects of different concentrations of airborne particle extract collected in 1992 on the CYP1A1 activity were studied in three lymphoblastoid cell lines (three different subjects). Concentrations tested ranged from the extracts of 25 m^3 to 500 m^3 air of 10 ml of culture medium and at none of concentrations was there any evidence of toxicity in the cells, as indicated by cell viability (usually over 90%).

Table 1. Dose-response Relationship Between CYP1A1 Activity and Airborne Particle Extracts Collected in 1992

	Cell line 1	Cell line 2	Cell line 3
0	0.033 ± 0.003	0.015 ± 0.001	0.020 ± 0.002
25 m^3	0.068 ± 0.007	0.043 ± 0.003	0.034 ± 0.004
50 m^3	0.093 ± 0.011	0.070 ± 0.005	0.050 ± 0.007
100 m^3	0.150 ± 0.009	0.128 ± 0.009	0.083 ± 0.010
200 m^3	0.260 ± 0.018	0.237 ± 0.015	0.145 ± 0.013
500 m^3	0.134 ± 0.042	0.312 ± 0.031	0.266 ± 0.024
6.7 μg MC	0.109 ± 0.007	0.077 ± 0.004	0.050 ± 0.009
37.5 pg TCDD	0.223 ± 0.015	0.156 ± 0.010	0.102 ± 0.011

Each value represents the mean ± S.E. of 4 experiments and is expressed in terms of 3-hydroxy-B[a]P (pmol/min/10^6 cells) formed.

For comparison, the cells were treated with either 37.5 pg of 2,3,7,8-tetrachlorinated-*p*-dibenzo dioxin (TCDD) or 6.7 μg of 3-methylcholanthrene (MC). CYP1A1 activity increased with increased the air volume up to 200 m^3 (Table 1), although three cell lines had different slopes of the dose-response relationship curve. The cells were treated with the extract from 100 m^3 of air, unless states otherwise. A similar inducing potency was observed between 37.5 pg of TCDD and the extract from 100 m^3 of air. Likewise, an inducing potency of 6.7 μg of MC was a similar to that of the extract from 50 m^3 of air.

Relative seasonal change of CYP1A1 inducibility and mutagenicity of the samples of 1992 were shown in Table 2. As compared with the mutagenicity of the extract sample collected in spring, that in summer was lower and that in autumn or winter was higher. There was no difference on seasonal change between indirect and direct mutagenicity or TA98 and TA100 strain. As for CYP1A1 inducibility, the winter sample had the strongest inducing potency of CYP1A1 while the summer sample had the weakest. Relative seasonal change was similar between the mutagenicity and the CYP1A1 inducing potency.

Spearman's rank correlation coefficients of the concentrations of seven PAHs with CYP1A1 inducibility or with the mutagenicity during the 25-year period were shown in Table 3. B[k]F, B[a]P, B[g,h,i]P, B[a]A and Chr were strongly related to CYP1A1 inducing potency. Pyr and Flu were moderately correlated with CYP1A1 inducing potency. The mutagenic activity in TA98 without S9 was not related to either PAHs concentration while that in TA100 without S9 was moderately, significantly related. The mutagenic activities in both TA98 and TA100 with S9 were positively and significantly correlated with PAHs concentrations.

Table 4 presents correlation coefficients of the concentrations of seven PAHs during the 25-year period. Concentrations of these PAHs showed a strong and significant correlation to each other. Particularly, B[a]P,

Table 2. Seasonal Change of CYP1A1 Inducing Potency and PAH Mutagenicity of the Samples of 1992

	Spring	Summer	Autumn	Winter
CYP1A1 inducing potency[a]	5.8[b] (100)	4.8 (83)	7.3 (126)	9.3 (160*)
TA98 (-S9)	12 (100)	7.5 (63*)	16 (133)	25 (210*)
TA98 (+S9)	15 (100)	7.5 (50*)	20 (133)	28 (187*)
TA100 (-S9)	14 (100)	7.5 (54*)	19 (136)	33 (236*)
TA100 (+S9)	21 (100)	11 (54*)	28 (133)	47 (224*)

* P < 0.05

Values in parentheses indicate the relative change with Spring value defined as 100.

[a] Obtained from cell line 1 treated with the extract from 100 m^3 of air.

[b] Number of revertants/m^3 air

Each value represents the mean number of net revertants of duplicate plates or the mean CYP1A1 inducing potency based on 4 experiments.

Table 3. Correlation Coefficients of Concentrations of PAHs in Every Year's Sample with CYP1A1 Inducibility and Mutagenicity for the Entire Period (1975 - 2000)

Substances in the air (ng/m^3)	CYP1A1 inducing potency	TA98 -S9	TA98 +S9	TA100 -S9	TA100 +S9
Benzo[a]pyrene	0.96	0.30†	0.93	0.64	0.95
Benzo[g,h,i]perylene	0.94	0.21†	0.92	0.59*	0.90
Benz[a]anthracene	0.93	0.23†	0.91	0.65	0.94
Chrysene	0.93	0.25†	0.91	0.65	0.91
Benzo[k]fluoranthene	0.97	0.35†	0.93	0.66	0.96
Pyrene	0.64	0.12†	0.66	0.65	0.69
Fluoranthene	0.54*	0.24†	0.57*	0.63	0.60*

Spearman's rank correlation coefficients.

All correlation coefficients except for marked * or † shown in the Table are significant at P < 0.0005. *P < 0.005 † Not significant

Table 4. Rank Corri\ation Coefficients of Concentrations of Seven PAHs in Air Samples (ng/m^3) for the Entire Period (1975 - 2000)

	Benzo [g,h,i]perylene	Benz [a]anthracene	Chrysene	Benzo [k]fluoranthene	Pyrene	Fluoranthene
Benzo[a]pyrene	0.98	0.98	0.98	1.00	0.71	0.55**
Benzo[g,h,i]perylene		0.97	0.97	0.97	0.69	0.53*
Benz[a]anthracene			1.00	0.98	0.79	0.66
Chrysene				0.98	0.80	0.65
Benzo[k]fluoranthene					0.71	0.58**
Pyrene						0.90

Spearman's rank correlation coefficients.

All correlation coefficients except for those marked * or ** shown in the Table are significant at P < 0.0005. *P < 0.01 **P < 0.005

Table 5. Average Change per Year by Seven PAHs and CYP1A1 Inducing Potency for the Entire Period (1975 - 2000)

PAHs	1975 (ng/m^3)	Change (%)	95% confidence interval
Benzo[a]pyrene	4.53	-9.0	-9.6 to -8.5*
Benzo[g,h,i]perylene	4.64	-9.7	-10.9 to -8.4*
Benz[a]anthracene	2.88	-10.8	-11.9 to -9.8
Chrysene	3.45	-12.5	-13.9 to-11.1
Benzo[k]fluorathene	2.89	-10.3	-11.1 to -9.5*
Pyrene	2.53	-9.7	-13.9 to -5.6
Fluoranthene	2.04	-9.7	-16.2 to -3.2
CYP1A1 inducibility	54.8	-9.9	-11.4 to -8.4

* Compared with chrysene, $P < 0.05$

B[g,h,i]P, B[a]A and B[k]F were strongly related to each other (Spearman's correlation coefficient (γ) > 0.97). Pyr and Flu were strongly correlated with each other ($\gamma = 0.90$) but were moderately related to the other five PAHs.

The time trend of the concentrations of seven PAHs and CYP1A1 inducing potency between 1975 and 2000 was shown in Table 5. Each PAH concentration showed a statistically significant decrease of 9.0 - 12.5% every year. Among those, a decrease of 12.5% (95% confidence interval = 11.1 - 13.9) of the concentration of Chr was significantly larger than that of B[a]P, B[g,h,i]P and B[k]F. Also a significant decrease of 9.9% of CYP1A1 inducing potency was observed.

Discussion

Human exposure to PAHs and their derivatives from inhalation of ambient air varies according to the degree of urbanization. The genotoxic potencies and the potential health risks of urban air pollutants, however, are different between cities because of the variation in air pollution sources. Emissions from traffic and domestic heating may be important sources of PAHs and their derivatives. Many research studies have indicated the mutagenic activity of air pollutants in some short-term bioassays (De Martinis et al., 1999; Lewtas et al., 1990; Nishioka and Lewtas, 1992) or carcinogenic activity in cell and animal experiments (Pott and Stober, 1983; Brune et al., 1978). Epidemiological investigations over the last 50 years suggest rather consistently that ambient air pollution may be responsible for increased risk of lung cancer (Nyberg et al., 2000; Katsouyanni and Pershagen, 1997; Cohen and Pope, 1995). The impact of air pollution on health has been studied in many cities throughout the world. The World Health Organization considers air pollution one of the environmental exposure factors that may affect human health (Maciel et al., 1999).

The *Salmonella*/microsome assay has made it possible to assesss the mutagenic activity of these extracts, made from environment exposures which contain variety of compounds including PAHs and PAH derivatives. These organic compounds may originate from combustion processes associated with industrial activities and especially from the incomplete burning of fuels of automotive vehicle emissions in urban and industrial areas (Crebelli et al., 1995; DeMarini et al., 1995). CYP1A1 is a component of the microsomal mixed function oxidase system dependent upon NADPH and CYP. The CYP gene is up-regulated (induced) by PAHs, 2,3,7,8-TCDD and so on. As the inducibility of CYP1A1 increases, so does the metabolism of procarcinogenic PAHs to reactive carcinogenic intermediates; enhanced metabolism often leads to a higher risk of malignancies in mice (Nebert and Jones, 1989). CYP1A1 inducing potency may be an appropriate indicator used for health-related environmental surveillance. B[a]P and other PAHs are suspected to cause cancers, including cancer of the human lung. Especially, B[a]P has been widely used over the past 40 years as an indicator of lung cancer risk because of its strong carcinogenicity (Menichini et al., 1999; Pott, 1983).

A close correlation among the concentrations of B[a]P, B[g,h,i]P, B[a]A, Chr and B[k]F were shown in Table 4. Many studies indicated that B[a]P is an indicator of the secular change of total PAHs (Air Quality of Guidelines for Europe, 2000). As shown in Table 3, the CYP1A1 inducing potency was strongly related with B[a]P during the 25-year period. The CYP1A1 inducing potency may be an excellent overall indicator, from which we could identfy the long-term trend of total PAHs in the air.

CYP1A1 inducing potency in each year was strongly related with the amount of B[a]P, B[g,h,i]P, B[a]A, Chr and B[k]F in the year air sample, while the enzyme activity was not so strongly related to the amount of Pyr and Flu. B[a]P concentration in the air has been decreasing every year together with a decrease of other PAHs (Table 5). The amount of each PAH in the airborne particulates showed a significant decrease of 9.0-12.5% every year and CYP1A1 inducing potency reflected this. These results suggest that the seven PAHs investigated may be representative of unmeasured PAHs, as unmeasured PAHs may also induce CYP1A1 enzyme. It may be that unmeasured PAHs at least not have been increasing.

Table 2 contributes to the evaluation of mutagenic activity of the organic fraction extracted from airborne particulate matter as a measure indicating risk to human health during annual seasonal conditions resulting in different CYP1A1 inducing potency. It should be stressed that the samples that presented lower mean CYP1A1 activity in summer had a lower percentage of extracted organic matter. In general, the mutagenicity was observed high in the autumn-winter period and low in spring-summer period in the northern hemisphere (Cassoni et al., 2004; Binkova et al., 2003). This was mainly caused by the influence of heating systems.

Various indirect-acting mutagens/carcinogens, which are catalyzed by various drug-metabolizing enzymes, as well as various direct-acting mutagens/carcinogens, exist in the airborne particles. Many of the enzymes involved in the activation/detoxification of PAHs, nitro-PAHs and aromatic and heterocyclic amines are polymorphic. The activation of

PAHs and nitro-PAHs, present in diesel exhaust is catalyzed by CYP isozymes resulting in formation of reactive diol epoxides, which can form DNA and protein adducts. The results of Table 5 indicated that there may be no synergistic effect of combined PAHs on CYP1A1 activity. Metabolites of PAHs are inactivated by conjugation with glutathione, glucuronide or sulphate. Nitro-PAHs, especially dinitro-PAHs, are among the most mutagenic compounds in the *Salmonella*/microsome assay and some of them are also potent carcinogens. Another metabolic activation pathway for nitro-PAHs is reduction of the nitro-group by nitro-reductase to produce reactive N-hydroxyarylamine intermediates, which in the case of dinitro-pyrene are O-acetylated by acetyltransferase to reactive N-acetoxy arylamines (Djuric et al., 1985). Other CYPs or metabolic enzymes including phase I enzymes other than CYPs and phase II enzymes should be applied to our samples.

Another class of potent mutagenic compounds found in diesel exhaust and airborne particles is likely to be a key factor in contributing to human lung cancer. Diesel exhaust contains soot particles which are carriers of mutagenic and carcinogenic substances. The particles are deposited in the lungs when inhaling the exhaust fumes. During our study period (1975 - 2000), several changes occurred. The number of vehicles in the Sapporo area increased, particularly diesel-powered vehicles. The percentage of diesel-powered vehicles in Sapporo increased from less than 10% in 1975 to 32% in 1992 (Matsumoto et al., 1998). After the mid-1970s, stricter regulations on the sulfur content in fuel oil were imposed. Annual coal consumption in the Sapporo area based on delivery records, showed a large downward trend after the mid-1970s. It is likely that these changes have influenced the long-term changes in air pollution. In fact, the extract from the former airborne particulates (collected before 1977) had stronger CYP1A1 inducing potency of more than 20 than those from the later samples (enzyme inducing potency of 3.4 in 2000) (data not shown). The increase in the number of diesel-powered vehicles is thought to have led to an increase in the concentration of nitro-PAHs through both primary emission and secondary formations, and many of these compounds are direct mutagens which show mutagenic activity in the absence of S9 mix. There have been many studies on the -S9/+S9 ratio in diesel emission particles, all of which report a value of over 1 (Crebelli et al., 1991; Matsumoto et al., 1998). While the -S9/+S9 ratio has been reported to be more than 1 in diesel emission particles, smaller values have been reported for particles emitted from gasoline powered vehicles (Jones et al., 1985; Crebelli et al., 1991). We are now studying on the concentrations and the mutagenicity of nitro-PAHs.

In conclusion, the mutagenicity and CYP1A1 inducing potency of airborne particulate matter in Sapporo during the 25-year period (1975-2000) were investigated. CYP1A1 inducing potency in each year was strongly related to the amount of B[a]P and other PAHs in each year. As the proportion of total PAHs to total mutagens/carcinogens in the extracts from airborne particles might have been decreasing with time, CYP1A1 inducing potency has been decreasing with time. CYP1A1 inducing potency may be considered a simple and comprehensive indicator in the evaluation of total mutagenicity/carcinogenicity of some PAHs in the airborne particles. However, the increase in the number of diesel-powered may lead an increase in the concentration of nitro-PAHs. To establish a good quality monitoring system of both PAHs and nitro-PAHs, it may be useful to conduct epidemiological studies of the impact of air pollution on the health of the population and for the use of decision makers in their efforts to improve our environment.

Acknowledgements

This study was funded in part by a grant from the Smoking Research Foundation, Japan.

References

Air Quality Guidelines for Europe (2000). WHO Regional Office for Europe, European Series No. 91, Copenhagen, pp. 92-96.

Atkinson R, Arey, J (1994). Atmospheric chemistry of gas-phase polycyclic aromatic hydrocarbons: formation of atmospheric mutagens. *Environ Health Perspect*, **102 (Suppl 4)**, 117-26.

Binkova B, Cerna M, Pastorkova A, et al (2003). Biological activities of organic compounds adsorbed onto ambient air particles: comparison between the cities of Teplice and Prague during the summer and winter seasons 2000-2001. *Mutat Res*, **525**, 43-59.

Brune H, Habs M, Schmahl D (1978). The tumor-producing effect of automobile exhaust condensate and fractions thereof. Part II: animal studies. *J Environ Pathol Toxicol*, **1**, 737-45.

Casson F, Bocchi C, Martino A, et al (2004). The Salmonella mutagenicity of urban airborne particulate matter (PM2.5) from eight sites of the Emilia-Romagna regional monitoring network (Italy). *Sci Total Environ*, **324**, 79-90.

Cohen AJ, Pope CA, 3rd (1995). Lung cancer and air pollution. *Environ Health Perspect*, **103 (Suppl 8)**, 219-24.

Crebelli R, Fuselli S, Conti G, et al (1991). Mutagenicity spectra in bacterial strains of airborne and engine exhaust particulate extracts. *Mutat Res*, **261**, 237-48.

Crebelli R, Conti L, Crochi B, et al (1995). The effect of fuel composition on the mutagenicity of diesel engine exhaust. *Mutat Res*, **346**, 167-72.

De Martinis BS, Kado NY, de Carvalho LR, et al (1999). Genotoxicity of fractionated organic material in airborne particles from Sao Paulo, Brazil. *Mutat Res*, **446**, 83-94.

DeMarini DM, Shelton ML, Stankowski LF Jr (1995). Mutation spectra in Salmonella of sunlight, white fluorescent light, and light from tanning salon beds: induction of tandem mutations and role of DNA repair. *Mutat Res*, **327**, 131-49.

Djuric Z, Fifer EK, Beland FA (1985). Acetyl coenzyme A-dependent binding of carcinogenic and mutagenic dinitropyrenes to DNA. *Carcinogenesis*, **6**, 941-4.

Heussen GA (1991). Inhibition of intercellular communication by airborne particulate matter. *Arch Toxicol*, **65**, 252-6.

IARC (1988) IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Vol. 46, Diesel and Gasoline Engine Exhausts and Some Nitroarenes, Lyon.

Jones E, Richold M, May JH, Saje A (1985). The assessment of

the mutagenic potential of vehicle engine exhaust in the Ames Salmonella assay using a direct exposure method. *Mutat Res*, **155**, 35-40.

Katsouyanni K, Pershagen G (1997). Ambient air pollution exposure and cancer. *Cancer Causes Control*, **8**, 284-91.

Katsuki T, Hinuma Y (1976). A quantitative analysis of the susceptibility of human leukocytes to transformation by Epstein-Barr virus. *Int J Cancer*, **18**, 7-13.

Kellermann G, Shaw CR, Luyten-Kellerman M (1973). Aryl hydrocarbon hydroxylase inducibility and bronchogenic carcinoma. *N Engl J Med*, **289**, 934-7.

Kiyohara C, Mohri N, Hirohata T, et al (1990). In vitro effect of methylsulfonyl polychlorinated biphenyls and 7,8-benzoflavone on aryl hydrocarbon hydroxylase activity in human lymphoblastoid cells. *Pharmacol Toxicol*, **66**, 273-6.

Kiyohara C, Nakanishi Y, Inutsuka S, et al (1998). The relationship between CYP1A1 aryl hydrocarbon hydroxylase activity and lung cancer in a Japanese population. *Pharmacogenetics*, **8**, 315-23.

Kouri RE, Salero RE, Whitmire CE (1973). Relationships between aryl hydrocarbon hydroxylase inducibility and suscepitibility to chemically induced subcutaneous sarcoma in various strains of mice. *J Natl Cancer Inst*, **50**, 363-8.

Kouri RE, Ratire H, Whitmire C (1974). Genetic control of susceptibility to 3-methylcholanthrene-induced subcutaneous sarcoma. *Int J Cancer*, **13**, 714-20.

Lewtas J, Chuang J, Nishioka M, Peterson B (1990). Bioassay-directed fractionation of the organic extract of SRM1649 urban air particulate matter. *Int J Environ Anal Chem*, **39**, 245-54.

Maciel AA, G_es CD, Cancio JA, et al (1999). Health environmental surveillance indicators. *Informe Epidemiol_gico do SUS Bras*, **8**, 59-67.

Matsumoto Y, Sakai S, Kato T, et al (1998). Long-term trends of particulate mutagenic activity in the atmosphere of Sapporo. 1. Determination of mutagenic activity by the conventional tester strains TA98 and TA100 during an 18-year period (1974-1992). *Environ Sci Technol*, **32**, 2665-71.

Menichini E, Monfredini F, Merli F (1999). The temporal variability of the profile of carcinogenic polycyclic aromatic hydrocarbons in urban air: a study in a medium traffic area in Rome, 1993-1998. *Atmos Environ*, **33**, 3739-50.

Moller L, Lax I, Eriksson LC (1993). Nitrated polycyclic aromatic hydrocarbons: a risk assessment for the urban citizen. *Environ Health Perspect*, **101 (Suppl 3)**, 309-15.

Nebert D, Jones JE (1989). Regulation of the mammalian cytochrome P-1450 (CYP1A1) gene. *Int J Biochem*, **21**, 234-52.

Nishioka MG, Letas J (1992). Quantification of nitro and hydroxylated nitro-aromatic/polycyclic aromatic hydrocarbons in selected ambient air daytime ainter samples. *Atoms Environ*, **26**, 2077-87.

Nyberg F, Gustavsson P, Jarup L, et al (2000). Urban air pollution and lung cancer in Stockholm. *Epidemiology*, **11**, 487-95.

Pott F (1983). Environmental contamination by PAH-air. In, Environmental Carcinogens: Polycyclic Aromatic Hydrocarbons'Ed Grimm G. CRS Press, Boca Ratonm FL, USA, pp84-101.

Pott F, Stober W (1983). Carcinogenicity of airborne combustion products observed in subcutaneous tissue and lungs of laboratory rodents. *Environ Health Perspec*, **47**, 293-303.

Yaffe D, Cohen Y, Arey J, Grosovsky AJ (2001). Multimedia analysis of PAHs and nitro-PAH daughter products in the Los Angeles Basin. *Risk Anal*, **21**, 275-94.

Yahagi T, Nagao M, Seino Y, et al (1977). Mutagenicities of N-nitrosamines on Salmonella. *Mutat Res*, **48**, 121-9.