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Is 1-hydroxypyrene a reliable bioindicator of measured dietary polycyclic aromatic hydrocarbon under normal conditions?

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Abstract

Five healthy volunteers consumed similar amounts of identical foods for 5 consecutive days. The concentration of pyrene and of benzo(*a*)pyrene was determined in each of the 15 meals by a short analytical method that included sample saponification, solvent extraction, and HPLC analysis. The volunteers also provided three daily total volume 8-h urine samples for the duration of the study for the assessment of 1-hydroxypyrene, a biomarker of pyrene and polycyclic aromatic hydrocarbon (PAH) exposure. Mean recoveries were 83 and 75%, respectively, for pyrene and benzo(*a*)pyrene in food. Daily dietary pyrene doses varied from 0.7 to 3 μg. Excluding two outliers consisting of meals containing charbroiled pork and beef, pyrene content in the meals estimated from the published literature data was correlated to the measured pyrene, but overestimated the actual concentration by ca. 70%. Despite the identical ingested doses of pyrene, there was a 50–76% (coefficient of variation) interindividual variability in the daily-excreted amount of 1-hydroxypyrene. Urinary excretion of this metabolite was not correlated with ingested dose of pyrene under the normal feeding conditions used in this study. Bioavailability, enzymatic polymorphism, and differences in enterohepatic cycling of the metabolite may contribute to the observed variability. It was calculated that dietary pyrene intake accounts for between 87.5 and 99.8% of the sum of dietary and inhalation intake. From the presented data, unless the above-mentioned factors are taken into account, 1-hydroxypyrene might not be a reliable bioindicator of ingested pyrene (PAHs) under normal feeding conditions.
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1. Introduction

In many workplaces, 1-hydroxypyrene (1-OHP) has been successfully used as a bioindicator of exposure to polycyclic aromatic hydrocarbons (PAHs) [1]. Occupational PAH exposure doses exceed by far those encountered in the general environment. For non-smoking people with no occupational exposure to these contaminants, the main source of PAH exposure comes from ingested food and from the breathing of both indoor and outdoor air. PAHs are indeed known contaminants of food arising from both their atmospheric deposition on crops and pyrolysis of food components during cooking [2–5].

Contradictory results, however, have been published regarding the relative contribution of food and air sources to the internal dose of PAHs. A few years

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ago, Lioy et al. [6] measured benzo(*a*)pyrene (BaP) in food and indoor air from ten houses located near a metal pipe foundry during 2 consecutive weeks. The authors reported that half of the results indicated that inhalation was the major pathway of exposure to BaP, while food intake was the major source for the other half. More recently, Phillips argued that diet was the major source of human exposure to PAHs, the largest contribution arising from cereals and vegetables rather than meat [7]. Van Rooij et al. estimated the relative contribution of food and air to the internal dose of PAHs as assessed by the measurement of urinary 1-OHP in 76 non-occupationally exposed people [8]. These authors concluded that in non-smokers dietary PAHs account for 99% of the observed urinary 1-OHP. It should be noted, however, that food contribution to PAH intake was estimated (not actually measured) from a food diary. In a study conducted on eight volunteers, Chénier and Viau collected all micturitions for a full week along with diary information on all food consumed by the volunteers [9] and estimated PAH intake. There was no relationship between the estimated PAH intake and the measured urinary 1-OHP. Similarly, Scherer et al. assessed the relationship between the diary-estimated dietary PAH intake and three biomarkers of PAH exposure, namely BaP-hemoglobin adducts, BaP-albumin adducts and urinary 1-OHP in 69 subjects [10]. They found no correlation between the estimated dietary PAH intake and any of the biomarkers.

In polluted areas of China [11] and Poland [12], increased urinary 1-OHP has been associated with ambient air contamination by PAHs. Increased urinary 1-OHP was also observed in non-occupationally-exposed people living near a Söderberg aluminum electrolysis plant compared to a control area in Canada [13]. The ambient concentrations of PAHs were, however, lower than in the cited studies from China and Poland. By contrast, in other studies by our group, no relationship between PAH air contamination and urinary 1-OHP was found in either a group of 22 physical exercise students in the Czech Republic [14] or in small kindergarten children in Montreal [15]. This may be attributed to the small contribution of airborne PAHs, compared with food PAHs, to the internal dose assessed by this biomarker.

The small-scale controlled study described in this report was therefore undertaken to: (a) verify whether an estimate of PAH dose from a food diary using existing data yields a reliable estimate of PAH dose from diet, (b) determine if there is a correlation between measured pyrene dietary intake and urinary 1-OHP, and (c) determine the interindividual variability in 1-OHP excretion in people fed exactly the same food in identical amounts for 5 consecutive days.

2. Experimental

2.1. Volunteers

Initially, five male volunteers were recruited for the study, and the Ethics Committee of our University approved the protocol. They were informed of the purpose and design of the study and they all signed a consent form. One week before the start of the study, the food menus to be served were discussed among them in order to take into consideration food allergies or other types of intolerance to specific foods or ingredients. The volunteers were instructed to restrain from eating any type of food outside the meals prepared for them and were only allowed to drink water ad libitum. However, shortly after the noon meal on the first day of the study, one volunteer indicated not feeling well (for reasons unrelated to the food served in the frame of the study) and decided to withdraw. A female volunteer immediately replaced him. During the evening of the first day, a second volunteer felt hungry and ate a meal at home, which excluded him from participating in the rest of the study. Another female volunteer replaced him. Thus, the final group of volunteers included three males and two females aged 25–53. They were all non-smokers and all reported not using any drug. Furthermore, data from a physiologically based pharmacokinetic model of urinary 1-OHP excretion currently under development (not shown) indicated that a three-fold increase in body fat would result in less than a 5% difference in 24-h 1-OHP excretion.

2.2. Meals

Personnel from the Department of Nutrition at the University of Montreal prepared all meals. Six

identical weighed portions of solid and liquid food were prepared and served at fixed hours: breakfast at 0800 h, lunch at 1200 h and dinner at 1700 h. An evening snack was provided to take home. Each volunteer ate all the contents of the served plates. One of the portions was taken back to the laboratory and kept at −20°C until ready to be processed for analysis. The content of the evening snack was combined to that of the dinner meal. Our collaborators from the Department of Nutrition were instructed to design menus with increasing PAH content based on their general knowledge of low and high PAH-containing food. The detailed menus served to the volunteers during the study appears in Appendix A. The choice of the daily menus was made such as to result in a gradual increase in estimated PAH content over the course of the study. Barbecued food items were cooked on a Hibachi using charcoal briquettes. At the end of the study, tables giving pyrene content of various foodstuffs were provided to the collaborators who were responsible for designing the menus [2,9]. They were asked to assess from these tables the total pyrene content of the food prepared for the volunteers and to calculate the resulting total estimated pyrene dose for each meal.

2.3. Analysis of PAH in food

2.3.1. PAH extraction

Liquids were extracted directly with *n*-hexane and ethyl acetate. However, preliminary results indicated that the doses of both pyrene and benzo(a)pyrene from the various beverages consumed by the volunteers (coffee, milk, fruit juices, beer, wine) made an insignificant contribution to the total ingested dose as previously observed in other studies (e.g., Ref. [4]). Hence, the contribution of beverages to the PAH dose was not considered in this study.

The solids from each frozen meal were lyophilized to a constant weight (approximately 60 h) in a Freeze Dryer 5 (Lab Con Co., Fisher Scientific, Nepean, Ontario, Canada). The lyophilized material was subsequently homogenized in a blender to a fine powder of uniform appearance. When required, a mortar and a pestle were used to complete the operation. The homogenates were kept at −20°C until ready to be extracted.

Because the composition of the meals varied widely, namely in terms of lipid, proteins, and fiber contents, a number of published analytical methods were considered. The objective pursued was to use a single method for all matrices. A combination of elements from these procedures [7,16] was finally adopted as follows. Five grams of lyophilized homogenate were weighed in a 42-ml Teflon tube equipped with a screw cap. A 25-ml aliquot of a 1:9 (v/v) mixture of aqueous sodium hydroxide (10 *M*) and methanol were added and placed in a shaker (Eberbach Ann Arbor, Michigan, USA) and extracted for 3 h at 180 oscillations per min. Following this saponification step, the samples were centrifuged at 3500 rpm for 10 min at room temperature. The volume of the liquid phase was measured and extracted three times with 15 ml of *n*-hexane using the oscillating shaker for 30 min each time followed by 10 min of centrifugation at 3000 rpm. The combined *n*-hexane fractions were evaporated to ca. 5 ml in a rotary evaporator. The residual volume was transferred in a 13×100 mm glass tube, centrifuged, and the clear supernatant transferred into a second 13×100 mm glass tube. The solvent was evaporated to dryness under a gentle stream of nitrogen and the residue dissolved in 1.0 ml of acetonitrile. The analyte content of food was calculated as:

$$[\mathrm{PAH}]_{\mathrm{food}} = \frac{[\mathrm{PAH}]_{\mathrm{extract}} \times V_{\mathrm{ACN}} \times 25}{V_{\mathrm{NaOH-MeOH}} \times W_{\mathrm{sample}}}$$

where $[\mathrm{PAH}]_{\mathrm{food}}$ is the concentration in the lyophilized meal of either pyrene or benzo(*a*)pyrene (μg/kg), $[\mathrm{PAH}]_{\mathrm{extract}}$ is the concentration (μg/ml) of PAH measured in the final volume of acetonitrile extract, V_{ACN} is the volume (ml) of acetonitrile in which the PAH are redissolved following extraction, 25 is the volume (ml) of aqueous sodium hydroxide and methanol used to perform the saponification, $V_{\mathrm{NaOH-MeOH}}$ is the recovered volume (ml) of aqueous sodium hydroxide and methanol following saponification, and W_{sample} is the weight of sample used to perform the analysis (kg). Both original and spiked samples were analyzed in duplicate, which allowed for the calculation of analytical recovery. Determination of enrichment was based on preliminary analysis of the original lyophilized samples. Samples having a pyrene concentration below 4 μg/kg were spiked (per 5 g of lyophilisate) with 5 ng of pyrene–

5 ng of benzo(*a*)pyrene, while samples having concentrations above that threshold were spiked with 10 ng of pyrene–5 ng of benzo(*a*)pyrene. Results reported in this article were corrected for recovery.

2.3.2. HPLC analysis

The analysis of PAH was performed using HPLC with fluorescence detection. The system consisted of an AS-100 autoinjector (Bio-Rad, Richmond, CA, USA), a model 1100 quaternary pump (Hewlett-Packard, Kirkland, Quebec, Canada), a 4.5×25 cm Supelcosil LC-PAH column (Supelco, Oakville, Ontario, Canada) maintained at 32°C, a LS-40 fluorometer (Perkin-Elmer, Buckingham, UK). Excitation/emission wavelength pairs were 333/390 and 296/405 nm for pyrene and benzo(*a*)pyrene, respectively. The detector signal was recorded and treated with PE Turbochrom 3 software. The injection volume was 40 μl. Elution conditions were (flow-rate of 1.6 ml/min): acetonitrile–water (90:10, v/v) for 6 min, then changing in one step to 96:4 (v/v) with a return to original conditions at 12.5 min. Approximate retention time for pyrene and benzo(*a*)pyrene under these conditions were ca. 4.2 and 9.7 min. For both analytes, a series of five standards were used: 6.25, 12.5, 25, 50, and 100 nmol/l. Calibration curves were linear and detection limits varied from 1 to 14 nmol/l for pyrene and from 4 to 14 nmol/l for benzo(*a*)pyrene [17].

2.4. Analysis of 1-hydroxypyrene in urine

2.4.1. Urine collection

Volunteers were asked to collect the total volume from all micturitions in 1-l plastic bottles containing a few crystals of thymol used as a bacteriostatic agent. The micturitions were combined for three 8-h periods starting approximately at 0600 h, 1400 h, and 2200 h. Exact time was recorded at the end of each period. The collection bottles were kept at 4°C at all times. For each volunteer, the urine volume of each collection period was recorded and a suitable aliquot kept at −20°C for subsequent analysis.

2.4.2. 1-Hydroxypyrene analysis

1-Hydroxypyrenene was analysed in urine samples according to Jongeneelen [18], as modified by Bouchard et al. [19]. Briefly, 25 ml of the urine samples were adjusted to pH 5 and enzymatically hydrolyzed overnight at 37°C with aryl sulfatase and β-glucuronidase. The hydrolysates were passed through a C_{18} Sep-Pak (Supelco, Oakville, Ontario, Canada) and eluted with methanol. The eluate was evaporated to dryness under nitrogen and redissolved in 2 ml of methanol. The same chromatographic system as that used for PAH analysis was used except that the LC-PAH column was replaced with a LC_{18} Supelcosil column (Supelco, Oakville, Ontario, Canada) and solvent gradient was as described in the above-referenced publications. In our laboratory, long term (3 years) measurement of inter-assay variability ($N=180$) of a positive control sample of rat urine indicated a coefficient of variation of 7.5%. Repeated measurements of 1-OHP in a positive control human urine ($N=6$) gave a coefficient of variation of 3.8%. Results were reported both as total daily excretion (in μg with 1 μg=5 nmol) or as μmol/mol creatinine. Creatinine was measured by the classical Jaffe reaction.

2.5. Statistics

The relationships between pyrene and benzo(*a*)pyrene in food, between estimated and measured pyrene in food, and between urinary excretion of 1-hydroxypyrene and measured dietary pyrene dose were examined with the Pearson correlation test. Despite the small numbers of data points in these correlations, Pearson was preferred to non-parametric Spearman statistics since the hypothesis tested was that of a useful linear correlation between the studied variables. The interindividual dispersion of daily urinary 1-hydroxypyrene excretion was described from the arithmetic mean and coefficient of variation. The level of significance was $P=0.05$.

3. Results

3.1. PAH in food

Mean(±SD) analytical recoveries were 83±20% for pyrene and 75±13% for benzo(*a*)pyrene. The

mean relative spans (100 × max − min/mean) within duplicates measured on all samples were 15% for pyrene and 13% for benzo(*a*)pyrene. For pyrene, the intra-assay ($N=5$) coefficients of variation on two samples were 13 and 20% while inter-assay ($N=3$) coefficients of variation on the same samples were 19 and 18%. Fig. 1 shows typical chromatograms of an original sample and of the same sample that had been spiked with pyrene and benzo(*a*)pyrene. Results of pyrene and benzo(*a*)pyrene doses measured in individual meals appear in Table 1. Pyrene concentrations varied between 1.1 and 9.4 μg/kg (dry weight basis). The total pyrene doses (per meal) ranged from 0.1 to 1.5. μg. Benzo(*a*)pyrene concentrations varied between not detected (seven samples) and 1.6 μg/kg, while doses ranged from below detection limit (seven samples) to 0.18 μg/meal. There was a statistically significant correlation between measured benzo(*a*)pyrene and pyrene concentrations ($r^2=0.52$, $P=0.045$; Fig. 2). Using either the slope of the regression or the ratio of individual data points indicates that concentration of dietary pyrene was on average between four and eight times that of benzo(*a*)pyrene. The relationship between the measured and estimated doses of pyrene, as assessed from the nature of the food consumed and published tables of pyrene content in various foodstuff is shown in Fig. 3. Overall, there is no statistically significant correlation between estimated and measured pyrene doses. However, visual

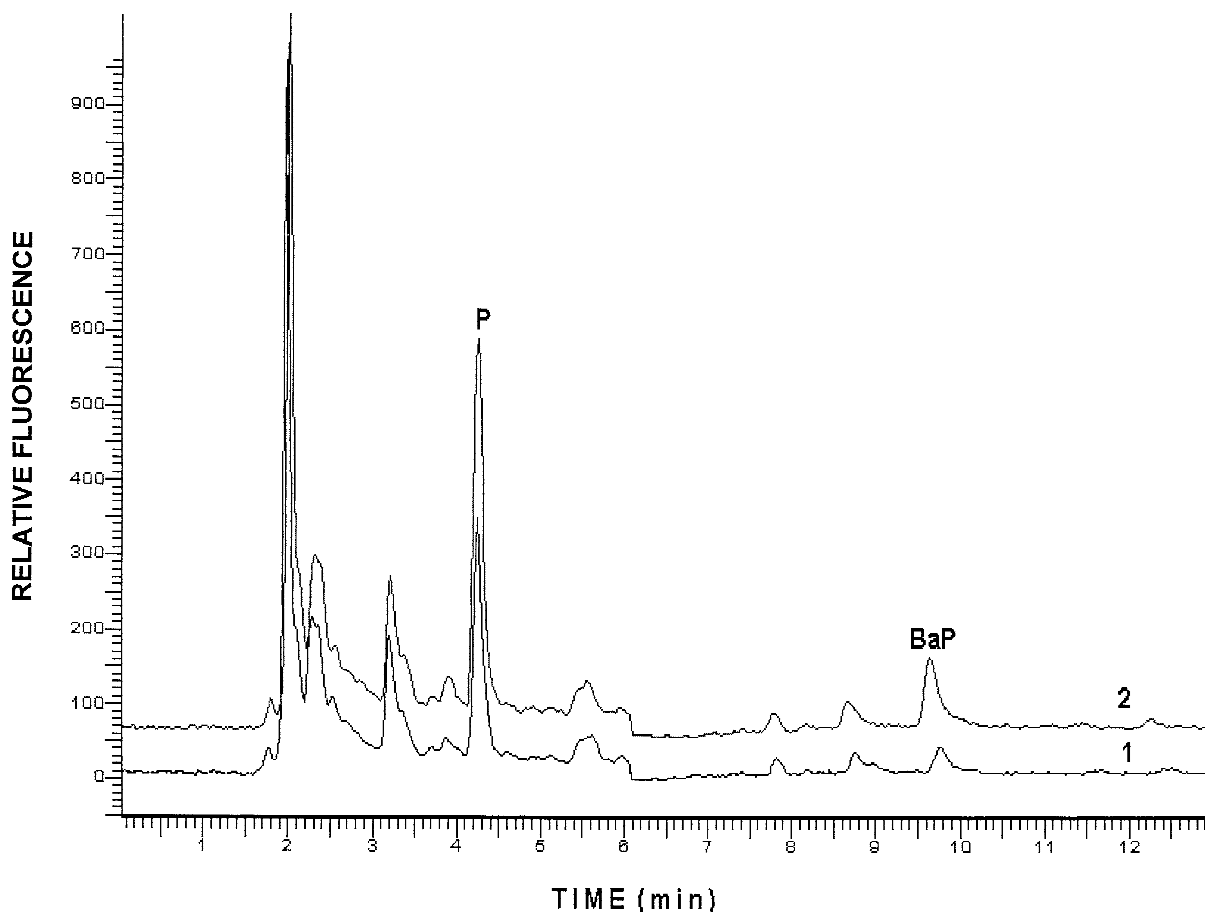

Fig. 1. Typical HPLC chromatograms of (1) a food sample and (2) the same food sample spiked with pure pyrene and benzo(*a*)pyrene before the extraction procedure. P = Pyrene peak, BaP = Benzo(*a*)pyrene peak.

Table 1
Measured pyrene and benzo(*a*)pyrene in food

Day	Meal	Pyrene		Benzo(*a*)pyrene	
		Concentration (μg/kg)	Dose (μg)	Concentration (μg/kg)	Dose (μg)
Monday	Breakfast	1.8	0.14	<LOD[b]	–
	Lunch	6.3	1.07	<LOD	–
	Dinner[a]	2.3	0.58	<LOD	–
Tuesday	Breakfast	1.4	0.19	<LOD	–
	Lunch	4.0	0.58	0.20	0.029
	Dinner	2.4	0.28	1.17	0.137
Wednesday	Breakfast	1.1	0.08	0.48	0.036
	Lunch	2.6	0.41	<LOD	–
	Dinner	1.5	0.24	<LOD	–
Thursday	Breakfast	1.7	0.17	0.33	0.032
	Lunch	9.4	1.49	1.55	0.246
	Dinner	6.5	1.35	<LOD	–
Friday	Breakfast	1.3	0.10	0.36	0.028
	Lunch	3.5	0.44	0.47	0.059
	Dinner	5.0	0.74	0.96	0.144

[a] Includes evening snack.
[b] Smaller than the limit of detection as defined by Miller and Miller [17].

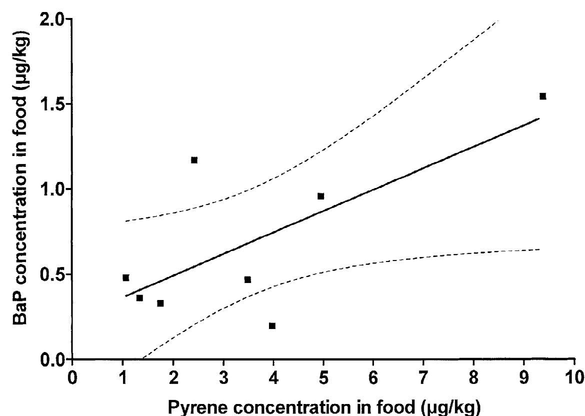

Fig. 2. Correlation between measured food pyrene and food benzo(*a*)pyrene in individual samples for which benzo(*a*)pyrene was detectable in the assay described in this article. $r^2=0.52$, $P=0.045$. The dotted lines represent the 95% confidence interval of the correlation.

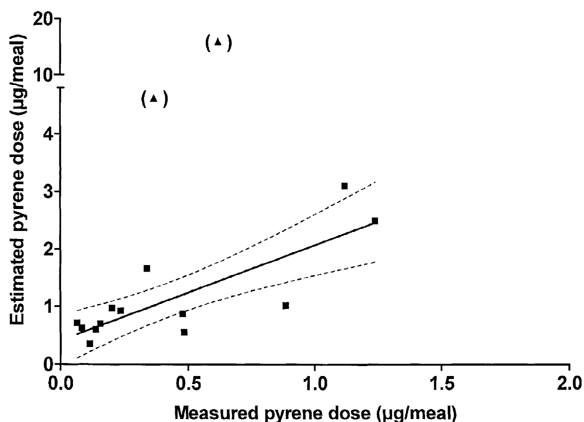

Fig. 3. Comparison of food pyrene content as estimated from published tables and as analysed in 15 meals. A significant correlation ($r^2=0.67$, $P=0.0006$) is found only after removing two barbecued meat meals (triangles within parentheses on the graph). The dotted lines represent the 95% confidence interval of the correlation.

inspection of the graph shows that two points are outliers. These correspond to values from Friday lunch and dinner meals where barbecued steak and barbecued pork chops were served. If these points are removed, a significant statistical correlation emerges ($r^2 = 0.67$, $P = 0.0006$) with a slope of 1.7, indicating that the estimated values are, on average, 70% higher than the actual measured values.

3.2. 1-Hydroxypyrene in urine

Fig. 4 shows two chromatograms of urine samples from the same individual analysed for 1-hydroxypyrene and displaying ''low'' and ''high'' concentrations of the analyte. As can be seen from Fig. 5, there was a large interindividual variability in the profiles of urinary 1-hydroxypyrene excretion, expressed in μmol/mol creatinine. The large interindividual variation in the urinary concentration of the metabolite in these individuals exposed to the same dietary doses of pyrene is noteworthy. A choice was made here to represent excretions as creatinine-corrected concentrations since this is a very frequent mode of expression of 1-hydroxypyrene concentrations when only spot urine samples are available, which occurs most of the time. Finally, Fig. 6 shows that no correlation was observed between the mean daily urinary excretion of 1-hydroxypyrene and the daily measured dose of ingested pyrene. The interindividual coefficient of variation in daily excretion of 1-hydroxypyrene was comprised between 50 and 76%.

4. Discussion

4.1. Analysis of pyrene and benzo(a)pyrene in food

This study was designed to allow the quantification of ingested doses of pyrene and benzo(*a*)pyrene. Most of the published analytical methods on food PAH determination aim at the determination of PAHs

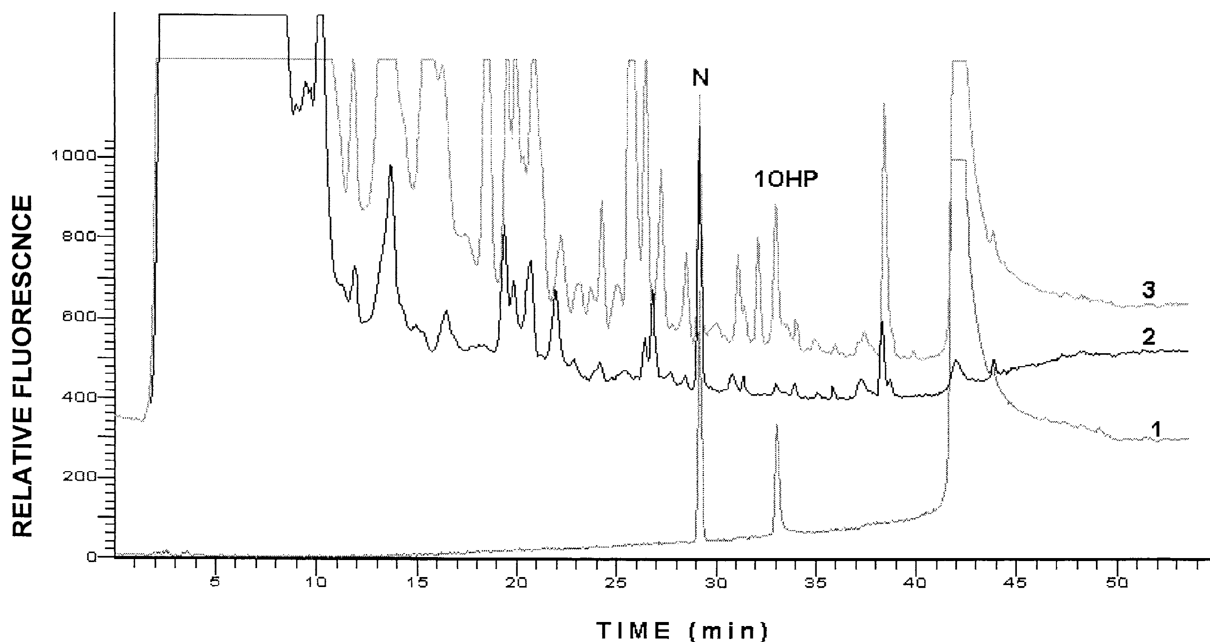

Fig. 4. Typical HPLC chromatograms of two urine samples from the same volunteer displaying a low (2) and a high (3) concentration of 1-hydroxypyrene. N = Naphthalene added to the final extract as a retention time reference, 1-OHP = 1-hydroxypyrene peak. Chromatogram (1) is the standard.

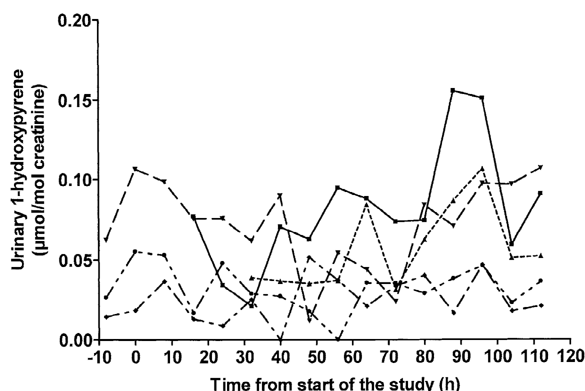

Fig. 5. Individual 1-hydroxypyrene excretion expressed as µmol/mol creatinine for each of the five volunteers of the study.

in specific matrices, e.g., vegetable oils [20], smoked food products [16,21], etc. Since the meals had variable protein and lipid contents, it was deemed necessary to use a saponification step combined with the extraction per se [7]. The mean recoveries obtained for both pyrene (83%) and benzo(*a*)pyrene (75%) were satisfactory given the variety of matrices to which the method was applied. Recently published methods optimized for smoked food gave recoveries of 88% for pyrene and of 84% and 94% for BaP [16,21]. The reproducibility, assessed by the measurement of the span of the results obtained for the duplicate determinations [15% for pyrene and 13% for benzo(*a*)pyrene], intra-assay (13.5, 20%) and inter-assay (19.5, 18%) coefficients of variations for pyrene, also suggests that this simple method is reliable.

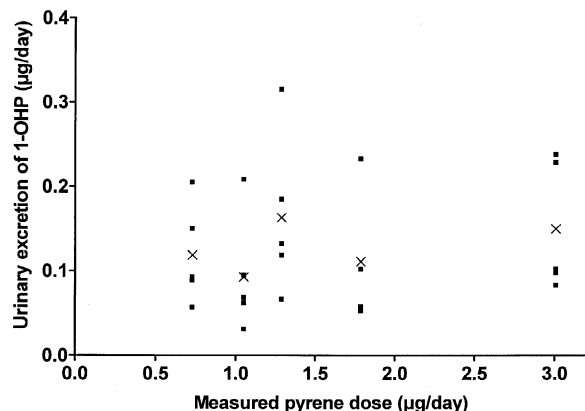

Fig. 6. Examination of the relationship between the daily excretion of 1-hydroxypyrene and the measured dietary daily dose of pyrene. Squares show the individual values whereas X represents the arithmetic mean.

4.2. Pyrene as an indicator PAH for overall PAH exposure

Previous studies indicated that the major contribution to dietary PAH intake comes from cereals (about one third) and from oils and fats (also one third), with fruits and vegetables contributing much of the remainder [7]. Meat, unless it is charbroiled to a very dark state, is considered to contribute relatively little to the daily-ingested dose. In Italy, it was found that the highest levels of PAHs were observed in pizza baked in wood-burning ovens and barbecued beef and pork [4]. Some authors found little difference in total PAH content between low and high temperature-cooked meat [5]. This study indicates that estimating pyrene concentration in charbroiled meat may largely overestimate measured pyrene content, suggesting that these types of food may pose the greatest problems in studies relying only on a diary to evaluate dietary PAH intake. For the other types of food, there seems to be a correlation between estimates and actual measured values but there was still a 70% overestimation of the estimated vs. measured food pyrene. Pyrene and fluoranthene often account for about half the total PAHs [7]. This observation and the fact that a significant linear correlation between pyrene and benzo(*a*)pyrene in food can be calculated from this and other published studies [2,22] suggests that, a priori, a bioindicator of exposure to dietary pyrene might be an acceptable surrogate to estimate dietary exposure to overall, and carcinogenic, PAHs.

4.3. Interindividual variation in urinary 1-hydroxypyrene excretion

All possible measures were taken in this study to ensure that all volunteers would consume exactly the same daily doses of dietary PAHs. For that purpose,

all meals were prepared, weighed, and served similarly by the same person, and an additional serving of each meal was saved for the quantitative determination of pyrene and benzo(*a*)pyrene. All volunteers took their meals at exactly the same time and they all consumed the weighed amount of food provided to them. Since, additionally, dietary PAH represents the bulk of PAH exposure in these individuals, one would expect that a reliable bioindicator of exposure would present minimal interindividual variations during the course of the study. But the results obtained indicated a 50–76% daily interindividual coefficient of variation in total (24-h) 1-hydroxypyrene excretion. Furthermore, there was no significant correlation between the measured ingested dose of pyrene and the 24-h excretion of 1-hydroxypyrene, which agrees with the recent observations by Scherer et al. in 69 non-occupationally exposed subjects [10]. Correlation was also absent between urinary 1-OHP and estimated pyrene dose from published tables. Using high doses [ca. 25 ng of benzo(*a*)pyrene per gram of meat] of dietary PAHs from charbroiled beef consumed by ten healthy volunteers for 5 consecutive days, Kang et al. also observed large interindividual variations in urinary 1-hydroxypyrene in the first morning voided urine samples [23]. One of the problems with the study of a real life situation such as that examined in the current report lies in the selection of sampling times for urine samples. Data from one of our previously published studies [24] allowed us to calculate that around 80% of the ''total'' (48-h) excretion of 1-OHP following ingestion of pyrene is excreted within 12 h. It was, therefore, estimated that the urine samples corresponding more closely to a given daily dose of dietary pyrene were those collected from one morning to the next. Indeed, the period ending in the morning was taken about 13 h after the previous meal. Any sample taken after breakfast would contain 1-OHP arising from pyrene present in this breakfast meal. As a corollary, the first morning urine also contained a fraction of the 1-OHP from dietary pyrene absorbed the day before. The planned gradual rise in food pyrene content over the course of the study limited the impact of the previous day's pyrene absorption on 1-OHP excretion.

Excluding variable interindividual doses, three main factors might account for these observations. First, there might be interindividual differences in the bioavailability of ingested pyrene. Second, there might be variations between individuals in the extent of pyrene biotransformation to 1-hydroxypyrene, which has been related to enzymatic polymorphism. Indeed, CYP1A1 Ile/Val, CYP MspI-homozygous, GSTM1-null and GSTT1+ genotypes and CYP1A2 phenotype have been associated with an increased 1-hydroxypyrene excretion when other variables are corrected for in a multivariate analysis [25–29]. However, other authors found no effect of CYP1A1 MspI, GSTM1 and GSTT1 on urinary 1-hydroxypyrene [30]. The third element that could account for interindividual differences in urinary 1-hydroxypyrene is that this metabolite undergoes intense enterohepatic cycling so that interindividual differences in the cycling efficiency would affect the urinary output of the metabolite [31,32]. Finally, the fact that for a given individual there was no clear relationship between the daily-ingested dose and the daily-excreted amount of 1-hydroxypyrene suggests that other elements may also contribute to the variable excretion of the metabolite. Whatever the precise contribution of each of these mechanisms, the present results confirm our previous observation made in two volunteers on the urinary yield of 1-hydroxypyrene from a given ''high'' oral bolus pyrene dose (500 μg) taken in olive oil varying by a factor of almost two between these two individuals [24].

4.4. Dietary intake vs. other sources

In non-occupationally exposed individuals, most published studies indicate that food is by far the main source of PAH exposure [7,15]. The total daily pyrene intake calculated in the present study (between 0.7 and 3 μg) is roughly the same as that reported in the UK (1.1 μg) [22]. Considering the extreme values of daily-ingested pyrene observed in this study (0.7 and 3 μg) and assuming ambient pyrene concentrations between 0.5 and 5 ng/m^3 [14] (corresponding to inhaled doses of between 0.01 and 0.1 μg for 20 m^3 inhaled volume per day), one can

calculate the relative contribution of each route of exposure excluding other sources. Dietary pyrene would contribute between 87.5% [0.7/(0.7+0.1)] and 99.8% [5/(5+0.01)] of the total pyrene dose.

Bioavailability is a potentially important factor that has rarely been considered in studies of exposure to PAHs. In experimental animals, comparing the molar percentage of 1-hydroxypyrene excreted in the rat following intravenous and oral administration (gavage in corn oil), Bouchard and Viau calculated a 100% bioavailability for orally administered pyrene [33]. However, pyrene bioavailability might be very different when ingested with solid food, which contains potential adsorption sites. Other authors have suggested a 12.5% bioavailability for ingested and 84% for inhaled pyrene [34]. Consideration of bioavailability could modify the percentage contribution of each route to total pyrene dose calculated above, possibly making ambient pyrene contribute more to the total absorbed dose, hence to the ultimate 1-hydroxypyrene excretion, if the latter bioavailability numbers were correct.

5. Conclusion

Healthy volunteers non-occupationally exposed to PAH and ingesting identical food in similar amounts show a 50–76% (coefficient of variation) interindividual variability in the urinary excretion of 1-hydroxypyrene that is not correlated to the relatively small variation in a measured dietary pyrene intake typical of the Canadian diet. Unless factors such as bioavailability, polymorphism in the biotransformation enzymes, and enterohepatic cycling are taken into account, 1-hydroxypyrene might not be a reliable bioindicator of ingested pyrene (PAHs) under normal feeding conditions.

Acknowledgements

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Appendix A. Detailed description of the meals consumed by the volunteers

Day	Meal	Menu	Weight (kg)
Monday	Breakfast	Orange juice	0.236
		Muslix Cereal	0.07
		Milk	0.2
		Cheese	0.022
		Coffee	0.175
	Lunch	Vegetable juice	0.34
		Bean salad and oil	0.29
		Whole wheat roll	0.045
		Butter	0.07
		Fruit purée	0.113
		Blueberry turnover	0.075
	Dinner	Red wine	0.125
		Baked lasagna	0.3
		Steamed broccoli	0.07
		Whole wheat roll	0.045
		Butter	0.007
		Fruit salad	0.014
		Maple and nut cookies	0.053
	Snack	Apricot fruit bar	0.042

Appendix A. Continued

Day	Meal	Menu	Weight (kg)
Tuesday	Breakfast	Fruit juice	0.2
		Carrot and blueberry muffins	0.156
		Butter	0.007
		Cheese	0.021
		Coffee and milk	0.2
	Lunch	Vegetable juice	0.236
		Oven broiled chicken souvlaki	0.1
		Tzatziki	0.05
		Pita bread	0.055
		Tabbouleh salad	0.1
		Cookies	0.03
	Dinner	Beer	0.2
		Pan fried Italian sausages	0.12
		Steamed green beans	0.08
		Potato salad	0.1
		Date square	0.075
		Dijon mustard	0.015
	Snack	Nut mix	0.03
Wednesday	Breakfast	Orange juice	0.236
		Toasted English muffin	0.085
		Butter and jam	0.044
		Milk	0.2
		Coffee	0.175
	Lunch	Vegetable juice	0.236
		Pan fried smoked ham steaks	0.1
		White rice	0.125
		Olive oil	0.01
		Tomato and cucumber salad	0.1
		Fruit purée	0.113
		Oatmeal cookies	0.053
	Dinner	Red wine	0.125
		Pan fried steak	0.135
		Baked potato	0.055
		Butter	0.007
		Broccoli and carrots	0.13
		Chocolate cake	0.055
	Snack	Granola bar	0.03

Appendix A. Continued

Day	Meal	Menu	Weight (kg)
Thursday	Breakfast	Fruit juice	0.2
		Toastedd bagel	0.09
		Smoked salmon	0.05
		Cream cheese	0.04
		Coffee and milk	0.215
	Lunch	Lemonade	0.2
		Hamburger (barbecued meat)	0.225
		Toasted hamburger bun	0.055
		Cole slaw	0.1
		Cherry strudel	0.06
	Dinner	Beer	0.2
		Barbecued chicken salad	0.1
		Mixed salad and dressing	0.085
		BBQ grilled sesame roll	0.025
		Key lime pie	0.14
		Cucumber, carrots	0.06
	Snack	Smoked meat	0.05
		Sliced rye bread	0.04
		Dijon mustard	0.01
Friday	Breakfast	Orange juice	0.236
		Toasted bagel	0.09
		Smoked trout	0.05
		Cream cheese	0.04
		Coffee and milk	0.215
	Lunch	Vegetable juice	0.236
		Barbecued steak	0.115
		Mixed salad with bacon	0.085
		BBQ grilled sesame roll	0.025
		Canned pears	0.14
	Dinner	Beer	0.2
		Barbecued pork chops	0.12
		Barbecued vegetables	0.08
		BBQ grilled bread	0.025
		Queen Elisabeth cake	0.06
		White rice	0.06
	Snack	Smoked meat	0.05
		Sliced rye bread	0.04
		Dijon mustard	0.01

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