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Urinary 1-hydroxypyrene and PAH exposure in 4-year-old Spanish children

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ABSTRACT

Aims: Exposure to polycyclic aromatic hydrocarbons (PAH), among the main compounds present in polluted urban air, is of concern for children's health. Childhood exposure to PAH was assessed by urinary monitoring of 1-hydroxypyrene (1-OHP), a pyrene metabolite, investigating its association with exposure to air pollution and other factors related to PAH in air.

Methods: A group of 174 4-year-old children were recruited and a questionnaire on their indoor and outdoor residential environment was completed by parents. At the same time, environmental measurements of traffic-related air pollution (NO_2) were carried out. A urine sample was collected from each child in order to analyze 1-OHP using HPLC with fluorescence detection, correcting for creatinine concentrations. Non-parametric tests and regression analyses were used to identify environmental factors that influence 1-OHP excretion.

Results: Mean urinary 1-OHP concentration was 0.061 μmol/mol creatinine, ranging from 0.004 to 0.314 μmol/mol. Non-parametric tests and regression analysis showed positive and significant associations ($P \leq 0.05$) between 1-OHP and predicted residential exposure to NO_2 (which was based on outdoor environmental measurements and geo-statistical analysis), self-reported residential vehicle traffic, passive smoking and cooking appliance. 1-OHP levels tended to be higher among children living in urban areas (0.062 μmol/mol *vs.* 0.058 μmol/mol for children living in rural areas) but differences were not significant ($P = 0.20$).

Conclusion: In Southern Spain, concentrations of urinary 1-OHP were in the lower range of those generally reported for children living in non-polluted areas in Western Europe and the USA. Traffic-related air pollution, passive smoking and cooking appliance influenced urinary 1-OHP level in the children, which should be prevented due to the health consequences of the inadvertent exposure to PAH during development.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAH) are released into the environment as a complex mixture of compounds during incomplete combustion of organic matter (IARC, 2003). PAH are found in multiple environmental media, including indoor and ambient air, soil, water and food. The main sources of human exposure to PAH are occupation, passive and active smoking, food and water and air pollution (Jongeneelen, 1997). Pollution of air by PAH is mainly due to fuel-combustion emissions from motor vehicle (e.g., diesel and gasoline vehicles), heating and power sources (e.g., including coal, oil and biomass) and indoor sources, such as cooking, residential heating and tobacco smoke (IARC, 1989, 2004; Straif et al., 2006; Lewtas, 2007). Thus, exposure of the general population to PAH from inhalation of ambient air varies according to the degree of urbanisation and industrialisation and the level of

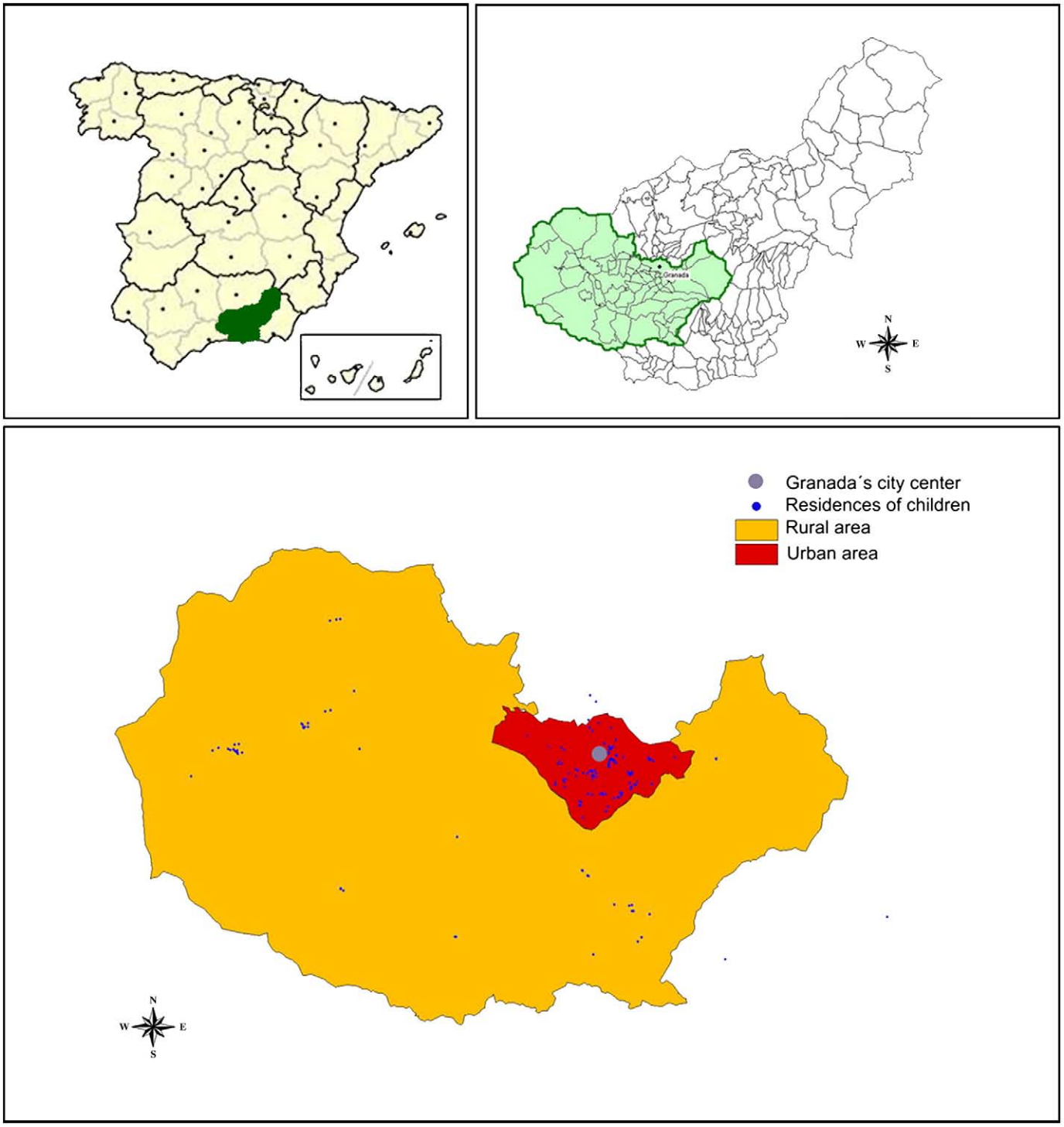

Fig. 1 – Study area and residences of children.

traffic, and it is influenced by the home environment and life style factors (Hansen et al., 2005, 2008).

PAH are human health hazards and a number of them are known carcinogenic compounds (IARC, 1983). It is well known that children are at particular risk for health effects of air pollutants since their respiratory and immune systems are not fully developed, they inhale relatively more air per kilogram of body weight than adults and they generally spend more time outdoors (Schwartz, 2004). Exposure to PAH from air pollution has been associated with increased risk of adverse health effects in children, such as asthma and cancer, though the extent to which this excess risk can be attributed to exposure to PAH remains unclear (Crosignani et al., 2004; Kim et al., 2005; Knox, 2005).

Pyrene is one of the most produced PAH in emissions from the combustion of petrol and diesel, the main source of PAH in urban environments (Castaño-Vinyals et al., 2004). Urinary 1-OHP, a major metabolite of pyrene, is considered an appropriate surrogate biomarker for total PAH-exposure of human populations (Levin, 1995; Jongeneelen, 1997; Siwinska et al., 1998) and is reported to reflect levels of PAH exposure from different sources such as ambient air, food and indoor air (Gilbert and Viau, 1997; Vyskocil et al., 1997, 2000). It has been suggested that urinary 1-OHP reflects exposure to PAH even at low air pollution levels (Castaño-Vinyals et al., 2004), and it is increasingly being used to biomonitor human exposure to air pollution (Hansen et al., 2008). In addition, the human biomonitoring of environmental exposures to PAH is considered a priority in the European Environmental and Health Program (Casteleyn et al., 2007).

Although more information is becoming available on urinary 1-OHP concentrations among the general population, levels in children are less well known. Epidemiological studies on childhood exposure to PAH over the past decade have shown a wide range of 1-OHP levels (Siwinska et al., 1998, 1999; Vyskocil et al., 2000; Fiala et al., 2001; Grainger et al., 2005), which were associated with local sources of outdoor exposure, diet, smokers at home and cooking with wood/coal (van Wijnen et al., 1996; Siwinska et al., 1999; Fiala et al., 2001; Mucha et al., 2006; Kollosa-Gehring et al., 2007). In urban areas with heavy traffic, motor vehicle emissions have been suggested as an important source of childhood exposure to PAH that, consequently, significantly affects 1-OHP excretion (Mielzynska et al., 2006; Ruchirawat et al., 2007; Tuntawiroon et al., 2007). Thus, in a study of 3–13 year-old Dutch children, 1-OHP concentrations in those living in urban residences were significantly higher than those in children living in rural areas (Hansen et al., 2005). Therefore, it is crucial to take into consideration the area of residence when studying 1-OHP levels in children.

The present study was carried out as part of the "Environment and Childhood Research Network" (INMA network), a population-based cohort study in different regions of Spain that focuses on prenatal environmental exposures in relation to growth, development and health from early foetal life until childhood (Ribas-Fitó et al., 2006). The cohort established in Granada province (Southern Spain) consists of 700 boys born at the San Cecilio University Hospital between October 2000 and June 2002 (Lopez-Espinosa et al., 2007). One of the objectives of the INMA study was to assess prenatal and postnatal exposure to air pollution *via* environmental measurements, questionnaires, geographical information systems (GIS) and the monitoring of urinary 1-OHP during pregnancy and infancy (Esplugues et al., 2007). With this background, the objectives of the present study were to determinate urinary 1-OHP concentrations in children living in Granada province and to evaluate their association with exposure to air pollution and other factors related to PAH in air.

2. Material and methods

2.1. *Study area*

The study was conducted in an area that covers the health district of the San Cecilio University Hospital, with a total population of 512,000 inhabitants and an extension of approximately 4000 km^2. This area includes part of the city of Granada (236,000 inhabitants) and 50 towns and villages. Two areas were differentiated: a) an urban area, corresponding to the central districts of the city of Granada and the metropolitan area around the city, with high population and high traffic densities (main roads with mean daily frequency about 20,000–40,000 vehicles), and b) a rural area, with lower traffic density (below 5000 vehicles/day), where the population resides mainly in small villages and the main activities are agriculture and forestry (Fig. 1).

2.2. *Study population*

From October 2000 to July 2002, mother–son pairs registered at the San Cecilio University Hospital of Granada were recruited, in order to investigate chronic exposure to environmental chemicals (Fernandez et al., 2007). The inclusion and exclusion criteria were published elsewhere (Lopez-Espinosa et al., 2007). The INMA study protocol included the medical follow-up of the children at the age of 4 years, as well as questionnaires and biological sample collection. Between April 2005 and June 2006, 1 out of 3 mothers was randomly contacted by phone and they agreed to complete a questionnaire on residential and home environment. A total of 220 children were contacted. Urine samples were collected and analysed for 1-OHP from 174 children, 118 living in the urban area and 56 in the rural area. Written informed consent was obtained from parents of children before the study, which was approved by the Ethics Committee of San Cecilio University Hospital.

2.3. *Questionnaires*

Parents completed a structured questionnaire on possible sources of children's exposure to air pollution and PAH, which included information on smoking habits, perception of residential traffic density and gas combustion-related sources in the house such as heating, water heating and cooking appliances.

2.4. Environmental measures

The INMA air pollution-study protocol included an estimate of individual air pollution exposure based on outdoor nitrogen dioxide (NO_2) measurements, following the methodology published by Esplugues et al. (2007). NO_2, like PAH, is generated by combustion of organic material and is a typical urban air pollutant that has been used as an indicator of vehicle emissions (Krämer et al., 2000; Hochadel et al., 2006). Sampling was done in two 7-day periods, one in winter and the other in summer. In brief, ambient air NO_2 was monitored in 76 locations throughout the study area using Radiello® passive samplers (Environmental Research Center, S. Maugeri Foundation, Padova, Italy) and concentrations were determined at the "Centro Nacional de Sanidad Ambiental" of the "Instituto de Salud Carlos III" in Madrid, Spain. NO_2 was adsorbed onto a cartridge coated with triethanolamine, desorbed with a sulphanilamide reactive and then quantified by spectrophotometry 537 nm. Children addresses were geocoded and then, using a methodology based on spatial interpolation methods (*kriging* technique) (Jerrett et al., 2005), the NO_2 residential level was estimated for each child and used as a proxy of individual exposure to traffic-related air pollution.

2.5. Urinary 1-OHP measurements

Urine samples (30 ml) were collected in the afternoon during the working week at the Hospital and stored in three 10 ml polypropylene containers at −20 °C until analysis. Samples were analysed for 1-OHP in the Laboratory of the Department of Public Health at Bilbao, Basque Country (Spain). Analytical procedure has been published elsewhere (Llop et al., 2008). In brief, 10 ml of acetic–acetate buffer 0.2 M and 100 μl of β glucuronidase arylsulfatase enzyme were added to 10 ml of urine, which was then mixed and placed in a stove at 37 °C during 18 h. 1-OHP was extracted after hydrolyzation of the urine by solid–liquid extraction using RP-18 cartridges. The

Table 1 – Descriptive analysis of 1-OHP concentrations (μmol/mol)

	All children			Exposed to ETS at home			Not exposed to ETS at home		
	N (%)	Mean ± SD	P	N (%)	Mean ± SD	P	N (%)	Mean ± SD	P
Exposure to ETS									
Not exposed to ETS	93 (53.4)	0.052 ± 0.050	0.17	–	–	–	–	–	–
Exposed to ETS	81 (46.6)	0.063 ± 0.055							
Smoking habit (cigarettes/day)									
Not exposed to ETS	93 (53.4)	0.060 ± 0.055	0.25	–	–	–	–	–	–
1–10	52 (29.9)	0.063 ± 0.055							
11–20	19 (10.9)	0.076 ± 0.044							
>20	10 (5.7)	0.052 ± 0.021							
Sampling season									
Spring	64 (36.8)	0.058 ± 0.057	0.14	26 (32.1)	0.056 ± 0.060	0.24	38 (40.9)	0.063 ± 0.063	0.32
Summer	16 (9.2)	0.087 ± 0.077		9 (11.1)	0.103 ± 0.092		7 (7.5)	0.061 ± 0.053	
Autumn	42 (24.1)	0.053 ± 0.035		27 (33.3)	0.060 ± 0.037		15 (16.1)	0.038 ± 0.028	
Winter	52 (29.9)	0.065 ± 0.042		19 (23.5)	0.071 ± 0.044		33 (35.5)	0.063 ± 0.046	
Area of residence									
Rural	56 (32.2)	0.054 ±0.055	0.20	30 (37.0)	0.063 ± 0.061	0.26	26 (31.2)	0.053 ± 0.059	0.23
Urban	118 (67.8)	0.060 ±0.040		51 (63.0)	0.068 ± 0.053		67 (68.8)	0.060 ± 0.046	
Predicted exposure to NO_2 ($\mu g/m^3$)									
<22.50	81 (46.6)	0.055 ± 0.051	**0.04**	38 (46.9)	0.060 ± 0.052	0.17	43 (48.4)	0.049 ± 0.053	0.07
≥22.50	93 (53.4)	0.067 ± 0.050		43 (53.1)	0.074 ± 0.059		50 (51.6)	0.064 ± 0.047	
Self-reported traffic-density									
Very low frequency	13 (4.6)	0.046 ± 0.034	0.20	6 (7.4)	0.040 ± 0.025	0.43	7 (7.5)	0.046 ± 0.046	**0.008**
Low frequency	63 (33.9)	0.049 ± 0.022		28 (9.9)	0.042 ± 0.025		35 (37.6)	0.054 ± 0.057	
Medium frequency	25 (12.1)	0.051 ± 0.038		12 (14.8)	0.070 ± 0.062		13 (14.0)	0.058 ± 0.015	
High frequency	21 (8.0)	0.079 ± 0.044		8 (9.9)	0.068 ± 0.045		13 (14.0)	0.083 ± 0.021	
Very high frequency	52 (27.0)	0.084 ± 0.057		27 (33.3)	0.074 ± 0.062		25 (26.9)	0.091 ± 0.038	
Cooking appliance									
Electric	91 (45.4)	0.056 ± 0.046	0.09	40 (49.4)	0.057 ± 0.044	**0.05**	51 (54.8)	0.056 ± 0.048	0.89
Gas	83 (40.2)	0.069 ± 0.059		41 (50.6)	0.076 ± 0.064		42 (45.2)	0.059 ± 0.053	
Water heater									
Electric	53 (23.0)	0.052 ± 0.033	0.37	17 (21.0)	0.048 ± 0.025	0.15	36 (38.7)	0.055 ± 0.038	0.67
Gas	121 (62.6)	0.066 ± 0.058		64 (79.0)	0.071 ± 0.060		57 (61.3)	0.059 ± 0.055	
Domestic heating									
None	22 (12.6)	0.061± 0.041	0.95	13 (16.0)	0.063 ± 0.044	0.97	9 (9.7)	0.057 ± 0.040	0.99
Electric	61 (35.0)	0.069 ± 0.069		32 (39.5)	0.075 ± 0.075		29 (31.2)	0.062 ± 0.061	
Gas	76 (43.5)	0.051 ± 0.036		26 (32.0)	0.055 ± 0.029		50 (53.8)	0.049 ± 0.037	
Open fire	15 (8.7)	0.053 ± 0.029		10 (12.3)	0.058 ± 0.028		5 (5.4)	0.047 ± 0.031	

1-OHP: 1-hydroxypyrene; ETS: Environmental tobacco smoke; SD: Standard deviation.
P: P-value in Mann–Whitney or Kruskal Wallis test.
Significant associations in bold ($P \leq 0.05$).

solvents used in the extraction were acetonitrile and water. Eluates were evaporated to dryness in a stream of N_2 and re-dissolved in acetonitrile for analysis by high resolution liquid chromatography (HPLC) with fluorimetric detection, using an RP-18 column and UV fluorescent spectrophotometer (excitation and emission wavelengths of 242 nm and 388 nm, respectively). 1-OHP concentrations (ng/ml) were corrected by the creatinine concentration in each urine sample and expressed in μmol/mol creatinine. The quantification limit (LOQ) for 1-OHP was 0.030 ng 1-OHP/ml urine. For samples with 1-OHP≤LOQ, a value of half the LOQ was taken.

2.6. Statistical analysis

A descriptive analysis of 1-OHP levels was performed in terms of the covariates considered in the study: urine sampling season (spring/summer/autumn/winter), residential area (rural/urban), smoking habit of parents at home (both "not exposed/exposed" and "number of cigarettes smoked daily at home"), residential traffic density perceived by parents (very low frequency/low frequency/medium frequency/high frequency/very high frequency), cooking (electric/gas), water heating (electric/gas) and domestic heating (none/electric/gas/open fire) appliances and predicted residential exposure to NO_2. Because 1-OHP and predicted NO_2 levels had an abnormal distribution, non-parametric tests were used to test the differences among groups. The significance level was set at 95% ($P \leq 0.05$).

Spearman test was used to evaluate the correlation between 1-OHP level and smoking habit of parents (number of cigarettes smoked daily at home), as continuous variable. Exposure to environmental tobacco smoke (ETS) was evaluated with two different categorical variables: children not living/living with smokers ("not exposed"/"exposed") and number of cigarettes smoked by parents at home (0; 1–10; 11–20; and >20 cigarettes per day). Predicted residential exposure to NO_2 (μg/m^3) was divided into two groups (categorical variable), finding a higher adjusted R^2 for its association with 1-OHP than when it was treated as a continuous variable. A cut-off point of 22.50 μg/m^3 was established based on the change in the slope of the relationship between 1-OHP concentration and predicted NO_2 in the localized weight regression (LOESS; Hastie and Tisbhirani, 1990).

Multivariate linear regression was used to examine the association between 1-OHP values and potential explanatory variables. 1-OHP concentration was treated as continuous variable on a log-transformed scale. All covariates associated with the outcomes at a P-value≤0.20 in univariate analysis were introduced in the models. Following a backward procedure, variables with P-value>0.10 were sequentially excluded from the model. F test for the change in R^2 in linear regression was used to check the exclusion (or not) of covariates step by step. Statistical analysis was performed using SPSS software package, version 15.0 (SPSS Inc., Chicago).

Table 2 – Regression analysis between 1-hydroxypyrene levels and factors of exposure to PAH

	β coefficient*	95% CI	P
Predicted NO_2	0.401	0.12 to 0.68	0.006
Exposure to ETS	0.264	−0.02 to 0.55	0.07
Cooking appliance	0.235	−0.04 to 0.52	0.10

CI: Confidence intervals.
Children exposed to <22.50 μg/m^3 NO_2, not exposed to ETS at home, and living in a house with electric cooking appliance as reference category.
* Coefficients correspond to 1-OHP on log-transformed scale as dependent variable.

3. Results

Mean age (±standard deviation [SD]) of the children participating in this study was 4.3 years (±0.2). Concentration of 1-OHP was above the LOQ in 78.8% of urine samples. Mean (±SD) of unadjusted and adjusted 1-OHP concentrations were 0.070 ng/ml urine (±0.070 ng/ml) and 0.061 μmol/mol creatinine (±0.051 μmol/mol), respectively. Concentration of 1-OHP ranged from 0.004 to 0.314 μmol/mol (0.015–0.553 ng/ml). In urban and rural areas, 43 and 54% of the children, respectively, were exposed to ETS, i.e., a total of 81 out of 174 children were living with smokers. Mean (±SD) predicted residential exposure to NO_2 was 20.96 μg/m^3 (±5.94 μg/m^3) and significantly different between children living in urban and rural areas (mean 24.45 *vs.* 13.42 μg/m^3; $P<0.001$).

Table 1 shows the distribution of urinary 1-OHP levels as a function of the covariates considered in the study. First, considering all children together, urinary 1-OHP levels tended to be higher among children exposed to ETS (mean 0.063 μmol/mol *vs.* 0.052 μmol/mol), although differences did not reach statistical significance ($P=0.17$). Moreover, when smoking was analysed in terms of number of cigarettes smoked daily at home (continuous), a positive but weak correlation was found between smoking at home and 1-OHP excreted by the children ($r=0.133$; $P=0.108$). Second, urinary 1-OHP concentrations of children living in urban areas were higher compared to rural children, however differences were not significant ($P=0.20$). Third, a significant difference ($P=0.04$) was found in 1-OHP urine concentration as a function of predicted residential exposure to NO_2, so that children exposed to residential $NO_2 \geq 22.50$ μg/m^3 showed higher 1-OHP concentrations compared with children below this level. Fourth, a close-to-significant difference ($P=0.09$) was found between children living in a house with gas cooking appliance and those in a house with electric cooking appliance.

In order to explore more deeply the effect of ETS exposure on urinary 1-OHP levels, we performed a stratified analysis of 1-OHP urinary levels and potential explanatory variables. Among the children living with non-smokers, there were no differences in 1-OHP levels between urban and rural children ($P=0.23$). Close-to-significant and significant differences were found for predicted NO_2 levels ($P=0.07$), and self-reported traffic density ($P=0.008$), respectively, so that children exposed to residential $NO_2 \geq 22.50$ μg/m^3 or living near streets with continuous traffic showed higher 1-OHP levels. In contrast, urinary 1-OHP levels of the children living with smokers were only significantly affected by cooking appliance ($P=0.05$).

In univariate regression analysis, variables associated with 1-OHP at a P-value≤0.20 were: predicted residential exposure

to NO_2 ($P=0.02$), self-reported residential traffic ($P=0.05$), smoking habit ($P=0.07$) cooking appliance ($P=0.10$), exposure to ETS ($P=0.11$), and area of residence ($P=0.13$). In multivariate analysis, the variables included in the model were: predicted NO_2 ($P=0.006$), exposure to ETS ($P=0.07$) and cooking appliance ($P=0.10$). Table 2 shows regression coefficients and 95% confidence intervals.

4. Discussion

This study detected an association between urinary excretion of 1-OHP and traffic-related air pollution estimated by outdoor NO_2 measurements, suggesting that motor vehicle emissions play an important role in the exposure to PAH of children in Granada. Accordingly, parents' perception of traffic density was also associated with 1-OHP levels but did not influence 1-OHP when considered together with exposure to NO_2, suggesting that NO_2 is better marker of traffic-related air pollution than is traffic perception. Concentrations of 1-OHP were slightly lower in the children living in a rural setting, characterised by a low density of traffic. This is consistent with previous findings by Hansen et al. (2005) in Denmark and Chuang et al. (1999) in the USA of a possible association between PAH from traffic-related air pollution and childhood exposure. In our study area, vehicle emissions appear to be the main source of PAH in ambient air because the area is mostly devoted to agriculture practices and services (Lopez-Espinosa et al., 2007) and contains little industrial activity.

In the framework of the INMA study, 1-OHP levels have been reported in pregnant women from Valencia, Eastern Spain (Llop et al., 2008), and they were also associated with outdoor NO_2 levels. A few studies have also reported 1-OHP urinary levels in workers occupationally exposed to PAH in Spain (Domingo et al., 2001; Schuhmacher et al., 2002; Mari et al., 2007). However, to our best knowledge, this is the first Spanish study to evaluate childhood exposure to environmental PAH by this approach. The urinary 1-OHP levels in these Southern Spanish children were in the lower range of those reported for young European and North American children who do not live in industrial areas and are exposed to low air pollution levels (Chuang et al., 1999; Vyskocil et al., 2000; Cirillo et al., 2006; Huang et al., 2006), and much lower than findings in Eastern Europe and Asian countries with high levels of industrial activity and vehicle traffic (Siwinska et al., 1998; Mielzynska et al., 2006; Mucha et al., 2006) (Fig. 2). Finally, the urinary 1-OHP levels found in 95% of the present children were below the reference value of 0.30 μg/g (0.155 μmol/mol) established by the German Environmental Survey (GerES III and IV) for children aged 3–14 years who are not exposed to ETS (Wilhelm et al., 2008).

Besides outdoor pollution due to traffic, indoor activities appeared to affect the children's exposure to PAH as reflected in their urinary excretion of 1-OHP. We found some association between urinary 1-OHP in children and indoor air pollution due to ETS, confirming previous reports (Siwinska et al., 1999; Kollosa-Gehring et al., 2007). As suggested by Rogger et al. (1993), the present data also support an association between the use of a gas cooking appliance and higher PAH exposure. The above-cited German survey indicated that ETS is an important predictor of urinary 1-OHP levels in children and found residential setting to be of minor importance (Becker et al., 2006; Kollosa-Gehring et al., 2007). However, in Thailand, 1-OHP levels were shown to be higher in rural versus urban residents due to the use of wood/coal to cook and heat water in rural areas (Chetinyanukornkul et al.,

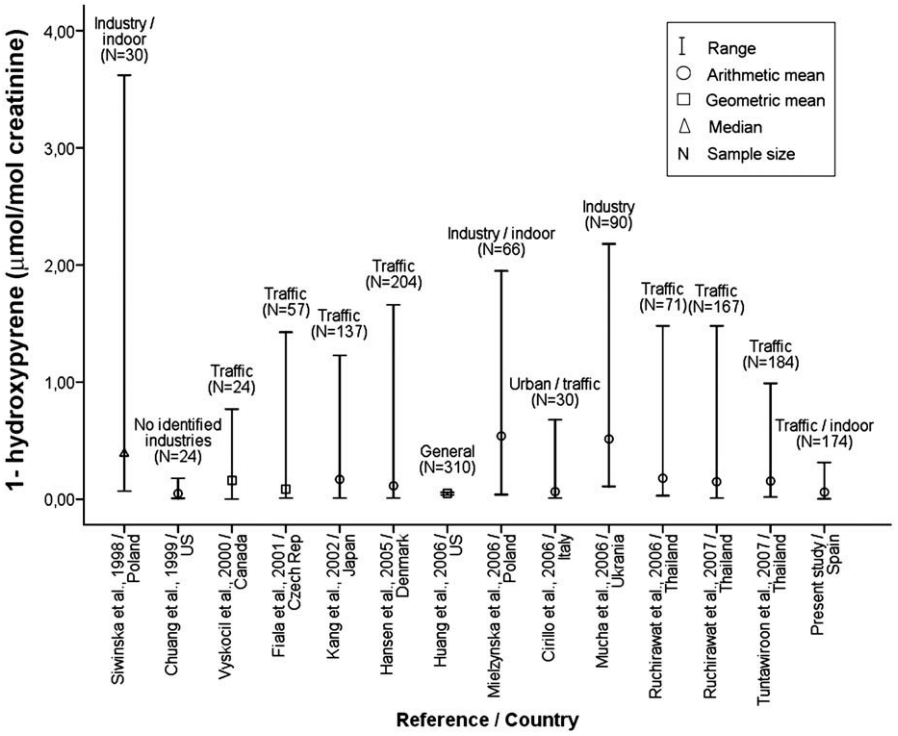

Fig. 2 – Review of reported children's 1-OHP concentrations.

2006), underlining the need to take into consideration the geographic area of studies.

There most important limitation in this study of chronic exposure to air pollution is that 1-OHP reflects short-term exposure and may be less reliable as an indicator of prolonged exposure to PAH. Because of the absence of direct environmental data on PAH concentrations, exposure was indirectly assessed by using predicted NO_2 concentrations at children's residences and analysing factors proposed to be related to PAH sources. Another important study limitation is that dietary exposure was not evaluated. Several studies have found that diet is the most significant contributor (about 90%) to PAH intake in children (Vyskocil et al., 1997, 2000; Fiala et al., 2001) and, consequently, to their 1-OHP levels. However, others have indicated that traffic density, industry or other sources of ambient air pollution play a major role in exposure assessment to PAH in children (Kanoh et al., 1993; Chuang et al., 1999; Hansen et al., 2005; Mucha et al., 2006). In the present study, 1-OHP levels were associated with pollution from traffic in the urban area, passive smoking and cooking with gas, suggesting that the contribution of air pollution to PAH intake in these children may not be negligible. Nevertheless, the possible influence of diet on 1-OHP in the study sample may have contributed to the absence of stronger associations with air pollution.

Account should also be taken of children's exposure to PAH through ingestion of contaminated soil, especially in young children playing outside near heavy traffic or industrial sources (Tang et al., 2006). In the present study, lower levels of traffic-related air pollution in the rural area may lead to lower AH deposition in soil and a lesser intake of PAH by this route in the rural versus urban children.

According to our findings, indoor sources and traffic-related air pollution may influence 1-OHP excretion, which has been proposed as a good marker of exposure to PAH and air pollution. Urinary 1-OHP levels were associated with predicted residential exposure to NO_2 and with traffic density in children living with non-smokers, suggesting that ETS exposure should be considered a confounding factor in this type of exposure.

Given the ubiquitous presence of PAH in the environment and their effects on children's development, wide research efforts are warranted into the biomonitoring of exposure to low environmental levels of PAH during vulnerable periods, e.g., pregnancy and childhood, and its possible association with adverse health effects.

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