Research Note

Polycyclic Aromatic Hydrocarbons in Food Samples Collected in Barcelona, Spain


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ABSTRACT

This study reports on the concentrations of eight polycyclic aromatic hydrocarbons (PAHs) in food samples collected in the city of Barcelona (Catalonia, Spain) from 2003 to 2004. Food samples included meat products, fish (fresh and smoked), other seafood (cephalopods, crustaceans, and bivalves), vegetable oil, and tea. Concentrations of benz[a]anthracene, benzo[h]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylen, benzo[a]pyrene, benzo[e]pyrene, dibenz[a,h]anthracene, and indeno[1,2,3-c,d]pyrene were determined by reversed-phase high-performance liquid chromatography with fluorescence detection. PAHs were detected in most tea samples (94%), which had the highest concentration of total PAHs (mean concentration of 59 μg/kg). Other food groups with a high presence of PAHs were bivalves (present in 34% of the samples; mean value of 2.7 μg/kg) and meat products (present in 13% of the samples; mean value of 1.7 μg/kg). The PAHs detected most frequently were benzo[e]pyrene and benzo[h]fluoranthene. No sample had levels above current regulation standards. Nevertheless, the frequent presence of PAHs in bivalves, tea samples, and meat products, together with the fact that dietary sources are the main exposure to these carcinogenic compounds, suggests the need for some monitoring scheme to follow up on these trends.

Polycyclic aromatic hydrocarbons (PAHs) are a large group of organic compounds that are formed during the incomplete combustion of organic substances. Experimental studies on animals show several adverse health effects, and their carcinogenicity and genotoxicity potential has attracted the most attention (27). The International Agency for Research on Cancer (13) determined that benzo[a]pyrene (BaP), benzo[a]anthracene, and dibenz[a,h]anthracene are probably carcinogenic in humans, while benzo[h]-fluoranthene, benzo[k]fluoranthene, dibenz[a,e]pyrene, dibenz[a,h]pyrene, dibenz[a,l]pyrene, 5-methylchrysene, and indeno[1,2,3-c,d]pyrene are possibly carcinogenic in humans.

The incomplete combustion of organic matter can occur spontaneously in nature (forest fires and volcanic eruptions), but the main source of environmental PAHS is human activities. Most come from fossil fuel combustion sources (such as automobiles, power plants, and residential heating), aluminum, iron and steel production, or refuse incineration (7). PAHs are mostly chemically inert, hydrophobic, and soluble in organic solvents (14). Consequently, environmental PAHs can be introduced along the food chain by plants and animals. Also, food can be contaminated during thermal treatment in food preparation or manufacture (drying or smoking) or cooking procedures (roasting, baking, or frying) (24).

Different studies have reported traces of PAHs in different food categories and beverages, including tea (15), fruits (22), cereals, oils (19), smoked meat (24), and fish (25), and have generally reported concentrations as parts per billion or micrograms per kilogram. It is generally thought that the predominant route of human exposure to environmental PAHs is food (estimated at more than 70%) for persons who are nonsmokers and nonoccupationally exposed (18, 26). Legal regulation is limited, partly because of the difficulty of defining safe levels of such a complex mixture. Recently, a European Union (EU) recommendation highlighted 15 PAHs as carcinogenic following the opinion of the Scientific Committee on Food (1, 23). An EU regulation, in force since 2005 (2), set limits only for benzo[a]pyrene (BaP) contents. It defines a threshold level for oils, edible fats, and fresh fish at 2 μg/kg. For smoked meat and fish products, the maximum level is set at 5 μg/kg, the same as for crustaceans and cephalopods. Levels for mollusks, bivalves, and infant foods have been set at 10 μg/kg. In addition, the government of Spain set a threshold level of 5 μg/kg for the sum of several PAHs in olive pomace oils after a media scare in 2001 due to the detection of BaP in these oils.

The presence of PAHs in food is a matter of concern, and health protection requires a sound information system. Following the opinion of the Scientific Committee on Food of the EU, which concluded that a safe threshold of exposure for PAHs in food could not really be defined, monitoring programs should not only control compliance with regulations, but should also control the actual presence of...
these different substances (5, 9). This article examines the results for PAHs with the aid of a monitoring program to determine the presence of several environmental pollutants in food in a large city within the EU.

MATERIALS AND METHODS

Food samples. Samples were collected as part of the IQSA program (Investigación de la Qualitat Sanitaria dels Aliments, Food Health Quality Research), developed by the public health services of the city of Barcelona (Catalonia, Spain) (3). This surveillance program started in 1984 with the aim of monitoring the presence and amount of additives and pollutants, be they chemical or microbiological, in food sold in the city of Barcelona. From 2003 to 2004, food samples were obtained from the retail commerce of the city (municipal markets, supermarkets, and grocery stores). Unpacked fresh products were obtained from a diversity of sources covering all city districts. Packed foodstuffs were selected to cover a variety of brands rather than different neighborhoods. Altogether, 291 samples pertaining to eight food groups were obtained for this project. They were classified in two groups, according to the most likely source of hydrocarbon pollution: environmental and technological. The environmental source group included 144 samples from fresh fish (27), cephalopods (44), bivalves (38), and crustaceans (35), while the technological source group included 147 samples from meat products (46), smoked fish (44), vegetable oils (40), and tea (17).

PAHs monitored. Following the regulation of PAHs in olive pomace oil implemented by the Government of Spain in 2001, the IQSA program included the control of eight PAHs in edible oils: benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[g,h,i]perylene, BaP, benzo[ghi]pyrene, dibenzo[a,h]anthracene, and indeno[1,2,3-c,d]pyrene. After a major oil spill (the sinking of the Prestige tanker) in 2002 in Galiza, a seafood-producing area in the northwest of Spain, monitoring was extended to seafood and fresh fish. Laboratory analyses were also extended to other food samples to obtain baseline data for the purpose of this study.

Analysis. The method used for PAH determination in food was developed in our laboratory and has been fully validated through parameters such as precision, recovery, and linearity; it was accredited by the National Accreditation Body (ENAC [Entidad Nacional de Acreditacion]) in 2002. Prior to extraction with dichloromethane, samples were homogenized. Sample sizes were 4 g for oils and fats and 10 g for other foods. Minced samples were extracted with dichloromethane, except for oil and fat samples, which were only diluted in dichloromethane. Gel permeation chromatography was used as a clean-up method. Analyses of PAHs were carried out by high-performance liquid chromatography on a reverse-phase column (RP-18 column Vydac 210TP54, 250 by 4.6 mm, with a particle size of 5 μm) at 25°C in a Peltier furnace with a water-acetonitrile gradient elution program. The linear gradient went from 60 to 100% acetonitrile in 30 min and was kept at 100% for 10 min. Reference standards used were PAH-mix 13 and BaP from Dr. Ehrenstorfer (Wesel, Germany) and National Institute of Standards and Technology standard reference materials no. 2978 (freeze-dried mussels). Moreover, our laboratory has been taking part in the FAPAS proficiency test, participating in series 6 (environmental contaminants) for selected fats and fatty foods since 2003.

A fluorescence detector programmed to different wavelengths of excitation and emission based on the time of elution was used to optimize the chromatographic results. Concentrations were ranked from the quantification limit (1 μg/kg) to 10 μg/kg. Samples with higher concentration values were fitted to these ranks by performing corresponding dilutions. Concentrations above 1 μg/kg were confirmed by analyzing the fluorescence emission spectrum. The limit of quantification (LOQ) was determined on different types of samples (e.g., oil, seafood, sausage, smoked fish). Six blank samples were spiked at a level of 1 μg/kg of each PAH. Recovery values at this level were between 60 and 80%, and precision values met Horwitz criteria. The limit of detection was calculated as one third of the LOQ and was checked chromatographically, obtaining a signal-to-noise ratio of over 3.

Statistical procedures. This is essentially a descriptive study. Standard descriptive quantitative methods (frequencies, means and standard deviations, and maximum levels) were calculated for each compound as well as for the sum of all eight PAHs. A concentration equal to zero was assumed for samples with PAH levels below the LOQ. Because of the number of negative results and because of the sensitivity of means to extreme values, we also grouped the proportion of samples within given concentration ranks.

RESULTS

Overall detection of PAHs in the samples was around 13%, and distribution by food groups is shown in Table 1. The highest percentage of total PAHs was found in tea samples (94%) and bivalves (34%). No sample of fish (either fresh or smoked) had measurable levels of PAHs. Benzo[e]pyrene and benzo[b]fluoranthene were the PAHs most frequently detected in the PAH-positive samples: 12 and 10%, respectively. Dibenzo[a,h]anthracene was the least frequent (1%). Table 2 shows that levels of PAHs in these samples were relatively low. The highest mean value was found in the tea group, with 59 μg/kg (maximum level of 188.6 μg/kg), followed by bivalves with 2.7 μg/kg (maximum level of 24.9 μg/kg) and meat products with 1.7 μg/kg (maximum level of 21.7 μg/kg). As can be seen, tea samples had much higher levels of total PAHs than the rest of the food groups. None of the food groups subject to regulation had values above the currently accepted levels of BaP. The food group with highest mean BaP value was bivalves, because of a single high value of 5.2 μg/kg in a mussel sample.

When the potential source of PAH contamination was considered, the distribution of PAH compounds varied among food groups (Table 3). Both in the environmental source group and the technological source group, benzo[e]pyrene was the most frequently detected PAH, and it also had the highest mean levels. On the other hand, BaP was the second most frequent PAH in the technological source group, but it was less present among foods susceptible to contamination from an environmental source. Up to 10% of the environmental source group samples had detectable levels of PAHs, with a mean level of 0.9 μg/kg, while 16% of the technological food samples had a median level of 7.6 μg/kg.

DISCUSSION

Fish and other seafood are exposed to a wide range of PAHs, mainly from oil spills at sea. However, fish have a mixed-function oxidase enzyme system that is more effec-
TABLE 1. Proportion of food samples with presence of selected polycyclic aromatic hydrocarbons (PAHs) by food group, Barcelona, 2003 to 2004

<table>
<thead>
<tr>
<th>Food groups (no. of samples)</th>
<th>Fish</th>
<th>Crustaceans</th>
<th>Cephalopods</th>
<th>Bivalves</th>
<th>Smoked fish</th>
<th>Meat products</th>
<th>Edible oils</th>
<th>Tea</th>
<th>All groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n = 27)</td>
<td></td>
<td>(n = 35)</td>
<td>(n = 44)</td>
<td>(n = 38)</td>
<td>(n = 44)</td>
<td>(n = 46)</td>
<td>(n = 40)</td>
<td>(n = 17)</td>
<td>(n = 291)</td>
</tr>
<tr>
<td>Benzo[a]pyrene (BaP)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>11</td>
<td>0</td>
<td>11</td>
<td>3</td>
<td>88</td>
<td>9</td>
</tr>
<tr>
<td>Benzo[a]anthracene (BaA)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>13</td>
<td>0</td>
<td>82</td>
<td>9</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene (DbahA)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene (BbF)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>26</td>
<td>0</td>
<td>4</td>
<td>3</td>
<td>94</td>
<td>10</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene (BkF)</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>13</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>82</td>
<td>8</td>
</tr>
<tr>
<td>Benzo[e]pyrene (BeP)</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>32</td>
<td>0</td>
<td>11</td>
<td>3</td>
<td>94</td>
<td>12</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene (BghiP)</td>
<td>0</td>
<td>3</td>
<td>2</td>
<td>13</td>
<td>0</td>
<td>9</td>
<td>3</td>
<td>82</td>
<td>9</td>
</tr>
<tr>
<td>Indeno[1,2,3-c,d]pyrene (IP)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>53</td>
<td>4</td>
</tr>
</tbody>
</table>

Total PAHs

0% 3% 2% 34% 0% 13% 3% 94% 13%

The limit of quantification (LOQ) for all compounds was 1 ppb or µg/kg.

TABLE 2. Distribution of total polycyclic aromatic hydrocarbons (PAHs) and benzo[a]pyrene (BaP) levels by food group, Barcelona, 2003 to 2004

<table>
<thead>
<tr>
<th>Food group (no. of samples)</th>
<th>Mean ± SD (µg/kg)</th>
<th>Maximum value (µg/kg)</th>
<th>No. (%) of samples with values within the concn ranks</th>
<th>&lt;LOQ</th>
<th>1–5 ppb</th>
<th>5–10 ppb</th>
<th>10–20 ppb</th>
<th>20–40 ppb</th>
<th>&gt;40 ppb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total PAHs</td>
<td>Fish (n = 27)</td>
<td>&lt;LOQ &lt;LOQ</td>
<td>27 (100) -- -- -- --</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crustaceans (n = 35)</td>
<td>0.3 ± 1.8</td>
<td>10.5</td>
<td>34 (97)</td>
<td>--</td>
<td>--</td>
<td>1 (3)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Cephalopods (n = 44)</td>
<td>0.3 ± 1.8</td>
<td>12</td>
<td>43 (98)</td>
<td>--</td>
<td>--</td>
<td>1 (2)</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Bivalves (n = 38)</td>
<td>2.7 ± 5.8</td>
<td>24.9</td>
<td>25 (66)</td>
<td>7 (18)</td>
<td>1 (3)</td>
<td>4 (11)</td>
<td>1 (3)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Smoked fish (n = 44)</td>
<td>&lt;LOQ &lt;LOQ</td>
<td>44 (100)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Meat product (n = 46)</td>
<td>1.7 ± 4.8</td>
<td>21.7</td>
<td>40 (87)</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>3 (7)</td>
<td>1 (2)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Edible oils (n = 40)</td>
<td>0.7 ± 4.8</td>
<td>30.6</td>
<td>39 (98)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>1 (3)</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Tea (n = 17)</td>
<td>59 ± 51</td>
<td>188.6</td>
<td>1 (6)</td>
<td>1 (6)</td>
<td>--</td>
<td>4 (24)</td>
<td>--</td>
<td>11 (65)</td>
</tr>
<tr>
<td>BaP</td>
<td>Fish (n = 27)</td>
<td>&lt;LOQ &lt;LOQ</td>
<td>27 (100) -- -- -- --</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crustaceans (n = 35)</td>
<td>&lt;LOQ &lt;LOQ</td>
<td>35 (100)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Cephalopods (n = 44)</td>
<td>0.05 ± 0.35</td>
<td>2.3</td>
<td>43 (98)</td>
<td>1 (2)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Bivalves (n = 38)</td>
<td>0.26 ± 0.95</td>
<td>5.2</td>
<td>34 (90)</td>
<td>3 (8)</td>
<td>1 (3)</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Smoked fish (n = 44)</td>
<td>&lt;LOQ &lt;LOQ</td>
<td>44 (100)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Meat product (n = 46)</td>
<td>0.24 ± 0.7</td>
<td>2.8</td>
<td>41 (89)</td>
<td>5 (11)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Edible oils (n = 40)</td>
<td>0.03 ± 0.19</td>
<td>1.2</td>
<td>39 (98)</td>
<td>1 (3)</td>
<td>--</td>
<td>--</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td></td>
<td>Tea (n = 17)</td>
<td>10.25 ± 9.05</td>
<td>30.4</td>
<td>2 (12)</td>
<td>4 (24)</td>
<td>3 (18)</td>
<td>5 (29)</td>
<td>3 (18)</td>
<td>--</td>
</tr>
</tbody>
</table>

The limit of quantification (LOQ) for all compounds was 1 ppb or µg/kg.
TABLE 3. Mean level and proportion of samples with detectable concentrations of selected polycyclic aromatic hydrocarbons (PAHs) by likely source of contamination, Barcelona, 2003 to 2004.a,b

<table>
<thead>
<tr>
<th>Technological source (n = 147)</th>
<th>Environmental source (n = 144)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (µg/kg)</td>
<td>%</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>1.5</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>1.2</td>
</tr>
<tr>
<td>Benzo[k]anthracene</td>
<td>1.0</td>
</tr>
<tr>
<td>Benzo[b]fluoranthene</td>
<td>1.4</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>1.0</td>
</tr>
<tr>
<td>Benzo[k]fluoranthene</td>
<td>0.6</td>
</tr>
<tr>
<td>Indeno[1,2,3-c,d]pyrene</td>
<td>0.6</td>
</tr>
<tr>
<td>Dibenz[a,h]anthracene</td>
<td>0.1</td>
</tr>
<tr>
<td>Total PAHs</td>
<td>7.6</td>
</tr>
</tbody>
</table>

a The limit of quantification (LOQ) for all compounds was 1 ppb or µg/kg.

b Environmental group includes food groups polluted by the environment (basically the marine ecosystem), such as fresh fish, cephalopods, bivalves, and crustaceans; technological group includes foods that have undergone treatments that potentially produce PAHs, such as meat products, smoked fish, vegetable oils, and tea.

Inferences drawn from comparisons must thus be taken with caution.

We would expect the presence of PAHs in smoked fish: the traditional process for smoking fish products involves the treatment of presalted, whole, eviscerated, or filleted fish with wood smoke, which contains a large number of PAHs. A relatively new alternative for traditional smoking is the use of smoke flavors, which have been produced commercially since the mid-20th century. These are generally smoke extracts that have been filtered and separated from the resinous material that contains most of the PAHs (25), which could explain the absence of PAHs in smoked fish samples in the present study. Much in the same way, meat products may also be exposed to different smoking (24) or drying processes: our results suggest that actual smoking is infrequent (1.7 ppb and nearly 90% not quantified).

Tea leaves have a high surface area; hence, they may accumulate PAHs, especially from air. Moreover, the drying or roasting process may increase their PAH content (17). A Chinese study that analyzed green and black tea leaves for 16 different PAHs also found high levels (497 to 1,048 µg/kg) (10). One should keep in mind that despite these results for tea leaves, tea is consumed in dilution. Another study in Spain determined PAH contents in tea samples that had been prepared as infusions: PAHs were present, but concentrations were only a few nanograms per liter (or parts per ton) (15).

Edible fats and oils are usually considered a potential source of PAHs in the diet because of their lipophilic nature and their importance in the diet (12, 19). In the 2000 total diet study performed in the United Kingdom, fats and oils were among the food groups with highest concentrations of PAHs, with an average value of 11.05 µg/kg (11). BaP concentrations in food samples collected across the EU in 2004 reached levels of 17.7 µg/kg for olive pomace oil and 3.12 µg/kg for sunflower oil. Other studies report PAH levels up to 19.8 µg/kg for olive oil and 1.7 µg/kg for sunflower oil (4). Our data show less contamination in these food groups (mean value of 0.7 µg/kg). For total PAHs, only olive pomace oil is regulated in Spain (with the limit set at 5 µg/kg). In our data, a single sample of olive oil (not olive pomace oil) had detectable levels of PAHs with a concentration of 30 µg/kg, clearly not an acceptable level. The occurrence of PAHs in edible oils is attributed mainly to the environmental contamination of the vegetable raw material, but it is also attributed to the contamination from some processing stages, such as seed drying when gases from direct combustion contribute to the contamination. Fortunately, PAH contamination of oils can be reduced to a great extent during the refining process, although these treatments do not affect all PAHs in the same way (12). The deodorization process seems to have little effect on high molecular PAHs and removes mainly light PAHs (up to four aromatic rings), while more highly condensed heavy PAHs, such as BaP, need charcoal treatment to be removed.

There have been proposals to use BaP as a global marker for PAH contamination of food (23). The low presence of BaP in food samples in which PAHs were most

likely of environmental origin (fish and seafood) suggests that it is not a good indicator for this source of PAHs, although it may be a good tracer for PAHs generated during technological food processing. This issue requires further study.

In conclusion, this study found indicators of PAH contamination in food samples marketed in Barcelona, with higher levels found in tea and bivalves. However, most food samples were free from PAH contamination, and detected levels were in the lower range. As single markets in the EU are a reality, they reflect not only local conditions, but also what may be expected in other parts of the EU. These findings contribute to the baseline data, providing further knowledge of PAH levels in food, and thus follow a recommendation of the European Commission (4 February 2005). They also suggest that BaP is not a good tracer for PAH contamination of fish and seafood originating from environmental sources.

ACKNOWLEDGMENT
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REFERENCES