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Assessment on dietary habits and polycyclic aromatic hydrocarbon exposure in primary school children.

Teresa Cirillo^{1*}, Paolo Montuori², Pierangela Mainardi², Immacolata Russo², **Evelina Fasano¹**

Maria Triassi² and Renata Amodio-Cocchieri¹

University of Naples “Federico II”, Italy.

¹ Department of Food Science.

² Department of Preventive Medical Sciences.

*Correspondence: Teresa Cirillo Department of Food Sciences; Facoltà di Agraria, Università degli Studi di Napoli “Federico II”, via Università, 100 - 80055 Portici - Naples, ITALY; e-mail: tcirillo@unina.it, Phone: +39 081 2539450 Fax: +39 081 2539407.

Paolo Montuori pmontuori@unina.it
Pierangela Mainardi pmainardi@unina.it
Immacolata Russo imrusso@unina.it
Evelina Fasano evafas@libero.it
Maria Triassi triassi@unina.it
Renata Amodio Cocchieri amodio@unina.it

Abstract

Thirty Italian children, 7-9 year aged, living in Naples were investigated on their dietary habits and on polycyclic aromatic hydrocarbon (PAH) exposure by a food diary-questionnaire and one week duplicate diet sample analyses.

Daily total food consumption mean value was 632 ± 215 g day⁻¹, median value 613 g day⁻¹. The daily energy intake and the diet composition meanly agreed with the official guidelines for the Italian children.

16 PAHs were simultaneously detected and, according to the EFSA approach, benzo[a]pyrene; benzo[a]pyrene+chrysene (PAH2); PAH2+benz[a]anthracene+benzo[b]fluoranthene (PAH4); PAH4+benzo[k]fluoranthene+benzo[ghi]perylene+dibenz[a,h]anthracene+indeno[1,2,3-cd]pyrene (PAH8) were considered in evaluating the children's dietary exposure to PAHs. The benzo[a]pyrene (BaP) median concentrations in foods varied from 0.06 to 0.33 µg kg⁻¹. Only three samples of cooked foods (one fish and two meat samples) exceeded legal limits fixed by EU for BaP.

Daily median intakes of benzo[a]pyrene, PAH2, PAH4, and PAH8 were 153; 318; 990; 1776 ng day⁻¹; their median exposure values were 5; 10; 28 ; 54 ng kg⁻¹ bw day⁻¹. The Margins of Exposure (MOEs) in median consumers agreed with the EFSA safety values except for PAH8.

Key words: Italian children, dietary habits; dietary polycyclic aromatic hydrocarbon exposure;

1. Introduction

A correct nutrition for children is very important for the prevention of many chronic diseases of adulthood. Throughout childhood, it is important to include in the diet a variety of foods for proper development. A diet containing a variety and appropriate quantitative of breads and grains, meats, fruits and vegetables, and dairies will help to prevent nutrient deficiencies. The nutritional principles have to be applied to children's as well as to adults' diet, although portions and number of servings per day may obviously be different. After the age of 2, a diet moderately low in fat and salt is recommended, because diets high in fat and salt may contribute to hypertension, heart disease, obesity, and other health problems later in life.

Diet, further that introduction way for nutrients, may be a significant source of chemical contaminants. The contamination of food by chemical hazards is a worldwide public health concern and is a leading cause of trade problems internationally. Contamination may occur through environmental pollution of the air, water and soil or through the intentional use of various chemicals or from food treatments, processing, storing and cooking methods. Among organic contaminants, polycyclic aromatic hydrocarbons (PAHs) represent an important class of food contaminants and diet represents the most important way of exposure for no occupationally exposed populations (EFSA, 2005).

PAH occurrence in foods belongs from the environmental pollution and the processing procedures. While the transportation of PAHs in the atmosphere is influenced by their volatility, in the terrestrial environment the PAH distribution is influenced by their lipophilicity and solubility in water that determine their capacity for transport and distribution between the different compartments, their uptake and accumulation by living organisms and their bioaccumulation in the food chain, in particular in lipid tissue of plants and animals. Low molecular mass PAHs can be mainly concentrated by adsorption through the waxy surface of vegetables and fruits. PAH concentrations are generally greater on plant surface (peel, outer leaves) than on internal tissues.

Careful washing may remove up to 50 % of the total surface adsorbed PAHs (Edwards, 1983; Nielsen *et al.*, 1996). The average background values are usually in the range of 0.01-1 $\mu\text{g kg}^{-1}$ in uncooked foods (Guillén *et al.*, 1997; Phillips, 1999). Processing procedures, such as smoking and drying, and cooking of food are commonly thought to be the major source of contamination by PAHs (Chen and Lin, 1997). Levels as high as 200 $\mu\text{g kg}^{-1}$ food have been found for individual PAH in smoked fish and meat (SCF, 2002). Jira (2004) found benzo[a]pyrene concentrations ranging from 0.05 to 0.35 $\mu\text{g/kg}$ (mean 0.12 $\mu\text{g/kg}$) in smoked ham and sausage. Duedahl-Olesen *et al.* (2006) reported benzo[a]pyrene levels for Danish smoked products such as bacon, small sausages and salami below 0.6 $\mu\text{g/kg}$. Cooking practices, as grilling, frying, roasting, can influence the production of PAH in the food, the type, number and amount of which depend on various parameters as temperature, time, kind of fuel used, distance from the heat source, drainage of fat, etc. (Saint-Aubert *et al.*, 1992; Mottier *et al.*, 2000). Lodovici *et al.* (1995) found in foods typical of Italian diet values of PAHs (9 compounds) ranging from 0.52 to 42.00 $\mu\text{g kg}^{-1}$. In barbecued meat, total PAHs were found to be present at levels up to 164 $\mu\text{g kg}^{-1}$, with benzo(a) pyrene present at levels as high as 30 $\mu\text{g kg}^{-1}$ (Phillips, 1999). In Europe an average benzo[a]pyrene concentration of 0.2 $\mu\text{g/kg}$ was reported for fresh fish and ranging from 1.4 $\mu\text{g/kg}$ to 5.3 $\mu\text{g/kg}$ for smoked fish obtained, respectively, by unknown or traditional smoking methods (European Commission, 2004).

Contamination of vegetable oils (including olive residue oils) with PAH usually occurs during technological processes like direct fire drying, where combustion products may come into contact with the oil seeds or oil (Larsson *et al.*, 1987; Speer *et al.*, 1990; European Standing Committee on Foodstuffs, 2001).

In the past decade the effects deriving from the PAH ingestion were evaluated by the International Programme on Chemical Safety (IPCS) (WHO/IPCS, 1998), the Scientific Committee on Food (SCF) (EC, 2002) and by the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2005). SCF concluded that for 15 PAHs, (benz[a]anthracene, benzo[b]fluoranthene,

benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, benzo[*a*]pyrene, chrysene, cyclopenta[*cd*]pyrene, dibenz[*a,h*]anthracene, dibenzo[*a,e*]pyrene, dibenzo[*a,h*]pyrene, dibenzo[*a,i*]pyrene, dibenzo[*a,l*]pyrene, indeno[1,2,3-*cd*]pyrene and 5- methylchrysene exists clear evidence of mutagenicity/genotoxicity in somatic cells in experimental animals *in vivo* and of carcinogenic effects in various types of bioassays in experimental animals. These compounds may be consequently regarded as potentially genotoxic and carcinogenic to humans and considered a priority group in the assessment of the risk of long-term adverse health effects following dietary intake of PAHs. Benzo[*a*]pyrene was suggested as a marker of occurrence and effect of the carcinogenic PAHs in food, based on the examination of the PAH profiles in food. Furthermore studies on experimental animals showed various toxic effects, such as haematological effects, liver, reproductive and developmental toxicity and immunotoxicity for others PAHs (acenaphthene, anthracene, fluoranthene, fluorene, naphthalene, pyrene) with NOAELs ranging from 70 to 1000 mg/kg bw/day in subchronic studies (SCF, 2002). **On 2007 the EFSA Panel on Contaminants in the Food Chain reviewed the available data on occurrence and toxicity of PAHs highlighting that about 30% of all the foods analyzed resulted negative for benzo[*a*]pyrene but positive for other carcinogenic and genotoxic PAHs. Eight PAHs, benz[*a*]anthracene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, benzo[*a*]pyrene, dibenz[*a,h*]anthracene and indeno[1,2,3-*cd*]pyrene, individually or in combinations, were considered to be currently the only possible indicators of the carcinogenic potency of PAHs in food. As an alternative to the NOAEL and LOAEL approach a new limit dose that provides a more quantitative alternative to the first step in the dose-response assessment, the Bench Mark Dose (BMD), was proposed, according to what suggested by Crump (1984).** The BMD lower limit (BMDL) is the bench mark dose lower confidence limit for a 10% of increasing in the number of tumour bearing animals compared to control animals (BMDL10). According to the EFSA, the CONTAM Panel calculated the BMDL10 values **for benzo[*a*]pyrene; the sum of benzo[*a*]pyrene and chrysene (PAH2); the sum of**

PAH2,benz[a]anthracene and benzo[b]fluoranthene (PAH4); the sum of PAH4, benzo[k]fluoranthene, benzo[ghi]perylene, dibenz[a,h]anthracene and indeno[1,2,3-cd]pyrene (PAH8) using a range of statistical models, and selected the lowest BMDL10 values from the statistical models that fit adequately the data. These values are 0.07; 0.17; 0.34 and 0.49 mg kg⁻¹bw day⁻¹, respectively. The CONTAM Panel defined a Margin of Exposure (MOE) approach based on dietary exposure to benzo[a]pyrene, PAH2, PAH4 and PAH8 for average and high level consumers and their corresponding BMDL10 values (EFSA, 2008).

The PAH exposure of general population by diet can be a public health concern and particularly in children. Children may be more susceptible to the effects of exposure, as they have higher rates of metabolism, less mature immune system, and different patterns of activity and behaviour than adults (Olden & Guthrie, 2000). Moreover, children eat more food per body mass and tend to eat different foods than adults. A knowledge of children's dietary intake is central to assess exposure and cumulative risk deriving from chemicals children are exposed to through the food they eat. Exposure assessment is a fundamental step in risk assessment, that represents the first component in risk analysis. The degree of exposure is determined by the amounts of contaminated foods consumed and by the levels of chemical contained in those foods. The estimation of intake is based on a number of assumptions and subjects to considerable uncertainty and variability. Several methodologies, ranging from the simple but inaccurate "Budget Method" to complex but relatively accurate "Total Diet Study", have been developed by the joint FAO/WHO consultations for exposure assessment. Risk assessments are based on an intake toward the upper extreme of the range (e.g. 90th or 95th percentile), in order to ensure that the majority of consumers are protected from possible adverse effects. Intake of a chemical is normally expressed as the amount ingested per unit time (e.g. mg day⁻¹) while exposure, expressed as mg kg⁻¹ bw day⁻¹, considers also the body weight. Exposure assessment can be achieved by: food concentration data; data at "as consumed level", on meals eaten really day by day; consumption data; more days of data to better estimate chronic exposure (FAO/WHO, 2005).

Personal monitoring is considered a good option for estimating exposure. This can be achieved through the “one week duplicate diet” method, where study participants prepare and collect duplicate portions of all the foods and beverages consumed during the monitoring period. One week duplicate diet method allows calculation of the quantity of contaminants actually ingested by an individual (WHO/FAO, 2005) and accounts for the effects of food production, storage and preparation (Tomerlin et al. 1997; Thomas et al. 1997; Akland et al. 2000).

Duplicate portion studies may especially be useful to assess dietary exposures for well defined population subgroups, such as children (Wilhelm et al., 2002; Murakami et al., 2003), vegetarians (MAFF, 2000; Clarke et al., 2003), breastfeeding mothers (Gulson et al., 2001), adult women (Tsuda et al., 1995), or people who consume catering establishment meals (Leblanc et al., 2000), as they provide dietary exposure information at the individual level, based on the diet “as consumed” (FAO/WHO, 2005).

The aims of the present study were: to achieve information about the diet in children in primary school; to determine the PAH’s contamination levels of foods consumed; to evaluate the children’s daily average PAH intake and exposure from food pathway.

2. Materials and methods

Sampling

The recruiting of the children for the survey was conducted in the metropolitan area of Naples, the major city in Campania Region and in Southern Italy. Three public primary schools, selected on the basis of their urban localization, the first in the ancient historical centre, the second in a residential area and the third in the suburbs, were contacted and a meeting was organized with children and parents to explain the objectives of the study. Initially 78 families participated in the meeting. Finally, the parents of 30 children, 12 males and 18 females 7-9 years aged, 10 from each school, that didn’t participate in school meal service, agreed to participated in the study.

201 Informed consent was obtained from parents of children and the project was approved by the
202 Ethics Committee of Medical Faculty, University of Naples “Federico II”.

203 On the selected children the body weights were initially recorded and the average calculated. It
204 resulted to be 33 ± 7 Kg (ranging 23-52 Kg).

205 All the 30 **volunteer** families involved carried out simultaneously the activities concerning the
206 study during a week. A food-diary questionnaire was structured to obtain from children’s parents
207 daily records of the type and the amounts of each food and beverage effectively ingested by their
208 child and some information about the food cooking techniques adopted. Sampling protocols and
209 food-diary questionnaire, as quality assurance and quality control procedures, were designed
210 specifically. Initial instructions combined with a visit during the first meal collection provided the
211 participant parents with training to properly collect samples. Collection procedures were designed
212 to minimize participant burden. Each participant’s parents were given verbal and written
213 instructions on how to use the food-diary questionnaire over a continuous 24-h monitoring day and
214 on how to collect the foods and beverages. Finally, every day participants were invited to prepare
215 a duplicate of every food and beverage consumed, collecting them in a 2-L, wide-mouth,
216 polyethylene containers and storing them in the refrigerator. The food and beverage samples
217 collected and refrigerated by the parents at home in the day before have been taken on the morning
218 and transported to the laboratory by the field team personnel.

219 Wet solid foods were homogenized, divided in aliquots of 5 g and lyophilized; dry foods were
220 ground and divided in aliquots of 5 g; liquid foods were divided in aliquots of 10 g and
221 lyophilized. Composite foods, as ham or salami sandwiches, were considered as an unique sample.
222 Among fresh fruit, pears, oranges, peaches, etc. were peeled before homogenized; apples, apricots,
223 grapes, strawberries, etc. have been analyzed with the skin, after tap water washing. All the
224 analytical samples were stored in the dark at 4 °C until processed.

225
226 ***PAH detection***

16 PAHs were simultaneously detected, **8 non carcinogenic: naphthalene (NAP), acenaphthylene (ACEN), acenaphthene (AC), fluorene (FLU), phenanthrene (PHEN), anthracene (ANT), fluoranthene (FLUO), pyrene (PYR), and 8 carcinogenic: benz[a]anthracene (BaA), benzo[b]fluoranthene (BbFA), benzo[k]fluoranthene (BkFA), benzo[ghi]perylene (BghiPE), chrysene (CHR), benzo[a]pyrene (BaP), dibenz[a,h]anthracene (DBahA), indeno[1,2,3-cd]pyrene (IP).**

Analytical samples were hydrolyzed at 100° C for 150 min with 20 ml of ethanol, 3.5 ml of 50 % aqueous KOH (cod.n.159222 Merck. Darmstadt GmbH, Germany) and 0.4 ml of 2.5 M aqueous Na₂S (cod.n.159340 Merck). The alkaline mixture was cooled and transferred in separatory funnel rinsing the flask by n-hexane, ethanol and distilled water. The funnel was vigorously shaken for 5 min. Organic phase was collected in a flask extracting twice more the aqueous phase with n-hexane; the three n-hexane combined extracts, filtered through anhydrous Na₂SO₄ (cod.n.106639 Merck), were concentrated to about 3-5 ml and purified on a glass column packed with 8 g of silica gel (70/230 mesh ASTM, 0.063-0.200 mm Merck) activated at 140 °C for 180 min. PAHs were eluted as rapidly as possible by 30 ml of ethyl ether-n-hexane (10:90 v/v). After elution, the solvent was evaporated to 1-2 ml, transferred in a vial and dried under a mild stream of N₂. The dried extract was reconstituted by 1 ml of acetonitrile. All the glassware used was protected by light by aluminium wrapping. The PAH detection was carried out by a Shimadzu LC10 HPLC chromatograph (Shimadzu ATVP Tokio, Japan) on a Restek Pinnacle II PAH stainless steel column 25 cm length, 4.6 mm i.d. (Restek Co., Bellefonte PA USA) with an UV-Visible detector (mod.SPD-10AVP, Shimadzu) set at 254 nm, and a fluorescence detector (mod.RF-10AVP, Shimadzu) set at 280 nm excitation and at 425 nm emission wavelengths.

A linear gradient from 50% acetonitrile 50% deionized water to 100% acetonitrile at 1 mL/min over 25 min was applied.

Water was obtained from a Milli-Q plus ultrapure water system (Millipore Corporate Billerica, MA, USA). The PAH pool standard solution, Superchrom 610 PAH calibration mix B cod. n°

31455, was purchased to Superchrom S.r.L. Milan, Italy. It consisted of a solution in methylene chloride-methanol (50:50, vol/vol) of ACEN at 2000 µg/ml; NAP and AC at 1000 µg/ml; FLU, FLUO, BbFA, DBahA and BghiPE at 200 µg/ml; PHEN, ANT, PYR, BaA, CHR, BkFA, BaP and IP at 100 µg/ml. Chromatographic standard solutions at seven dilutions, from 1:100 to 1: 1000 v/v in acetonitrile were employed to obtain calibration curves. The 1:500 standard dilution was adopted as working solution. PAHs were identified and quantified by comparing, respectively, the retention times and the areas of the peaks in the samples with those of the standard compounds. At least three standard solutions, with PAH concentrations in the interior of the calibration curve, were measured at the beginning, on running and at the end of each batch of sample analysis. Plot of UV/fluorimetric response (peak area) vs amount of each standard compound was obtained.

Polyethylene containers used for food sampling were previously extracted twice with hot n-hexane, and the extracts tested for PAHs. All the extracts were PAH non detectable.

About the quality assurance (QA) and control (QC) and the recovery, for each food group one sample in which PAH's levels were all non detectable was selected as blank matrix. Fresh fruit+fruit juices and bread, crackers, etc. group+cornflakes were considered together. The performance of the analytical procedure was examined by extracting sample blank matrixes fortified with three known amounts of the PAH pool standard solution (2 ml of 1:250; 1:500; 1:1000 v/v dilutions). Three replicates at each of the three fortification levels were analyzed along with the blank matrix. At the three levels of fortification the recoveries were: for NAP-ACEN-PY-DBahA, $67\pm4\%$; $69\pm6\%$ and $71\pm5\%$ (mean = $69\pm5\%$); for BaA-IP-ANT-AC-FLU-PHEN-FLUO $80\pm4\%$; $85\pm6\%$ and $90\pm3\%$ (mean = $85\pm5\%$); for CHR-BkFA-BaP-BbFA-BghiPE, $87\pm6\%$; $90\pm7\%$ and $96\pm2\%$ (mean = $90\pm5\%$).

Each batch of samples also contained one fortified sample to verify method performance during the extraction set.

The blank matrix samples were tested and the mean blank values and SD are calculated. The limits of detection (LOD) were defined as the mean blank values plus 3SDs.

LODs and quantification limits (LOQ) for PAHs in ng g^{-1} were, respectively, 0.03 and 0.09 for ANT, BbFA, BkFA, BaP, BghiPE, IP; 0.05 and 0.15 for BaA, CHR, DBahA; 0.10 and 0.30 for AC, FLU, PHEN, FLUO; 0.20 and 0.60 for NAP, PYR; 0.40 and 1.20 for ACEN.

The concentrations of the 16 PAHs detected, corrected for recoveries (mean recovery values), were listed in two separate Tables, on the basis of their toxicological properties; they are presented as number of samples analyzed; median concentration values and ranges; % of the prevalence of contamination, assuming that, when a result was below the LOQ, for individual PAH the value was equal to the quantification limit (Upper bound value). The same procedure

was adopted in combining PAH concentrations as PAH2; PAH4 and PAH8. (Table 4).

The Margins of Exposure (MOEs) were calculated by dividing the lowest BMDL10 values of BaP, PAH2, PAH4 and PAH8 by the median and highest levels of dietary exposures (EFSA,2008).

3. Results and discussion

In Table 1 the children's typical diet with the distribution meals and the medium portion sizes consumed, as registered by parents in the food diary-questionnaires, and their daily food consumptions are shown. For each child the mean daily food consumption was calculated as arithmetic mean of the consumptions obtained every day during the week studied. The daily total food consumptions ranged from 241 to 1277 g day^{-1} , with a median value of 613 g day^{-1} .

The dietetic model described reproduces the traditional Southern Italian habits, with a quite light breakfast early in the morning, a more substantial meal (lunch) at 13-14 hours, in which a first course based on pasta or rice with tomato sauce or with legumes as beans, lentils, pies, chick peas is always present; a more simple meal (dinner) in the evening, consisting of pizza or meat or fish or a cold dish made with eggs, ham, salami or cheese served with vegetables; these two principal meals are generally concluded with fresh fruit. However, analyzing the food-diary questionnaire,

some new dietetic habits have been evidenced, as the consumption of cornflakes in the breakfast, a quite large daily intake of sweet or salted commercial snacks, the increasing use of fresh or frozen composite ready-to-cook dishes, as poultry-spinach cutlets, fish-fingers, etc.

The daily composition of the children's diet appears on average quite according to what suggested by the official Recommended Dietary Allowances for Italian people (SINU 1996), that, for the children aging from 7 to 9 years, suggest a daily energy intake of 80 kcal kg^{-1} body weight (bw) for males and $70.4 \text{ kcal kg}^{-1}$ bw for females. The energy intake has to be divided in 15 % on breakfast, 35 % on lunch, 30 % on dinner and 10 % each on the morning and the afternoon snacks. Considering the mean body weight of children involved in the study, 33 kg, and the average of the suggested daily male-female energy intakes, $75.2 \text{ kcal kg}^{-1}$ bw, the total energy intake would be $2482 \text{ kcal day}^{-1}$. In the above cited dietary scheme the calculated energy is meanly about $2200 \text{ kcal day}^{-1}$, distributed in 13 % on breakfast, 36 % on lunch, 27 % on dinner and the remaining 24 % divided on the morning/afternoon snacks.

The % of the prevalence of contamination, the median and the ranges of concentrations of single PAHs were **shown** in **Tables 2a; 2b**. FLU, PHEN, ANT, FLUO and PYR were the compounds **most commonly detected** in all the foods **analyzed**. Candies showed the highest median level for NAP and PHEN; chocolate and cornflakes presented highest median level for PHEN, while fruit juices for BaA and FLUO (2.61 and $2.23 \text{ } \mu\text{g kg}^{-1}$, respectively. Milk was the less contaminated food.

In milk, fruit juices, candies and fresh fruit BaP was never found at detectable levels; in the other foods analyzed the prevalence of detection varied from 24 % (fish) to 60 % (cakes, biscuit, pastries and meat); the medians of concentrations varied from 0.06 to $0.33 \text{ } \mu\text{g kg}^{-1}$; the highest concentrations were found in an egg based course ($17.77 \text{ } \mu\text{g kg}^{-1}$) and in a barbecued beefsteak ($15.79 \text{ } \mu\text{g kg}^{-1}$).

Lodovici et al. (1995) in a study on Italian diet found in raw and oil free cooked foods and in beverages BaP concentrations ranging from 0.001 to $1.445 \text{ } \mu\text{g Kg}^{-1}$; in this study BaP ranged from $< \text{LOD}$ to $17.77 \text{ } \mu\text{g Kg}^{-1}$, with a median value of $0.65 \text{ } \mu\text{g Kg}^{-1}$, a

value quite similar to those referred in the Reports by European Commission and "Istituto Superiore di Sanità" (EC, 2002; 2004; ISS, 2003) (Table 3).

The EU Regulation 1881/2006 (Commission of the European Parliament, 2006) fixed the maximum admissible concentrations for BaP in many foods. In this study only in one sample of grilled swordfish ($6.21 \mu\text{g kg}^{-1}$) and in two samples of beefsteaks, one barbecued ($15.79 \mu\text{g kg}^{-1}$) and one grilled ($7.19 \mu\text{g kg}^{-1}$), BaP exceeded the limits of 2 and $5 \mu\text{g kg}^{-1}$ set, respectively, for fresh fish and smoked meat.

The highest mean values of the PAH4 and PAH8 combinations were found in fresh fruit (Table 4), for the high concentrations of BaA (median value $8.51 \mu\text{g kg}^{-1}$) found in apple samples (n.20) (data not shown). This result is much more higher than that reported for BaA in apples by Lodovici et al. (1995) ($0.333 \mu\text{g kg}^{-1}$). We aren't able to explain this finding; a specific environmental pollution in the harvesting zones or a post harvest contamination, deriving from a surface treatment of the fruits, could have been affected the level of BaA contamination. Egg and meat based courses too showed values of PAH4 and PAH8 quite higher than the other food categories. According to EFSA, CHR and BaA were the dominating compounds while DBaHA appeared the lowest.

In Table 5 are reported the average contributions of a medium portion of each food to dietary BaP, PAH2, PAH4 and PAH8 daily intake (ng day^{-1}) of the children participant in the study, calculated multiplying the medium portion size of each food eaten for the mean concentration of each PAH found. Fresh fruit and fruit juices, followed by meat and egg based

products, showed the highest PAH4 and PAH8 contributions. The contribution from cereal based foods was about 34 %, according to the European available data referred by ISS (2003) and EFSA (2008).

The means, medians and ranges of BaP, PAH2, PAH4 and PAH8 dietary intakes and exposures in the children participant in the study were reported in Table 6. The BaP daily intake ranged from 16 to 1429 ng, with a median value of 153 ng; the daily exposure varied between 0.5

and 38 ng kg⁻¹ bw day⁻¹, with a median value of 5 ng kg⁻¹ bw day⁻¹. This median value of exposure to BaP was about twice higher than what reported in an evaluation of temporal trends in PAH dietary intakes in UK (foods collected on 1979 and on 2000; mean exposure for British children 7-10 years old 4.1 ng kg⁻¹ bw day⁻¹ on 1979 and 1.9-2.6 ng kg⁻¹ bw day⁻¹ on 2000.) (COT, 2002), but similar to the mean value of 5 ng kg⁻¹ bw day⁻¹ observed by Falcò et al. (2003) in children 4-9 years old (mean body weight 24 kg) from Catalonia (Spanish).

EFSA reported that the median BaP, PAH2, PAH4 and PAH8 dietary exposures, referred to an adult of 60 Kg bw and calculated both for mean and high dietary consumers, across European countries varied for BaP between 3.9-6.5 ng kg⁻¹ bw day⁻¹; for PAH2, between 10.7-18.0 ng kg⁻¹ bw day⁻¹; for PAH4, between 19.5-34.5 ng kg⁻¹ bw day⁻¹; for PAH8, 28.8-51.3 ng kg⁻¹ bw day⁻¹. These values are quite lower than those obtained in the present study as expected considering the children eat more food per body mass than adults. Margins of Exposure (MOEs) at the median of dietary exposure to benzo[a]pyrene, PAH2, PAH4 (Table 7) agreed with the safety values (≥10,000) proposed by the EFSA Scientific Committee, while PAH8 MOE resulted <10,000. However the MOEs obtained for high level consumers were all very lower than 10,000, indicating a potential concern for children's health and a possible need for risk management action.

Regarding the PAHs other than the carcinogenics, the EU Scientific Committee on Food has estimated that in Europe the dietary exposure to each of the most abundant compounds, such as PHEN, ANT, FLUO and PYR, corresponds mainly, for a person weighing 70 kg, to about 70, 80, 60 and 60 ng kg⁻¹ bw day⁻¹, respectively. The Committee stated that at these levels of intake non-carcinogenic effects are not to be expected and the risk of genotoxic effects from dietary exposure to PAH can be considered low (EC, 2002). On the basis of the consumption data (Table 1), of the median concentrations of non carcinogenic PAHs found in the food samples examined (Table 2a) and of the children's mean body weight (33 ± 7 kg), the exposures were estimated to amount to 89 13, 46 and 26 ng kg⁻¹ bw day⁻¹, respectively.

The 24-h intakes of foods and beverages by the participants measured in this study are considered representative of their dietary exposures during the monitoring period. However, it is important to note that the brief monitoring period may hinder data interpretation in several ways. The duplicate food collections over a week may not be representative of the amounts or types of foods consumed over time (month or year) or by other children residing in Naples. Besides it measures only immediate diet, but do not take into account the variability of diet due to e.g. festivities day, season, etc. (Lightowler and Davies, 2002). Moreover, underestimates may occur if the parents do not provide to collect duplicate portions of all the foods eaten by their children. In addition, it is possible that a participant may not eat breakfast, lunch or dinner on a day, or may consume foods and beverages away. Also as likely is a single food item that contains unusually high concentrations of the contaminants considered may cause the 24-h diet to measure high levels. Results of analyses of 24-h food collections may then suggest lower or higher than actual or typical intake, or estimated intake by other food sampling approaches. The diet diary/questionnaire is considered a powerful method in combinations with duplicate portion technique but it is not without problems. In fact it is recognised that asking individuals to collect a duplicate diet does affect their dietary habits and this must be taken into consideration. Moreover participants may record food consumption referred to portions served but not completely eaten by child. For these reasons, the results of the study should be interpreted cautiously with regard to PAH dietary exposure of Italian children.

4. Conclusions

In conclusion about the dietary habits of the children involved this study have shown that the composition of the diet and the subdivision of the energy intake among the meals were on average according to those suggested for the children aging from 7 to 9 years by the Recommended Dietary Allowances for Italian people. The results obtained confirm that the food **can** represent an important source of exposition to PAHs. Because the children's food consumptions were highly

variable day by day and child by child, the levels of exposure to PAH by food can be considerably different, achieving values significant in increasing the health risk. Joint FAO/WHO Food Standards Programme proposed draft code of practice for the reduction of the PAH contamination of food. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) conducted a comprehensive risk assessment of PAH in 2005 (WHO 2007). JECFA recommended keeping the content of these contaminants in food as low as reasonably achievable. Contamination of food with PAHs via environmental contamination should be controlled either by source-directed measures like filtering the smoke from relevant industries (e.g., cement work, incinerator and metallurgy) and limiting the exhaust of PAHs from cars. Good agricultural practices (GAPs), including the selection of appropriate farmland, could also decrease the environmental contamination of foods with PAHs. For reducing the contamination with PAHs during food processing the Codex Alimentarius Commission (CAC) have developed a Code of Practice (COP) as a mean of disseminating strategies that will facilitate the reduction of PAHs in internationally traded foodstuffs and of identifying critical points of importance for a reduction of contamination of food with PAH during commercial smoking and direct drying processes.

Foods marketed are eaten both by adults and by children besides it's need more watching and is necessary to better define legal limits of PAH concentrations in foods. The children have lower body weight and fast metabolism and are more exposed at risk due to the action of these contaminants. Furthermore it is important to underline the necessity of deeping the scientific knowledges also on the adverse effects of these pollutants on children and young people health. These results can be used to adopt a specific evaluations methodology for the young populations and to define safety values expressed in MOEs for children. Considering that children are a category that need to be protected, it's important to adopt preventive measures for reduce every risk for their health. The importance of modifying the cooking practices that can influence the contamination of foods with PAHs and of avoiding to consume too frequently foods grilled or

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2 432 barbecued has to be underlined by an adequate information of the population and, particularly, of
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References

1. Akland GG, Pellizzari ED, Hu Y, Roberds M, Rohrer CA, Leckie JO and Berry MR. 2000. Factors influencing total dietary exposures of young children. J Expo Anal Environ Epidemiol. 10: 710-722.
2. Chen BH, and Lin YS. 1997. Formation of Polycyclic Aromatic Hydrocarbons during processing of duck meat. J. Agric. Food Chem., 45, 1394-1403.
3. Clarke DB, Barnes KA., Castle L, Rose M, Wilson LA, Baxter MJ, Price KR, and DuPont MS. 2003. Levels of phytoestrogens, inorganic trace-elements, natural toxicants and nitrate in vegetarian duplicate diets. Food Chemistry, 81(2), 287-300.
4. Commission of the European Parliament. 2006. Commission regulation (EC) 1881/2006 of 20 December 2006 setting maximum levels for certain contaminants in foodstuffs. Off J Eur Union. L 364: 5-24.
5. COT. 2002. Polycyclic Aromatic Hydrocarbons in the 2000 Total Diet Study. Reports TOX/2002/26, TOX/2002/26 Annex A (Draft) and TOX/2002/26 Annex B. United Kingdom: Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment; pp 1-22. <http://www.food.gov.uk/multimedia/pdfs/31pah.pdf>
6. Duedahl-Olesen L, White S, and Binderup ML. 2006. Polycyclic Aromatic hydrocarbons (PAH) in Danish smoked Fish and Meat products. Polyc Arom Comp 26: 163-184.
7. EC (European Commission). 2002. Opinion of the Scientific Committee on Food on the risks to human health of Polycyclic Aromatic Hydrocarbons in food expressed on 4 December 2002. http://europa.eu.int/comm/food/fs/sc/scf/index_en.html
8. EC (European Commission). 2004. Report on tasks for scientific cooperation (SCOOP). Collection of occurrence data on polycyclic aromatic hydrocarbons in food. Report of experts participating in task 3.2.12. European Commission, Brussels. http://europa.eu.int/comm/food/food/chemicalsafety/contaminants/out153_en.pdf

9. **Edwards NT. 1983. Polycyclic hydrocarbons (PAHs) in the terrestrial environment. A review. J Environ Qual. 12: 427-441.**
10. EFSA (European Food Safety Authority). 2005. Opinion of the Scientific Committee on a request from EFSA related to a harmonized approach for risk assessment of substances which are both genotoxic and carcinogenic. The EFSA J. 282: 1-31.
http://www.efsa.europa.eu/EFSA/Scientific_Opinion/sc_op_ej282_gentox_en3.0.pdf
11. EFSA (European Food Safety Authority). 2008. Polycyclic Aromatic Hydrocarbons in Food Scientific Opinion of the Panel on Contaminants in the Food Chain (Question N° EFSA-Q-2007-136) Adopted on 9 June 2008. The EFSA J. 724: 1-114
12. European Standing Committee on Foodstuffs. 2001. Outcome of the expert group meeting on 3 October on ways to prevent contamination of olive residue oil and other oils with polycyclic aromatic hydrocarbons (PAH). Summary record of the 85th meeting of the Standing Committee on Foodstuffs, 25th October 2001, agenda item 9.
http://ec.europa.eu/food/fs/rc/scfs/rap09_en.pdf
13. Falcò G, Domingo JL, Llobet JM, Teixidò A, Casas C, & Muller L. 2003. Polycyclic aromatic hydrocarbons in food: human exposure through the diet in Catalonia, Spain. J Food Prot. 66: 2325-2331.
14. FAO/WHO (Food and Agriculture Organization of the United Nations/World Health Organization). 2005. Joint FAO/WHO Expert Committee on Food Additives (JECFA). Sixtyfourth meeting, Rome, 8-17 February 2005. Summary and Conclusions.
http://www.who.int/ipcs/food/jecfa/summaries/summary_report_64_final.pdf
15. **Gulson, BL, Mizon KJ, Korsch MJ, Mahaffey KR, and Taylor AJ. 2001. Dietary intakes of selected elements from longitudinal 6-day duplicate diets for pregnant and nonpregnant subjects and elemental concentrations of breast milk and infant formula. Environ Res 87(3): 160–174.**

16. ISS Istituto Superiore di Sanità. 2003. Bocca BV, Crebelli R, & Manichini E. Presenza degli idrocarburi policiclici aromatici negli alimenti. Rapporti ISTISAN 03/22: 1-45.
17. Jira W. 2004. A GC/MS method for the determination of carcinogenic polycyclic aromatic hydrocarbons (PAH) in smoked meat products and liquid smokes. *Eur Food Res Technol* 218: 208-212.
18. Leblanc JC, Malmauret L, Guérin T, Bordet R, Boursier B, and Verger P. 2000. Estimation of the dietary intakes of pesticides residues, lead, cadmium, arsenic and radionuclides in France. *Food Add Contam* 17: 925-932.
19. Lightowler H J and Davies G J. 2002. Assessment of iodine intake in vegans: weighed dietary record vs duplicate portion technique *Eur J Clinical Nutr* 56, 765-770.
20. Lodovici M, Dolara P, Casalini C, Ciappellano S, and Testolin G. 1995. Polycyclic aromatic hydrocarbon contamination in the Italian diet. *Food Add Contam* 12: 703-713.
21. MAFF 2000. Duplicate diet study of vegetarians—dietary exposures to 12 metals and other elements. London, United Kingdom, Ministry of Agriculture, Fisheries and Food, Food Standards Agency, January (Joint Food Standards Group Food Surveillance Information Sheet No. 193; (<http://archive.food.gov.uk/maff/archive/food/infosheet/2000/no193/193vege.htm>).
22. Mottier P, Parisod V, Turesky RJ, 2000. Quantitative determination of polycyclic aromatic hydrocarbons in barbecued meat sausages by gas chromatography coupled to mass spectrometry. *J. Agric. Food Chem.* 48: 1160-1166.
23. Murakami T, Narita N, Nakagaki H, Shibata T, and Robinson C. 2003. Fluoride intake in Japanese children aged 3-5 years by the duplicate-diet technique. *Fluoride* 36(1): 49.
24. Nielsen T, Jørgensen HE, Larsen JC, and Poulsen M. 1996. City air pollution of polycyclic aromatic hydrocarbons and other mutagens: occurrence, sources and health effects. *Sci. Tot. Environ* 189/190, 41-49.

25. Olden K, & Guthrie J. 2000. Children's health: a mixed review. *Environ Health Persp.* 108: 250-251.
- 26. Petersen BJ & Barraj LM (1996): Assessing the intake of contaminants and nutrients: an overview of methods. *J. Food Comp. Anal.* 9: 243 – 254.**
- 27. Phillips DH. 1999. Polycyclic Aromatic Hydrocarbons in the Diet. *Mutat. Res.*, 443: 139-147.**
- 28. Saint-Aubert B, Cooper JF, Astre C, Spiliotis J, and Joyeux H, 1992. Evaluation of the induction of polycyclic aromatic hydrocarbons (PAH) by cooking on two geometrically different types of barbecue. *J. Food Compos. Anal* 5: 257-263.**
29. SINU (Società Italiana di Nutrizione Umana). 1996. Livelli di Assunzione raccomandati di energia e nutrienti. Istituto Nazionale della Nutrizione. Rome. Italy
30. Speer K, Steeg E, Horstmann P, Kühn Th and Montag A. 1990. Determination and distribution of polycyclic aromatic hydrocarbons in native vegetable oils, smoked fish products, mussels and oysters, and bream from the river Elbe. *J High Resol Chromatogr.* 13: 104-111.
31. Thomas KW, Sheldon LS, Pellizzari ED, Handy RW, Roberds JM and Berry MR. 1997. Testing duplicate diet sample collection methods for measuring personal dietary exposures to chemical contaminants. *J Expo Anal Environ Epidemiol.* 7: 17-36.
32. Tomerlin JR, Berry MR, Tran NL, Chew SB, Petersen BJ, Tucker KD and Fleming KH. 1997. Development of a dietary exposure potential model for evaluating dietary exposure to chemical residues in food. *J Expo Anal Environ Epidemiol.* 7: 81-101.
- 33. Tsuda T, Inoue T, Kojima M, and Aoki S. 1995. Market basket and duplicate portion estimation of dietary intakes of cadmium, mercury, arsenic, copper, manganese, and zinc by Japanese adults. *J AOAC Int* 78(6): 1363–1368.**
34. WHO/FAO Codex Alimentarius Commission Agenda Item 9. 2007. JOINT FAO/WHO food standards programme Codex committee on contaminants in foods. Proposed draft

code of practice for the reduction of contamination of food with polycyclic aromatic hydrocarbons (PAH) from smoking and direct drying processes.
<http://www.codexalimentarius.net>

35. WHO/FAO.2005. Dietary exposure assessment of chemicals in food Report of a Joint FAO/WHO Consultation Annapolis, Maryland, USA 2–6 May 2005: pp. 1-88

36. WHO/IPCS (World Health Organization/International Programme on Chemical Safety).1998. Selected Non-heterocyclic Polycyclic Aromatic Hydrocarbons. Environmental Health Criteria 202. International Programme on Chemical Safety, World Health Organization, Geneva.

37. Wilhelm M, Wittsiepe J, Schrey P, Budde U, and Idel H. 2002. Dietary intake of cadmium by children and adults from Germany using duplicate portion sampling. Sc Tot Environ 285: 11–19.

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Table 1. **Composition of the typical diet** of the children participant in the study, **median** portion size (g) of food consumed and **daily food consumption (g/day).**

Meal/Food	Medium portion size (g)
<i>Breakfast (between 7 and 8:30 a.m.)</i>	
Milk	150
Cakes, biscuits, pastries, etc	25
Cornflakes	25
<i>Snack (about 10 a.m. and about 4 p.m.)</i>	
Fruit juices	150
Ham or salami sandwiches	70
Cakes, pastries, etc	25
Chocolate	20
Candies	20
<i>Lunch (between 12.30 and 2 p.m.)–Dinner (between 7:30 and 8:30 p.m.)</i>	
Pasta or rice with tomato sauce or legumes	100
Meat and meat products	90
Fish and fish products	100
Dairy products	70
Egg based products	60
Pizza	70
Fresh or cooked vegetables	100
Bread, crackers, bread sticks, rusks, etc	65
Fresh fruit	150

Daily food consumption (g/day)		Median	Range
Mean ± S.D.	632 ± 215	613	241- 1277

Table 2a. Prevalence of contamination (%) and concentrations of non carcinogenic PAHs (median and range $\mu\text{g Kg}^{-1}$) in foods consumed by the children participant in the study.

PAH	FOOD															
	Milk (Samples n.80)		Cakes, biscuits, pastries, etc (Samples n. 120)		Cornflakes (Samples n. 81)		Fruit juices (Samples n.65)		Ham or salami sandwiches (Samples n. 67)		Chocolate (Samples n. 67)		Camdies (Samples n.28)		Pasta or rice with tomato sauce or legumes (Samples n. 203)	
	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)
NAP	20	0.33 (0.33-2.16)	31	0.33 (0.33-42.24)	50	2.46 (0.33-10.76)	30	0.33 (0.33-5.01)	30	0.33 (0.33-19.80)	29	0.33 (0.33-9.90)	67	3.10 (0.33-12.27)	28	0.33 (0.33-21.10)
ACEN	—	<LOD	27	1.32 (1.32-39.60)	17	1.32 (1.32-38.94)	32	1.32 (1.32-5.65)	27	1.32 (1.32-50.32)	14	1.32 (1.32-8.05)	33	1.32 (1.32-22.44)	10	1.32 (1.32-19.46)
AC	—	<LOD	18	0.54 (0.54-23.07)	—	<LOD	48	0.54 (0.54-5.81)	20	0.54 (0.54-39.56)	43	0.54 (0.54-13.60)	—	<LOD	14	0.54 (0.54-13.60)
FLU	48	0.14 (0.14-0.41)	41	0.14 (0.14-2.49)	67	0.54 (0.14-0.87)	33	0.14 (0.14-1.02)	40	0.14 (0.14-2.90)	43	0.14 (0.14-3.13)	67	0.59 (0.14-0.76)	70	0.18 (0.14-3.98)
PHEN	70	0.92 (0.14-2.60)	93	2.35 (0.04-15.85)	100	4.04 (1.53-11.70)	100	1.74 (0.04-8.52)	87	2.26 (0.04-20.10)	86	4.78 (0.04-8.84)	67	2.86 (0.04-5.20)	94	2.00 (0.04-12.70)
ANT	50	0.34 (0.04-3.58)	89	0.21 (0.01-4.00)	100	0.58 (0.11-2.24)	100	0.30 (0.01-1.11)	84	0.24 (0.01-2.24)	86	0.46 (0.01-8.29)	67	0.22 (0.11-2.25)	93	0.18 (0.01-4.48)
FLUO	50	0.57 (0.14-2.21)	69	1.01 (0.14-15.71)	67	0.88 (0.14-6.75)	67	2.23 (0.14-3.29)	60	1.30 (0.14-3.38)	86	1.89 (0.13-5.40)	67	0.94 (0.13-5.37)	46	0.14 (0.14-29.40)
PYR	40	0.06 (0.06-1.08)	62	0.40 (0.06-8.86)	83	1.05 (0.06-1.86)	67	0.51 (0.06-1.81)	67	0.71 (0.06-3.48)	71	0.60 (0.36-2.04)	35	0.06 (0.06-4.98)	47	0.06 (0.06-24.70)

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PAH	FOOD															
	Meat and meat products (Samples n. 126)		Fish and fish products (Samples n. 58)		Dairy products (Samples n. 75)		Egg based products (Samples n. 79)		Pizza (Samples n. 57)		Fresh or cooked vegetables (Samples n. 91)		Bread, crackers, bread sticks, rusks, etc (Samples n. 42)		Fresh Fruit (Samples n. 88)	
	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)
NAP	33	0.33 (0.33-89.76)	47	0.33 (0.33-11.73)	30	0.33 (0.33-18.08)	44	0.33 (0.33-101.50)	30	0.33 (0.33-4.82)	31	0.33 (0.33-63.36)	30	0.33 (0.33-27.72)	20	0.33 (0.33-30.10)
ACEN	12	1.32 (1.32-31.10)	24	1.32 (1.32-7.19)	—	<i><LOD</i>	6	1.32 (1.32-85.80)	—	<i><LOD</i>	10	1.32 (1.32-27.72)	22	1.32 (1.32-68.64)	12	1.32 (1.32-5.65)
AC	20	0.54 (0.54-17.49)	12	0.54 (0.54-6.80)	18	0.54 (0.54-8.16)	25	0.54 (0.54-29.92)	10	0.54 (0.54-8.20)	20	0.54 (0.54-18.12)	11	0.54 (0.54-7.34)	36	0.54 (0.54-14.34)
FLU	47	0.14 (0.14-11.40)	41	0.14 (0.14-3.55)	47	0.14 (0.14-1.13)	44	0.14 (0.14-3.54)	70	0.45 (0.14-1.10)	69	0.16 (0.14-2.25)	56	0.18 (0.14-5.18)	40	0.14 (0.14-7.70)
PHEN	94	2.60 (0.04-27.53)	95	2.86 (0.04-25.27)	88	2.43 (0.04-22.07)	88	3.28 (0.04-18.72)	90	1.69 (0.04-9.47)	87	1.83 (0.03-13.00)	96	2.00 (0.04-13.00)	88	1.96 (0.04-7.41)
ANT	80	0.26 (0.01-7.84)	82	0.22 (0.01-2.65)	82	0.21 (0.01-1.66)	75	0.25 (0.01-1.68)	90	0.24 (0.01-1.12)	76	0.20 (0.01-11.95)	74	0.22 (0.01-6.15)	84	0.31 (0.01-1.18)
FLUO	61	1.20 (0.14-18.00)	65	1.35 (0.13-13.66)	53	0.57 (0.14-3.58)	67	0.70 (0.13-3.92)	60	0.48 (0.14-2.30)	87	0.18 (0.14-40.50)	56	1.01 (0.14-62.10)	56	0.66 (0.14-3.38)
PYR	65	0.68 (0.06-13.80)	58	0.60 (0.06-8.79)	59	1.03 (0.06-3.84)	56	0.54 (0.06-5.29)	40	0.06 (0.06-2.40)	54	0.09 (0.06-3.60)	44	0.06 (0.06-2.81)	68	0.71 (0.06-6.00)

Table 2b. Prevalence of contamination (%) and concentrations of mutagenic/carcinogenic PAHs (median and range $\mu\text{g Kg}^{-1}$) in foods consumed by the children participant in the study.

PAH	FOOD															
	Milk (Samples n. 80)		Cakes, biscuits, pastries, etc (Samples n. 120)		Cornflakes (Samples n. 81)		Fruit juices (Samples n.65)		Ham or salami sandwiches (Samples n. 67)		Chocolate (Samples n. 67)		Camdies (Samples n.28)		Pasta or rice with tomato sauce or legumes (Samples n. 203)	
	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)
BaA	—	<LOD	29	0.05 (0.05-7.69)	33	0.05 (0.05-1.89)	100	2.61 (0.995-24.20)	40	0.05 (0.05-2.34)	57	0.37 (0.05-1.75)	67	1.23 (0.05-3.62)	32	0.05 (0.05-33.70)
CHR	28	0.03 (0.03-0.85)	33	0.03 (0.03-15.00)	33	0.03 (0.03-0.74)	35	0.03 (0.03-5.00)	30	0.03 (0.03-0.84)	42	0.03 (0.03-1.13)	67	0.23 (0.03-0.45)	30	0.03 (0.03-1.25)
BbFA	25	0.02 (0.02-0.74)	33	0.02 (0.02-2.22)	17	0.02 (0.02-0.43)	26	0.02 (0.02-1.80)	30	0.02 (0.02-12.62)	28	0.02 (0.02-0.94)	33	0.02 (0.02-1.00)	10	0.02 (0.02-4.68)
BkFA	25	0.06 (0.06-1.00)	20	0.06 (0.06-2.46)	17	0.06 (0.06-1.14)	32	0.06 (0.06-2.45)	—	<LOD	42	0.06 (0.06-1.40)	35	0.06 (0.06-2.95)	6	0.06 (0.06-2.73)
BaP	—	<LOD	60	0.30 (0.06-2.60)	50	0.33 (0.06-0.90)	—	<LOD	40	0.06 (0.06-2.37)	29	0.06 (0.06-0.63)	—	<LOD	45	0.06 (0.06-1.50)
DBahA	25	0.24 (0.24-0.55)	4	0.24 (0.24-3.54)	17	0.24 (0.24-2.10)	7	0.24 (0.24-1.24)	33	0.24 (0.24-18.90)	14	0.24 (0.24-18.88)	33	0.24 (0.24-8.00)	4	0.24 (0.24-7.55)
DBahP	—	<LOD	11	0.18 (0.18-16.00)	34	0.18 (0.18-2.20)	10	0.18 (0.18-3.88)	10	0.18 (0.18-6.50)	14	0.18 (0.18-4.18)	32	0.18 (0.18-15.00)	8	0.18 (0.18-30.84)
IP	—	<LOD	13	0.06 (0.06-3.00)	—	<LOD	14	0.06 (0.06-2.89)	7	0.06 (0.06-4.80)	14	0.06 (0.06-3.25)	34	0.06 (0.06-1.80)	10	0.06 (0.06-25.20)

PAH	FOOD															
	Meat and meat products (Samples n. 126)		Fish and fish products (Samples n. 58)		Dairy products (Samples n. 75)		Egg based products (Samples n. 79)		Pizza (Samples n. 57)		Fresh or cooked vegetables (Samples n. 91)		Bread, crackers, bread sticks, rusks, etc (Samples n. 42)		Fresh Fruit (Samples n. 88)	
	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)	Pos %	Median (min-max)
BaA	49	0.05 (0.05-12.50)	64	0.45 (0.05-5.06)	18	0.05 (0.05-0.89)	37	0.05 (0.05-26.25)	40	0.05 (0.05-1.25)	38	0.05 (0.05-17.50)	30	0.05 (0.05-11.25)	56	0.56 (0.05-48.00)
CHR	43	0.03 (0.03-6.43)	35	0.03 (0.03-3.18)	53	0.15 (0.03-1.25)	37	0.03 (0.03-2.20)	20	0.03 (0.03-1.22)	25	0.03 (0.03-6.82)	41	0.03 (0.03-3.75)	40	0.03 (0.03-4.13)
BbFA	25	0.02 (0.02-30.28)	29	0.32 (0.02-1.26)	12	0.02 (0.02-0.54)	62	0.03 (0.02-9.83)	40	0.02 (0.02-0.70)	50	0.02 (0.02-1.26)	37	0.02 (0.02-2.05)	12	0.02 (0.02-0.51)
BkFA	10	0.06 (0.06-14.76)	46	0.06 (0.06-1.23)	5	0.06 (0.06-6.49)	62	0.08 (0.06-1.97)	40	0.06 (0.06-4.98)	31	0.06 (0.06-1.12)	15	0.06 (0.06-0.98)	8	0.06 (0.06-1.34)
BaP	60	0.09 (0.06-15.79)	24	0.06 (0.06-6.20)	35	0.06 (0.06-1.08)	38	0.06 (0.06-17.77)	50	0.07 (0.06-3.50)	46	0.06 (0.06-2.48)	40	0.06 (0.06-3.55)	—	<LOD
DBahA	6	0.24 (0.24-24.78)	29	0.24 (0.24-12.04)	5	0.24 (0.24-4.72)	19	0.24 (0.24-5.66)	20	0.24 (0.24-6.95)	25	0.24 (0.24-8.85)	11	0.24 (0.24-4.54)	16	0.24 (0.24-7.54)
DBahP	10	0.18 (0.18-11.64)	18	0.18 (0.18-13.69)	5	0.18 (0.18-2.90)	13	0.18 (0.18-18.00)	20	0.18 (0.18-10.00)	10	0.18 (0.18-1.20)	7	0.18 (0.18-26.88)	15	0.18 (0.18-13.84)
IP	18	0.06 (0.06-6.00)	6	0.06 (0.06-4.15)	5	0.06 (0.06-4.54)	25	0.06 (0.06-4.45)	10	0.06 (0.06-19.00)	5	0.06 (0.06-14.40)	7	0.06 (0.06-16.80)	16	0.06 (0.06-12.00)

Table 3. Comparisons between the BaP concentrations ($\mu\text{g Kg}^{-1}$) obtained in foods analyzed in the present study and the literature data.

Meals	Benzo(a)pyrene $\mu\text{g Kg}^{-1}$				
	Present study	SCF, 2002	ISS, 2003	Falcò et al.2003	European Commission, 2004
	Median value	Mean or Range mean	Mean or Range mean	Mean	Mean
Milk	<0.06	0.1	n.d. – 0.01	0.011	–
Cakes, biscuits, pastries, etc.	0.30	0.25	0.3- 0.4		
Bread, crackers, bread sticks, rusks, etc.	0.06	0.10	0.01- 0.11		
Cornflakes	0.33	0.12	0.04	0.262	0.16
Pasta or rice with tomato sauce or legumes	0.06	–	n.d. – 0.02		
Pizza	0.07	–	0.02-0.2		
Chocolate	0.06	0.1	–	–	–
Meat and meat products	0.09 (15.79)*	0.1 (157.00)*	n.d.- 0.05 (212.00)*	0.098	(157.00)*
Fish and fish products	0.06	0.1	n.d.- 4.5	0.235	0.2
Dairy products	0.06	0.04		0.078	–
Egg based products	0.06	–	0.01	0.023	–
Fresh or cooked vegetables	0.06	0.5- 4.2	n.d. – 1.4	0.013	–
Fresh fruit	<0.06	–	n.d – 0.5	0.014	–

* grilled meat

Table 4. Concentrations **mean, range and geometrics mean** ($\mu\text{g kg}^{-1}$) of BaP, PAH2, PAH4 and PAH8 in the foods analyzed in the present study.

Foods		BaP		PAH2		PAH4		PAH8	
		Mean ± S.D.	Geometric Mean Range	Mean ± S.D.	Geometric Mean Range	Mean ± S.D.	Geometric Mean Range	Mean ± S.D.	Geometric Mean Range
		µg kg ⁻¹							
Milk		<0.06 —		0.12 ± 0.14 0.09-0.91	0.10	0.26 ± 0.20 0.16- 2.00	0.12	0.87 ± 0.95 0.70 -3.49	0.54
Cereal based foods	Cakes, biscuits, pastries, etc	0.72 ± 0.78 <0.06-2.60	0.30	0.84 ± 2.90 0.09- 16.50	0.58	1.59 ± 3.50 0.16-17.88	1.07	3.44 ± 5.05 0.70-21.53	2.43
	Bread, crackers, bread sticks, rusks, etc	0.27 ± 0.71 <0.06-3.55	0.11	0.73 ± 1.10 0.09-3.81	0.27	1.98 ± 2.93 0.16-13.84	0.80	4.95 ± 8.27 0.70-29.65	2.03
	Cornflakes	0.41 ± 0.39 <0.06-0.90	0.26	0.58 ± 0.40 0.09-0.96	0.38	0.72 ± 0.50 0.16-1.44	0.53	1.44 ± 0.87 0.70-3.06	1.26
	Pasta or rice with tomato sauce or legumes	0.42 ± 0.45 <0.06-1.50	0.20	0.59 ± 0.57 0.09-2.05	0.32	1.75 ± 4.27 0.16-35.00	0.70	3.43 ± 6.90 0.70-40.00	1.77
	Pizza	0.41 ± 1.08 <0.06-3.50	0.14	0.57 ± 1.10 0.09-3.52	0.19	0.98 ± 1.32 0.16-3.90	0.42	2.25 ± 2.33 0.70-9.35	1.33
Fruit juices		<0.06 —		0.19 ± 1.06 0.09-5.06	0.10	4.11 ± 3.89 1.10-8.51	2.93	4.65 ± 3.90 1.63-9.05	3.63
Ham or salami sandwiches		0.19 ± 0.48 <0.06-2.37	0.09	0.31 ± 0.65 0.09-3.21	0.15	1.23 ± 2.92 0.16-15.88	0.44	2.79 ± 4.45 0.70-19.36	1.48
Chocolate		0.14 ± 0.21 <0.06-0.63	0.08	0.37 ± 0.41 0.09-1.19	0.22	1.09 ± 1.05 0.16-3.00	0.70	4.57 ± 7.07 0.70-20.41	2.26
Candies		<0.06 —		0.32 ± 0.21 0.09-0.51	0.25	1.97 ± 1.66 0.42-3.74	1.40	2.51 ± 1.66 0.96-4.27	2.11
Meat and meat products		0.75 ± 2.44 <0.06-15.78	0.09	1.17 ± 3.00 0.09-18.37	0.27	3.97±8.29 0.16-44.54	1.03	6.27± 10.67 0.70-60.00	2.62
Fish and fish products		0.58 ± 1.49 <0.06-6.20	0.20	1.00 ± 1.07 0.09-6.23	0.32	2.22 ± 2.74 0.16-8.44	0.94	3.06 ± 2.89 0.70-10.00	2.06
Dairy products		0.13 ± 0.24 <0.06-1.08	0.10	0.36 ± 0.41 0.09-1.31	0.21	0.60 ± 0.60 0.16-1.84	0.40	2.05 ± 3.00 0.70-12.78	1.30
Egg based products		1.17 ± 4.42 <0.06-17.77	0.09	1.62 ± 4.55 0.09-18.49	0.27	4.74 ± 8.09 0.16-26.71	0.89	7.20 ± 9.17 0.70-29.21	3.06
Fresh or cooked vegetables		0.18 ± 0.41 <0.06-2.48	0.08	0.50 ± 1.75 0.09-6.90	0.17	2.39 ± 4.08 0.16-18.06	0.72	3.41 ± 5.31 <0.70-24.70	1.71
Fresh fruit		<0.06 —		0.52 ± 0.98 0.09-4.19	0.18	6.03 ± 12.16 0.16-48.10	1.24	7.27 ± 12.12 0.70-49.00	2.81

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Table 5. **Mean** contributions of a **average** portion of foods to dietary BaP, PAH2, PAH4 and PAH8 daily intake (ng day⁻¹) of the children participant in the study.

Foods		Average portion size (g day ⁻¹)	DIETARY INTAKE (ng day ⁻¹)			
			BaP	PAH2	PAH4	PAH8
Milk		150	9.00	13.50	24.00	105.00
Cereal based foods	Cakes, biscuits, pastries, etc	25	18.00	21.00	39.75	86.00
	Bread, crackers, bread sticks, rusks, etc	65	17.55	47.45	128.70	321.75
	Cornflakes	25	10.25	14.50	18.00	36.00
	Pasta or rice with tomato sauce or legumes	100	42.00	59.00	175.00	343.00
	Pizza	70	28.70	39.90	68.60	157.50
Fruit juices		150	9.00	13.50	616.50	697.50
Ham or salami sandwiches		70	13.30	21.70	86.10	195.30
Chocolate		20	2.80	7.40	21.80	91.40
Candies		20	1.20	6.40	39.40	50.20
Meat and meat products		90	67.50	105.30	357.30	564.30
Fish and fish products		100	58.00	100.00	222.00	306.00
Dairy products		70	9.10	25.20	42.00	164.00
Egg based products		60	70.20	97.20	284.40	432.00
Fresh or cooked vegetables		100	18.00	50.00	239.00	341.00
Fresh fruit		150	9.00	78.00	904.50	1090.50

Table 6. BaP, PAH2, PAH4, PAH8 daily intake (mean $\pm$ S.D., median and range ng day⁻¹) and exposure (mean $\pm$ S.D., median and range ng kg⁻¹ bw day⁻¹) in the children participant in the study.

	INTAKE (ng day ⁻¹)		EXPOSURE (ng kg b.w day ⁻¹)	
	Mean $\pm$ S.D.	Median (Range)	Mean $\pm$ S.D.	Median (Range)
BaP	196 $\pm$ 205 (16. -1429)	153	6 $\pm$ 6 (0.5 – 38)	5
PAH2	461 $\pm$ 460 (23 – 3149)	318	14. $\pm$ 14 (0.7 – 77)	10
PAH4	1562 $\pm$ 1751 (43 – 9065)	990	47 $\pm$ 53 (2 – 336)	28
PAH8	2535 $\pm$ 2264 (188 – 9930)	1776	78 $\pm$ 77 (8– 396)	54

Table 7. Margins of exposure (MOEs) of dietary exposure to benzo[a]pyrene, PAH2, PAH4 and PAH8 at median and high level in the children participant in the study.

Marker for the carcinogenic PAH in food	median dietary exposure (ng kg ⁻¹ b.w. day ⁻¹)	high level dietary exposure (ng kg ⁻¹ b.w. day ⁻¹)	BMDL10 (mg kg ⁻¹ b.w. day ⁻¹)	MOE median dietary exposure	MOE high level dietary exposure
BaP	5	39	0.07	15474	1812
PAH2	10	77	0.17	17798	2213
PAH4	28	336	0.34	12128	1012
PAH8	54	396	0.49	9108	1236