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Hepatic enzyme induction and mutagenicity of airborne particulate matter from Santiago, Chile in the nourished and malnourished rat

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1. Respirable, airborne particles in the ambient air in downtown Santiago, Chile, have been characterized for the seasonal variation in total polycyclic aromatic hydrocarbon content, 13 of which have been identified including the mutagens (benzo(*a*)pyrene, dibenzo(*a,h*)anthracene, benzo(*a*)anthracene, benzo(*b*)fluoranthene and indeno(1,2,3, *c,d*)pyrene amongst others.

2. Organic extracts derived from these particles were administered to both the nourished and malnourished rat and resulted in modulation of the hepatic mixed function oxidase system including induction of NADPH-cytochrome P450 reductase, cytochrome P4501A as determined by Western blot analysis and the associated ethoxyresorufin *O*-deethylase and aryl hydrocarbon hydroxylase activities.

3. The cytochrome P4504A1-dependent 12-hydroxylation of lauric acid was induced in the malnourished state, but this activity was significantly inhibited by treatment of the animals with particle extracts in both nutritional states.

4. The particle extracts contained both direct and indirect-acting mutagens in the Ames test, and depending on the relative complement of both, resulted in either increased or decreased mutagenicity in the presence of S9 activation systems derived from both nourished and malnourished animals.

5. These results are discussed in the context of the interindividual risk assessment of airborne, particulate matter to man.

Introduction

Environmental pollution and its impact on human health is an ever growing problem in both developed and under-developed countries, and epidemiological studies have implicated the importance of urban air pollution in the aetiology of human respiratory diseases, including cancer (Doll 1978, Perera *et al*. 1993). Of particular concern is the adsorption of combustion-derived polycyclic aromatic hydrocarbons (PAH) to respirable airborne particles ($< 10\ \mu$m) as these ubiquitous environmental contaminants are well known to be metabolized by the cytochrome P450-dependent mixed-function oxidase (MFO) system in various tissues, producing mutagenic and carcinogenic metabolites in experimental animal models (Epstein *et al*. 1992, Adonis and Gil 1993, Ioannides and Parke 1993). This concern for the human health effects of environmental particular matter is reflected in new proposals from the US Environmental Protection Agency to regulate smaller particles ($\leqslant 2.5\ \mu$m) for the first time and to restrict their concentrations to 15 $\mu g/m^3$ annually and 50 $\mu g/m^3$ daily (Editorial 1996, Reichhardt 1996).

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As the prevailing complement of tissue cytochrome P450s is crucial in dictating the toxicological response to many xenobiotics (Hodgson and Levi 1994), then factors which alter this complement are highly likely to alter the predisposition to xenobiotic-induced toxicity. In addition to the well-described induction of CYP1A enzymes by PAHs (Gonzalez 1993), nutritional status has become an increasingly recognized factor that can modulate MFO activity and hence toxicological responses to xenobiotics (Hietenan 1980, Gil *et al*. 1988, Gibson 1992). For example, Salazar *et al*. (1983) reported that rat liver benzo(*a*)pyrene hydroxylase activity was markedly reduced in protein energy malnutrition, but rapidly recovered after feeding malnourished rats with a high protein diet. In addition, the metabolism of endogenous substrates including testosterone and arachidonic acid (both metabolized by several different cytochrome P450s) are substantially influenced by the nutritional status of the animal (Gil *et al*. 1988, Orellana *et al*. 1989).

We have chosen to study the airborne particles in downtown Santiago, Chile, because the city is heavily polluted as a result of poor ventilation in a valley surrounded by the Andes, very little wind or rain, a high content of dust, particles and smoke, a high density of industry and poorly controlled vehicle exhaust emissions and a layer of trapping, thermal inversion (900 m), which can be as low as 150 m in the winter months (Adonis and Gil 1993). Our previous studies have examined the ability of airborne particle extracts in Santiago to modulate MFO activity in the rat (Quinones and Gil 1995). We now extend these studies to report the variation in PAH content in airborne particles from Santiago and to examine the influence of these particle extracts on MFO activities and mutagenicity. In light of the above nutritional effects on MFO activities, we additionally decided to examine both normal and malnourished animals.

Materials and methods

Chemicals

Benzo(*a*)pyrene (B{*a*}P), 3-hydroxy-benzo(*a*)pyrene, NADPH H^+, cytochrome c, and bovine serum albumin were purchased from Sigma (St Louis, MO, USA) and ethoxyresorufin and resorufin from Molecular Probes (Eugene, OR, USA). A polyclonal antibody to rat CYP1A (recognizing both 1A1 and 1A2) was kindly supplied by Dr Costas Ioannides, University of Surrey, UK.

Airborne particulate matter sampling and processing

Airborne-derived particulate matter (total particles) was collected in downtown Santiago (Plaza Italia) using a HighVol atmospheric sampler (General Metal Work, model GMW L-2000H) placed at a height of 2 m and between 1223 and 2481 m^3 air was filtered per day. Airborne particles were also collected in a similar manner from San Felipe, a rural area 75 km north-west of Santiago. The amount of particulate matter so collected was determined by weighing the filters before and after filtration. The glass fibre filters (Whatman GS-A) were removed every 24 h and stored in the dark at $-20\,^{\circ}C$. In order to avoid photodecomposition of labile materials, all of the following operations were performed under yellow light. Each filter was extracted with 100 ml dichloromethane (three times) under ultrasonic vibration (Brausonic 1510), the combined extracts filtered through Teflon filters to remove fibres and large particles (yielding particles of $\leqslant 10\,\mu m$) and reduced to 3 ml under vacuum. The resultant concentrate was analysed for PAH content by hplc and was used in Ames test assays and enzyme induction studies in the rat.

The PAH present in the particulate matter extracts were separated and quantitated by hplc as described by Gil and Adonis (1995). Briefly, the hplc system consisted of a Rheodyne injector, a Merck-Hitachi L-6200 intelligent pump, a Shimadzu variable wavelength UV detector (SPD2A) or a Waters fluorescence detector (430AC), and a Shimadzu Chromatopac integrator (CR4A). Chromatography and separation of PAH was carried out on a Supelcosil LC-PAH column (length 25 cm, diameter 4.6 mm). The solvent elution programme was initially for 2 min with water:acetonitrile (50:50) followed by a

linear gradient (water:acetonitrile, 50:50 to 100% acetonitrile over 16 min) and finally with 100% acetonitrile for 16 min. PAHs were detected by UV detection at 254 nm and by fluorescence detection (260–300 nm excitation and 385–455 nm emission, depending on the individual PAH). The identification of individual PAHs in the organic extracts was determined by comparison of retention times of samples versus authentic standards and by comparison of their diode array spectra (Merck-Hitachi model L4500). PAHs positively identified in this way included naphthalene, acenaphthene, acenaphthylene, fluorene, pyrene, benzo(*a*)anthracene, chrysene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)anthracene, benzo(*g,h,i*)perylene and indeno (1,2,3,*c,d*)pyrene.

Animals

Immature female Wistar rats (University of Chile Breeders) were used throughout and the following groups studied. Normal nourished group: after parturition, eight offspring from the same litter were nursed by one mother for 21 days and for the subsequent 14 days were fed a standard rat diet (Rodent Laboratory Chow, Kimber Co., Santiago, Chile) and water *ad libitum*. Malnourished group: protein energy malnutrition was induced by the following experimental model. After birth, 16 offspring from the same litter were nursed by one mother for 21 days and for the subsequent 14 days were fed a protein free diet, as previously described (Salazar *et al*. 1983).

Thirty-five-day-old control (90–100 g) and malnourished (20–22 g) rats were killed by decapitation 48 h after a single intraperitoneal injection of either the particulate extract (40 mg/kg) or benzo(*a*)pyrene (40 mg/kg), using corn oil as vehicle. Control animals (normal or malnourished) received a single dose of corn oil. After killing, the livers were removed, perfused with 10 mM Tris-HCl–40 mM NaCl (pH 8.0), weighed, homogenized in ice-cold 100 mM Tris-HCl–5 mM EDTA (pH 7.5) and microsomes isolated by differential ultracentrifugation (Ioannides and Parke 1993). Microsomes were resuspended in 10 mM Tris-HCl–5 mM EDTA (pH 7.5) to a concentration of 1–2 mg protein/ml, protein concentrations being determined by the method of Lowry *et al*. (1951) using bovine serum albumin as standard.

Enzyme assays

The amount of total microsomal cytochrome P450 was determined by difference spectra (Shimadzu UV+210A spectrophotometer) using the difference absorption coefficient (450–490 nm) of 91 mM^{-1} cm^{-1} (Omura and Sato 1964). NADPH-cytochrome P450 reductase activity was determined by the method of Williams and Kamin (1962) using cytochrome c as the electron acceptor and ethoxyresorufin *O*-deethylase and aryl hydrocarbon hydroxylase activities assayed by the fluorometric methods of Burke and Mayer (1974) and Nebert and Gelboin (1968) respectively on a Shimadzu RF-540 spectrofluorimeter. The omega (12)- and omega-1 (11)-hydroxylation of lauric acid was assayed by hplc as previously described (Sharma *et al*. 1988).

Western blotting of liver microsomes was carried out according to the method of Towbin *et al*. (1979) using a polyclonal antibody to CYP1A, the antibody recognizing both 1A1 and 1A2 (Rodrigues *et al*. 1987).

Mutagenicity assays

The *Salmonella* (TA98)/plate incorporation assay was performed as described by Maron and Ames (1983) in the absence/presence of an S9 fraction from male Wistar rats pretreated with Arochlor 1254 (Ames *et al*. 1975). The particle organic extracts were suspended in DMSO and tested at five concentrations (10–200 μg per plate) in triplicate at a S9 protein concentration of 1 mg/plate. Revertant colonies were scored after 72-h incubation at 37 °C and all assays contained positive controls (2-aminoanthracene and 2-nitrofluorene). Extracts of the filter blanks and solvent blanks (DMSO), with and without metabolic activation were tested as negative controls.

Results and discussion

One of the major problems in assessing the chemical composition and related biological activities of organic, airborne environmental xenobiotics is that there is almost a constantly varying flux of pollution input to the atmosphere, the trapping and retention of which is moderated by the prevailing climatic conditions. For example, figure 1 shows the monthly variation of total PAHs over a calendar year in downtown Santiago, peaking in the cold months (May–July, Southern hemisphere)

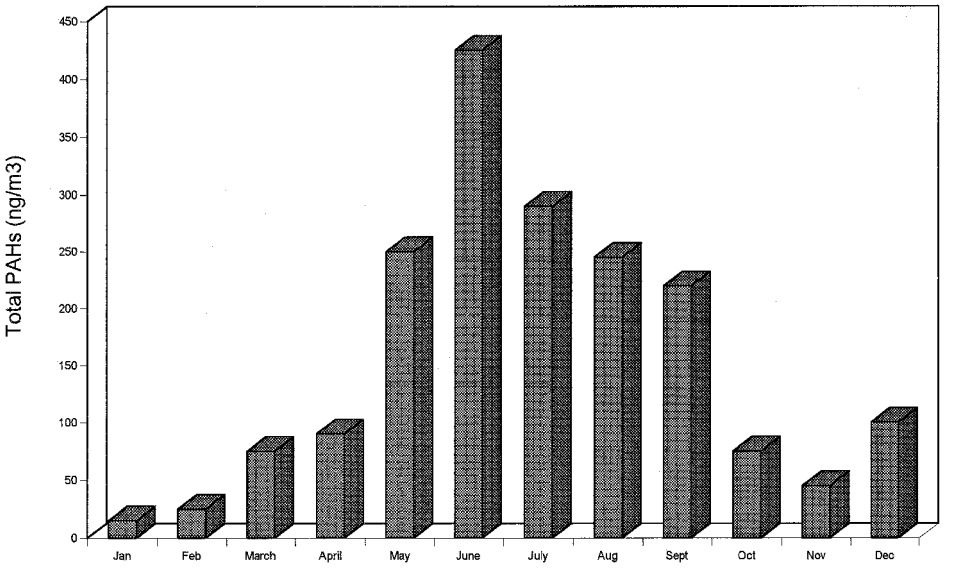

Figure 1. Monthly average of total polycyclic aromatic hydrocarbons in airborne particles in downtown Santiago, Chile. Particles were collected from five separate monitoring stations in 1991 and analysed for PAHs as described in the Materials and methods. Data are reported as the mean of five values and differ from each other by $< 15\%$.

at values approximately 20 times higher than in the hot season (November–February), presumably as a result of the lower level thermal inversion layer (150 m) in the winter months. Similarly, the chemical composition of particle extracts can vary quite substantially over short periods of time, as shown for the months of June and July (table 1). In addition, it should be noted that the total and carcinogenic PAHs appear to be substantially lower in the rural area (San Felipe) as compared with downtown Santiago.

Irrespective of the above variations however, it is important to note that most particle extracts contain a substantial amount of PAHs, and hence have potential biological activity. We have previously demonstrated that when particle extracts are administered to the rat, the mixture has the ability to influence liver microsomal MFO activity (Quinones and Gil 1995) and we have now extended these studies to protein-malnourished rats. As shown in table 2, malnourished rats generally respond similarly to rats fed on a normal diet in that cytochrome P450, NADPH-cytochrome P450 reductase and EROD and AHH activities are significantly increased in response to extract treatment, although the latter two enzyme activities appear to be induced to a greater extent in malnourished rats as compared with the normal fed controls. The induction of EROD and AHH activities in malnourished animals by extract treatment is further substantiated by the Western blot data for CYP1A, using benzo(*a*)pyrene treatment as a positive control (figure 2A). Although the polyclonal antibody recognises both CYP1A1 and 1A2 (Rodrigues *et al.* 1987), we could not identify which of these enzymes were induced as the gel did not resolve the two proteins. However, it should be noted that previous studies with extract treatment of nutritionally normal rats have established that CYP1A1 is substantially induced, with little influence on the levels of CYP1A2 (Quinones and Gil 1995).

Table 1. Polycyclic aromatic hydrocarbon content of organic extracts from airborne particles in Santiago and San Felipe, Chile.[a]

PAH	San Felipe concentration (ng/m³)	San Felipe region (ng/m³)	Santiago concentration (ng/m³)	Santiago range (ng/m³)
Pyrene	0.5	n.d.–0.9	33.7	10.9–77.3
Chyrysene	0.1	n.d.–0.1	27.3	0–66.6
Benzo(*a*)pyrene[b]	0.8	0.7–0.9	20.3	3.4–82.5
Fluorene	n.d.	n.d.	19.6	0–54.5
Indeno(1,2,3-*c*,*d*)pyrene[b]	1.6	1.4–1.8	18.5	0–26.0
Dibenzo(*a*,*h*)anthracene[b]	1.3	1.2–1.3	15.3	0–26.8
Benzo(*k*)fluoranthene[b]	0.1	0.1–0.2	11.7	0.5–17.2
Benzo(*b*)fluoranthene[b]	1.7	0.4–3.0	7.7	1.1–16.2
Benzo(*g*,*h*,*i*)perylene	2.4	2.1–2.7	3.2	0–29.0
Anthracene	0.1	0.1–0.1	2.6	0–8.0
Fluoranthene	0.3	0.2–0.3	2.6	0–18.2
Benzo(*a*)anthracene[b]	0.2	0.2–0.2	2.1	0–10.4
Phenanthrene	0.1	0.1–0.1	0.9	0–7.8
Acenaphthene	n.d.[c]	n.d.	n.d.	n.d.
Acenaphthylene	n.d.	n.d.	n.d.	n.d.
Naphthalene	n.d.	n.d.	n.d.	n.d.
Total PAHs	9.2		158.4	
Carcinogenic PAHs[b]	5.7		75.6	

[a] Concentration and range of PAHs in original air samples from Plaza Italia, downtown Santiago (mean of nine separate collection samples) and a rural area located 75 km north-west of Santiago (San Felipe, average of two collection samples). Note that the average person breathes approximately 20 m³ air per day.

[b] Carcinogenic PAHs are the sum of the air concentrations (ng/m³) of benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo(*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a*,*h*)anthracene, indeno(1,2,3,*c*,*d*)pyrene.

[c] n.d., not detected.

Table 2. Influence of airborne particle organic extracts on the cytochrome P450 MFO system in the normal and malnourished rats.[a, b]

	Control animals		Malnourished animals	
	Untreated	Extract-treated	Untreated	Extract-treated
Cytochrome P450 (nmol/mg)	0.72±0.01	0.83±0.02***	0.31±0.01	0.41±0.03**
NADPH-cytochrome P450 reductase (nmol/min/mg)	15.4±1.03	22.8±1.74*	14.7±0.70	26.2±1.20***
EROD (pmol/min/mg)	55±4	233±23**	25±2	206±34***
AHH (pmol/min/mg)	130±7	290±52*	86±8	322±60**
Laurate hydroxylase (nmol/min/nmol P450)				
12-Hydroxylase	4.51±1.00	3.11±0.63***	13.41±2.35	8.15±0.71***
11-Hydroxylase	2.44±0.36	1.55±0.25***	4.30±0.70	3.42±0.91**

[a] All animals were administered a single i.p. injection of the extract (40 mg total weight/kg), killed 48 h later, and enzyme activities determined as described in the Materials and methods. Values are the mean±SEM for nine animals, each treated with the nine separate pollution samples described in table 1. Values are significantly different from the corresponding control value at * $p < 0.05$, ** $p < 0.01$ or *** $p < 0.001$.

[b] Some of the data in control animals have been published previously (Quinones and Gil 1995), but are included for a fuller appreciation of the data for the malnourished animals.

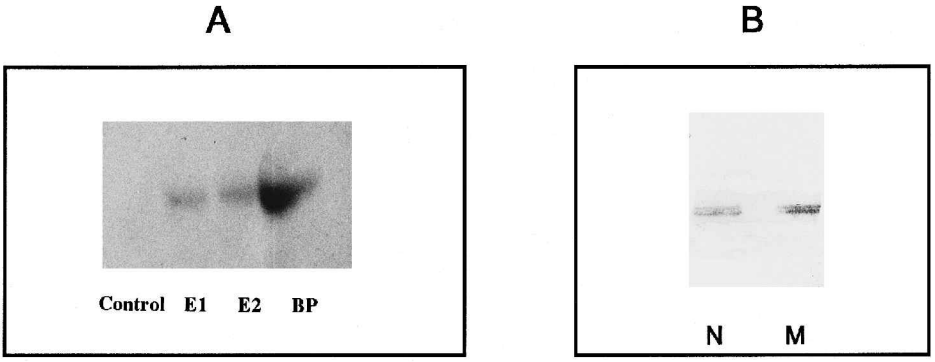

Figure 2. Influence of environmental organic pollutant extracts and the malnourished state on the expression of CYP1A and 4A1 in rat liver microsomes. (A) Microsomal protein (50 μg) from the pooled livers of three rats per group were resolved by SDS-PAGE and transferred electrophoretically to nitrocellulose. The immunoblot was carried out with sheep anti-rat CYP1A1 (1:10000 dilution) followed by peroxidase-linked donkey anti-sheep IgG (1:1000) with diaminobenzidine as substrate. Liver microsomes were derived from malnourished rats (control), and malnourished rats treated with either a single dose (40 mg/kg) of extract 13200 (E1), extract 13127 (E2) or benzo(*a*)pyrene (BP). (B) Western blot analysis for CYP4A1 expression was carried out as in (A) but using an antibody to CYP4A1. Normal (N) and malnourished (M) rat liver microsome samples were derived from pooled groups (n = 3).

Table 3. Mutagenic activities of organic extracts from airborne particles in the normal and malnourished rat.

	Sample 13127 (revertants/m^3)	Sample 13200 (revertants/m^3)
No S_9	42±2	177±42
Control S_9	166±21	68±4
Malnourished S_9	207±8	87±2

Samples 13200 and 13127 had total PAh contents of 173 and 186 ng/m^3 respectively, and when extracts of the samples were administered to rat (40 mg/kg) this resulted in hepatic microsomal EROD activities of 97 and 290 pmol/min/mg for 13200 and 13127 respectively.

Both control and malnourished animals were pretreated with a single dose of Arochlor 1254 (500 mg/kg, i.p.), S_9 fractions prepared and Ames test mutagenicity of particle extracts determined as described in the Materials and methods. Samples 13200 and 13127 were separately collected on 11–12 and 30–31 May 1991 and contained 173 and 186 ng/m^3 total PAHs respectively. Data shown are revertants per m^3 and are the means of triplicate determinations at each of five extract concentrations (10–200 μg/plate).

Accordingly, the increased EROD and AHH activities after extract treatment likely arise from an increase in both NADPH-cytochrome P450 reductase activity and the increase in CYP1A protein. Again, we must emphasize that the variability in chemical composition of the air samples results in a variable MFO response to treatment. For example, when normal nourished rats are separately treated with different extracts (numbers 13127 and 13200; table 3), EROD activity is induced 5.3-fold and 1.8-fold respectively. It is instructive to compare semi-quantitatively the CYP1A induction produced by the extracts and benzo(*a*)pyrene (figure 2). For example, benzo(*a*)pyrene concentrations in the extracts are approximately 1 μg per mg dry wet, and for the rats and dose used in our study (100 g for the normally

nourished group and 40 mg/kg total extract injected), this represents a benzo(*a*)pyrene dose in the extract of 4 μg, compared with 4 mg for the pure compound. Although we have not established the dose–response relationship for benzo(*a*)pyrene induction of CYP1A1 in our studies, the above dose estimations probably rationalizes the lower level of induction observed with the extracts.

The malnourished state significantly increases the 12-hydroxylation of lauric acid approximately three-fold as compared with the normal nourished animals (table 2), consistent with the increase in CYP4A1 (the enzyme responsible for lauric acid 12-hydroxylation), as assessed by Western blot analysis (figure 2B). This induction of CYP4A1 in the malnourished animal is in general agreement with previous reports wherein alterations in nutritional status modulates the MFO-dependent metabolism of drugs, carcinogens and steroid hormones (Hietenan 1980, Gil *et al*. 1988). Although we have not examined the precise molecular mechanisms of CYP4A1 induction in the malnourished animals, it is plausible that, in protein-deficient malnutrition, there is a significant increase in the nutritionally triggered, metabolic flux of endogenous fatty acids in the liver, the latter providing the proximal stimulus for CYP4A1 induction via the peroxisome proliferator activated receptor (Gottlicher *et al*. 1992). Table 2 also shows that the 11-hydroxylation of lauric acid is also increased in the malnourished state. As CYP2E1 is considered to be the major catalyst for this reaction (Imai 1988), it is conceivable that the hyperketonaemic state induced by malnourishment induces this activity, a hypothesis that requires further experimental investigation.

In view of the fact that the extracts contain PAHs, including significant amounts of carcinogenic PAHs such as benzo(*a*)anthracene, benzo(*b*)fluoranthene, benzo (*k*)fluoranthene, benzo(*a*)pyrene, dibenzo(*a,h*)anthracene and indeno(1,2,3,*c,d*) pyrene, it was of relevance to examine if the extracts were associated with mutagenic activity as assessed by the Ames test. In preliminary screening of different particle extract samples, it became clear that there was both a substantial variation in mutagenic activity and the relative abundance of direct and indirect (requiring metabolic activation) mutagens in the particulate extracts. As shown in table 3, sample 13200 contained more direct-acting mutagens than sample 13127 as assessed by the higher mutagenicity in the absence of the S9 activation system. Hplc analysis of these extracts revealed similar contents of total PAHs and the mutagenic benzo(*a*)pyrene and dibenzo(*a,h*)anthracene (data not shown), but the direct acting mutagens are clearly of more relevance in the absence of S9. Although we have not identified the precise nature of the direct acting mutagen(s) in these extracts, it is known that polycyclic aromatic nitroarenes are ubiquitous, directly acting, environmental contaminants (IARC 1989) and may well have contributed to the observed direct mutagenicity in our studies. In support of this possibility, is that fact that our previous studies using nitroreductase-deficient strains of *S. typhimurium* (TA/98R and TA98/1,8DNP6) have additionally implicated nitro- and dinitro-arenes as direct acting mutagens present in particle extracts (Adonis and Gil 1993). Furthermore, the presence of direct-acting mutagens in urban air particulate extracts has also been reported in Rome (Crebelli *et al*. 1988, 1995). Addition of the above two particle extracts to the S9 fraction derived from both fully nourished and malnourished rats resulted in different effects on mutagenicity in that both S9 fractions produced a decrease in mutagenicity with extract 13200, but resulted in an increased mutagenic response with the 13127 extract. These observations may be rationalized by the fact that extracts 13200 and 13127 have high levels of direct and

indirect-acting mutagens respectively. For sample 13200, addition of the S9 fractions would result in enhanced metabolism and hence deactivation of the direct-acting mutagens, whereas for sample 13127 addition of the metabolic system would enhance the mutagenicity of the component, indirect-acting mutagens.

In summary, we have demonstrated that the malnourished state in the rat influences the liver MFO system, the response to induction by environmental particle extracts and the resultant mutagenicity of the extract components in the Ames test. As such, our data in the rat provide a platform for discussion of the risk assessment of environmental pollution to man. In terms of human exposure, the average PAH content in the samples studied is 158 ng/m^3 (table 1), and assuming a breathing rate of 20 m^3 per day, this would result in an exposure of 3.2 μg total PAH per day. Once inhaled by man, it is very likely that the adsorbed pollutants are rendered bioavailable via the lung, a conclusion supported by the observation that aqueous mixtures of dipalmitoyl phosphatidyl choline (the major component of pulmonary surfactant) have the ability to solubilize/disperse adsorbed mutagens in diesel exhaust particles, and exhibit a similar mutagenicity to dichloromethane extracts of the same particles (Keane *et al*. 1991). Although the human lung is known to express the CYP1A1 enzyme (Nebert *et al*. 1991), it is not clear if enzyme induction, and hence increased predisposition to mutagenicity, actually occurs in man. However, in this context, it is instructive to note that our previous studies on exposure of human lymphocytes to the particle extracts, either in the presence or absence of S9 activation fractions, resulted in substantial increases in chromosomal aberrations (Silva *et al*. 1992), indicative of human responsiveness to the particle components. This question of the human responsiveness to enzyme induction by particle extracts may be addressed by *in vitro* studies examining the influence of extracts on the regulatory, 5′-flanking elements of the human CYP1A genes (coupled to reporter gene constructs) in transactivation experiments, studies that are underway in our laboratories.

Our studies can be placed in the context of relative risk assessment to man in that the mutagenicity of Santiago particle extracts in the Ames test, is 7-fold higher than in Rome (Crebeli *et al*. 1995) and 4-fold higher than in Berkeley, California (Kado 1986). Although we are aware off possible interlaboratory variations in Ames test data, other confounding factors that may obscure a realistic risk assessment to man include the variation in chemical composition of the particles and the nutritional status of the individual, and the existence of interindividual pharmacogenetic differences in the germ line expression of both CYP1A1 (activation) and glutatione *S*-transferases (deactivation) as they relate to the susceptibility of human lung to xenobiotic-induced cancer (Kawajiri *et al*. 1996). In addition, our studies have emphasized the importance of examining the liver MFO responses and mutagenicity of xenobiotics as they occur in environmental pollutant mixtures. This is important as these real-life exposures to mixtures are encountered on a daily basis and provide more meaningful and relevant hazard identification information than that obtained from multiple, single exposures to different chemicals, any of which may either antagonise (van Birgelen *et al*. 1994) or synergize (Bannister and Safe 1987) the biological responses to other xenobiotic mixture components such as poly-halogenated aromatic hydrocarbons, as is becoming increasingly recognized for other many other classes of chemicals (Yang 1994).

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