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MONITORING OF URBAN AIR POLLUTION BY MUTAGENICITY ASSAYS

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Summary. - *Urban air particulate extracts were assayed for mutagenicity in* Salmonella typhimurium *strain TA98 and in the nitroreductase deficient derivatives TA98NR and TA98/1,8DNP$_6$. The results obtained indicate a low contribution of nitropyrenes from diesel exhausts to air particulate mutagenicity in the city of Rome. Fractionation of extracts into acidic, basic and neutral components showed that neutral compounds account for about two-thirds of the total mutagenic activity. No significant mutagenic activity was detected in body fluids of rodents treated with airborne particulate extracts nor in the micronucleus test in mice. Basic and neutral fractions of air particulate extracts proved to inhibit liver aminopyrine-N-demethylase in the mouse.*

Riassunto (Mutagenicità del particellato atmosferico urbano). - *L'estratto organico del particolato aereo della città di Roma è stato saggiato per l'attività mutagena con il ceppo di* Salmonella typhimurium *TA98 e con i derivati nitroreduttasi deficienti TA98NR e TA98/1,8DNP$_6$. I risultati ottenuti dimostrano che i nitropireni presenti nelle emissioni diesel non danno un contributo significativo alla mutagenicità del particolato aereo urbano. Il frazionamento dell'estratto organico in componenti acida, neutra e basica ha dimostrato che composti della frazione neutra rendono conto per circa due terzi dell'attività totale. La somministrazione dell'estratto organico del particolato a roditori non ha determinato alcuna rilevabile mutagenicità nei fluidi biologici né effetti mutageni (micronuclei) in cellule di midollo osseo ma ha inibito significativamente l'attività della aminopirina-N-demetilasi epatica.*

Introduction

Several epidemiological studies demonstrate a higher incidence of lung cancer in urban than in rural areas [1, 2]. This increased risk has been related to the exposure to volatile genotoxic compounds, mostly originating from the combustion of fossil fuels. Many well-known carcinogenic agents (e.g. benzo(a)pyrene, benzo(a)anthracene, nitropyrenes, etc.) have been identified among the variety of organic compounds adsorbed onto airborne particulate matter collected in polluted urban areas [3]. Accordingly, benzene extracts of urban air particulate matter have been known for decades to be carcinogenic in mammals [4, 5]. However, to what extent exposure to air pollutants may pose a significant human hazard, in comparison for example to smoking habits and occupational exposures, is still debated [6]. The evaluation of the health impact of air pollution is in fact an extremely difficult task. Hundreds of chemical compounds were identified in the organic material vehicolated by airborne particulate matter: therefore, in the case of such mixed concurrent exposure, synergistic or antagonistic interactions among carcinogens, cocarcinogens, promoters and inhibitors may occur in an unpredictable way.

Furthermore, for a proper evaluation of the genotoxic risks related to airborne particulate exposure, additional problems have to be taken into account, such as the undefined biological availability of the organic compounds adsorbed on particulate matter and the possible overestimation of their genotoxic potency due to the extraordinary mutagenic activity exerted in bacteria by some nitro compounds present as minor components in air particulate extracts [7].

We have recently completed a study on air particulate matter to address some of these questions, namely a) the relative contribution of nitro compounds to urban air mutagenicity, b) the biological availability of the adsorbed organic matter, c) the in *vivo* mutagenicity of particulate extracts and d) the effects of air particulate extracts on liver enzymes activities. The results of this work are summarized and briefly discussed in this paper.

Materials and methods

Sampling and samples preparation

Airborne particulate matter of inhalable size was collected by a high-volume cascade impactor located outside the Istituto Superiore di Sanità, Rome. The organic phase of particulate matter collected on glass fiber filters was

Table 1. - *Collection of air particulate matter in the urban area of Rome: weight values*

Period covered by sampling	Duration of sampling (days)	Air volumes (m^3)	Particulate collected (g)	Organic fraction (g)	Organic matter (%)	Particulate ($\mu g/m^3$)	Organic fraction ($\mu g/m^3$)
23/01/86-14/02/86	14	18.790	2.790	0.560	20	152	31
01/04/86-12/05/86	20	23.240	3.158	0.460	15	136	20
23/06/86-19/07/86	22	25.400	3.977	0.364	9	156	14

Soxhlet-extracted with dichloromethane. One half of the organic extract was fractionated into acidic, neutral and basic components. Serum extraction of mutagenic compounds from the particulate matter was performed by incubating glass fiber filters with calf serum at 37 °C with shaking for 24-96 h. Mutagenic components were concentrated from serum by chromatography on XAD-2 resin.

Mutagenicity assays

The Salmonella/microsome plate incorporation assay was performed as described by Maron and Ames [8] with aroclor induced rat liver S9 for metabolic activation. Mutagenic potencies were calculated from the linear portion of the dose response curve. The micronucleus test was performed according to the recommended EPA protocol [9] with male and female Swiss mice.

Collection and processing of body fluids

Airborne particulate extracts were dissolved in olive oil and administered by gastric intubation or intraperitoneal (i.p.) injection to male Sprague-Dawley rats housed in metabolic cages. Excreta were collected in the dark on solid CO_2. Blood samples were obtained by ventricular puncture. Mutagenic compounds from urines and faeces were concentrated by XAD-2 chromatography (urines) and diethyl ether extraction (faeces).

Experiments on liver microsomal monooxygenases

Airborne particulate extracts were administered in dimethylsulphoxide by i.p. injection to male Swiss mice. Aniline hydroxylase (AH) and aminopyrine-N-demethylase (APND) activities were determined in the 9,000 *g* supernatant of liver homogenates [10]. In *in vitro* assays, the 9,000 *g* liver supernatants of untreated Swiss mice and Sprague-Dawley rats were centrifuged at 100,000 *g* to obtain the microsomal fraction. Cytochrome P-450 content after incubation with test materials was determined as described by Mazel [10].

Results and discussion

Sampling of air particulate matter was performed over 2-3 weeks periods in winter, spring and summer 1986. Data on weight values and air concentration of particulate matter and organic compounds are shown in Table 1. The concentration of airborne particles was not strongly dependent on seasonal variations whereas a significant decrease in the amount of organic matter was observed. This trend was paralleled by a reduction in the air concentration of mutagens (Fig. 1) which was due to the concurrent decrease in the content of organic matter, rather than to the lower mutagenicity of organic extracts of spring and summer samples. These data demonstrate a similar or lower concentration of airborne mutagens in the city of Rome with respect to other European [11] and American [12] densely inhabited areas. Furthermore, the comparison of the results obtained in this study with *S. typhimurium* strains TA98 (highly sensitive to the mutagenic activity of all nitro compounds), TA98NR (resistant to 1-nitropyrene) and TA98/1,8DNP$_6$ (resistant to all nitro and dinitropyrenes) suggests that nitropyrenes of diesel origin [7, 13] only play a limited role in the mutagenicity of air samples of the city of Rome.

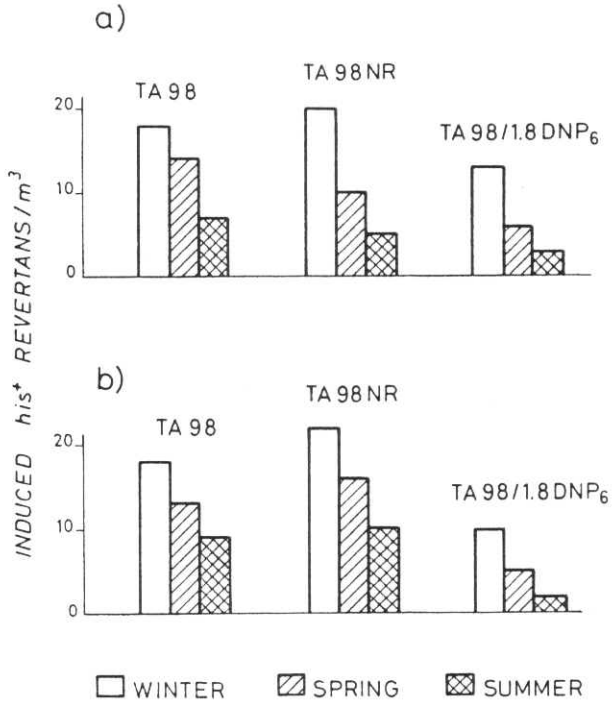

Fig. 1. - Mutagenic activity of airborne particulate extracts in *S. typhimurium* strains. Number of induced *his*$^+$ revertants/m^3 of samples air. (a) without S9; (b) with S9.

Table 2. - *Mutagenic activity of acidic, basic and neutral components of air particulate extracts in* S. typhimurium *strains*

Sample	Fraction	Strain: S9 Mix	TA98		TA98NR		TA98/1,8DNP$_6$	
			-	+	-	+	-	+
Winter	acidic		433 *(a)*	455	248	317	113	99
	neutral		511	589	719	820	474	366
	basic		ns	1,584	ns	1,892	ns	ns
Spring	acidic		788	809	358	551	123	93
	neutral		662	713	769	966	578	358
	basic		ns	462	ns	333	ns	ns
Summer	acidic		324	267	129	143	110	67
	neutral		557	624	345	431	188	123
	basic		ns	450	ns	385	ns	ns

(a) number of induced *his*$^+$ revertants/mg, calculated from the linear portion of the dose-effect relationship; ns: no significant mutagenic activity detected

Fractionation of air particulate extracts into acidic, basic and neutral components did not show large seasonal variations in weight values: the acidic fraction accounted for 21% of the whole extractable matter (range 16.8-25.2 %), the neutral one for 75.1% (range 73.0-77.3) and the basic one for 3.9% (range 1.8-5.9%). The specific mutagenic potency (number of induced *his*$^+$ revertants/mg) was determined for each fraction in *S. typhimurium* strains TA98, TA98NR and TA98/1,8DNP$_6$ in the presence and in the absence of exogenous metabolic activation (Table 2). Roughly, neutral compounds accounted for two-thirds of the observed activities, whereas the basic and, to a higher degree, acidic fractions contributed the remainder. Directly-acting mutagens were detected in acidic and neutral fractions, where aliphatic and polycyclic nitro compounds are expected. Mutagenic agents present in the basic fraction required exogenous metabolic activation and did not revert strain TA98/1,8DNP$_6$, strongly suggesting the involvement of aromatic amines or related compounds [14].

To assess the biological availability of the organic matter adsorbed onto air particles, serum extraction of mutagenic components was attempted. Only a minor fraction (about 7%) of the mutagenic activity exerted by dichloromethane extracts was observed in serum extracts of air particulate matter, suggesting limited availability.

To get further information on the *in vivo* fate of airborne organic matter, body-fluids mutagenicity was determined in Sprague-Dawley rats after oral administration and i.p. injection of dichloromethane extracts of air particulate. Urine concentrates, plasma samples and faecal extracts of animals treated at 80 mg/kg body weight were ineffective in bacterial mutagenicity assays. Taking into account dosage administered and the specific *in vitro* mutagenicity of the administered material, it can be calculated that the concentration of active/activable mutagens in body-fluids was below 2% of the administered dose in urines and faeces and below 5% in serum. Therefore the rapid and total disappearance of mutagens in body fluids of treated animals suggests an extensive *in vivo* inactivation, although liver postmitochondrial fractions (S9) were unable to reduce significantly the mutagenicity *in vitro*.

To gather further data on the biological consequences of the *in vivo* exposure to airborne particulate matter, the induction of micronuclei in mouse polychromatic erythrocytes and the effects on liver microsomal enzymes were studied.

No increase in the frequency of micronucleated polychromatic erythrocytes was observed in Swiss mice administered by i.p. injection with 200 and 400 mg/kg of particulate extracts, although toxicity to bone marrow, as shown by the decreased frequency of polychromatic erythrocytes, was observed.

Administration of extracts of winter and spring samples of air particulate (25 mg/kg, by i.p. injection) caused a significant ($p < 0.01$) decrease in aminopyrine-N-demethylase (APND) activity in mouse liver. Furthermore, a significant decrease in APND and AH (aniline hydroxylase) activities were observed after administration of neutral components, whereas the basic fraction only affected the APND activity. *In vitro* experiments suggest a direct interaction of test materials with microsomal cytochrome P-450, independently on the presence of NADPH in the incubation mixture.

In summary, the results of this study allow the following conclusions: a) nitro and dinitropyrenes, the most important genotoxic species in diesel exhaust, only play a limited role in the mutagenicity of airborne material in the city of Rome; b) most of the genotoxic organic compounds adsorbed onto air particulate matter are poorly available and quickly metabolized *in vivo* into inactive derivatives.

Consequently, they show no or low mutagenic activity in *in vivo* assays; c) the synergistic or antagonistic interaction of airborne materials with other xenobiotics should be taken into account. Inhibition of liver microsomal enzymes for example can modulate the (geno)toxic risks related to occupational exposure or life style.

Reliable estimation of the risks for humans related to air pollution therefore could be more adequately provided by *in vivo* experimental systems able to take into account the complex interactions and metabolic/pharmacokinetic features of genotoxicants vehicolated by airborne particles. On the other hand, due to the steadily increase in air pollution worldwide, this goal is at presence of primary importance.

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